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# Toward the development of predictive systems ecology modeling: MetaConnect and its use as an innovative modeling platform in theoretical and applied fields of ecological research

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# Summary/Sommaire:

# Figure, table and equation tables/Sommaire des figures, tableaux et équations :

## Figures:

## Tables/Tableaux :

## Equations:

## Abstract:

In a context of global change, scientists and policy-makers require tools to address the issue of biodiversity loss. Population viability analysis (PVA) has been the main tool to anderstand and plan for this problem. However, the tools developed during the 90s poorly integrate recent scientific advances in landscape genetics and dispersal. Here, I developed a flexible and modular modelling platform for PVA that addresses many of the limitations of existing software and in this way answer the call made by Evans et al. (2013) for predictive systems ecology models. MetaConnect is an individual-based, process-based and PVA-based modelling platform which could be used as a research or a decision-making tool. In my thesis, I present the modeling base core of MetaConnect and its validation and then present different uses of this plateform in theoretical and applied ecology.

## Résumé :

Dans un contexte de changements globaux, les scientifiques et les législateurs requièrent des outils leur permettant de traiter la question de la perte de biodiversité. L'analyse de viabilité de population (PVA) est l'outil principal pour traiter le problème. Cependant, les outils développés dans les années 90 n'intègrent que très peu les récents progrès réalisés en génétic du paysage et sur la compréhension de la dipsersion. Ici, j'ai développé une plateforme de modélisation flexible et modulaire pour réaliser des PVA qui palie à la plupart des limitations des logiciels existants et répondant de ce fait à l'appel fait par Evans et al. (2013) pour développer des modèles prédictifs des systèmes écologiques. MetaConnect est un modèle individu centré, basé sur le déroulement des processus biologiques et principalement basé sur la réalisation d'analyses de viabilités qui peut être utlisé à la fois comme un outil de recherche ou d'aide à la décision. Dans ma thèse, je présente le module central de MetaConnect et sa validation puis présente différentes application de cette plateforme à des fins théoriques et appliquées.

# Introduction

"***Global change*** *refers to planetary-scale changes in the Earth system. The system consists of the land, oceans, atmosphere, poles, life, the planet's natural cycles and deep Earth processes. These constituent parts influence one another. The Earth system now includes human society, so global change also refers to large-scale changes in society (International Geosphere-Biosphere Programme 2010). More completely, the term "global change" encompasses: population, climate, the economy, resource use, energy development, transport, communication, land use and land cover, urbanization, globalization, atmospheric circulation, ocean circulation, the carbon cycle, the nitrogen cycle, the water cycle and other cycles, sea ice loss, sea-level rise, food webs, biological diversity, pollution, health, over fishing, and more (Steffen et al. 2004).*"

The global change concept is recent (1980) and was initially introduced by climatologists who studied if the climate was changing, if this change was predictable and if humans are responsible for such a change (Bolin 1970; Bolin and Bischof 1970; Seiler and Crutzen 1980; Bruhl and Crutzen 1988; Crutzen and Andreae 1990). Human induced climate change as a scientific field has generalized to other scientific fields under the term of "global change". Global change concerns any change that occurs at the Earth scale under anthropogenic pressure (Wikipedia). The consequences of global change often represent an important direct and indirect risk for public health and/or population safety (Chan et al. 1999; Patz et al. 2000; Gubler et al. 2001; Reiter 2001; Rose et al. 2001; Walther et al. 2002; Townsend et al. 2003; Watson et al. 2005; Reaser et al. 2007; Lenton et al. 2008; Myers and Patz 2009; Nicholls and Cazenave 2010; Mimura 2013). Among the fields encompassed by the term 'global change', biodiversity loss is one of the less understood and only has recently been studied (Barbrault 2005; Cardinale et al. 2012). Habitat loss and habitat fragmentation in relation to human activities have become the major threats to biodiversity (IUCN 2013). Over the last centuries, habitat conversion from forest to agriculture has taken over large amounts of lands, leaving species with a shrinking world. This conversion has accelerated over the past decades, with tropical forest being destroyed at annual rates between 1 and 4% of their current areas (Dobson et al. 1997). This habitat destruction is believed to be a major cause of species extinction (Dobson et al. 1997). Habitat fragmentation, another side-effect of human activity, also has profound repercussions on species extinction (Fahrig 2003). Events and international structures like the Earth Summit in Rio de Janeiro (1992) or the Convention on Biological

Diversity, encourage awareness in politics and the public about the risks linked to biodiversity loss (Barbrault 2005; Cardinale et al. 2012). Such awareness has led supra-national and national stakeholders to produce legislative and operational tools to face the loss of biodiversity. In the European Union (EU), legislative tools are represented by directives that protect species and their habitat (European directives CEE 1979; 1992), and the operative tools consist on granting biodiversity conservation programs (LIFE, LEADER), and endowed the EU with a reserve site network (Natura 2000 network).

Habitat destruction and fragmentation has consequences from the ecosystem scale (Fahrig 2003; Cardinale et al. 2012; de Mazancourt et al. 2013) to the genetic scale (Ingvarsson 2001; Baguette et al. 2013b). Habitat fragmentation modifies landscape patterns in a four-step process: reduction in habitat ara, increase in number of habitat patches, decrease in size of habitat patches and increase in isolation of patches (Fahrig 2003). This alteration of landscape patterns has diverse effects on population dynamics. As patches become smaller, the size of the population supported decreases; this can increase the stochastic risk of extinction from demographic processes (Legendre et al. 1999; Reed et al. 2002), but also from genetic stochasticity: small populations are more subjected to risks of inbreeding depression (Brook et al. 2002b), loss of genetic diversity and mutation accumulation (Rowe and Beebee 2003). Moreover, by increasing the distances between patches and therefore the risks of dispersal, landscape fragmentation prevents individuals from moving from one population to another. This can hinder the recolonisation of patches where sub-populations have become extinct, leading to stochastic extinction of metapopulations (Fahrig 2003). Furthermore, by reducing gene flow between populations, isolation can lead to genetic structuring of sub-populations and impede genetic rescue of populations with high levels of inbreeding (Ingvarsson 2001; Keller and Waller 2002; Tallmon et al. 2004).

To assist policy makers and landscape planners, ecologist have developed models in order to rationalize conservation planning by conducting predictive studies following three main axes (Hanski and Gilpin 1997):

1. species survival: this approach consists of evaluating the extinction or quasi-extinction probability for a given species. The population viability analysis (PVA) is focused on a species' population dynamic and aims at estimating whether a species will survive in a given population or landscape (Lacy 1993; Hamilton and Moller 1995; Southgate and Possingham 1995; Brook et al.

1997; Beissinger and Westphal 1998; Letcher et al. 1998; Brook et al. 1999; Legendre et al. 1999; Scheller and Mladenoff 2004; Schtickzelle and Baguette 2004). This approach usually devotes little attention to the possible genetic complications that can occur with increasing inbreeding (Brook et al. 2002b) or linked to altered meta-population functioning and its demographic and genetic consequences (but see Lindenmayer et al. 1995; Schtickzelle and Baguette 2004).

2. population genetic structuring: PVA based on genetic viability assumes that inbreeding is deleterious to the individuals and must be avoided (Lande 1995; Grimm et al. 2004; Tallmon et al. 2004; Landguth and Cushman 2010). Indeed, increasing inbreeding is assumed to decrease population adaptability rendering the populations more sensitive to diseases and variations in their environments (Frankham 1998; Reed et al. 2002; Frankham 2005). Theoretical studies show that inbreeding may play a major role in species extinction (Lande 1995). However, the analysis of field data has shown that even though inbreeding plays a role in population viability, this role seems to be minor for most (non-island) populations (Frankham 2005; Pertoldi et al. 2007; Radwan et al. 2010). Indeed, the impact of inbreeding may occur at a long temporal scale (Keller and Waller 2002; Robert et al. 2004; Frankham 2005) and the impact on the immune system and its link to population extinctions remains uncertain (Frankham 2005; Radwan et al. 2010).
3. metapopulation functioning: this axis of PVA development is focused on the fluxes of individuals between patches of a meta-population. This axis takes care of the fact that in a given landscape, some suitable habitat may be sometimes empty of individuals (Hanski and Gilpin 1997). Based on a PVA focused on a single patch approach, this means that the population is extinct. However, individuals coming from other patches near by may colonize or reinforce populations of empty or nearly empty patches (Doak and Mills 1994). Similarly, genetic variability in a single patch may be maintained by individual flow coming from the surrounding patches (Hastings and Harrison 1994; Tallmon et al. 2004). Such a system may lead to cases where persistence of a species cannot be insured by a single patch but requires by a patch network (Hanski and Gilpin 1997; Pritchard et al. 2000).

During the 90s, decision making tools were developed on the basis of PVA models mostly build with matrix based models (Box A) (Caswell 2001; Pe'er et al. 2013). Several software packages like ALEX, RAMAS, GAPPS, INMAT, ULM or VORTEX were developed as generic tools to perform PVA analysis (Harris et al. 1987; Akçakaya and Ferson 1990; Lacy 1993; Akçakaya 1994; Mills and Smouse 1994; Legendre and Clobert 1995; Akçakaya et al. 2003) and used by the IUCN experts to determine species' threat level (Brook et al. 1997). In the 90s matrix based models were preferred to more complex and realistic individual processes based models due to the computational requirement of such models (Hanski and Gilpin 1997), although a few relational generic models were developed (ULM, VORTEX) (Box A). At the time of very limited computational power, matrix based models permitted to work at the population level using appropriate and powerful mathematical tools (Leslie 1945; Ferriere et al. 1996; Caswell 2001). Nowadays, even though matrix based models are still a very widespread tool, the development of computational power allows building more realistic models based on individual characteristics and behavior (individual based model: IBM) driven by biological processes (process based model) that can impact the global population/meta-population persistence and evolution (Doebeli and Koella 1994; Lindstrom and Kokko 1998; Doebeli and de Jong 1999; Hochberg et al. 2003; Sinervo and Clobert 2003; Sinervo and Calsbeek 2006; Cote and Clobert 2007; Duckworth 2009). The ability of integrating different components of individuals characteristics (i.e. the use of IBM) to perform PVA (Pe'er et al. 2013) permit to better understand how individual characteristics and behavior affect a species persistence and to elucidate some links between individual life history traits (LHTs) and their impact at the population level (Figure 1) (Travis and Dytham 1999; Travis et al. 1999; DeAngelis and Mooij 2005; Moulherat et al. submitted-b). However, if IBMs have permitted a better understanding of key processes involved in PVAs, they have also led to a high number of models with their specific output and metrics that are usually hard or impossible to compare with other models (DeAngelis and Mooij 2005; Kindlmann and Burel 2008; Pe'er et al. 2013). Such diversity could be a major constraint on the use of IBMs to perform PVAs and integrate the results into conservation planning because:

1. Conservation managers and policy makers have difficulties to find the best model for the question they seek to address (ITTECOP 2013)
2. It is recommended to use PVAs by making output comparison under different scenarios (scenarios of modeling assumptions) rather than absolute results of each independent scenario that may require multiple models with comparable outputs

(Brook et al. 1997; Burgman and Possingham 2000; Pe'er et al. 2013). However, usually, model outputs are specific to the software used and few software packages are flexible enough to allow the building of various scenarios (Brook et al. 1997; Grimm et al. 2004; DeAngelis and Mooij 2005; Pe'er et al. 2013).

3. IBMs require highly detailed information and a strong understanding of the model structure, applicability and limits which are often poorly detailed (DeAngelis and Mooij 2005; Grimm et al. 2006; Urban et al. 2009; Pe'er et al. 2013) as well as a solid theoretical background to limit misinterpretations of results (Ferriere et al. 1996; Burgman and Possingham 2000; Caswell 2001; Urban et al. 2009).

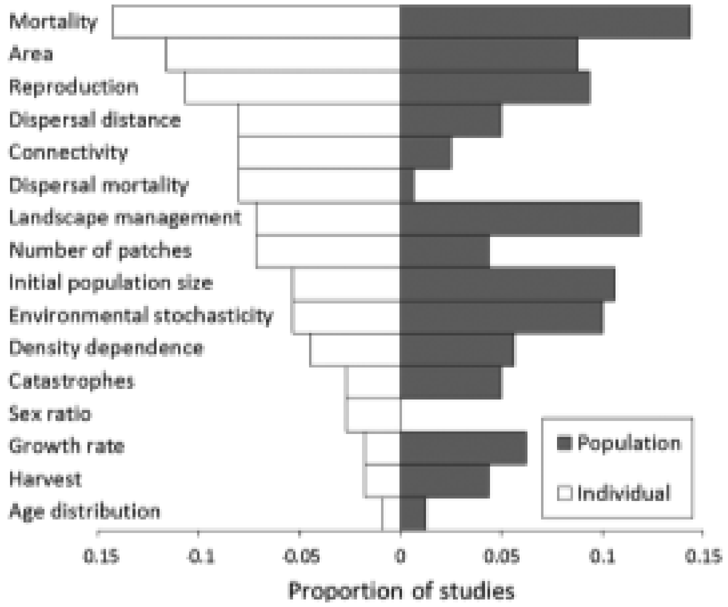

Figure 1: The frequency of papers reviewed in the SCALES project that explored the effect of key parameters on viability on the basis of individual-based (white) or population-based (shaded) models (Pe'er et al. 2013).

Box A: Matrix based model or relational models

Matrix based models are suitable for any system that can be represented as a graph (sensu Euler 1741 figure X.a) and used in population dynamics to model at the population scale the summary of individual characteristics (Caswell 2001). This approach was initially developed to work on age-structured population to outperform life table analysis (Leslie 1945). This framework permits numerous developments (two sex models, meta-population models … see Caswell 2001 for details and other developments) under an efficient computational environment. This approach is mainly driven by the definition of a transition matrix $A$ that will determine the population structure ($N$) at $t+1$ (Leslie 1945; Caswell 2001).

(A)

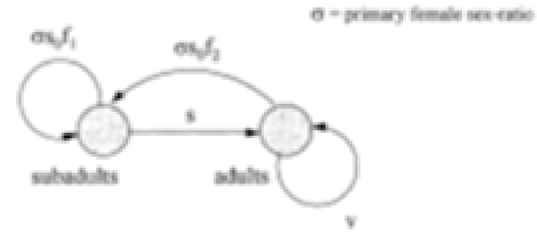

Figure A.1: Two age-class, one sex life-cycle graphs for passerines where $s_0$ is the juvenile survival rate, $s$ the subadult survival rate, $v$ the adult survival rate, $f_1$ the subadult fecundity, $f_2$ the adult fecundity and $\sigma$ the primary female sex-ratio (adapted from Legendre et al. 1999)

However, while any matrix based model can be translated into relation based models; all relation based models are not likely to be translated into matrix based models (Figure A.2). Indeed, all systems cannot be represented as a graph (i.e. mating systems based on individual characteristics, interactions genotype/phenotype …). In that sense, relational models are more flexible by their structure but require more computational power (DeAngelis and Mooij 2005; Pe'er et al. 2013) and many mathematical tools as sensitivity analysis cannot be used (Cross and Beissinger 2001; Naujokaitis-Lewis et al. 2009).

(B)

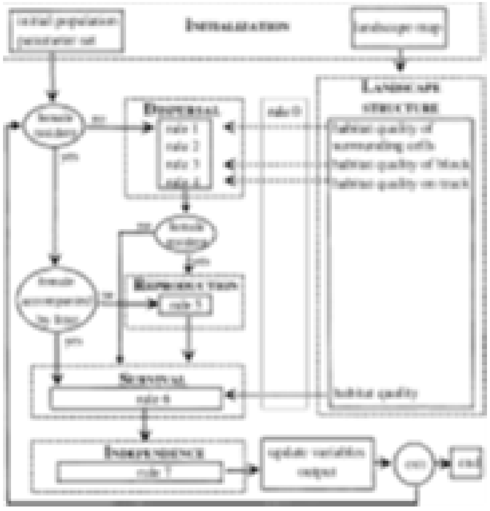

Figure A.2: Flow chart for the individual-based simulation model of a bear population (Wiegand et al. 1999). The flow chart shows each of the events that can happen to a female bear during the yearly step of the model: dispersal, reproduction, survival, and independence (from the mother). Each of these events is governed by one or more rules not shown here (adapted from DeAngelis and Mooij 2005).

Currently many non-generic models are built with a mixture of matrix based and relation based models especially in order to build non-linear and/or time variant $A$.

Currently, IBMs to perform PVAs have limited flexibility that restrains their application to the scientific field in which they were developed (DeAngelis and Mooij 2005) and models that deal with demography have little way of incorporating genetic factors and dispersal behavior into their analysis. Models based on genetic PVAs (e.g. SPLATCHE (Currat et al. 2004), CDPOP (Landguth and Cushman 2010), META-X (Grimm et al. 2004)) assume over-simplified demographic systems (Baguette et al. 2013b; Pe'er et al. 2013). The main goal of my PhD was to develop a generic IBM, that allows an integration of a large array of demographic, genetic and dispersal processes. In this respect, the idea was to develop a core base computational module based on a demographic PVA model where one could develop and plug in modules for genetic system, dispersal, or other features and then output results that contain similar metrics to permit comparisons between models, situations, scenarios, and so on (Figure 2). Since PVA models aims to determine extinction or quasi-extinction probabilities of a population, they have to simulate the population dynamic at a selected time horizon. This means that PVA models are population dynamic models with a specific output concerning extinction probability that can be ignored or removed for other purposes. The objective of this model is to be a modeling platform (MetaConnect) that could satisfy the needs of researchers from different disciplinary fields (conservation biology, landscape ecology, landscape genetic, evolutionary biology…) as well as conservation managers, policy makers and planning managers that also could be upgraded to incorporate new relevant modules and methods. In this respect, I will present in the first chapter the core base modeling structure of MetaConnect. Then the second chapter is devoted to the use of MetaConnect in evolutionary biology. The third chapter deals with the applied use of MetaConnect with respect to conservation programs. The use of MetaConnect in applied regulatory studies is showed in chapter four. The Fith chapter is focused on complementary studies that could allow “feeding” MetaConnect. Indeed, as MetaConnect is an IBM, it requires considerable input data that are mostly easy to obtain through the available literature. However other variables, such as maximum distance dispersal of species, which are known to be a critical to (met-)population dynamics can be explored through model sensitivity. In other words, a small variation of this variable may induce a large change in the simulation outcome (Ruckelshaus et al. 1997; Tischendorf 2001).

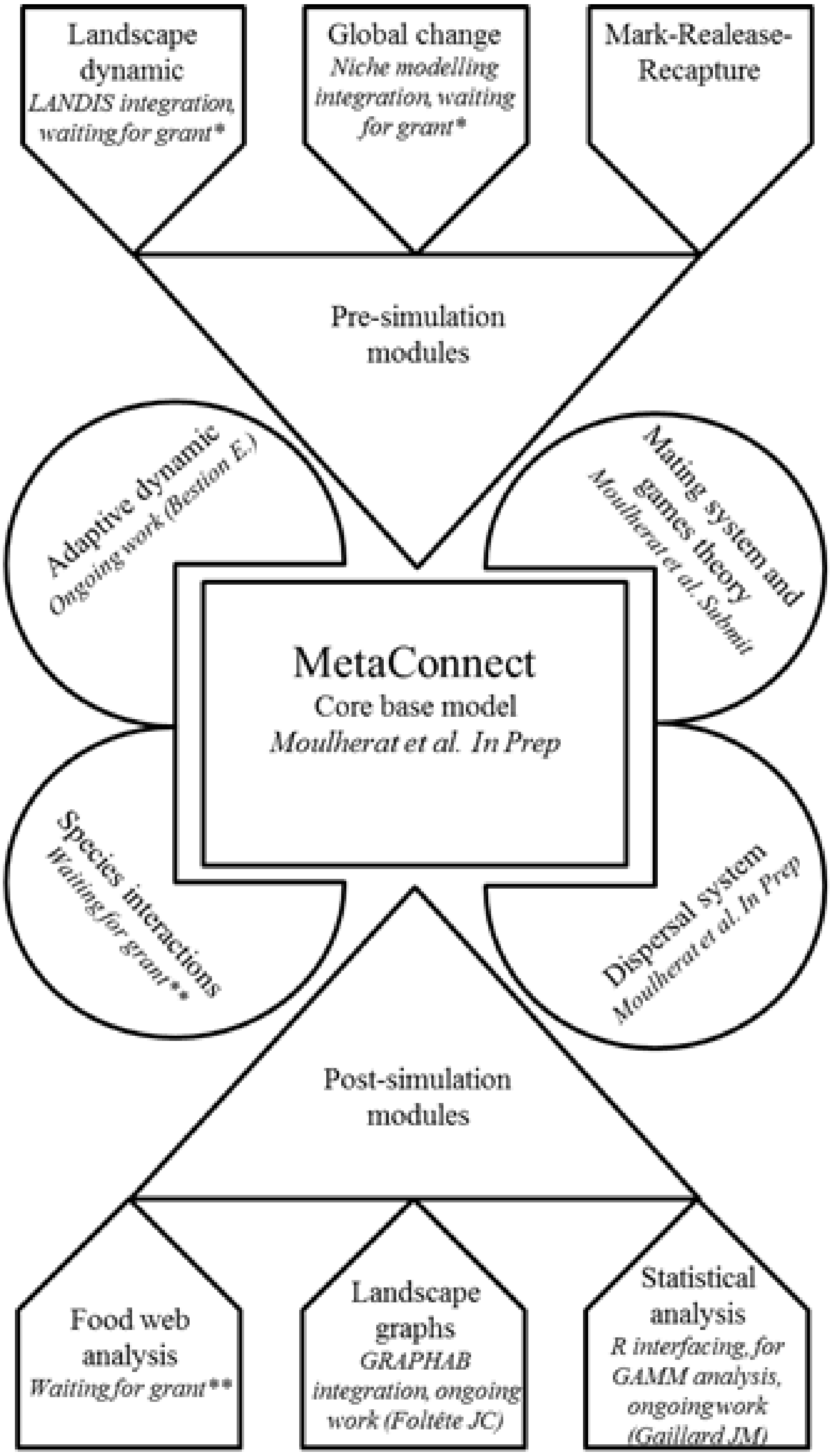

Figure 2: MetaConnect assemblage. The Figure presents the core based PVA model (Moulherat et al. submitted-a) with examples of additional plugins that increase and complete the core base model flexibility and applicability (Moulherat et al. submitted-a; Moulherat et al. submitted-b). Pre and post-simulation modules will allow using previously developed

models such as LANDIS (Scheller and Mladenoff 2004), GRAPHAB (Foltete et al. 2012) or n_w (Gauzens et al. 2013) and an interface to use R (R Development Core Team 2005).

[*] Bio-SUMCOSA project submitted to FRB (Fondation pour la Recherche pour la Biodiversité) 2013 grant.

[**] MetaSystem project submitted for an Eco-Innov 2013 grant (Regional Midi-Pyrénées grant).

*« **Les changements globaux** font références à des modifications systémiques à l'échelle planétaire. Les systèmes généralement considérés sont la vie des terres, des océans, de l'atmosphère, des pôles, mais aussi les cycles naturels planétaires ou les processus de la dynamique du manteau terrestre, ces différents éléments s'influençant les uns les autres. Les systèmes planétaires incluent dorénavant la société humaine. Les changements globaux font donc aussi références aux modifications des modifications sociétales à large échelle (International Geosphere-Biosphere Programme 2010). Plus complètement, le terme « changement global » recouvre les thématiques de : population, climat, économie, gestion des ressources, développement énergétique, transports, communication, utilisation des sols et végétation, urbanisation, globalisation, circulation atmosphérique, circulation des océans, cycle du carbone, cycle de l'azote, cycle de l'eau et autres cycles, disparition des banquises, niveau des mers, chaine alimentaires, diversité biologique, pollution, santé, surpêche, et bien d'autres (Steffen et al. 2004) . »* (Wikipedia).

Le concept de changement global est récent (1980) et a initialement été introduit par les climatologistes qui cherchaient à déterminer si le climat changeait, si ces changements étaient prévisibles et si l'Homme avait une responsabilité dans ces changements (Bolin 1970; Bolin and Bischof 1970; Seiler and Crutzen 1980; Bruhl and Crutzen 1988; Crutzen and Andreae 1990). La conception initiale du réchauffement globale d'origine anthropique a de nos jours été transposé à toutes les disciplines scientifiques sous le terme générique de « changement global ». Le terme de changement global, est utilisé pour toute modification d'origine anthropique ayant des répercussions à l'échelle planétaire (Wikipedia). Les conséquences d'un changement global représentent généralement un risque direct ou indirect sur la santé publique et/ou la sécurité des populations humaines (Chan et al. 1999; Patz et al. 2000; Gubler et al. 2001; Reiter 2001; Rose et al. 2001; Walther et al. 2002; Townsend et al. 2003; Watson et al. 2005; Reaser et al. 2007; Lenton et al. 2008; Myers and Patz 2009; Nicholls and Cazenave 2010; Mimura 2013). Parmi les thématiques scientifiques couvertes par le terme de changement global, l'érosion de la biodiversité est l'un des moins bien étudiés et des plus récemment perçu comme une préoccupation majeure (Barbrault 2005; Cardinale et al. 2012). La destruction et la fragmentation des habitats liés à l'activité humaine sont devenues les principales menaces pour la biodiversité (IUCN 2013). Durant les derniers siècles, l'extension des terres agricoles au détriment des espaces forestiers a contraints les espèces dans des espaces de plus en plus restreints. Cette conversion des espaces c'est accélérée au cours des dernières décennies avec notamment une destruction des forêts tropicales à un taux annuel moyen de 1 à 4% de leurs surfaces actuelles (Dobson et al. 1997). La fragmentation des habitats, un autre effet collatéral de l'activité humaine, a aussi des répercussions profondes sur les extinctions d'espèces (Fahrig 2003). Des évènements et des structures internationales tels que le « *Earth Summit* » à Rio de Janeiro (1992) ou la « *Convention on Biological Diversity* », participent à la sensibilisation du grand publique et des politiques aux problématiques liées à la perte de biodiversité (Barbrault 2005; Cardinale et al. 2012).Une telles prise de conscience a conduit les parties prenantes nationales et supranationales à se doter d'outils législatifs et opérationnels pour lutter contre l'érosion de la biodiversité. En Europe (EU), les outils législatifs sont représentés par les directives qui protègent directement les espèces et leurs habitats (European directives CEE 1979; 1992), et d'outils opérationnelles consistant à financer des projets de conservation (LIFE, LEADER), et se doter d'un réseau de sites protégés (réseau Natura 2000).

La destruction des habitats et leur fragmentation a des conséquences de l'échelle écosystémique (Fahrig 2003; Cardinale et al. 2012; de Mazancourt et al. 2013) à l'échelle génétique (Ingvarsson 2001; Baguette et al. 2013b). La fragmentation des habitats modifie le facies paysager *via* un processus en quatre étapes : réduction des surfaces habitables, augmentation du nombre de patches d'habitats, réduction de la taille des patches d'habitat et isolement des patches d'habitat (Fahrig 2003). Cette altération des paternes paysagers a diverses impactes sur les dynamiques populationnelles. Par la réduction des patches, la taille des populations y vivant se réduit ce qui peut conduire à une augmentation du risque d'extinction dues à la stochasticité démographique (Legendre et al. 1999; Reed et al. 2002), mais aussi dues à la stochasticité d'origine génétique : les populations les plus petites sont plus sujettes aux risques liés à la consanguinité (Brook et al. 2002b), la perte de diversité génétique et l'accumulation de mutations délétères (Rowe and Beebee 2003). De plus, en augmentant la distance entre les patches d'habitat, et par là même, la prise de risque pendant la dispersion, la fragmentation des habitats, limite les échanges d'individus entre patches d'habitat. Ceci peut raréfier la recolonisation des patches préalablement éteints augmentant les risques d'extinction stochastique de la metapopulation (Fahrig 2003). Qui plus est, en réduisant le flux d'individus entre les populations, l'isolement génétique peut mener à une structuration génétique qui limitera le potentiel de sauvetage génétique des populations fortement consanguines (Ingvarsson 2001; Keller and Waller 2002; Tallmon et al. 2004).

Afin d'assister le législateur et les aménageurs du territoire, les écologues ont développé des modèles visant à rationaliser les programmes de conservation en réalisant des études prospectives suivant trois axes principaux (Hanski and Gilpin 1997):

1. La survie des espèces : cette approche consiste à évaluer la probabilité d'extinction ou de quasi-extinction d'une espèce donnée. L'analyse de viabilité de population (PVA) est focalisée sur la dynamique populationnelle d'une espèce et cherche à prévoir si cette espèce parviendra à se maintenir dans un paysage donné (Lacy 1993; Hamilton and Moller 1995; Southgate and Possingham 1995; Brook et al. 1997; Beissinger and Westphal 1998; Letcher et al. 1998; Brook et al. 1999; Legendre et al. 1999; Scheller and Mladenoff 2004; Schtickzelle and Baguette 2004). Cette approche n'intègre généralement que peu ou pas les complications génétiques pouvant survenir pour des populations de petites tailles avec une forte consanguinité (Brook et al. 2002b) ou le lien avec le fonctionnement de la metapopulation et ses conséquences sur la démographie et la génétique (cependant voir Lindenmayer et al. 1995; Schtickzelle and Baguette 2004).
2. Structuration génétique des populations : les PVA basées sur la viabilité génétique supposent que la consanguinité est délétère pour la survie des individus et doit être évitée (Lande 1995; Grimm et al. 2004; Tallmon et al. 2004; Landguth and Cushman 2010). En effet, il est considéré que l'augmentation de la consanguinité réduit le potentiel adaptatif des populations les rendant plus vulnérable aux épidémies et variations de l'environnement (Frankham 1998; Reed et al. 2002; Frankham 2005). Des études théoriques ont montrées que la consanguinité pouvait jouer un rôle majeur dans l'extinction des espèces (Lande 1995). Cependant, l'analyse de données de terrain a montré que bien que la consanguinité joue un rôle dans la viabilité des populations, celui-ci est mineur dans la plupart des cas (excepté pour les systèmes insulaires) (Frankham 2005; Pertoldi et al. 2007; Radwan et al. 2010). En effet, l'impact de la consanguinité peut n'avoir une échéance qu'à long terme (Keller and Waller 2002; Robert et al. 2004; Frankham 2005) et son impact sur le système immunitaire ses implications sur l'extinction des populations reste incertain (Frankham 2005; Radwan et al. 2010).

3. fonctionnement des métapopulations : cet axe de développement des PVA est focalisé sur le flux d'individus entre les patches d'habitat favorable d'une métapopulation. Cet axe est attentif au fait qu'au sein d'un paysage donné, des habitats favorables sont parfois susceptible d'être inhabités (Hanski and Gilpin 1997). Une PVA basée sur une seule population, considèrerait que la population est éteinte. Cependant, des individus venant de patchs proches sont susceptible de coloniser ces patchs pour lesquels la population est éteinte ou de renforcer des populations en quasi extinction (Doak and Mills 1994). De façon similaire, la variabilité génétique peut être maintenue par le flux d'individus venant de patches alentours (Hastings and Harrison 1994; Tallmon et al. 2004). De tels fonctionnement peuvent mener à des cas où la persistance des espèces ne peut être assuré par une seule et unique population mais par un réseau de patch de populations (Hanski and Gilpin 1997; Pritchard et al. 2000).

Au cours des années 90, les outils d'aide à la décision ont été bâtis sur la base de modèle matriciels de PVA (Box A) (Caswell 2001; Pe'er et al. 2013). Des logiciels tels que ALEX, RAMAS, GAPPS, INMAT, ULM ou VORTEX ont été développés comme des outils de PVA génériques (Harris et al. 1987; Akçakaya and Ferson 1990; Lacy 1993; Akçakaya 1994; Mills and Smouse 1994; Legendre and Clobert 1995; Akçakaya et al. 2003) et utilisés par les experts de l'IUCN pour déterminer le statut de protection des espèces (Brook et al. 1997). Dans les années 90, les modèles matriciels étaient préférés à des modèles individu et processus centrés plus réalistes mais plus complexes en raison des limitations technologiques requises par de tels modèles (Hanski and Gilpin 1997) et seuls quelques modèles relationnels ont été développés (ULM, VORTEX) (Box A). A l'époque où la puissance de calcul était très limitée, les modèles matriciels ont permis de travailler à l'échelle populationnelles à l'aide d'outils mathématiques appropriés (Leslie 1945; Ferriere et al. 1996; Caswell 2001). De nos jours, bien que les modèles matriciels soient toujours très utilisés, l'augmentation de la puissance de calcul permet de construire des modèles plus réalistes basés sur les caractéristiques et comportements individuels des espèces (modèles individus centrés : IBM) sous tendus par des processus biologiques identifiés (modèle processus centré) qui peuvent affectés la persistance et l'évolution des populations/métapopulations à l'échelle globales (Doebeli and Koella 1994; Lindstrom and Kokko 1998; Doebeli and de Jong 1999; Hochberg et al. 2003; Sinervo and Clobert 2003; Sinervo and Calsbeek 2006; Cote and Clobert 2007; Duckworth 2009). La capacité d'intégration de différentes caractéristiques individuelles (i.e.

l’utilisation d’IBM) pour réaliser des PVA permet de mieux comprendre comment les caractéristiques et comportements individuels affectent la persistance des espèces (Pe'er et al. 2013) et de comprendre les liens existants entre traits d’histoire de vie (LHT) et leurs impacts à l’échelle populationnelle (Figure 1) (Travis and Dytham 1999; Travis et al. 1999; DeAngelis and Mooij 2005; Moulherat et al. submitted-b). Cependant, si les IBM permettent une meilleur compréhension des processus clés impliqués dans les PVA, ils mènent aussi à un nombre considérable de modèles avec leurs sorties et métriques spécifiques qui sont généralement difficiles voire impossible à comparer entre elles (DeAngelis and Mooij 2005; Kindlmann and Burel 2008; Pe'er et al. 2013). Une telle diversité peut être une contrainte à la généralisation des IBM pour réaliser des PVA et intégrer les résultats dans des programmes de conservation. Les raisons en sont :

1. Les gestionnaires de programmes de conservation et les législateurs ont des difficultés à trouver les modèles adaptés à leurs besoins (ITTECOP 2013).
2. Il est recommandé de réaliser des PVA en comparant les résultats issus de différents scenarios (scenarios d’hypothèses de modélisation) plutôt que les résultats absolus d’un unique scenario (Brook et al. 1997; Burgman and Possingham 2000; Pe'er et al. 2013). Cependant, généralement, les sorties de modèles sont spécifique à l’utilité du logiciel et seuls quelques rares logiciels sont suffisamment flexible pour réaliser des scenarios variés (Brook et al. 1997; Grimm et al. 2004; DeAngelis and Mooij 2005; Pe'er et al. 2013).
3. L’utilisation des IBM nécessitent une grande quantité de données détaillées et une solide compréhension de la structure des modèles, de leurs champs d’applications et de leurs limites qui sont généralement peut détaillés (DeAngelis and Mooij 2005; Grimm et al. 2006; Urban et al. 2009; Pe'er et al. 2013) ainsi que de solides bases théoriques pour limiter les interprétations des résultats erronées (Ferriere et al. 1996; Burgman and Possingham 2000; Caswell 2001; Urban et al. 2009).

Actuellement, les IBM existant pour réaliser des PVA ont une flexibilité limitée, ce qui restreint leur utilisation au champ disciplinaire scientifique pour lequel ils ont été conçu (DeAngelis and Mooij 2005) et les modèles traitant des aspects démographiques n’ont que peu de moyens d’intégrations des problématiques génétiques et de dispersion. Les modèles de PVA basés sur la génétique (SPLATCHE (Currat et al. 2004), CDPOP (Landguth and Cushman 2010), META-X (Grimm et al. 2004)) utilisent des présupposés démographiques présentant une simplification excessive (Baguette et al. 2013b; Pe'er et al. 2013). L’objectif principal de ma thèse a été de développer un IBM générique permettant une intégration large des processus démographique, génétique et de dispersion. Pour ce faire, l'idée a été de un corps de logiciel basé capable de réaliser des PVA et de greffer à ce corps des modules de génétique, de dispersion, mais aussi les modules d'analyse des résultats permettant la comparaison entre différents modèles et scenarios de modélisation (Figure 2). Comme les modèles de PVA ont pour objectif de déterminer les probabilités d'extinction ou de quasi extinction à un horizon donné, ils sont basés sur des modèles de dynamique de populations présentant une sortie spécifique permettant de calculer les probabilité d'extinction et qui, de fait peuvent être aisément ignorer ou supprimer pour des objectifs différents. L'objectif de mon modèle est de devenir une plateforme de modélisation (MetaConnect) satisfaisant à la fois les scientifiques de différentes disciplines (biologie de la conservation, écologie du paysage, génétique du paysage, biologie évolutive,…) aussi bien que les gestionnaires d'espaces naturels, les législateurs et les aménageurs du territoire et pouvant être mis à jour pour incorporer de nouveaux modèles et méthodes pertinents. Pour ce faire, je présente ici dans le premier chapitre, le corps de MetaConnect. Par la suite, le second chapitre est dédié à l'utilisation de MetaConnect en biologie évolutive. Le troisième chapitre quant à lui porte sur l'utilisation appliquée sur le terrain de MetaConnect dans des programmes de conservation. L'utilisation de MetaConnect dans les études réglementaire est traité en chapitre quatre. Le cinquième chapitre est focalisé sur des études connexes qui permettent de « nourrir » MetaConnect. En effet, comme MetaConnect est un IBM, il nécessite de nombreuses données d'entré qui peuvent être majoritairement extraites de la littérature mais certaines variables telles que la distance maximum de dispersion des espèces sont reconnues comme critiques au regard de l'analyse de sensibilité et doivent faire l'objet d'une attention particulière. En d'autres termes, une petite variation de cette variable est susceptible d'engendrer des résultats de simulations très différents (Ruckelshaus et al. 1997; Tischendorf 2001).

# I. **MetaConnect** *(adapted from Moulherat et al. submitted-a)*

The core base modeling of MetaConnect is a simple population dynamic model which can be used as a standalone application to perform PVAs. However, if the core base model estimates extinction probabilities, it also simulates the complete population dynamic tagging individuals with genetic tags (neutral alleles such as microsatellites).

## *Model design*

MetaConnect simulates metapopulation dynamics and genetics using the species life cycle and life history traits, the landscape characteristics and their interactions. The simulations allow inferring of local and global extinction probabilities, individual dispersal within the meta-population, local and global genetic diversity and local and global genetic differentiation (from classical Fst analyses or as input files for Structure software (Pritchard et al. 2000)).

MetaConnect is an individual and process-based-model which means that:

(1) all individuals in the model are independent and behave in respect to their phenotype (individual-based model)

(2) patterns emerging in the different outputs of the model are the products of flexible and adjustable rules implemented in the model (process-based-model).

### *Model structure*

Table I: Nomenclature of MetaConnect's main parameters and variables.

| Parameters and variables | Description | Distribution law of random variables |
|---|---|---|
| ***Demographic characteristics*** | | |
| $K$ | Carrying capacity* | |
| $k$ | Total competition coefficient | |
| $s_i$ | Survival of individual from class $i$ | Bernoulli |
| $F$ | Fecundity | Poisson |
| $\sigma$ | Primary sex ratio | Bernoulli |
| ***Mating system*** | | |
| Mating system | Mating system assumptions | |
| $H_m$ | Male harem size | Poisson |
| $H_f$ | Female harem size | Poisson |
| ***Genetics*** | | |
| $L$ | Number of loci | |
| $A$ | Number of alleles per locus | |
| $\mu$ | Mutation rate | |
| ***Dispersal*** | | |
| $p_d$ | Dispersal probability | Bernoulli |
| Dispersal rule | Dispersal algorithm | |
| ***Initialization and model parameterization*** | | |
| $N_0$ | Initial number of individuals | |
| $\sigma_0$ | Initial sex-ratio | |
| $f_a$ | Initial class structure | |
| $T$ | Time steps | |
| $MC_l$ | Number of landscape random generations | |
| $MC_d$ | Number of population dynamic simulations per landscape | |

* The carrying capacity $K$ is derived from the competition coefficient and the competition assumptions.

## 1 Landscape

The modeling platform MetaConnect requires three layers describing the landscape:

(1) Patches: locales of suitable habitat for the focal species. Each patch is identified by its name and is constituted by all the adjacent cells having the same name.
(2) Carrying capacities: provides the carrying capacity for each cell of the layer. The patch carrying capacity then corresponds to the mean value of its constitutive cell's carrying capacities.
(3) Costs: provides a coefficient representing preferences for each cell of the map

The landscape layers can be imported from GIS software as raster files.

## 2 Demography

The population dynamics are represented by a succession of individual states linked by transitions. The user can build the species life-cycle by assembling "bubbles" representing the individual state and "arrows" representing transition rules between individual states (Figure 3). The "bubbles" correspond to what we will call "class" along this manuscript and can correspond to age classes or sex or anything that can be defined as a group of individuals with the same demographic characteristics. Density dependence can be a scramble or contest and can be designed as a part of a transition. The mating system can be chosen from monogamy, polygamy, polyandry and/or polygyny (Legendre et al. 1999). The demographic parameters (Table 1) can be patch-specific. Environmental stochasticity has been included as random processes inducing normal variation around the patch's mean value of demographic parameters. As an example, the fecundity parameter follows a Poisson distribution (demographic stochasticity) with parameter λ equal to the average fecundity ($F \rightsquigarrow \mathcal{P}(\bar{F})$). The average fecundity can vary from one patch to another and within simulation time steps following a Gaussian distribution (Table 1).

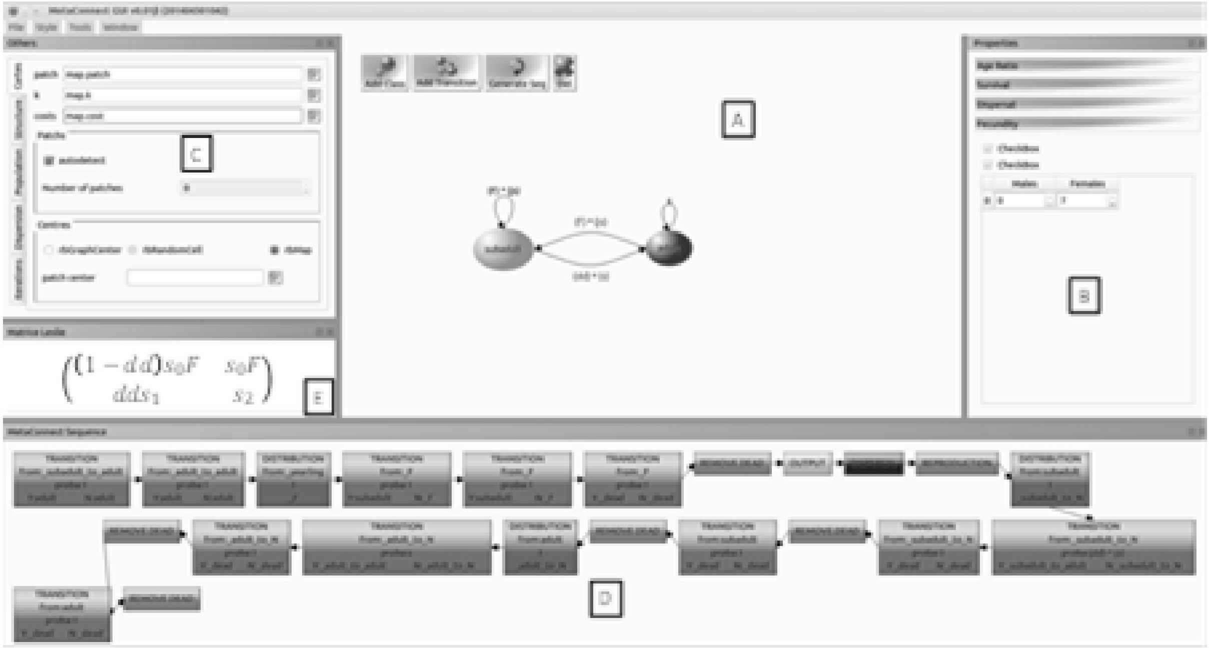

Figure 3: Example of the Legendre et al. (1999) passerine life cycle with two age-classes and two sexes that can be modeled with MetaConnect. On this MetaConnect screenshot, in the A section, "bubbles" correspond to reproductive status of individuals (subadult or adult) and *dd* is the density-dependent recruitment probability that depends on the competition assumption (contest or scramble competition respectively equations 1a and 1b). The user defines the species life-cycle as a combination of settable "bubbles" (add class) and "arrows" (add transition) (A). The species life history traits are set up in the B section and the run settings are defined in the C section. Then, the MetaConnect workflow (D) and the Leslie matrix (E) are generated automatically. The Leslie matrix and the MetaConnect workflow can be changed by the user which automatically adjusts the life-cycle graph.

Dispersal decision $p_d$ is implemented by setting a proportion of individuals (reproductive and/or non-reproductive) leaving a patch. The density-dependent recruitment probabilities $p_r$ is determined by equation 1 where $N_T$ can be a chosen combination of the number of individuals per class (i.e. $N_T$.could be the total population or just the reproductive individuals) (Caswell 2001).

Equation 1:

$$\text{If contest competition: } p_r = \frac{1}{1 + kN_T}$$

$$\text{If scramble competition: } p_r = e^{-kN_t}$$

Dispersal is age- and sex-dependent, and the process by which individuals disperse can be chosen from three families of movement rules:

1. The first family of dispersal rules does not take account of the preference coefficients. Dispersal between patches is modeled by a probability for an individual to reach another patch. The probability of reaching a patch can be equal between patches, or depend on the Euclidean distance between patch centers, or can be set manually.
2. The second family represents the interaction between individuals and their environment through the use of preference coefficients. This family comprises the random-walk dispersal rule (RW) and a correlated random-walk rule (CRW). The CRW assumes a degree of directional persistence, (i.e., the movement direction at time *t+1* depends on the direction taken at time *t*) and not solely an environmental based one.
3. The last family of rules assumes that individuals have knowledge of their environment. They are able to reach the other patches by taking the easiest route (Least-Cost-Path, LCP). From a focal patch, the LCP algorithm usually assumes that a single patch can be reached (Botea et al. ; Adriaensen et al. 2003; Pe'er and Kramer-Schadt 2008; Barraquand et al. 2009). Such an assumption is unrealistic, and to relax it we implemented a multiple LCP movement rule, in which we calculated all possible combinations of LCPs between the focal patch and all the other patches (Urban et al. 2009; Foltete et al. 2012). Then, for the reachable patches (i.e. LCP length less than the maximum dispersal distance), the probability to reach a patch is weighted by the LCP length (number of map cells crossed) or cumulative cost (total cost of all map cells crossed). We also adapted the Stochastic Movement Simulator (SMS) (Palmer et al. 2011; Coulon et al. in prep), which relaxes the assumption of omniscience inherent in the LCP approach. With the SMS rule, individuals make movement decisions based on the environment within a limited perceptual range and a tendency to directional persistence similar to that in a CRW. At each movement time step, the SMS algorithm calculates a movement probability for each cell surrounding the individual’s current cell. The calculation process evaluates the displacement cost for each surrounding cell

based on the coefficient of rugosity of the cells in the neighborhood and its distance to the current place (see Palmer et al. 2011 for details).

The dispersal event ends when the individual dies or reaches a patch different from his original patch regardless of the arrival patch quality.

### 3 Genetics

Individuals are genetically tagged using neutral polymorphic loci. The number of loci and number of alleles per locus can be specified by the user. A single mutation rate (probability of creating a new allele without possibility of reverse mutation) implemented in the model allows the production of new alleles at each locus during simulations, and can be specified by the user. Gene transmission is assumed to be Mendeleian and siblings are assumed to have the same father (randomly chosen from the female harem for polyandrous cases).

### *Model outputs*

The model provides many forms of outputs based on focal species life history traits and landscape maps, which are adaptable to various theoretical and applied contexts. The outputs report the results at three levels at a frequency specified by the user, allowing dynamic visualization of the simulations:

1. Demography: the model provides population size that can be split into the different classes implemented in the MetaConnect set up. The model also derives extinction probability, colonization probability and time before extinction and colonization. These indicators are calculated at the local (patch) and global (metapopulation) scales.
2. Dispersal: for each pair of patches, the model provides the number of individuals that reach a new patch or die during the dispersal process. In addition, maps of cell occupancy are drawn from successful dispersal events (number of individual who visited the map cells during the whole run).
3. Genetics: the model provides the genetic diversity and differentiation (Fst, Fis, Fit, He and Ho) at the local and global scale.

All these results can be directly plotted using the MetaConnect results viewer (Figure 4) or extracted as text files. In addition R (R Development Core Team 2005) has been incorporated to the viewer, which allows direct analysis of the MetaConnect outputs.

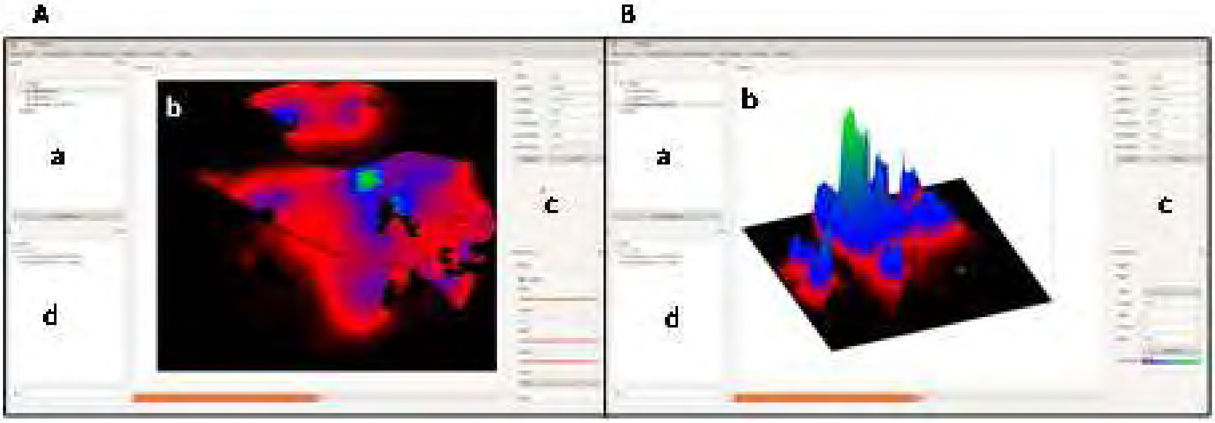

Figure 4: Screenshot of an example of MetaConnect results. Data to be displayed or analyzed are selected in the "a" section and displayed in the "b" section in two (A) or three (B) dimensions. Section "c" allows setting of the display options, and the "d" section is an R console to perform simple analysis and plotting of the MetaConnect outputs. An R package and a specific section of MetaConnect are under development for a complete integration of R into MetaConnect.

### *Model validation:*

As suggested by Pe'er et al. (2013), we validated the MetaConnect component by comparing its results when possible with results provided by equivalent analytical models and IBMs. Results from MetaConnect and previous models matched well, and we present here only the most important comparisons that pertain to MetaConnect PVA core modeling, namely the validation of (1) the demographic core modeling, (2) the mating system toolbox and (3) the genetic module.

#### *Population Viability Analysis*

Brook et al. (1997) compared the ability of 5 PVA packages (INMAT, GAPPS, RAMAS/age, RAMAS/metapop and VORTEX which is the only non-analytical model of this study) to predict the behavior of real populations by making a retrospective study on the Lord Howe

Island woodhen *Gallirallus sylvestris*. This study provides us the opportunity to compare in a single context the convergence between MetaConnect and the most used PVA software (Lindenmayer et al. 1995; Brook et al. 1997; Brook et al. 1999; Pe'er et al. 2013) as well as the ability of MetaConnect to estimate the behavior of a real species' population dynamics.

MetaConnect was set up based on the VORTEX system provided in Brook et al. (1997); a stable age structure was determined manually by calculating the eigenvector associated with the first eigenvalue of the Leslie matrix (Caswell 2001) provided for the RAMAS/metapop system and the competition type was assumed to be contest (Appendix A of related paper).

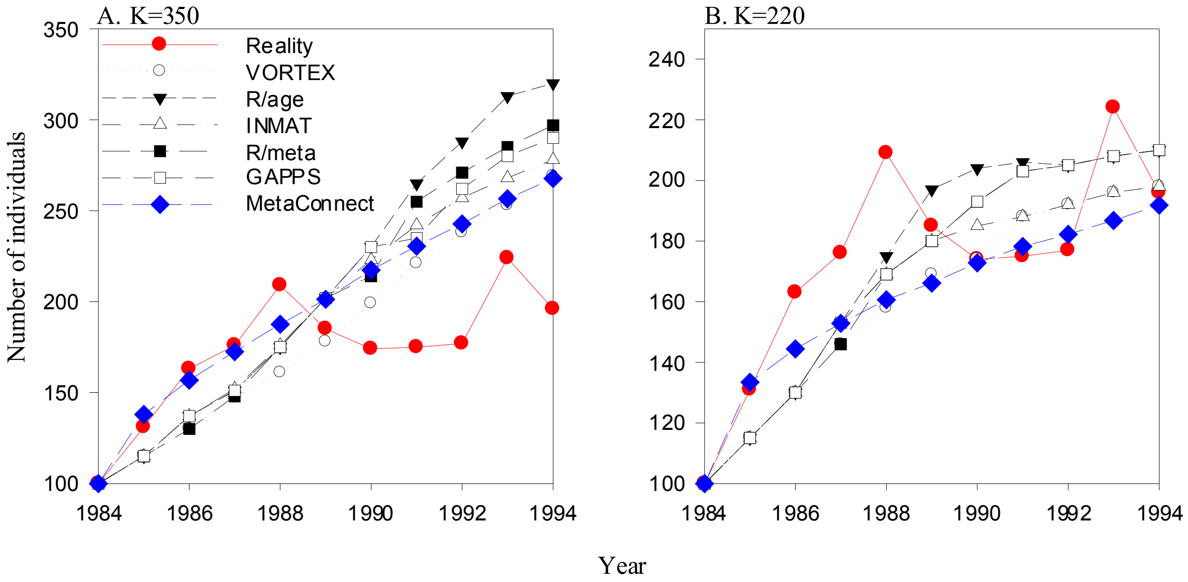

Figure 5: Simulated projections of the Lord Howe Island woodhen for the PVA software packages MetaConnect, GAPPS, INMAT, RAMAS/age, RAMAS/metapop and VORTEX under the effect of demographic and environmental stochasticity (derived from Brook et al. 1997). Initial population size set at stable age distribution of 100 individuals. Population history of the woodhen (1984-1994) is included for comparison. Models of density dependence were contest for MetaConnect and ceiling for models from Brook (1997) with carrying capacities set to K=350 (A) and K=220 (B).

Regardless of the carrying capacity, MetaConnect population dynamics predictions agreed with those provided by the other software packages (Figure 5) and the predicted population size after 10 years was similar to the size predicted by the other software (difference in mean number of individuals (± SD) between MetaConnect and the 5 PVA models for *K*=350: 21.8

± 22.2, for *K*=220: 9.2 ± 6.8, Table 2). Moreover, MetaConnect better captured the initial (1984-1988) strong growth of the population (Figure 5).

Table II: Estimates of final population size (after 10 years) predicted by software GAPPS, INMAT, RAMAS/age, RAMAS/metapop, VORTEX (Brook et al. 1997) and MetaConnect PVA core modeling. Mean ± standard deviation (as reported by Brook et al. 1997) are given for *K*=350 and *K*=220.

| Scenario | GAPPS | INMAT | R/age | R/metapop | VORTEX | MetaConnect |
|---|---|---|---|---|---|---|
| *K*=350 | 325±57 | 277±43 | 283±78 | 296±82 | 268±83 | 268±48 |
| *K*=220 | 211±28 | 199±20 | 194±49 | 205±40 | 197±37 | 192±35 |

### *Validation of the mating system toolbox*

Population dynamics model outputs are highly sensitive to the mating system (Doebeli and Koella 1994; Lindstrom and Kokko 1998; Legendre et al. 1999; Calsbeek et al. 2002). Legendre et al. (1999) related the colonization success of invasive passerine birds in New Zealand to their mating system (monogynous *vs* polygynous). To validate, the mating system toolbox of MetaConnect core modeling, we compared outputs of MetaConnect population dynamics to those obtained by Legendre et al. (1999).

MetaConnect provides results consistent with those provided by Legendre et al. (1999) (Figure 6). The main difference between the models comes from the assumption of the recruitment function. Indeed, Legendre et al. (1999) assume an infinite Malthusian growth function, which is not currently implemented in MetaConnect. Rather, we used a contest recruitment function with *K* set at 1000 (results not showed), and decreased it to 500 and 250 (Figure 6) to examine the role of setting a constraint on the Malthusian growth rate. Unsurprisingly, for *K*=1000 results from MetaConnect converged with those obtained by Legendre et al. (1999) under an infinite Malthusian growth function. As the carrying capacity was reduced, extinction probability increased and tended more closely to the pattern observed in the wild. Legendre et al. (1999) suggested that the difference observed between their modeling outputs and the natural observations were probably explained by environmental stochasticity. However, our results suggest that, even though environmental stochasticity may explain a significant part of the discrepancy, the carrying capacity of the population must play a major role in the observed pattern of invasive species extinctions (Figure 6). This agrees with a study of spider extinctions on island where carrying capacity was also found to play a significant role on extinction even in presence of environmental stochasticity (Schoener et al. 2003).

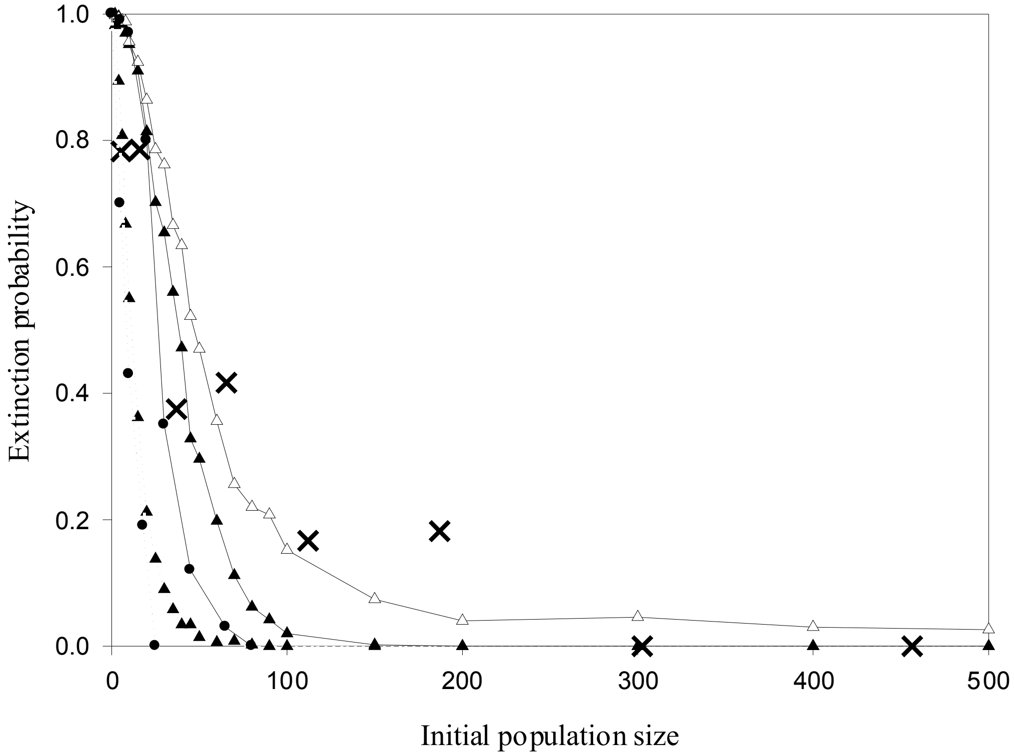

Figure 6: Predicted extinction probability of passerine bird species as a function of the mating system and initial population size. Filled circles correspond to the predictions made by the model used by Legendre et al. (1999) and crosses correspond to natural observations of passerine invasion success in New-Zealand. Triangles are extinction probability estimates provided by MetaConnect. Plain lines correspond to a monogamous mating system and dotted lines to polygynous mating system. Filled triangles are estimates assuming high carrying capacities ($K$=500) and empty triangles correspond to low carrying capacities ($K$=250).

### *Validation of the genetic module*

MetaConnect assumes panmixia in local populations (i.e. in patches). Regardless of initial conditions and model assumptions, MetaConnect provides estimates of Fis close to 0. Moreover, simulations considering two patches without dispersal generate a strong genetic structure at the metapopulation scale (Fst≈1) but no structure within local populations (Fis≈0). These two results confirm that the genetic core base of the platform behaves properly.

In addition, we validated the genetic part by comparing the MetaConnect genetic outputs to predictions of the CDPOP model (Landguth and Cushman 2010) in comparable situations. Landguth and Cushman (2010) explored with CDPOP the genetic outcome of three scenarios of landscape structures. The first corresponds to a panmictic population, the second assumes that two patches are isolated by an impassable barrier and the third assumes a simple diffusion model weighted by landscape preference coefficient. Figure 7 shows that results from MetaConnect converged with those obtained by Landguth and Cushman (2010).

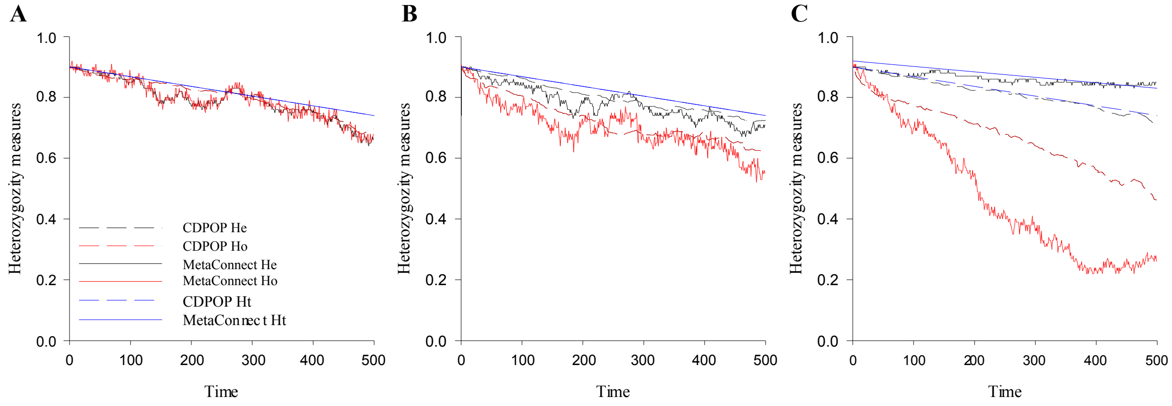

Figure 7: Heterozygosity measures derived from Landguth and Cushman (2010) (long dash) or estimated with MetaConnect (solid lines) under similar dispersal scenarios described in Landguth and Cushman (2010) (A. Panmictic scenario, B. patches are separated by an impassable barrier, C. Individuals navigate between patches in a displacement matrix where rugosity coefficients are comprised between 1 and 63, and dispersers are dispersing following the random walk dispersal rule). For CDPOP and MetaConnect runs, $H_e$ and $H_o$, the expected and observed heterozygosity can be compared with curves of decay of heterozygosity produced according to Equation 1. The differentiation patterns are similar and variations between MetaConnect results and CDPOP can be attributed to variability between runs and differences in the displacement matrix map used in scenario B and C:

$$H_t = \left(1 - \frac{1}{2N_e + 1}\right)^t H_o$$

Where:

$$N_e = \frac{4N_M N_F}{N_M + N_F}$$

$H_o$ is the observed heterozygosity and $N_M$, $N_F$ and $N_e$ are respectively the number of males, females and the effective population size.

## Model sensitivity:

MetaConnect is a highly flexible IBM which means that dozens of variables can be setup in various modeling context rendering a complete sensitivity analyses impossible to run as with most IBMs (Cross and Beissinger 2001; Naujokaitis-Lewis et al. 2009; Pe'er et al. 2013). Only the mating systems and the dispersal parts of MetaConnect were submitted to sensitivity analysis (see below).

### *Mating system*

Extinction probabilities and allele extinction probabilities are sensitive to the mating system assumption (see Legendre & Clobert 1999 in the validation part above and Moulherat et al. submitted). I will not develop this part here since a complete article is devoted to the implication of the mating system in the model output in a single population that could be reasonably extended to meta-population systems (Moulherat et al. submitted-b).

### *Landscape resolution, dispersal rule and life strategy impact the outcome linked to dispersal*

We studied how landscape structure and resolution will affect dispersal responses in two virtual species with contrasted ecological profiles. The effect of landscape structure was assessed with the virtual landscape designed by Adriaensen et al. (2003), which is a standard arena for testing tools in movement simulations (Chardon et al. 2003; Palmer et al. 2011). In this landscape, we created five suitable habitat patches (ten cells of the grid radius per patch for external patches and a single cell patch for the central one), one at the center and one at the middle of each side of the grid (Figure 8).

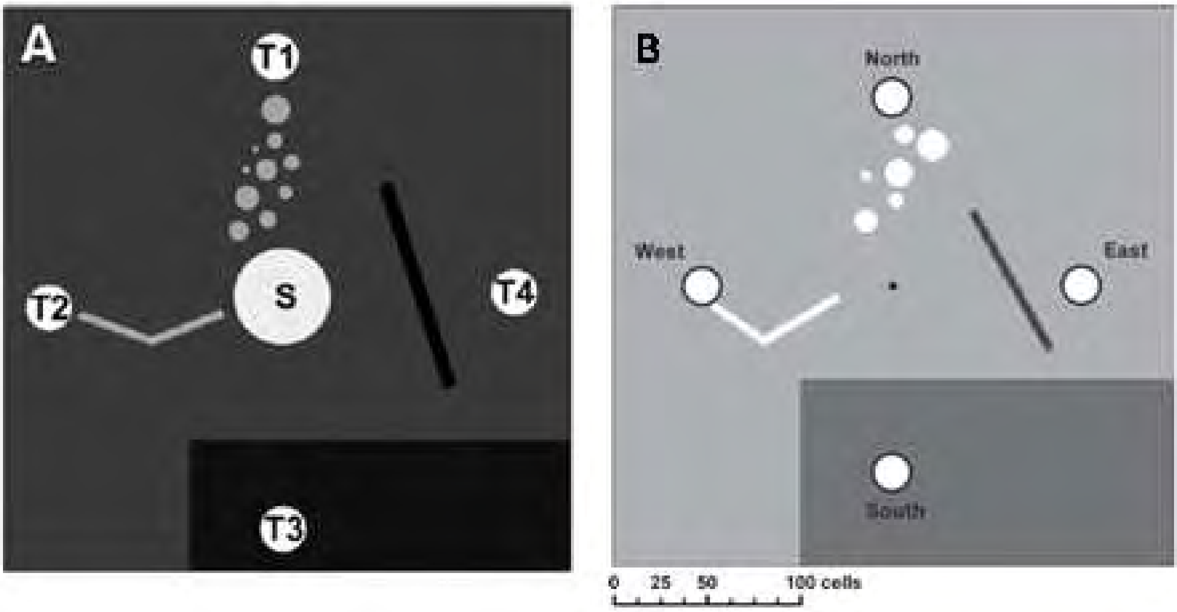

Figure 8: A. The initial virtual landscape designed by Adriaensen et al. (2003), 1000 X 1000 cells, cell size 1 unit, with four target patches (T1-T4) at equal Euclidean distance from S, the source patch. In the matrix (grid cell resistance R=20), the different structures are to the east a barrier (R=200), to the north a stepping stone like structure of small habitat patch (R=5), to the west a corridor (R=5), and to the south a high resistance zone (R=40). Concerning the habitat patches (S, T1-T4), R=1. B. The simplified virtual landscape used by Palmer et al. (2011) and in this study, 300 X 300 cells, cell size 1 unit. S becomes a one cell patch at equivalent Euclidean distance of T1-T4. The respective resistances of the matrix, the barrier, the stepping stone, the corridor and the high resistance zone are R=10, 100, 1, 1 and 20.

The central patch S was the only one with a permanent population with a carrying capacity of 500 individuals. The four other patches (T1-T4) can host immigrants but their carrying capacity is negative. We have thus a source sink system; the basic idea is to detect how landscape structures in between the central population and the four peripheral ones will affect the immigration rate in the four peripheral ones. Landscape structure are either corridors or stepping stones that are supposed to facilitate dispersal from the central population, or zones with a higher resistance to movements or barriers supposed to impede dispersal from the central population (Figure 8). To describe the sensitivity of MetaConnect to landscape resolution, we reduce the resolution of the modified map of Palmer et al. (2011) by aggregating pixels (Figure 9).

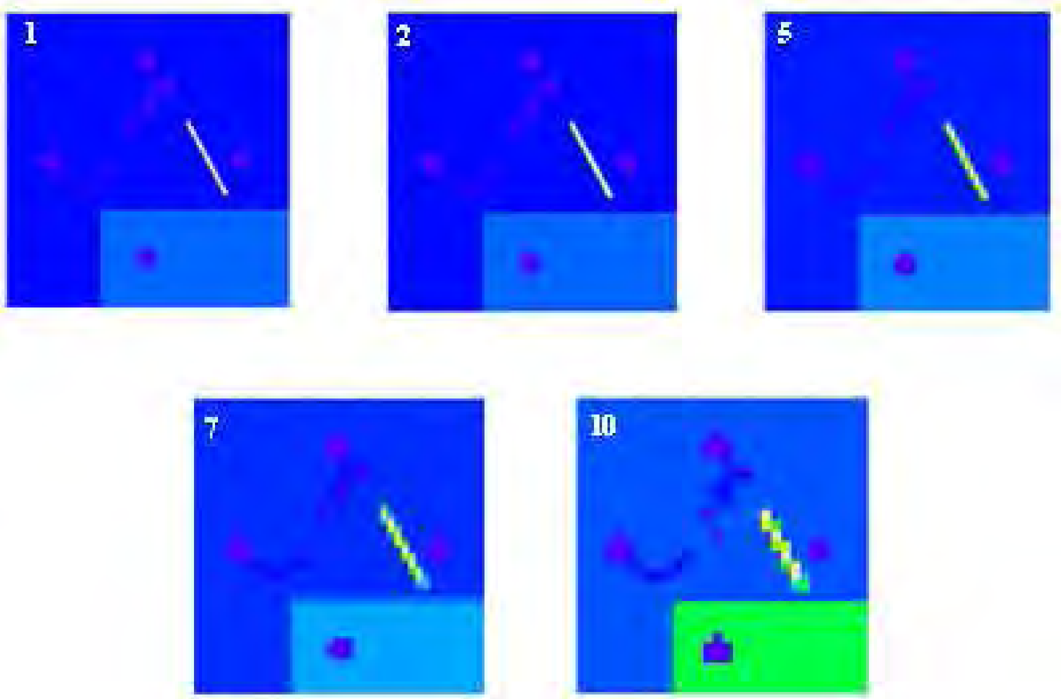

Figure 9: Virtual landscapes of decreasing resolution derived from the initial landscape used by Palmer et al. (2011). The resolution reduction was performed by aggregating respectively 2, 5, 7 and 10 pixels (2-10) of the original map (1). The resistance value of the landscape are represented as a gradient from high (yellow, initially $R_{1,2}$=100 that reduces in 5, 7 and 10, respectively $R_5$=85, $R_7$=75 and $R_{10}$=55) to low values (purple, R=0).

The two species released in the landscape differed only in their fecundity. Inter-specific analyses indeed showed that dispersal is traded-off against dispersal ability; species with a high reproductive output (fast species) dispersing less than species with a low reproductive output (slow species) (e.g. Clobert et al. 1998; Stevens et al. 2012). Given the existence of a dispersal polymorphism within species (Stevens et al. 2010), we created two different dispersal strategies for each species: in the short distance dispersal strategy, individuals are able to cross 10% of the grid cells before dying, whereas this threshold increases to 20% for the long distance dispersal strategy. The virtual species has a lizard-like life style with two stages, adults and sub-adults. The survival of both stages was fixed at 0.5, and the fecundity of the slow and the fast species was fixed at 5 and 10 respectively, which provides a stable age structure with 14% and 8% of adults, respectively. Dispersal occurred at the sub-adult stage, at a fixed rate of 30%. Without dispersal mortality with the LCP rule all the individuals that disperse will reach a patch and the recolonization time will be 1 which will mask the effect of the other parameters tested. To tackle this problem, we assumed that the probability to die during a RW (i.e. not reach a patch) is equal to the probability to die during a LCP or a SMS the computational translation of such assumption is that the dispersal mortality probability a LCP event is fitted on the probability of reaching a patch in RW and the probability of dying between two cells of a SMS dispersal is equal to the probability of dying during a RW divided by the number of steps that an individual can do during a dispersal event.

We tested the differences between the two species by comparing three different displacement modes. Individuals either moved following a random walk, or used least cost paths (LCP) (Adriaensen et al. 2003), or moved using the stochastic movement simulator (SMS) rule (Palmer et al. 2011). In the random walk model, dispersing individuals move across the landscape totally randomly. The costs of the cells in which they move is cumulated, and when a critical threshold is reached, they die if they are still in the matrix. In the least cost path model, they selected their trajectory by minimizing the total cumulated cost associated when moving in the different cells in the landscape. The dispersal probabilities from the source to the target patches are computed from the costs associated with the LCP: the higher the cost, the lower the probability to move towards the target patch (see equation 2).

<u>Equation 2:</u>

$$p_i = \frac{1}{n_{patch}} \times \left(1 + \frac{\bar{c} - c_i}{\sum_1^i |\bar{c} - c_i|}\right)$$

Where $p_i$ is the probability of reaching the patch i, $\bar{c}$ is the mean of the costs of all the least cost paths and $c_i$ is the cost of path i. If all the costs are equal, the probability of reaching a patch = 1/parch number. Dispersal mortality on a least cost path corresponds to the probability that the individual is unable to reach a patch when performing a random walk displacement in a homogeneous landscape (i.e. no landscape structure and R of the grid cells = 10). The dispersal mortality depends thus on the dispersal itself, and not on the length of the least cost path. The SMS rule (Palmer et al. 2011) was setup with a directional and target biases were fixed to medium values (5 and 2.5, respectively). The perceptual range was fixed to 10, the memory to 5. We used the arithmetic mean for calculating the effective cost within the perceptual range.

For each species, each dispersal strategy and each displacement mode, we ran 500 simulations of 100 time steps each. Results provide the mean and the standard error of two parameters after 100 time steps over the 500 simulations.

### 1 Sensitivity of trajectories to landscape resolution

Depending on the dispersal rule used in the MetaConnect runs, MetaConnect's output (especially dispersal issues) may differ with changes in the landscape resolution. Indeed, the dispersive individual simulated may change the path they are expected to use in the highest resolution landscape. Such changes affect the successful dispersal event occurrence and thus the functional connectivity estimations. Furthermore the stronger the relationship between the dispersal rule and the landscape characteristics, the more the dispersal estimators will be biased by the landscape resolution (Figures: 10, 11 and 12).

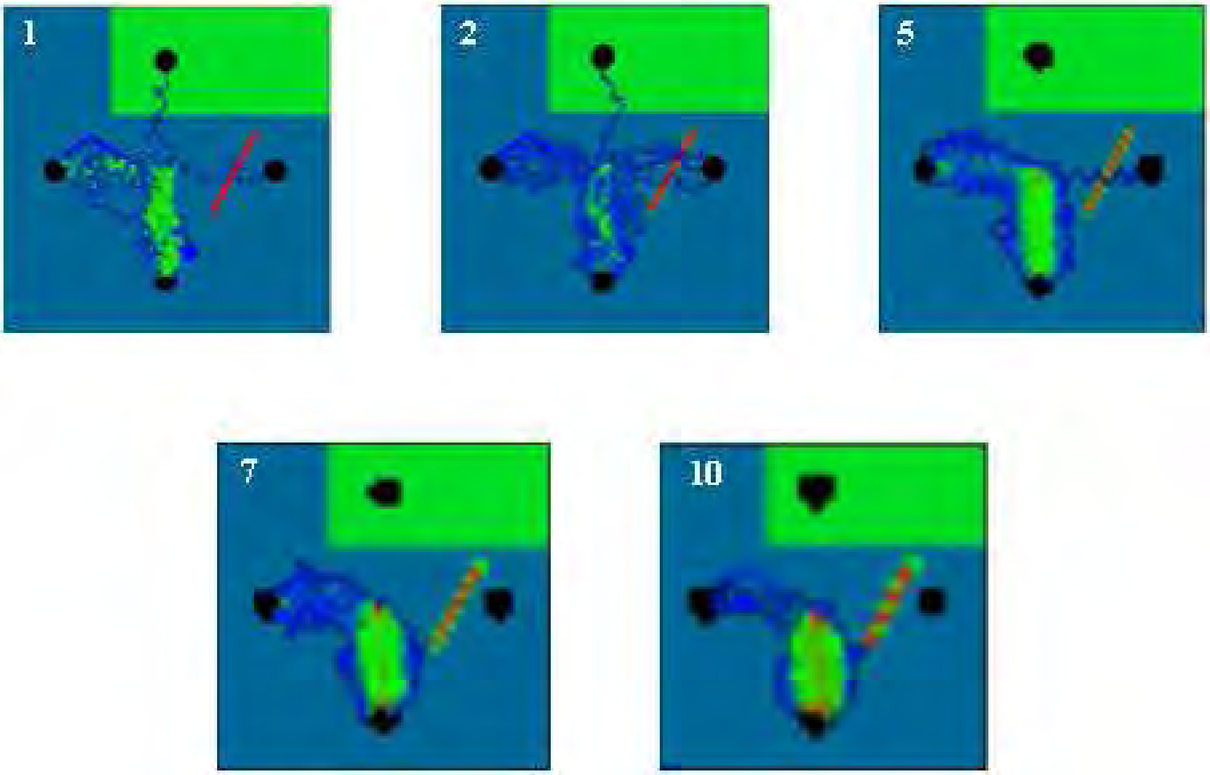

Figure 10: Random walks in the virtual landscape described by Palmer et al. (2011). Only successful dispersal events are drawn and visit frequency increases from dark blue (1) to red (>=100). This preliminary results tend to show that the resolution do not affect significantly the paths dispersing individual will use.

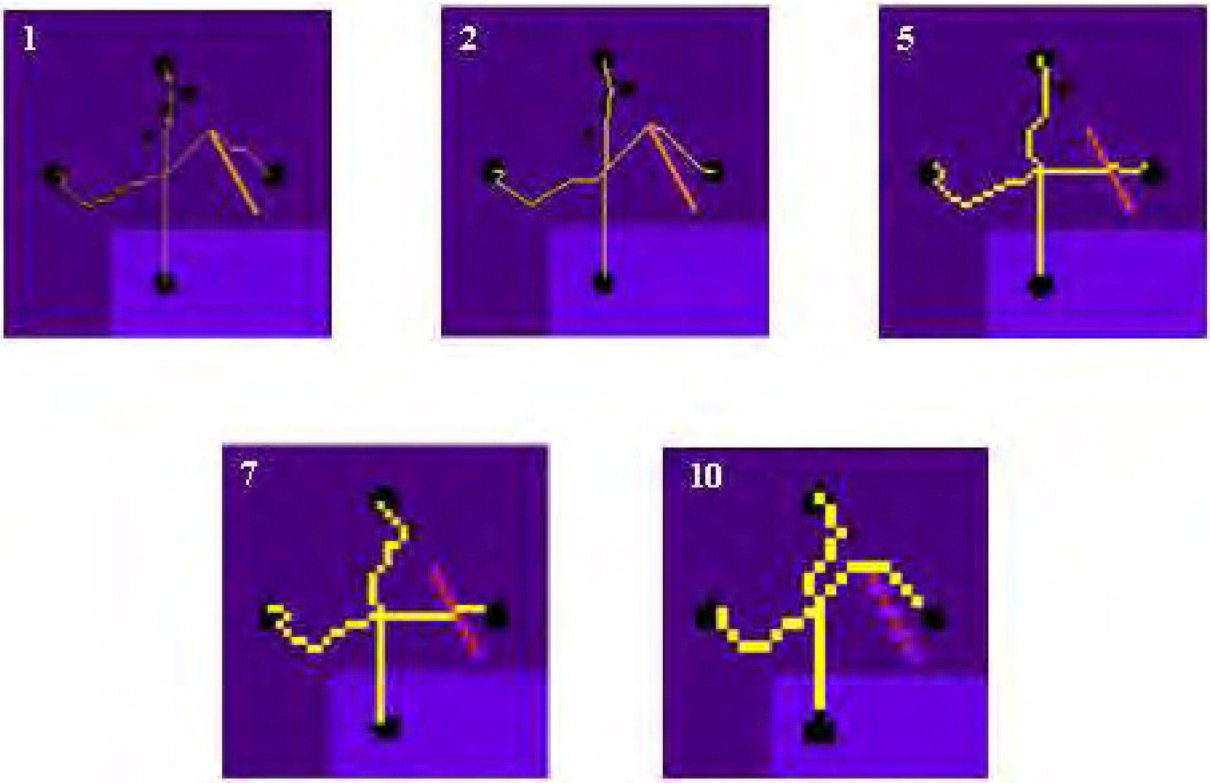

Figure 11: Least-Cost-Paths (yellow cells) computed with the A* algorithm (Botea et al.) in the virtual landscape described by Palmer et al. (2011). The paths change when the resolution changes. These trajectory shifts are linked to changes in the landscape structure with landscape resolution. Trajectory shift is particularly important and unpredictable in the barrier case since the LCP cross the barrier for pixel size = 5 or 7 and skirts the barrier when pixel size = 10. Rugosity coefficients are represented as a color gradient from black (1) to orange (100).

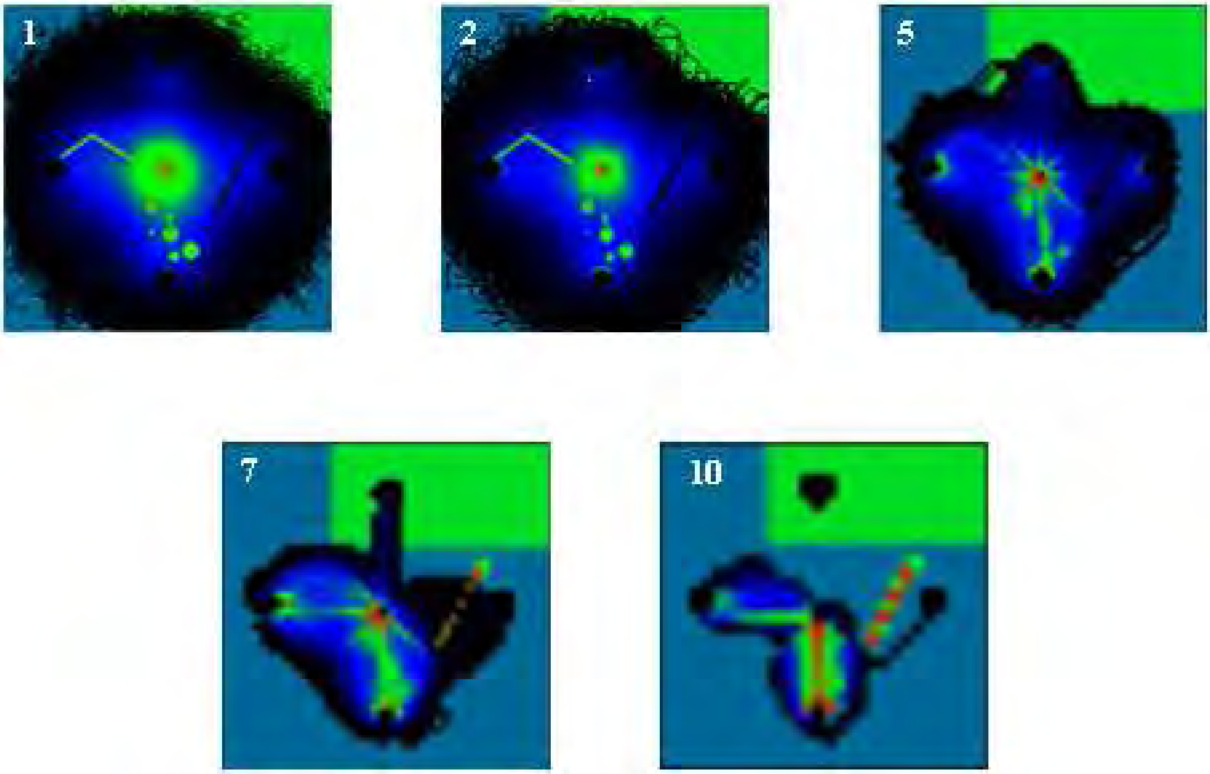

Figure 12: Successful dispersal event trajectories simulated with the Stochastic Movement Simulator (Palmer et al. 2011) in the virtual landscape described by Palmer et al. (2011). Only successful dispersal events are drawn and visit frequency increases from dark blue (1) to red (>=3000). This preliminary result tends to show that the resolution significantly affects the paths dispersing individuals will use. As in the LCP case, the landscape structure if affected by the resolution reduction which reduces a barrier's resistance and increases corridor's resistance. At the same time, the stepping stone structure essentially becomes a very large corridor.

## 2 Sensitivity on colonization time

We compare first the time needed to colonize each of the peripheral patches separated from the source population by a corridor, a stepping stone, a high resistance zone and a barrier, respectively. Results are shown at Figure 13, 14 and 15 for displacement modes using a random walk, least cost paths and SMS rule, respectively. Here, the colonization time corresponds to the time steps recorded between an extinction event (each time step) and a reproduction event in this previously empty patch.

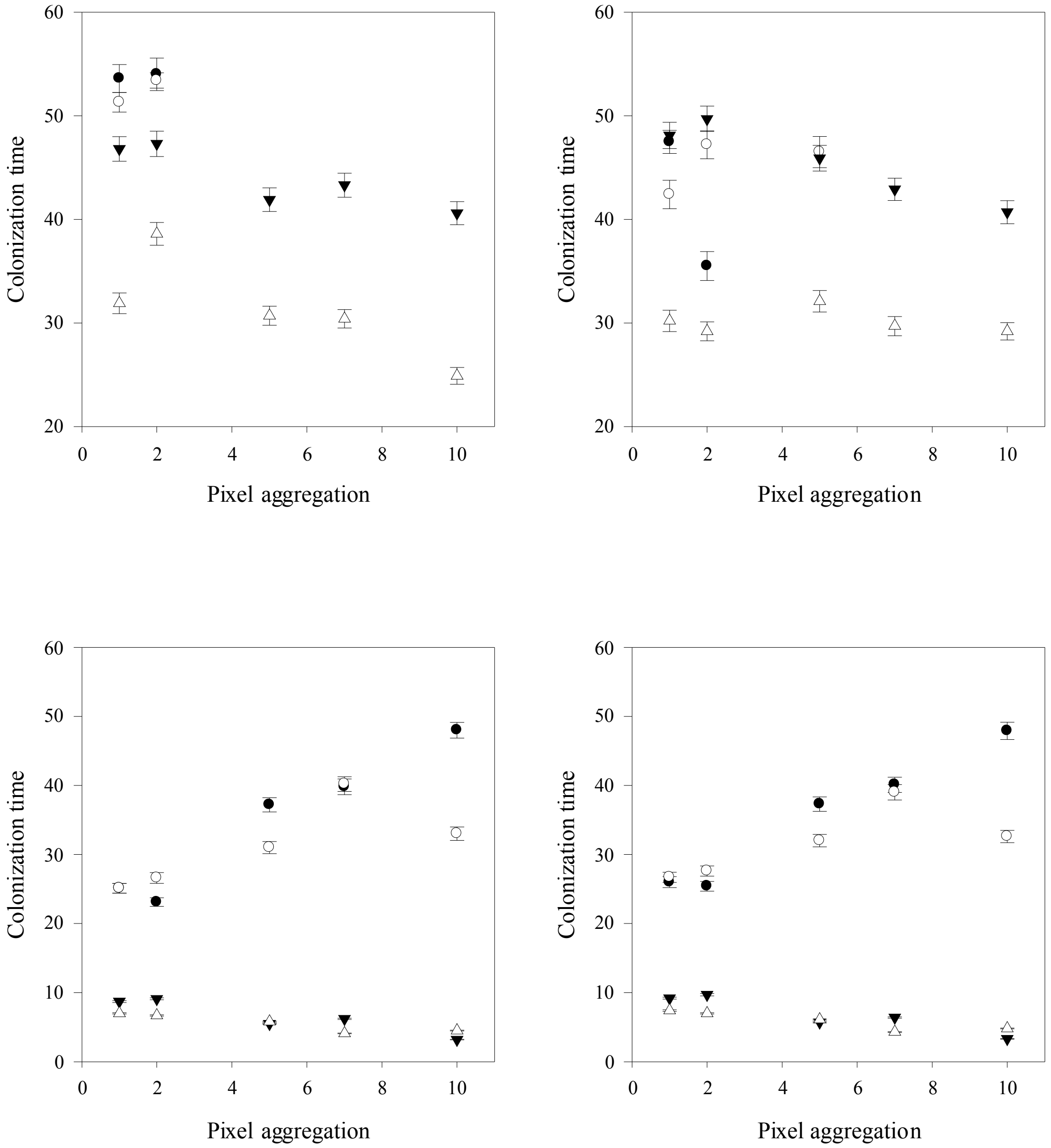

Figure 13: Random Walk (RW) and colonization time. Time between extinction and re-colonization of patches separated from the central patch by a barrier (black circles) or zone with a higher resistance (white circles) or a corridor (black triangles) or a stepping stone like structure (white triangles). The left column is for the slow species (fecundity = 5), the right column for a fast species (fecundity = 10). On the upper line, results for short dispersers (maximum 10% grid cell movement) and at the bottom for long dispersers (maximum 20% grid cell movement). All these results are function of the pixel aggregation (i.e. resolution) corresponding to the initial number of cells aggregated into a single one.

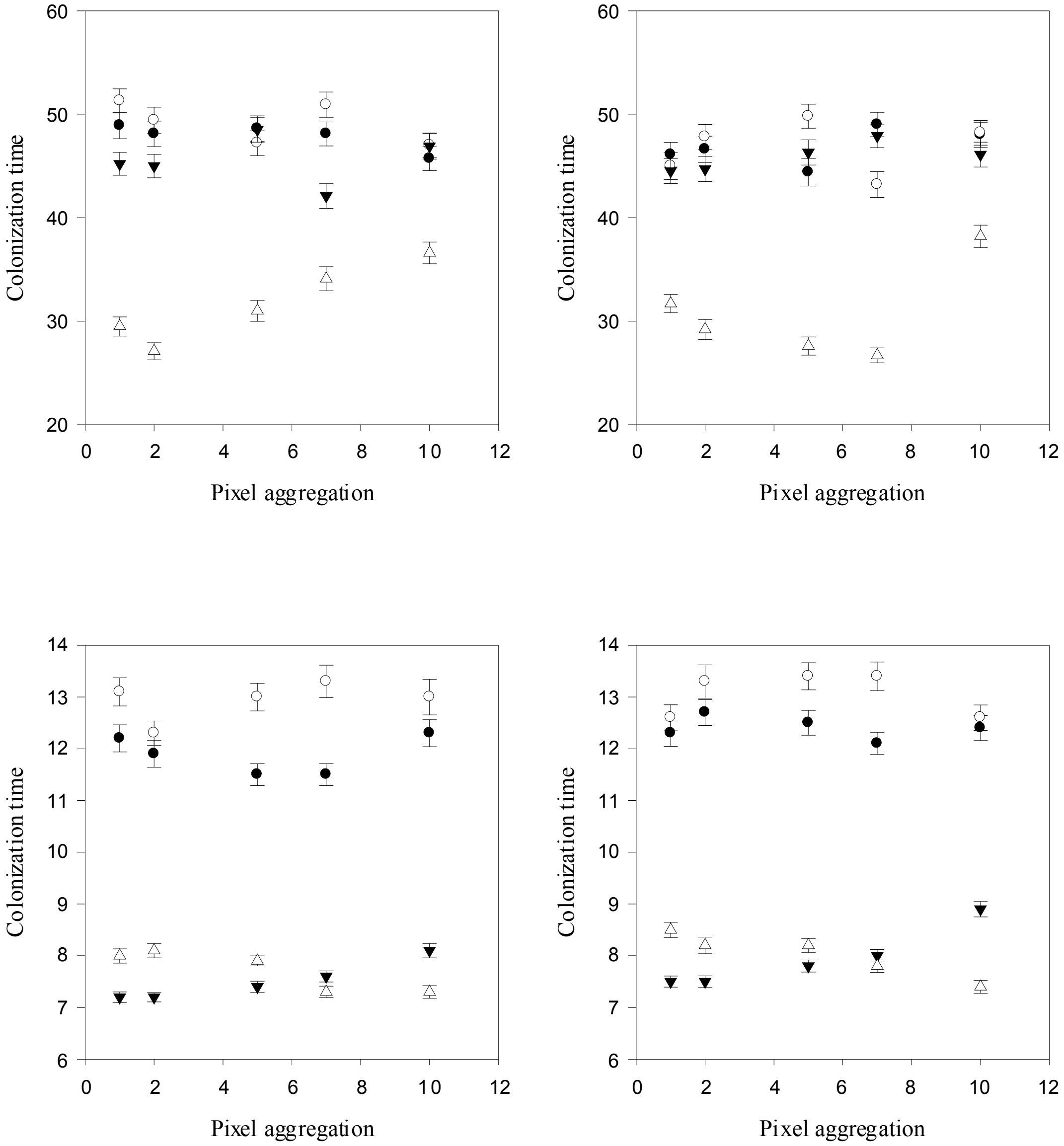

Figure 14: Least cost path model and colonization time in respect to landscape resolution. Time between extinction and re-colonization of patches separated from the central patch by a barrier (black circles) or zone with a higher resistance (white circles) or a corridor (black triangles) or a stepping stone like structure (white triangles). The left column is for the slow species (fecundity = 5), the right column for a fast species (fecundity = 10). On the upper line, results for short dispersers (maximum 10% grid cell movement) and at the bottom for long dispersers (maximum 20% grid cell movement). All these results are function of the pixel aggregation (i.e. resolution) corresponding to the initial number of cells aggregated into a single one.

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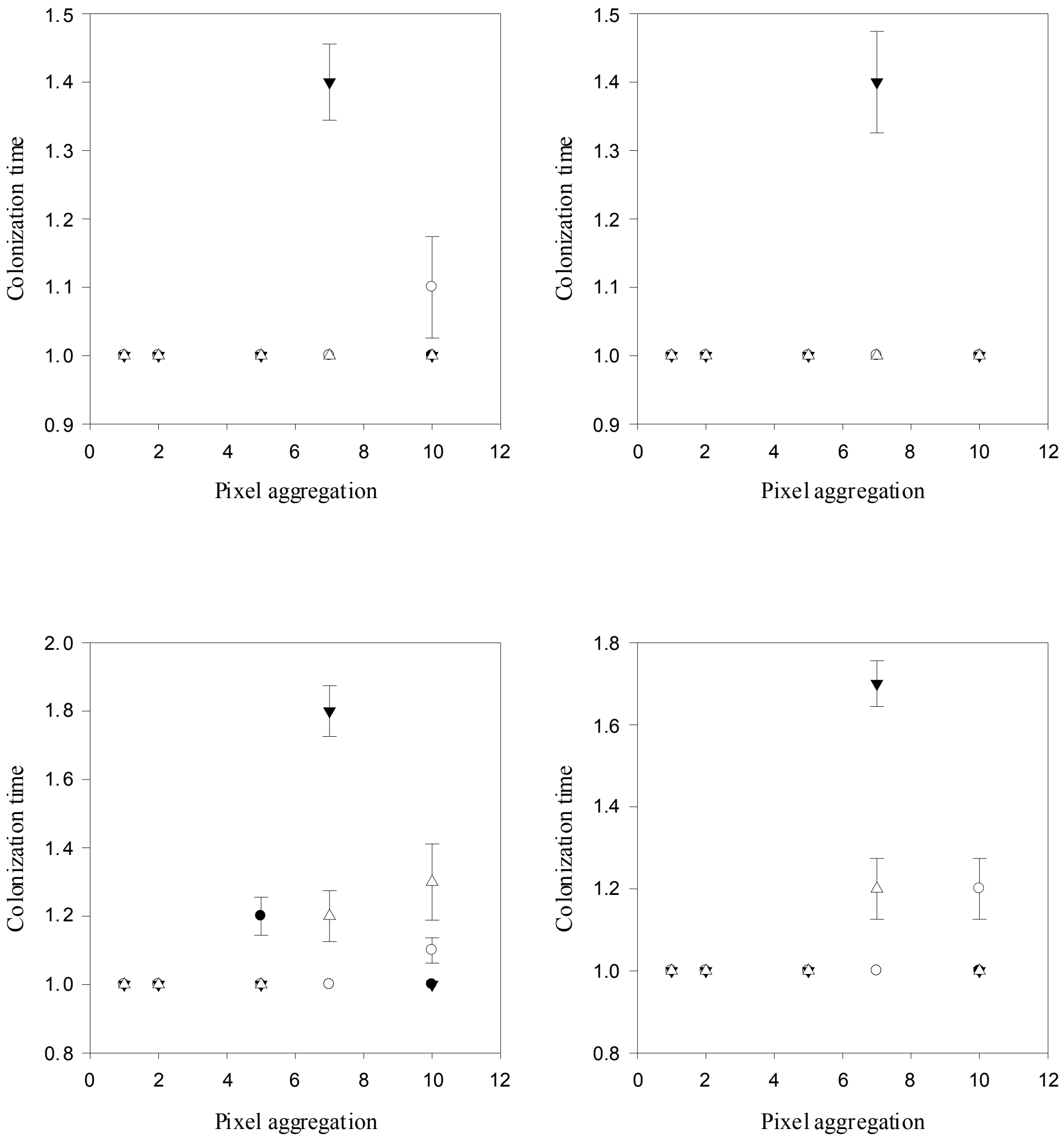

Figure 15: SMS rule and colonization time in respect to landscape resolution. Time between extinction and re-colonization of patches separated from the central patch by a barrier (black circles) or zone with a higher resistance (white circles) or a corridor (black triangles) or a stepping stone like structure (white triangles). The left column is for the slow species (fecundity = 5), the right column for a fast species (fecundity = 10). On the upper line, results for short dispersers (maximum 10% grid cell movement) and at the bottom for long dispersers (maximum 20% grid cell movement). All these results are function of the pixel aggregation (i.e. resolution) corresponding to the initial number of cells aggregated into a single one.

Focusing only on the results at the best resolution (pixel aggregation of 1), when dispersing individuals moved using a random walk, the effect of landscape structures on the colonization time is obvious for the slow species using the short dispersal strategy: the ranking of the patch colonization time is barrier > high resistance zone > corridor > stepping stone. The long colonization time of the patches separated by the barrier and the high resistance zone from the source patch corresponds to the resistance of both structures to the displacement. The much higher colonization time of the patch connected by a corridor relative to the patch connected by stepping stones depends on the random nature of the displacement. Individuals in the corridor did not maintain the directionality of their displacement, which means that they were often confronted with high resistance cell. Contrarily, those individuals that moved as a stepping stone model had higher chances to encounter the low resistance cells of a small neighbour patch, which means that their threshold of cumulated costs was less rapidly reached than the corresponding threshold of those individuals moving in the corridor. This effect disappears when individuals of the slow species use the long distance dispersal strategy. The ranking of patch colonization time (barrier = high resistance zone > corridor = stepping stone) corresponds to the resistance of these structures to the movement. For the fast species, the pattern is globally similar, with corridor a more pronounced effect of the corridor: the colonization time of the patch connected by the corridor is even higher than the colonization time of the patch separated by a high resistance zone. As for the slow species, this effect disappears when individuals used the long distance dispersal strategy. The effect of the species' strategy (low vs. high fecundity) is obvious on the colonization time of the short distance dispersal strategy: the higher number of dispersing individuals produced by the fast species decreases the colonization time for all structures except the corridor. Patches were connected two to three times faster when individuals of both species used the long distance dispersal strategy. When dispersing individuals used the least cost path, the effect of landscape on the colonization time perfectly corresponds to the resistance of the structures (barrier > high resistance zone > stepping stone > corridor) for both the slow species and fast species for those individuals that used the long distance dispersal strategy. However, for those individuals that used the short distance strategy, we detect the same negative effect of the corridor in the stepping stone relative to the random walk displacement. Finally, the colonization time is much shorter (almost four times) when individuals use the long distance dispersal strategy. When individuals moved using the SMS rules, their displacement was so efficient that they reached the four patches within one time step, regardless of their ecological profile, their dispersal strategy and the nature of the landscape structures.

The landscape resolution modifies the landscape structure. The modification begins with the 5 pixels aggregation; linear structures like corridors and barriers tend to look like a steppingstone for the first one and a more permeable structure for the second one. The shift in the landscape structure due to the decreasing resolution impacts the colonization time differently depending on the dispersal rule and dispersal abilities. Indeed, with RW, the impact of each group of structure (facilitator: stepping stone and corridor and inconvenience: barrier and high resistance) is amplified (i.e. reduction of colonization time for facilitating structure and increase of colonization time for inconvenient structure) with the resolution reduction (Figure 13). The LCP dispersal rule is sensitive to the landscape structure. The linear structures (barrier and corridor) tend to be unstructured. The barrier becomes more permeable leading to a reduction of the colonization time and the corridor tends to become a steppingstone. In contrast, the stepping stone tends to looks like a corridor (Figure 14). These transformations of the landscape representation impact the colonization time and the corridor tends to induce a pattern previously associated with the stepping stone and inversely, the stepping stone becomes a large corridor that reduces the colonization time (Figure 14). With the SMS dispersal rule, the resolution reduction has little influence on the colonization time (Figure 15) and as found by Palmer et al. (2011), stepping stones seems to be able to become some movement traps that increase the colonization time.

Except for the SMS dispersal rule, we found here as previous other studies that maximal distance that individual could travel during a dispersal event greatly influences the colonization time, reflecting a strong sensitivity to this parameter (Ruckelshaus et al. 1997; Mennechez et al. 2004; Naujokaitis-Lewis et al. 2009). Indeed, long dispersers colonize empty patches faster than short dispersers do (Figure 13, 14).

The colonization time is not sensitive to the species life strategy (i.e. short lived species or long lived species).

## 3 Sensitivity on dispersal

We now turn to the number of individuals reaching the four target patches according to the two species profiles, the two dispersal strategies and the three displacement modes (Figure 16, 17 and 18).

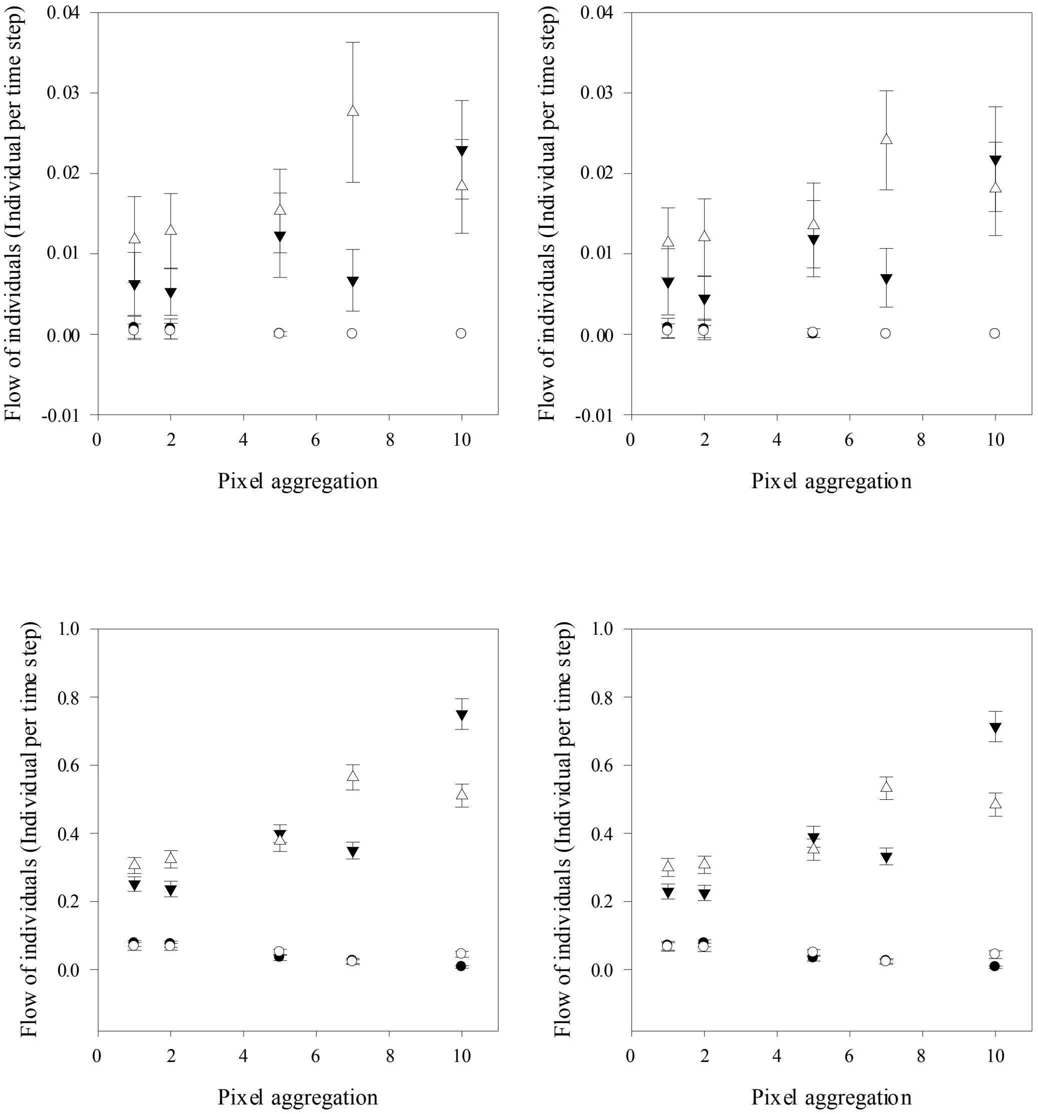

Figure 16: Random walk and flow of individuals in respect with landscape resolution. Number of individual reaching patches separated from the central patch by a barrier (black circles) or zone with a higher resistance (white circles) or a corridor (black triangles) or a stepping stone like structure (white triangles). The left column is for the slow species (fecundity = 5), the right column for a fast species (fecundity = 10). On the upper line, results for short dispersers (maximum 10% grid cell movement) and at the bottom for long dispersers (maximum 20% grid cell movement). All these results are function of the pixel aggregation (i.e. resolution) corresponding to the initial number of cells aggregated into a single one.

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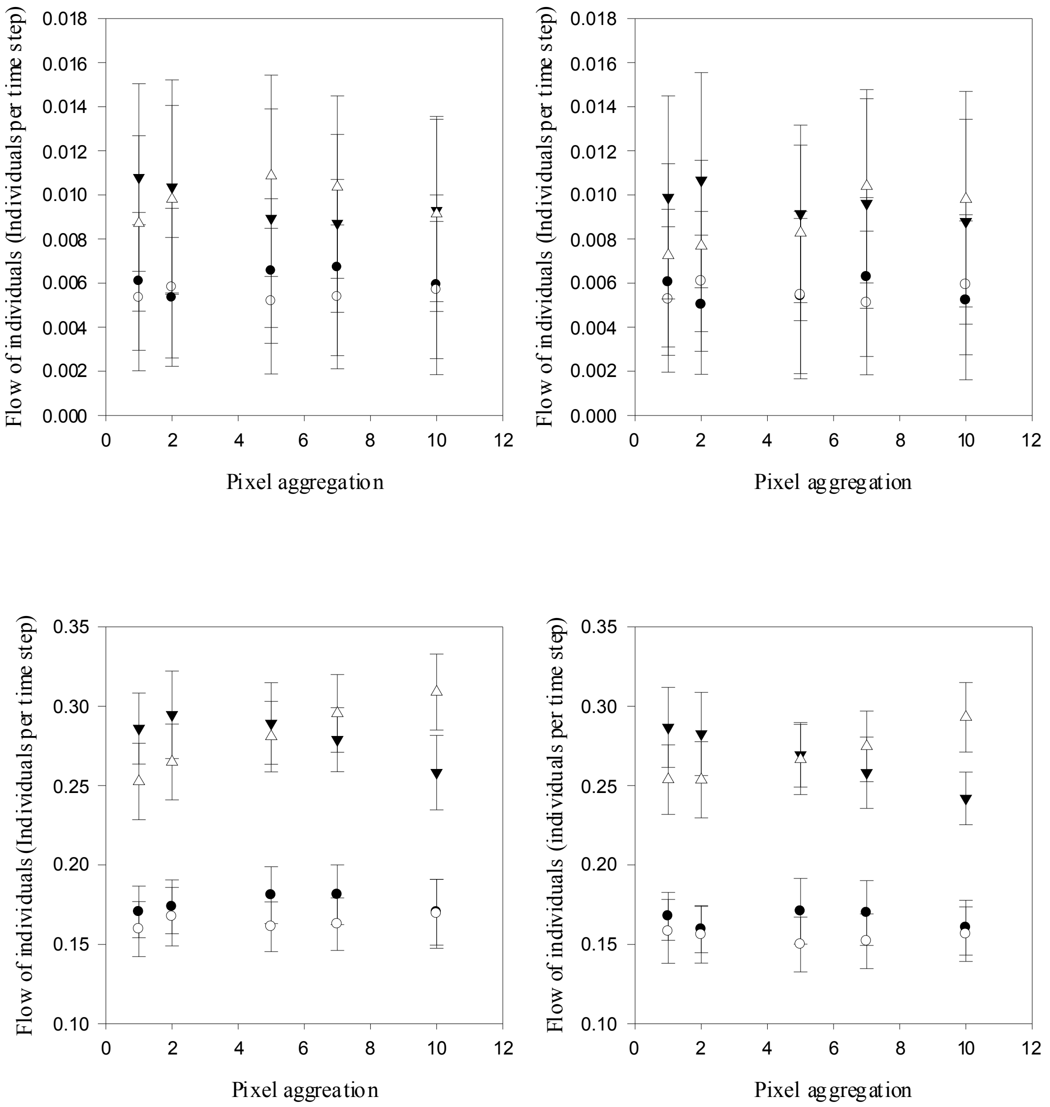


Figure 17: Least cost path model and flows of individuals in respect with landscape resolution. Number of individual reaching patches separated from the central patch by a barrier (black circles) or zone with a higher resistance (white circles) or a corridor (black triangles) or a stepping stone like structure (white triangles). The left column is for the slow species (fecundity = 5), the right column for a fast species (fecundity = 10). On the upper line, results for short dispersers (maximum 10% grid cell movement) and at the bottom for long dispersers (maximum 20% grid cell movement). All these results are function of the pixel aggregation (i.e. resolution) corresponding to the initial number of cells aggregated into a single one.

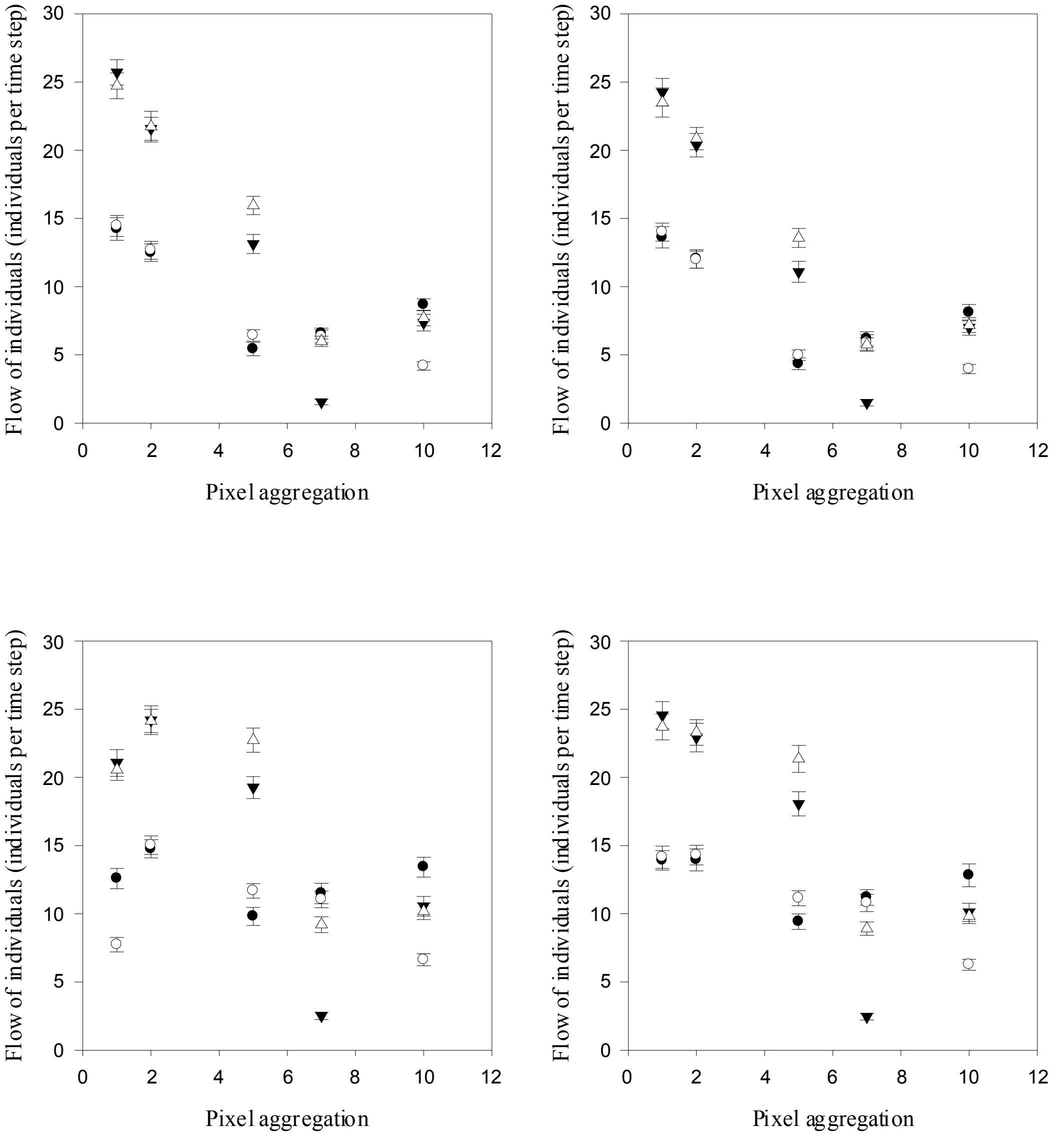


Figure 18: SMS rule and flows of individuals in respect with landscape resolution. Number of individual reaching patches separated from the central patch by a barrier (black circles) or zone with a higher resistance (white circles) or a corridor (black triangles) or a stepping stone like structure (white triangles). The left column is for the slow species (fecundity = 5), the right column for a fast species (fecundity = 10). On the upper line, results for short dispersers (maximum 10% grid cell movement) and at the bottom for long dispersers (maximum 20% grid cell movement). All these results are function of the pixel aggregation (i.e. resolution) corresponding to the initial number of cells aggregated into a single one.

With random walk, the numbers of individuals reaching each of the four target patches correspond to the resistance of the landscape structures that separate these patches from the source population (stepping stone > corridor > barrier = zone of high resistance). The precedence of the stepping stone with respect to the corridor comes from the process already mentioned: individuals in the corridors did not maintain the directionality of their displacement, whereas individuals moving in the stepping stone area encountered more often low resistance cell. The long distance dispersal strategy proved to be twenty times more efficient than the short distance dispersal strategy in corridors and stepping stones. No difference was found between the slow and the fast species. With the least cost path model, no significant effect of landscape structure on individual flows was found when individuals from both the slow and the fast species used the short dispersal distance strategy. However, when individuals used the fast strategy, the effects of landscape structure was markedly different. The ranking was then corridor = stepping stone > zone of high resistance = barrier. The numbers of individuals reaching the target patch in the stepping stone and corridor areas were ca. 30 times higher with the long distance dispersal strategy, whereas no difference between the slow and the fast species was detected. With SMS rule, many individuals reach the target patches at each time step (100 times more than the best random walk and LCP results). This difference between RW/LCP and SMS is due to the dispersal mortality assumption. Indeed, the dispersal mortality for LCP is fitted on the observed dispersal mortality in RW to allow comparisons for life history strategy, dispersal abilities and landscape resolution. Similarly, the dispersal mortality per movement step with SMS is fitted on the RW's. Since SMS needs less movement steps to reach a target, the flow of individuals is increased (Figure 18).

## 4 Conclusion on the sensitivity to landscape resolution

Sawyer et al. (2011) reviewed the application of least cost path modeling in landscape connectivity studies. A major bias in published studies on animals is that most models use coarse-grain environmental data layers to determine habitat connectivity, an approach that is often biased by researcher-perceived structural connectivity and runs the risk of missing important biological aspects. This bias is even more complicated by the fact that although the scale of analysis has been shown to greatly impact strength of detected relationships, study grain was typically dictated by the remotely sensed data available rather than by species perceptions of landscape features. Overall, the strength of the correlation between remotely sensed habitat layers and individual movement is relatively unknown and poorly validated

(Sawyer et al. 2011). Here we tackle this issue by changing the grain of our virtual landscape. We divided the size of our grid of cells by 2, 5, 7 and 10, and investigated how the two parameters (colonization time and flow individuals) evolved according to this modification. The resistance value of the new cell was the mean value of the initial cells. Altogether, we detected a non-linear and unpredictable response of the two parameters to this clustering test. The most impressive deviation was observed for the colonization time when individuals moved according to the SMS rule: whereas this colonization time was of 1 time step for all target patches, it climbs to 1.8 for the corridor structure in all the tested situations when the size of the cell grid was divided by 7. This effect disappeared when the grid was divided by ten. Overall it was the least cost path model that was the less affected by the changes in the landscape grain, whereas results from both the random walk and the SMS rule were substantially modified.

Model limitations:

By its structure, MetaConnect allows the user to take into account most of the requirements necessary for a complete and flexible PVA and decision-making tool (i.e. metapopulation dynamics and genetics) (Grimm et al. 2004; Baguette et al. 2013b; Pe'er et al. 2013).

Currently, its main limitations come from the landscape representation. In MetaConnect the landscape is represented using the patch-matrix approach, in which a pixel is a suitable habitat or not. This approach is slightly unrealistic for many species (Clobert et al. 2001a; Urban et al. 2009; Pe'er et al. 2011). Furthermore, reproduction in the suitable habitat is assumed to be panmictic which is usually not true because (1) patch shape and structure isolate or aggregate individuals within a patch and (2) individual behavior (territoriality, mating system, cooperation,…) may aggregate or isolate individuals within a patch (Doebeli and Koella 1994; Clobert et al. 2001a; Sinervo and Clobert 2003; Snyder and Chesson 2003; Ylonen et al. 2003; Nonacs and Kapheim 2007). Further development of MetaConnect toolboxes would allow tackling this limitation of intra-patch spatial structuring by splitting individual use of space into daily movements and dispersal events (Mueller and Fagan 2008; Roshier et al. 2008; Pe'er et al. 2013).

A second limitation is the way genetic mutation is modeled. Currently, the mutation model is very simple, just assuming that a new allele can occur at a given constant rate and that no reverse mutations are possible. Further toolbox development would allow various methods for modeling mutations to be incorporated (Willi et al. 2006; Neher 2013; Wray 2013) and in addition would permit simulation of the action of the genotypes on the individual phenotype (Montalvo et al. 1997; Baskett 2012; Mouquet et al. 2012; Chauvenet et al. 2013; Clobert et al. 2013; Moulherat et al. submitted-b).

### Model application and perspective

European regulation requires spatial planners to evaluate precisely the impacts of developments on ecological network functioning. Baguette et al. (2013b) recommended a robust workflow in that direction. The procedure comprises performing an analysis of metapopulation dynamics and dispersal over a landscape for each affected species to design sound ecological network functioning. MetaConnect is clearly a first step in this direction. Indeed, the user can easily build consensus networks for several species within the same study site and under the same framework with standardized and comparable outputs. However, such an approach does not yet incorporate the inter-specific interactions that could greatly change population dynamics and dispersal (Caswell 2001; Clobert et al. 2013). The next generation of MetaConnect (MetaSystem) will integrate basic inter-specific interactions such as competition, predation and parasitism.

Application of graph-theoretic connectivity is increasing at an exponential rate in ecology and conservation (Kindlmann and Burel 2008; Kadoya 2009; Urban et al. 2009; Moilanen 2011). In this framework, graph nodes represent habitat patches and graph edges represent the connectivity between patches (Urban and Keitt 2001; Taylor et al. 2006; Kindlmann and Burel 2008; Urban et al. 2009; Foltete et al. 2012). If the mathematical background of graph theory is promising to deal efficiently with the ecological connectivity (Urban and Keitt 2001), the oversimplification made by modeling a metapopulation with a graph (Urban et al. 2009; Moilanen 2011) leads to limited interpretation and operational efficiency (Kadoya 2009; Moilanen 2011). As an example, Kadoya (2009) concluded that graph modeling of metapopulations provides little congruence with connectivity inferred from population genetic structure. As shown is our case study, with MetaConnect we could parameterize nodes graphs with predictions of the demographic module and graph edges with dispersal assessed from dispersal simulation or derived from genetic estimates. In such an application of graph-theoretic connectivity, the graph does not model the metapopulation functioning as such, but is only used as a powerful analytical tool. Bergerot et al. (2013) obtain relevant assessments of landscape functional connectivity for a butterfly with a similar framework.

We conclude by highlighting the recent call for a new generation of models that begin to provide predictive systems ecology (Evans et al. 2012). This call argued that while in a few sub-disciplines such as dynamic vegetation modeling and climate change modeling we have already developed a capacity for simulating complex systems, typically we lack such a predictive modeling capability elsewhere in ecological and evolutionary disciplines (Moorcroft et al. 2001; Evans et al. 2012; Hoban et al. 2012; Bocedi et al. 2014). The climate modeling community and the dynamic vegetation modeling communities both possess several models with which they can conduct inter-model comparisons, conduct cross-validations, etc (Evans et al. 2012; Pe'er et al. 2013). This is also now true of the population genetics community, which has several software packages available (reviewed in Hoban et al. 2012). We believe that MetaConnect, together with the recently published software, RangeShifter (Bocedi et al. 2014), begin to address this challenge to develop complex systems models that can be used to inform policy in the sphere of spatial and landscape ecology. Our hope is that further modeling platforms will be developed that have similar philosophy and that over the next few years a strong community may grow involving multiple models and a broad range of user communities and stakeholders.

## II. MetaConnect in evolutionary biology: What genetic mechanisms underlying mating strategy expression maintain trimorphism in a rock-paper-scissors game? *(Moulherat et al. submitted-b)*

Although natural selection is expected to reduce variability, polymorphism is common in nature even when traits are under strong selection. Discrete polymorphism of mating strategies are widespread and offer a good opportunity to understand the genetic processes that allow the maintenance of polymorphism in relatively simple systems. In this chapter, I aim at exploring the genetic mechanism underling the expression of discrete mating strategies in the rock-paper-scissors (RPS) game. Heterozygotes carry the information for different strategies, yet little attention has been devoted to the mechanisms underlying heterozygote phenotype. I explored the maintenance of polymorphism under 1) genetic dominance or 2) phenotypic plasticity as mechanisms driving the expression of alternative strategies in males and females. In this respect, I built the *Mating system and game theory* "toolbox" of MetaConnect (Figure 2) and explore the maintenance of polymorphism in a single population system.

### Theoretical context

Evolutionary mechanisms such as natural selection and genetic drift are expected to reduce genetic variation within populations, yet polymorphism is surprisingly widespread in animal and plant populations. To explain this paradox, a number of mechanisms have been proposed, such as heterosis (Cook 2007), niche selection (Snyder and Chesson 2003; Bach and Kelly 2007) or negative frequency dependent selection (Gross 1996; Sinervo and Lively 1996; Punzalan et al. 2005; Bond 2007) which are all known to allow the stable maintenance of polymorphism in natural populations (Berry et al. 1991; Danchin et al. 2005). Discrete polymorphism such as trimorphism in mating strategies, which have been reported over the last two decades (reviewed in Sinervo and Calsbeek 2006) are particularly interesting in this respect since they offer the opportunity to study the maintenance of polymorphism in a very simple context. Theory suggests that trimorphism can be maintained by negative frequency dependent selection in a system as long as (1) each strategy is not an evolutionarily stable strategy (ESS) (Gross 1996) and (2) strategy pay-offs form a rock-paper-scissors game (RPS game) with the other two strategies (Maynard-Smith 1982; Gross 1996; Sinervo and Lively 1996). In the RPS game, each strategy beats another one but is beaten by the third one leading to an equilibrium of strategy frequencies (Box B). Theoretically, such a game can lead to a stable equilibrium of strategy frequencies or oscillations over time (Maynard-Smith 1982; Sinervo and Lively 1996).

Box B: The Rock-Paper-Scissor (RPS) game in ecology

The RPS game assumes that a trimorphism can be maintained if none of the three phenotypes is an evolutionary stable strategy (ESS) like in the children game rock-paper-scissor where the rock beats the scissors that beats the paper which finally beats the rock (Maynard-Smith 1982). If Maynard-Smith described this game as a theoretical hypothesis to explain polymorphism in 1982, it is later, in 1996 that Sinervo and Lively described the first biological (the side blotched lizard *Uta stansburiana*) system that exhibit a RPS game in the male mating strategy (Figure B.1). In this system, orange throat males are aggressive, polygynous and beat monogamous mate guarder blue throat males. The third male mating strategy consists in mimicking the female morph (yellow throat) to sneak orange polygynous males. However, blue males are able to distinguish sneaker males and prevent them to cuckoldry (Sinervo and Lively 1996). Nowadays, RPS game have been observed in various biological systems and context and reviewed by Calsbeek et al. (2002).

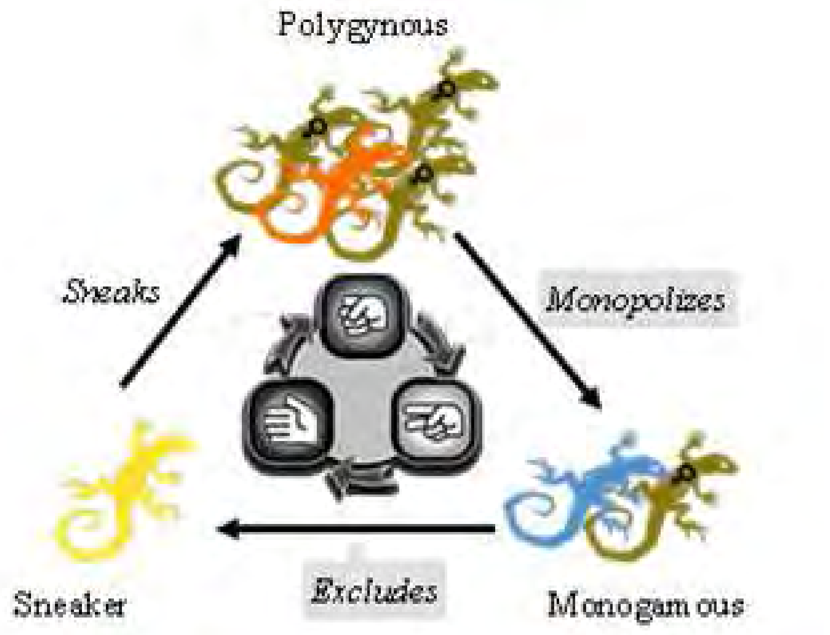


Figure B.1: Description of the RPS game male alternative strategies in *Uta stansburiana*. The polygynous orange aggressive male (rock) beats the blue throat, monogamous, mate guarder male and is beaten by the sneaky female like morph male. The mate guarding behavior of the blue male prevents him against cuckolfry from the sneaky male (Sinervo and Lively 1996).

The RPS game has been extensively modeled assuming clonal reproduction and pure phenotypic strategies (Maynard-Smith 1982; Sinervo and Lively 1996; Sinervo 2001a; Kerr et al. 2002) as well as diploid models (Sinervo 2001a; Svensson et al. 2005). Although theoretical models show long term maintenance of the RPS game, some discrepancies, such as strategy frequencies at equilibrium or strategy frequency oscillatory dynamics, remain between theoretical model predictions and field observations (Sinervo and Lively 1996; Sinervo 2001a; Kerr et al. 2002; Sinervo and Calsbeek 2006). For instance, while evidence from the field suggests a pattern of cycles of strategy frequencies with no attenuation (Sinervo and Calsbeek 2006), models predict the stability of strategy frequencies as cycles attenuate over time (Maynard-Smith 1982; Sinervo and Lively 1996; Sinervo 2001a). Differences between theoretical and empirical observations may have various origins. First, previous models of the RPS game rarely integrate demographic stochasticity (Sinervo and Lively 1996; Sinervo 2001a; Svensson et al. 2005); however, see (Calsbeek et al. 2002). But demographic stochasticity occurs in most biological systems and has an important impact on population dynamics (Legendre et al. 1999; Caswell 2001) and game stability (Maynard-Smith 1982; Caswell 2001). Secondly, interactions between the sexes are also known to influence game stability and population dynamics(Maynard-Smith 1982; Lindstrom and Kokko 1998; Alonzo and Sinervo 2001; Svensson et al. 2005; Alonzo and Sinervo 2007). In some lizards, including *Uta stansburiana*, females also express alternative reproductive strategies (Sinervo et al. 2000b; Sinervo et al. 2007). Female color types express an r/K-strategy set expressed as a trade-off between clutch size that is inversely correlated with offspring size and survival (Sinervo et al. 1992; Sinervo et al. 2000b; Svensson et al. 2005; Vercken et al. 2010).

Only a few studies have attempted to explore the genetic mechanisms underlying mating strategy games, usually assessing that a dominance relationship exists between the three alleles (Sinervo and Lively 1996; Sinervo 2001a; Svensson et al. 2005; Sinervo et al. 2008). Although there are some arguments for dominance (Cordero 1990; Sinervo et al. 2001; Svensson et al. 2005), field evidence such as specific morph expression in heterozygote (Sinervo and Lively 1996; Sinervo et al. 2007) or switches in individual reproductive strategies (Sinervo 2001a) suggest that a plastic mechanism may underlie the RPS game. Maynard-Smith (1982) showed that phenotypic plasticity could generate infinite cycles of strategy frequencies. Other than this work by Maynard-Smith (1982), very little theoretical attention has been devoted to phenotypic plasticity as a mechanism underlying expression of reproductive strategies in an RPS game (Sinervo 2001a; Sinervo and Calsbeek 2006), despite empirical evidence that suggests an endocrine basis to plasticity in male strategies (Sinervo et al. 2000a; Mills et al. 2008). Field evidence also suggests social-context dependant phenotypic plasticity could underlie the RPS game (Sinervo and Lively 1996; Sinervo 2001b;

Sinervo et al. 2001; Svensson et al. 2005; Sinervo and Calsbeek 2006). Heterozygotes carry genetic information for at least two strategies and thus would be the most likely candidates for expressing mating strategy plasticity. For both sexes, plasticity could be advantageous if the alleles coding the different strategies have no interactions or are synergetic (Sinervo 2001a). However, this information can be antagonistic and reduce a heterozygote's performance compared to homozygotes. For example, an individual with a territorial polygyny allele and a non-territorial sneaker allele could not express both strategies. Such a cost could impact the stability of the RPS game (Maynard-Smith 1982). Consequently, heterozygosity and social environment should be taken into account to describe and model the RPS game.

The first goal of this chapter is to explore how different genetic mechanisms leading to the expression of alternative reproductive strategies and demographic stochasticity could influence the maintenance of trimorphism in RPS games with polygynous (P coded by a *p* allele), monogamous (M coded by a *m* allele) and sneaker (S coded by a *s* allele) males (Sinervo and Lively 1996). In this regard, I developed a MetaConnect toolbox that allows modifying the mating system with respect to the RPS game outcomes (Figure 2). The individual-based-model structure of MetaConnect allows us to simulate effects of individual genetic information (i.e. are individuals homozygotes or heterozygotes for the gene coding reproductive strategy?), social environment and demographic stochasticity on individual expression of reproductive strategies. In this framework, I explored the maintenance of polymorphism under two hypotheses on the mechanisms leading to individual phenotype:

- Genetic dominance: we assessed genetic dominance of allele *p* on *s* and *s* on *m* ($p>s>m$) (Sinervo 2001a; Svensson et al. 2005; Sinervo et al. 2007). Past diploid models explicitly ignored over-dominance and only considered co-dominance to dominance (Sinervo 2001a).
- Plasticity: heterozygotes are plastic and able to apply the best strategy given their alleles for the current social context thereby providing an advantage to heterozygotes (Sinervo 2001a; Sinervo 2001b).

The second goal is to explore the impact on the maintenance of polymorphism of the genetic structure and life history traits under each genetic hypothesis, given a set of assumptions about the strategies: (1) alternative reproductive strategies in either one or both sexes and (2) costs linked to heterozygosity. Finally I tested the toolbox on a case study using parameters from *Uta stansburiana* to determine which processes most likely explain the maintenance of trimorphism in this species and predict strategy frequencies emerging from an RPS game.

Toolbox implementation

The RPS game implemented in the mating system toolbox modifies the workflow of the mating systems implemented in MetaConnect core base modeling as described in Figure 19.

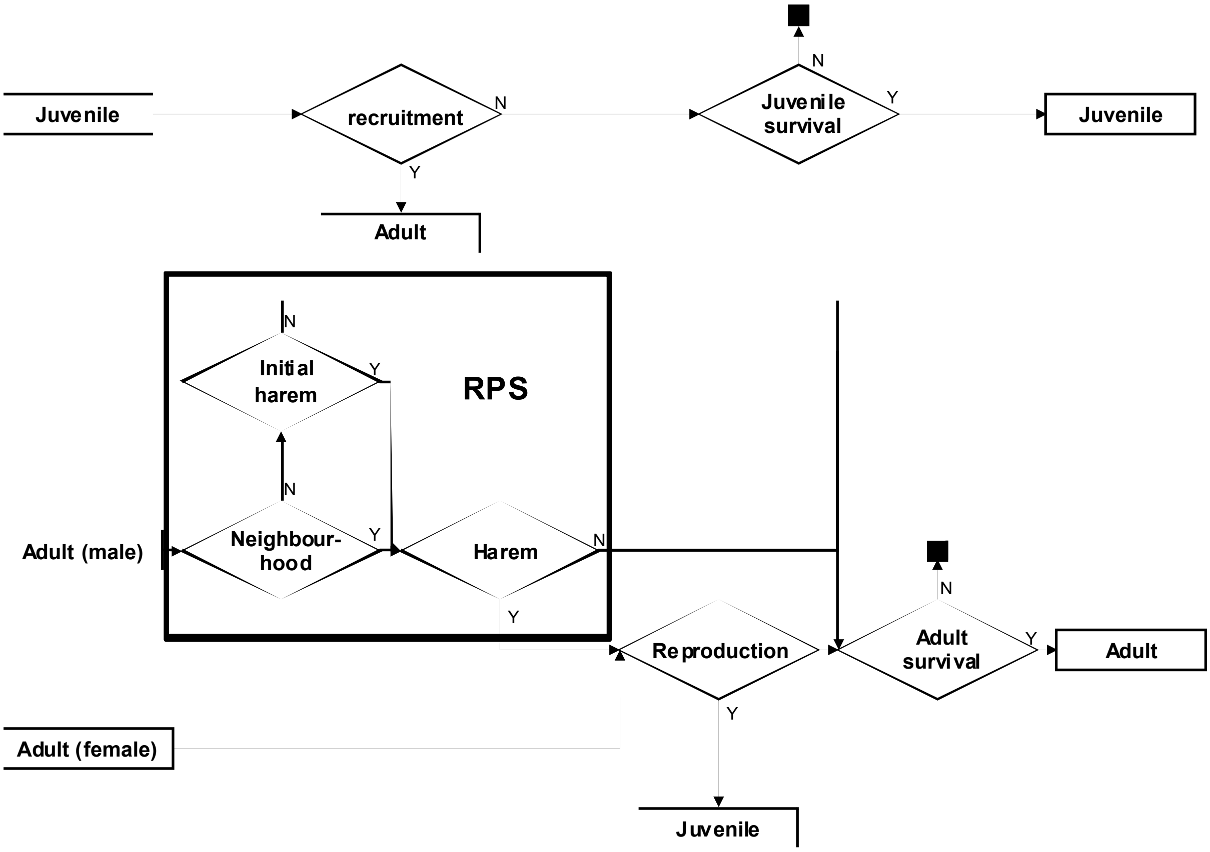


Figure 19: Life history of the model during a single time step showing the transitions between age classes (rectangles) and decisional rules (rhombus) regulating these transitions. Beginning with a juvenile individual, if in the recruitment phase (recruitment rhombus) the result is 'yes' (Y), the individuals is recruited as a reproductive adult and starts the mating phase. Else (the result is 'no' (N)), it stays in the juvenile compartment and will be tested for its survival. The black squares correspond to exclusion of individuals from the system due to their death. The mating procedure is composed by the three processes in the bold rectangle and includes the implementation of the RPS game.

*Male reproductive strategy phenotype and the RPS game*

The mating system is driven by male social strategies. The fitness depends on the capacity of any given male to monopolize a harem. A male's ability to win the RPS game against his neighbors is summarized in the dyadic pay-off matrix $w_0$ (table 3). A male's reproductive success is thus altered by the spatial structure of neighboring genotypes which is closely linked to the average frequency of each phenotype. For example, if we consider a sneaker male playing against a polygynous one, the sneaker male will probably win. I used equation 3 (derived from Sinervo and Lively 1996), where $W_i$ is the relative average gain of the strategy $i$, $f$ the frequencies of strategies $i$ and $k$ and $w_1$ the relative dyadic payoff matrix, to estimate at each time step the relative average fitness of each strategy playing against the beaten strategy (i.e. P *vs* M, M *vs* S, S *vs* P).

Equation 3:

$$W_i = f_i \sum_{1}^{k} f_k w_{1(i,k)}$$

The mating process is implemented in three steps. First, a male's neighborhood is determined. The neighborhood size is assumed to follow a Poisson distribution and neighbors are randomly allocated among other males regardless on their strategy. Then males of each strategy constitute their harem. Harem size also follows a Poisson distribution and females are allocated randomly among non-mated females. The constitution of initial harems begins with polygynous males and ends with sneakers. Next, the contests between male starts with S playing against P neighbors, then M playing against S neighbors and finally P playing against M neighbors. In this game phase, each pair of males plays the game where the winning strategy has a probability of acquiring each female of the loosing male's harem according to a Bernouilli event with $p = 0.5 + W_i$ where $W_i$ is calculated using equation 3.

### *Female reproductive investment as gradient of r-K strategies*

Female mating strategies might have some impact on the maintenance of male polymorphism (Lindstrom and Kokko 1998). I implemented female alternative reproductive strategies as an r-K gradient. We assumed that the alleles supporting female strategies are the same as in males (i.e. Polygynous, monogamous and sneaker male strategies are coding by genes leading to the expression respectively of r, intermediate and K reproductive strategies in females) following the empirical example in *Uta stansburiana* (Sinervo 2001a; Sinervo et al. 2007). I also assumed that the genetic mechanisms leading to female phenotypic strategies are the same as for males (allelic dominance or plasticity).

Offspring number and recruitment probability for intermediate females did not change from the model without the female game. However, the mean number of offspring ($F$) produced by K strategy females was 15% higher than for intermediate females, and 15% lower for r strategy females compared with intermediate females. Accordingly to an r-K gradient of strategies, the recruitment probability of offspring from r strategy females was reduced by a factor $\Delta_s$ while offspring from an K strategy female had a recruitment probability increased by a factor $\Delta_s$.

### *Cost of heterozygosity*

Heterozygotes might be less efficient than homozygotes in applying their strategy to reproductive competition. Indeed, in the well-known example of human sickle-cell anemia, even though heterozygotes have better global fitness than homozygotes when malaria is present, their hemoglobin is less efficient than that of homozygotes (Fleming et al. 1979). Using a similar effect in the toolbox, an aggressive homozygote polygynous male may be more aggressive than a heterozygote leading to a larger harem size than for the heterozygote individual. To determine the impact of this possible effect on the maintenance of trimorphism I implemented a cost ($c$) to heterozygotes in both male and female games. For males, I considered that a game between heterozygotes is equivalent to a game between pure strategists but that pure strategist had an advantage playing against heterozygotes which was implemented as an increased probability to win by a factor $c$. For heterozygotes, I assumed that the initial mean harem size was reduced by a factor $c$ as was the probability of winning in the RPS game against a pure strategist. In the female game, heterozygotes had their differential fecundity from pure intermediate strategy reduced by a factor $c$ and their offspring survival difference from intermediate female offspring was also reduced by a factor $c$.

## Running the model

### *Nomenclature and MetaConnect set up*

Table III: Nomenclature and default parameter value used in MetaConnect setup in Chapter II

| **Parameters, variables and their distributions** | **Value(s) of parameter for general model** | **Value(s) for *Uta stansburiana*** | **Description** | **References for *Uta stansburiana*** |
|---|---|---|---|---|
| **Demography** | | | | |
| $\sigma \rightsquigarrow \mathcal{B}(p)$ | 0.5 | 0.5 | sex ratio | (Sinervo 2001a) |
| $s_j \rightsquigarrow \mathcal{B}(p)$ | 0.2, 0.5 | 0.2 | juvenile survival | (derived from Sinervo and Lively 1996) |
| $s_a \rightsquigarrow \mathcal{B}(p)$ | 0.2, 0.5 | 0.2 | adult survival | (derived from Sinervo and Lively 1996) |
| k | 0.004, 0.002, 0.001 | 0.004, 0.002, 0.001 | competition coefficient | see text |
| $F \rightsquigarrow \mathcal{P}(\bar{F})$ | 14, 9.2, 6.5, 4.4 | 14 | fecundity | (derived from Sinervo and Lively 1996) |
| **Life history traits** | | | | |
| *Males* | | | | |
| $w_0$ | $\begin{array}{c\|c\|c\|c} & \mathbf{P} & \mathbf{M} & \mathbf{S} \\ \hline \mathbf{P} & 1 & 2 & 0.50 \\ \hline \mathbf{M} & 0.5 & 1 & 2 \\ \hline \mathbf{S} & 2 & 0.5 & 1 \end{array}$ | $\begin{array}{c\|c\|c\|c} & \mathbf{P} & \mathbf{M} & \mathbf{S} \\ \hline \mathbf{P} & 1 & 1.32 & 0.35 \\ \hline \mathbf{M} & 0.34 & 1 & 2.3 \\ \hline \mathbf{S} & 3 & 0.69 & 1 \end{array}$ | RPS game pay-off matrix<br>*(Mean number of females won by a focal male of a given strategy (by row: polygynous (P), monogamous (M) or sneaker (S)) when play against 3 other types of males (by columns))* | (Sinervo 2001b) |
| $h_p \rightsquigarrow \mathcal{P}(\overline{h_p})$ | 3 | 3 | Orange harem size | (Zamudio and Sinervo 2000) |
| $h_m \rightsquigarrow \mathcal{P}(\overline{h_m})$ | 1 | 1 | Blue harem size | (Zamudio and Sinervo 2000) |
| $h_s \rightsquigarrow \mathcal{P}(\overline{h_s})$ | 0.5 | 0.5 | Yellow harem size | (Zamudio and Sinervo 2000) |
| $n_p \rightsquigarrow \mathcal{P}(\overline{n_p})$ | 3 | 2.95 | Number of orange male neighbors | (Sinervo 2001b) |
| $n_m \rightsquigarrow \mathcal{P}(\overline{n_m})$ | 3 | 2.35 | Number of blue male neighbors | (Sinervo 2001b) |
| $n_s \rightsquigarrow \mathcal{P}(\overline{n_s})$ | 3 | 5.05 | Number of yellow male neighbors | (Sinervo 2001b) |
| *Females* | | | | |
| ΔF | ± 0.15 | ± 0.15 | Deviation from mean fecundity for r and K strategists | see text |
| Δs | ± 0.015 | ± 0.015 | Deviation from mean recruitment for juveniles from r or K females | see text |
| *Heterozygosity cost* | | | | |
| c | 0.2 | 0.2 | Cost factor | see text |

### *Model scenarios*

Under each genetic mechanism, I examined a range of possible life history trait values provided in table 3. In addition I applied the same framework to explore the specific case study of the side blotched lizard (*Uta stansburiana*) using parameters found in the extensive literature on this species. For each set of parameters, I considered several initial conditions of allele frequencies:

1. I aimed to determine if the genetic mechanism allowed maintenance of reproductive strategy trimorphism. Consequently, I assumed that all alleles were initially equally represented in the population.
2. I performed runs with only two alleles in the population to determine how long it took for each strategy to outcompete the other.
3. I simulated the "appearance" of a third allele (and therefore a third strategy) in a two allele population. Appearance of the new allele is implemented by replacing 20 heterozygote individuals (carrying alleles for the two initial strategies) into 20 heterozygotes who carry the new allele and the allele coding the strategy that is currently losing. For example, in a population with only '*p*' and '*m*' alleles, we removed 20 '*pm*' individuals and replaced them by 20 '*ms*'.

Simulations were run during 300 time steps which is sufficient to reach the stability of the population dynamics and of the genetic structure. All runs were repeated 200 times. I chose to analyze the model output using time series analysis (wavelet analysis see appendix A of related paper). Wavelet analysis is data consuming so I extended the number of time steps to 2050 for scenarios where polymorphism was maintained to be able to perform a consistent analysis (Box and Jenkins 1976).

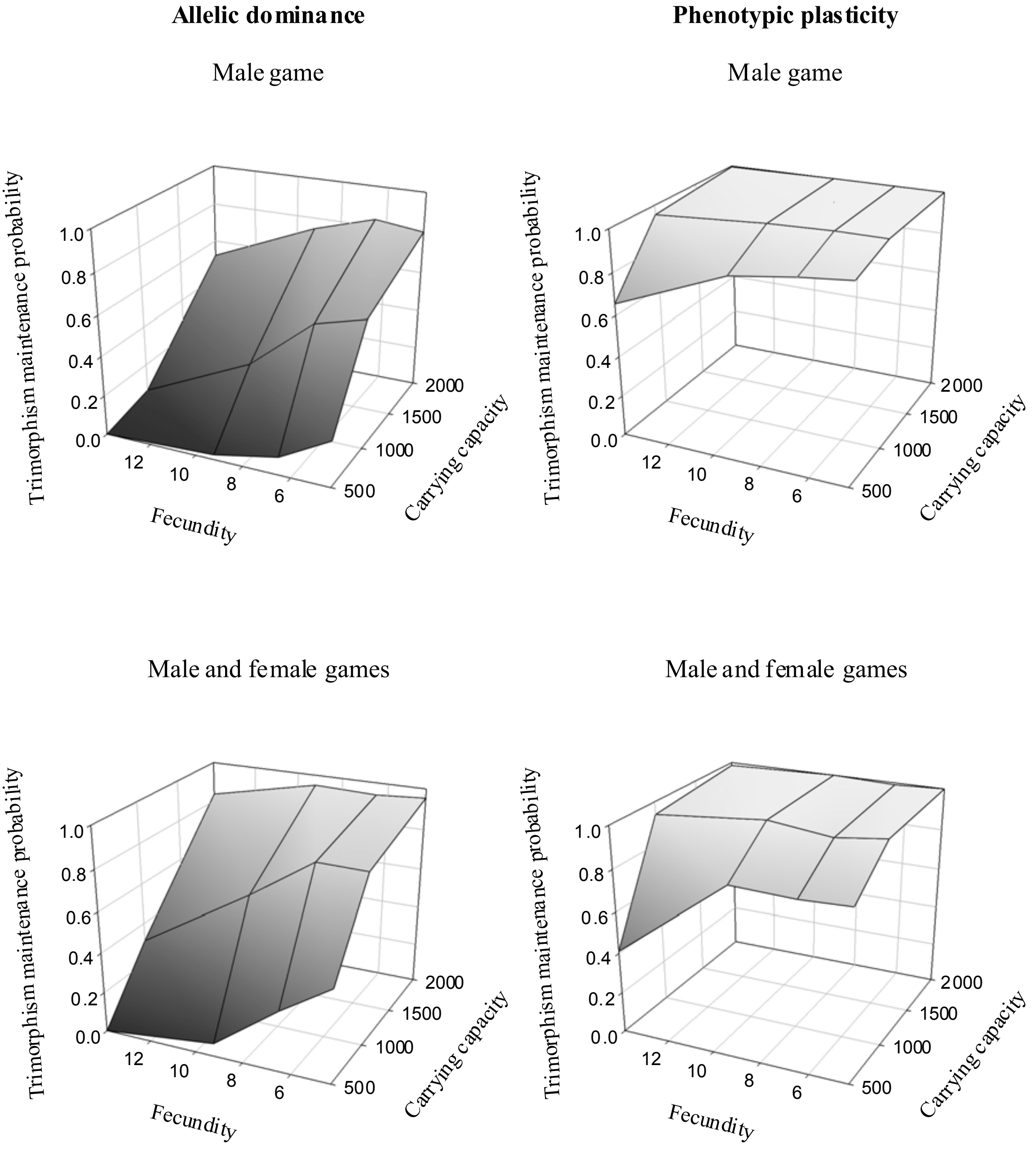
## Maintenance conditions of trimorphism in the RPS game context

The trimorphism maintenance probability is sensitive to the individual lifespan and to the population carrying capacity. The increase of both of these independent parameters reduces the risk of allele extinction through demographic stochasticity (Figures 20, 21).

When only the male game is modeled, the underlying genetic mechanism has a strong effect on the maintenance of polymorphism (table 4). Even though, the two genetic scenarios we modeled can maintain polymorphism, alternative reproductive strategies are more easily maintained under the hypothesis of plasticity than under the genetic dominance hypothesis (maintenance probabilities equal to 1 and 0.4 respectively) (Figures 20, 21).

Coupling male and female games have different effects depending on the genetic mechanism underlying the reproductive strategies (table 4). The female game increases the ability of the three morphs to coexist under the genetic dominance hypothesis. In our simulations, when a male strategy is rare, it is also rare for females and this means that the allele for this strategy will be favored in both sexes at the same time, thereby reinforcing the fitness advantages arising from negative frequency dependence. In contrast, when plasticity underlies the strategies, the female game reduces the maintenance probability of trimorphism (Figure 1). Time series analysis shows that the female game destabilizes the male game through an increase of the effect of environmental stochasticity (social interactions).

Not surprisingly, when heterozygosity has a cost, the maintenance probability of trimorphism decreases (table 4). This reduction is low regardless of the genetic mechanism underlying reproductive strategies when only males are playing (Figure 20). However, if both sexes present alternative reproductive strategies, the cost of heterozygosity drastically reduce the maintenance probability of trimorphism and does so regardless of the genetic mechanism underlying strategies (Figure 21). Under the dominance hypothesis, the reduced probability of maintaining the trimorphism when heterozygosity is costly seems to be explained by a change in average strategy frequencies. The polygynous strategy frequency is similar to other scenarios but the sneaking strategy frequencies fell from 0.4 to 0.3 thus increasing the risk of stochastic extinction. Under, the plasticity hypothesis, the amplitude of strategy frequency oscillations was increased, thereby increasing occurrences of extinctions due to stochastic events at low frequency phases of a rare allele.



**Allelic dominance**

**Phenotypic plasticity**

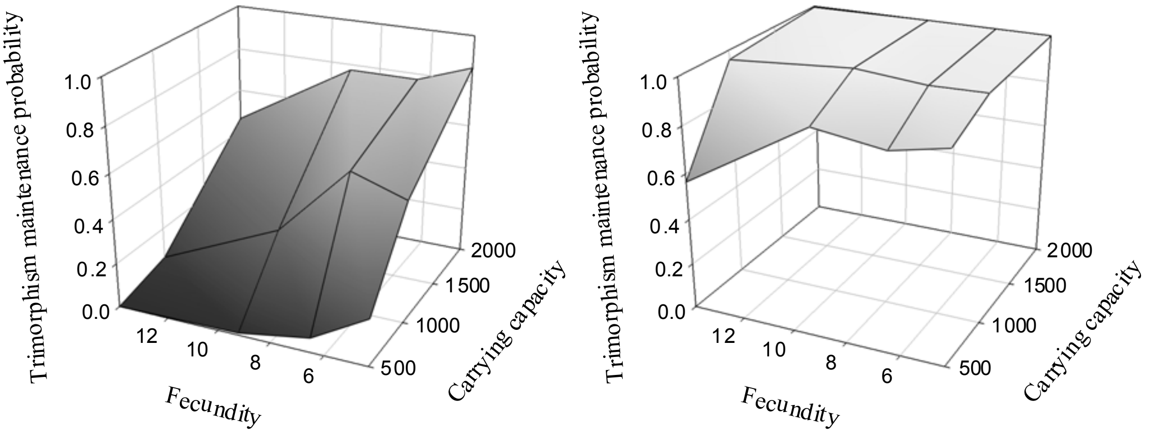
Male game

Male and female games

Figure 20: Trimorphism maintenance probability when males express alternative reproductive strategies or males and females express alternative reproductive strategies under the allelic dominance or phenotypic plasticity hypotheses. The maintenance of the trimorphism is more easily maintained when individuals are plastic than if the phenotype depends on an allelic dominance relationship. The female game sustains the maintenance of the trimorphism.

**Allelic dominance** **Phenotypic plasticity**

Male game with cost to heterozygosity Male game with cost to heterozygosity



Male and female games with cost to heterozygosity Male and female games with cost to heterozygosity

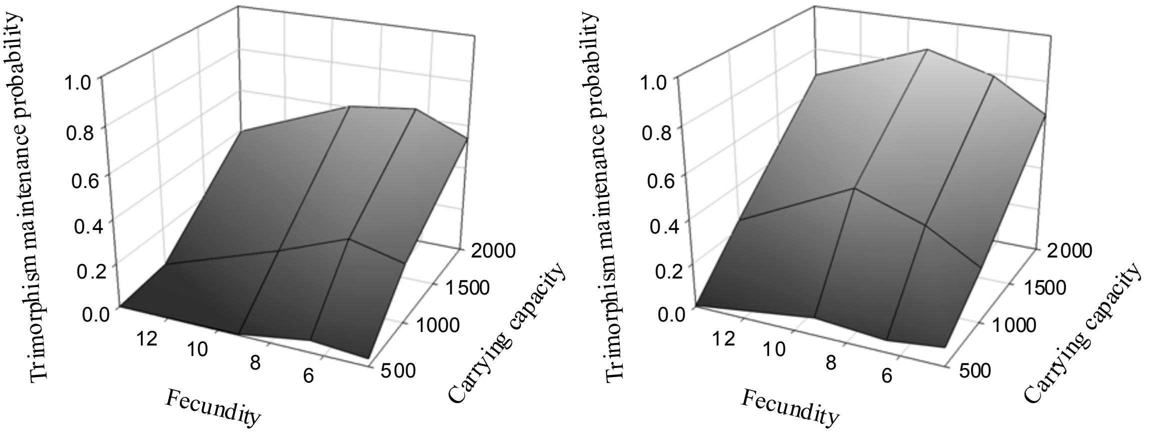


Figure 21: Trimorphism maintenance probability when males express alternative reproductive strategies or males and females express alternative reproductive strategies under allelic dominance or phenotypic plasticity hypotheses when heterozygote phenotypes are less efficient than homozygotes (cost of heterozygosity). The trimorphism is more easily maintained if individuals are plastic. The heterozygosity cost induces a strong decrease of probability of maintenance of trimiorphism when females are affected by the cost.

Table IV: Relative sensitivity of the maintenance of trimorphism in regard with the scenarios assumptions (i.e. who plays? And how do they play?).

| Mechanism | Scenario | ♂ | ♂+♀ | ♂+cost | ♂+♀+cost |
|---|---|---|---|---|---|
| Genetic dominance | | - - | - | - - - | - - - - |
| Plasticity | | + + + | + + | + + | - |

As in previous studies (Sinervo and Calsbeek 2006; Bond 2007; Dijkstra et al. 2008), I found that discrete polymorphism can be maintained by negative frequency dependent selection (Maynard-Smith 1982). However, my results suggest that the underlying genetic control of the mating strategy phenotype strongly affects the probability that polymorphism is maintained. Previous genetic models studying the properties of the RPS game assumed that a dominance relationship existed in alleles coding for the three strategies (Gross 1996; Svensson et al. 2005; Sinervo and Calsbeek 2006). However, even though trimorphism can be maintained under the allelic dominance hypothesis, the conditions for its maintenance are restrictive when compared to the plasticity hypothesis. Indeed, inclusion of demographic stochasticity under the genetic dominance hypothesis will likely lead to a loss of one of the three alleles underlying the RPS game, especially when compared to the plasticity hypothesis. This would be especially important since evolution of a third strategy will most likely require some length of time where the two other strategies are stable. From my results, I should expect that the trimorphism will be maintained by dominance and negative frequency dependent selection only in large populations or in long-lived species with high dispersal rates, where these trimorphisms are apparently rare (Sinervo and Calsbeek 2006), or in small populations of a metapopulation as suggested by Corl et al. (2010).

In contrast, when heterozygotes are able to adopt the best strategy with respect to their social environment, polymorphism can be maintained regardless of the population size or the species life cycle. The plasticity hypothesis (or an advantage to the heterozygote) seems to allow the maintenance of polymorphism for a wider range of species life strategies and environments; and accordingly we might expect the plasticity mechanism to be more wide-spread in trimorphic species and by extension in simpler mating systems such as r-K gradient of strategies (Vercken et al. 2010). In this respect, previous empirical as well as theoretical studies have shown that the advantage to the heterozygote often underpins the maintenance of polymorphism of reproductive strategies in natural populations (Fleming et al. 1979; Sinervo and Zamudio 2001; Sinervo 2001a; Cook 2007; Vercken 2007; Vercken et al. 2010).

### RPS game functioning: disentangling RPS game structuring from demographic structuring of morph frequencies

The cross-correlation functions (see appendix A of related paper) between frequencies of pairs of strategies show that the polygynous strategy has a negative effect on the monogamous strategy and a positive effect on sneakers. Surprisingly, the frequency of the monogamous strategy does not influence the sneaker strategy frequency suggesting that the game is largely driven by the polygynous strategy (Figure 22).

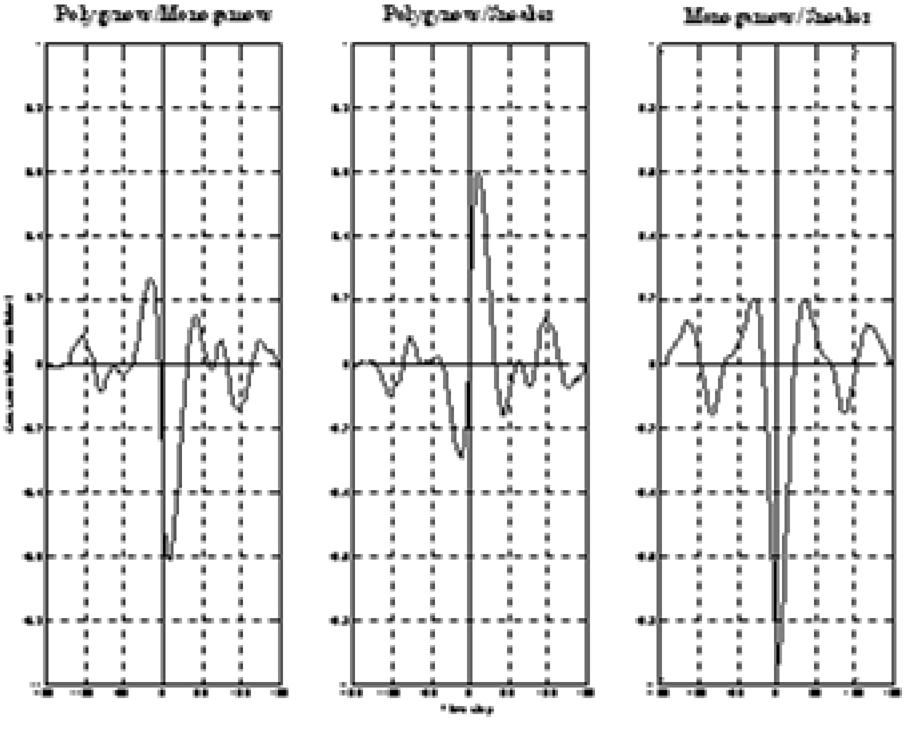


Figure 22: The influence that two time series impose on each other is detected by cross-corelation plots. If no causal relation exist between them, the cross-correlation function is symetric and centered on 0. The existence of a causal relation between the two series generates an asymmetry in the cross-correlation function (Box and Jenkins 1976). Moreover,

the direction of the deviation along the ordinate axis provides the direction of the correlation (see appendix A of related paper for more details). Here, asymetry and the direction of deviation in the cross-correlation function between the polygynous strategy and the monogamous strategy as well as between the polygynous strategy and the sneaker strategy, means that the polygynous strategy has a negative effect on monogamous individuals and a positive effect on sneakers. In contrast, the cross-correlation function between the monogamous strategy and the sneaker shows that the monogamous strategy does not affect the allele frequency dynamic of the sneaker strategy. At the global scale of the RPS game, the polygynous strategy appears to drive the game.

Regardless of the specific model configuration, wavelet analyses and multi-resolution analyses show that strategy frequency time series are structured on different time scales. Time scales of the different processes described above increase with increasing lifespan. For short-lived species ($S_a = 0.2$ and $S_j = 0.2$), the interpretable emerging structures are situated between 4 and 32 time steps (Figure 23). The fastest structure (a 4 time step period) is generated by density-dependence of recruitment. Indeed, these structures are independent of the alternative strategy interactions (Figure 23) and appear in the analysis of the population dynamic (result not show). The structures emerging from the RPS game occur at time scales between 16 and 32 time steps corresponding to the maximum correlation between pairs of strategy frequencies (Figure 23). Moreover, strategies do not influence each of the other strategies at the same time scale. For example, the polygynous strategy dynamic influences the monogamous and the sneaker strategies at a 16 time step scale whereas monogamous and sneaker strategies influence each other and the polygynous strategy at a 32 time step scale (Figure 23). Wavelet power spectra of the strategy frequency dynamic show that cyclical structures are detected at the 16 and 32 time scales (Figure 23). The multi-resolution spliting confirms that these structures that make up the overall dynamic are cyclical (Figure 23) and that the relative frequency of strategies exhibit a consistent pattern with those expected for the RPS game (see appendix B of the related paper for a detailed view of the pattern). A third category of structure exists at the 8 time step scale due to the link between the mating system and the density-dependence of the recruitment (Figure 23).

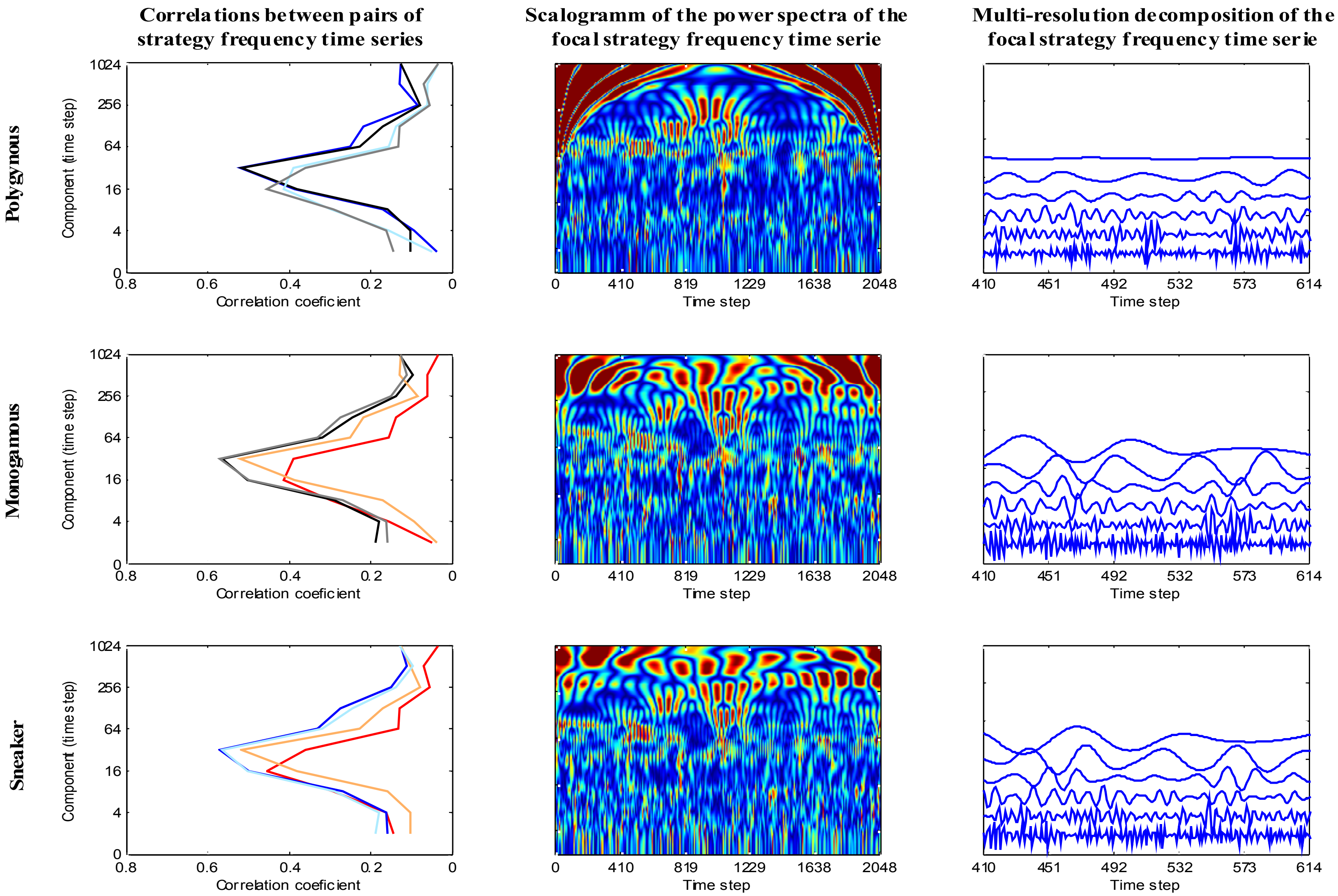

Correlations between pairs of strategy frequency time series
Scalogramm of the power spectra of the focal strategy frequency time serie
Multi-resolution decomposition of the focal strategy frequency time serie
Polygynous
Monogamous
Sneaker
Component (time step)
1024
256
64
16
4
0
0.8
0.6
0.4
0.2
0
Correlation coeficient
0
410
819
1229
1638
2048
Time step
410
451
492
532
573
614
Time step

Figure 23: Wavelet ("Morlet" wavelet) and multi-resolution analyses (see appendix A of the related paper for a description of the analysis) of strategy frequencies dynamics. From top to bottom, results are provided for focal time series of respectively polygynous, monogamous and sneaker strategy frequency dynamics. The time series are outputs from a simulation with allelic dominance, both the males and females exhibit alternative reproductive strategies and heterozygosity is not costly. Left panels correspond to the correlation between the non focal time series of strategy frequencies and the multi-resolution decomposition of the focal time series (see appendix A of the related paper). Dark blue, black and red lines represent the effect of each of the two non-focal time series on the component of the focal time series listed on the left of the graph and grey, orange and light blue lines are the effects of the focal time series on each of the non-focal time series. Non-focal time series are red, blue or black for polygynous, monogamous and sneaker strategy frequency dynamics respectively and are orange, light blue and grey respectively when considering the effect of focal time series on non-focal time series. Central panels are the power spectra analysis of the strategy frequencies. The colors code for power values from relative low values (dark blue) to relative high values (dark red). The nature (period, stationarity) of the cycles detected in the time series are determined using their specific signatures of alternation between red and blue regions (see appendix A of the related paper for details of the signatures). Right panels are the multi-resolution decomposition of the strategy frequencies which correspond to the time series decomposition at each time scale. The analyzed time series is the sum of the multi-resolution decomposition.

Theory says that in the RPS game, all strategies must have an equivalent global fitness over the long time scales to constitute an ESS (Maynard-Smith 1982; Gross 1996). This would intuitively suggest that the maintenance of the cyclical structures generated by the RPS game may be equally sensitive to frequency variation of the three strategies. My time series analyses have shown that, in the general case, the genetic dynamic emerging from the RPS game is driven by a single strategy, the polygynous strategy. This suggests that even though all the strategies have equivalent global fitness, the system will be more sensitive to perturbation of the polygynous strategy and that trimorphism is closely linked to the frequency dynamic of the polygynous strategy. While most empirical systems do not have a sufficiently long time series to investigate the influence of each strategy on population frequency dynamics, the time series analysis techniques used here suggest that social game payoff structure may not reflect the actual importance of each social strategy when demographic stochasticity is present.

## *RPS game in* Uta stansburiana

Because empirical pay-off matrices differ from the generic RPS game, I also tested the whole set of scenarios on the specific case of *Uta stansburiana*. When alleles are initially at the same frequency, the dominance hypothesis leads to monomorphic populations for $K<2000$. Trimorphism is easily maintained under the allelic dominance hypothesis when $K\geq2000$ or under the plasticity hypothesis. Under the plasticity hypothesis, the trimorphism is easily maintained even in small populations (Figure 24).

Cross-correlation function analyses show that the RPS game generates cyclical structures with periods of 24 time steps for the male game under the dominance hypothesis and 51 time steps when considering both the male and female games under the plasticity hypothesis.

As in the general case, the female game has a stabilizing effect on cyclical structures emerging from the RPS game under the dominance hypothesis and a destabilizing one under the plasticity hypothesis. The result is an increase of the probability of maintenance of trimorphism under the dominance mechanism and a reduction of this probability under the plasticity hypothesis.

The cost of heterozygosity has little effect on the allele extinction probability when considering only the male game but increases this probability when the female game is added suggesting that in the wild the cost of heterozygosity may be low or non-existent as the trimorphism seems to be stable.

When a given allele is initially rare, no allele is able to invade the population when allelic dominance underlies the strategies. In contrast, *p* and *m* alleles but not *s* can invade when rare under the plasticity mechanism thereby leading to the maintenance of trimorphism. This suggests that the evolution of this trimorphism could only have occurred under the plasticity hypothesis in very large population sizes, or if the payoff matrix has evolved.

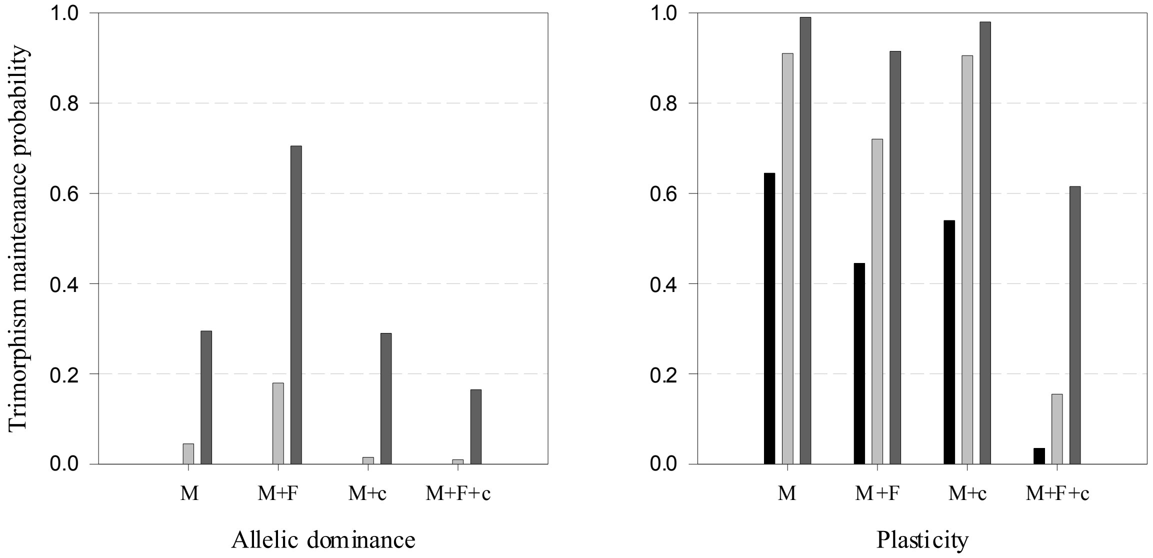


Figure 24: Trimorphism maintenance probabilities under the dominance hypothesis (left panel) and the plasticity hypothesis (right panel) for *Uta stansburiana* for the different combinations of two sex game assumptions (M: male game, F: female game and c: heterozigosity cost). Probabilities under the genetic dominance hypothesis are zero for K=500 (black and not shown) and low for K=1000 (light grey). The trimorphism seems to be maintained only in large populations (K=2000 in dark grey). Moreover, the female game increases the maintenance probability while the heterozygosity cost hypothesis reduces it (see text).

In the *Uta stansburiana* case, my model results did not fully allow me rejecting one of the two genetic mechanisms underlying the RPS game that I simulated. While the trimorphism maintenance probabilities for *Uta stansburiana* estimated by my model (but also by some empirical results) provide support for the plasticity hypothesis, the analysis of the population dynamic (the cyclical periodicity) arising from this hypothesis does not match the observed cycles, since my model predicted cycles with a 33-51 time steps cycle versus a 5-6 time steps cycle in nature (Sinervo and Lively 1996; Sinervo 2001a; Sinervo and Calsbeek 2006). The inclusion of the two sex scenario provided support for the genetic dominance hypothesis since it gives both a high probability of maintaining the game over long periods of time and a shorter time period for the cycle (16-32 time steps), which is closer to the periodicity seen in nature.

If some biological processes like female mate choice (Alonzo and Sinervo 2001) or computational constraints (see related paper), may explain a part of the discrepancies between RPS modeling and field results, a statistical bias cannot be excluded. Indeed, field observation of strategy frequencies in the side blotched lizard do not extend for a sufficiently long time period to perform time series analysis to calculate the period of cycles analytically rather than graphically (Sinervo 2001a; Sinervo and Calsbeek 2006). If we graphically measure the time period of cycles, we obtain similar results as observed in the field (~4 year cycles regardless on the genetic mechanism scenario). Time series analysis is very powerful even with noisy data (Box and Jenkins 1976; Abarbanel 1996; Abry 1997) and for this time period, simulation outcomes are explained by population dynamic density-dependence rather than the frequency dependent mating game. This suggests that the periods usually measured graphically (Sinervo and Lively 1996; Sinervo 2001a; Svensson et al. 2005; Sinervo and Calsbeek 2006; Sinervo et al. 2006) are likely due to demographic density-dependence rather than induced by the RPS game and that cycles observed in the field might not correspond to cycles emerging from the RPS game per se.

## Chapter conclusion

In this chapter, I began the implementation of the first operational MetaConnect toolbox. The mating system and game theory toolbox is currently very simple since it only includes a male type of alternative reproductive strategy (RPS game), a female type of alternative reproductive strategy (r-K discret gradient) and the possibility of including a cost linked to the individual genotype in its phenotype expression. However, thanks to the MetaConnect structure, the implementation of such a basic toolbox would easily allows extension of the study I did in single population to a meta-population system with the ability of testing the sensitivity to dispersal mode and providing comparables outputs. In the *Uta stansburiana* exemple, such a possibility would allows us to test the Corl et al.'s (2010) hypothesis of trimorphism maintenance in a meta-population system.

In parallel, I started the implementation, with Elvire Bestion, of a genetic toolbox to integrate adaptive dynamic to examine the evolution of strategies. Since MetaConnect core base model, simulates explicitely gene transmission, it is very simple to implement a toolbox that allows the mutation of genes (currently basic: simple mutation only, no recombination nor reverse mutation,….) and the interaction genotype/phenotype. The idea in this toolbox is to simulate the mutation/selection processes on a set of chosen life history traits. The mutation/selection proces then occurs on the traits mean values and/or on its raction norm. This possibility will permit to work with the same toolbox on the evolution of life history traits as well as on the evolution of their plasticity. I expect this toolbox to be of a high interest to evolutionary biologists.

# III. MetaConnect in conservation biology: Corridor functionality for dragonflies and butterflies within the Montselgues (Ardèche, France) bog network

Study context

In 2010, I was contacted by the Parc Naturel des Monts d'Ardèche (PNR MA) and the Conservatoire des Espaces Naturels de Rhône-Alpes (CEN RA) because they needed a population viability analysis for dragonflies within the bog network of the Montselgues plateau in France (Figure 25). The bog network of the *plateau de Montselgues* (France, Ardèche, 44°30'13.63"N, 4°00'32.29"E, alt. 1032 m) is composed of 4 main bogs (Narcettes, Chabreille, Cham du Joux, Rouveyrettes). This bog network is a Natura 2000 site that benefited from a LIFE Nature conservation program from the EU (LIFE-Nature 2005-2010 n° LIFE05NAT/F/000135). In that site, in 2007, the Parc Naturel des Monts d'Ardèche (PNR MA) and the Conservatoire d'Espaces Naturels de Rhône-Alpes (CEN RA) conducted a mark release recapture (MRR) survey on dragonflies and damselflies species and concluded that the site needed a restoration of bog connectivity (Jullian and Coïc 2002). In 2009, they aimed at restoring the functional connectivity as required by the Art. R.371-20 – I of the Décret n° 2012-1492 du 27 décembre 2012 of the French legislation, by building a biological corridor through the forestry plantations (Sutcliffe and Thomas 1996) between les Narcettes and la Cham de Chabreille (Figure 25). A second aspect of my work for the CEN RA and the PNR MA was to evaluate whether the artificial corridor they built is currently efficient. To do so, I had to compare the flow of individual before corridor construction with the current (2011) flow. In 2007, the MRR session objective was to 1) inventory dragonflies and damselflies species 2) survey the population of *Phengaris alcon* (butterfly) 3) count the number of dragonflies, damselflies and butterflies that move from a site to another. All this work was done by a single M2 student. Such a survey was neither optimal to perform the PVA of dragonflies nor measure the flow of individuals between bogs.

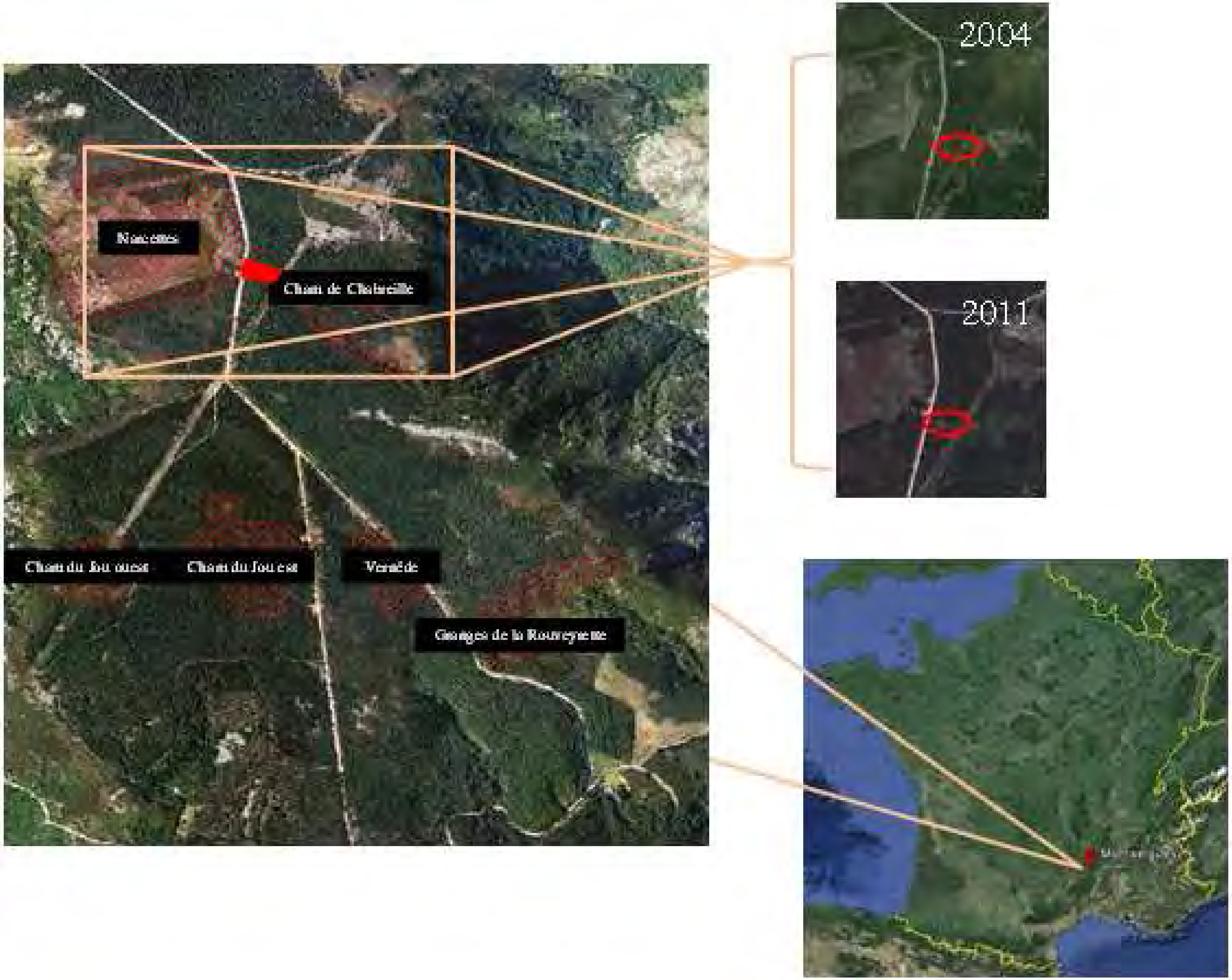


Figure 25: Bog network of the Montselgues plateau in France. The red polygones and circles show the landscape modifications corresponding to the corridor built by the CEN RA in 2009 to connect the Narcettes to the Cham de Chabreille.

*Orthetrum coerulescens functional connectivity (Moulherat 2013b; Moulherat et al. in prep)*

## 1 Dragonflies 2007 MRR overview and 2011 field work design

During the MRR session of 2007 (47 days), 1451 dragonflies from 17 species were marked (Figure 26). Among the 17 species, only *Orthetrum coerulescens* presents a sufficient sample size to perform a robust MRR analysis. In addition, the sample was strongly biased toward mature adult males. The 2007 sampling induces restrictions in the analysis. The interpretation of results relies on the following information:

1. In dragonflies, dispersal occurs in non-mature individuals (Dumont and Hinnekint 1973; Michiels and Dhondt 1991; Jakob and Suhling 1999; Angelibert and Giani 2003).
2. Males are less mobile than female (Michiels and Dhondt 1991; Angelibert and Giani 2003) especially in *O. coerulescens* where males are territorial (Ladet et al. 2012).
3. MRR in dragonflies is highly biased toward male capture (Jakob and Suhling 1999; Kery and Juillerat 2004) but Kery and Juillerat (2004) showed that this bias is behavioral (i.e. more cryptic females) and that the real population sex ratio is equal in *O. coerulescens*.
4. The time devoted to capture in each bog was independent from the bog surface.

Finally, the data set available to perform a PVA analysis and the analysis of the corridor functionality concerns the sex and the age class that disperses the least.

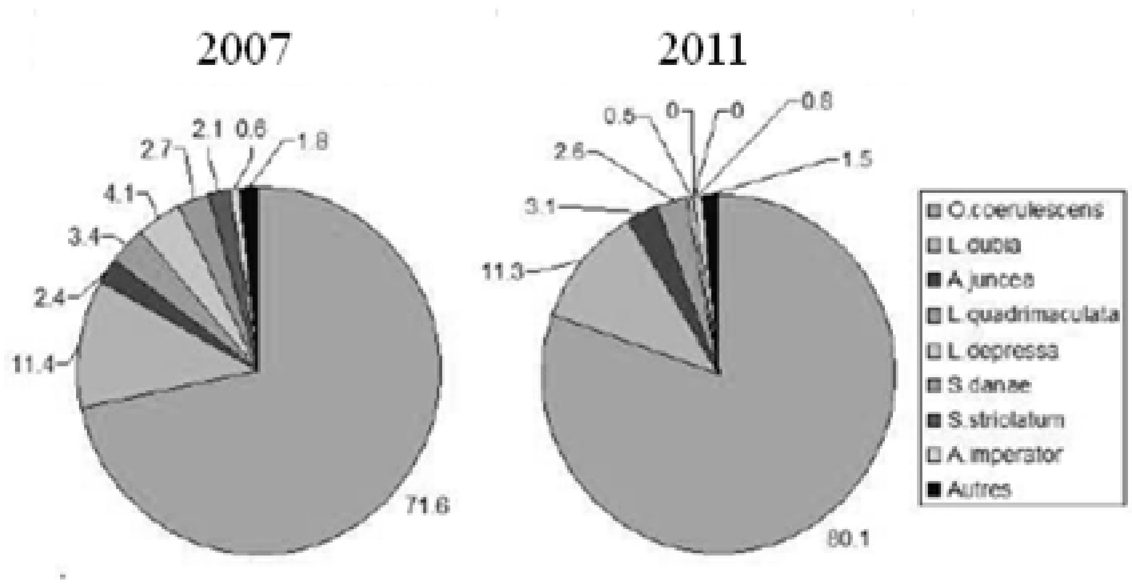


Figure 26: Repartition (% of marked species) of dragonflies marked in 2007 and 2011 within the bog network of the plateau de Montselgues (France).

Given the available datas, we decided in 2010 to perform a new MRR survey in 2011 focused on *Orthetrum coerulescens*. The rational for this is that if we only consider adult male dispersal (theoretically rare) as an input of the PVA, it should be conservative. If this flow is sufficient to sustain the population viability and the population genetic diversity, this scenario would correspond to a very pessimistic case, and by extention, the population must be viable and the genetic diversity sufficient. The survey then had two objectives:

1. Realizing a MRR analysis with the POPAN model to estimate demographic characteristics of the *O. coerulescens* population (adult male survival, catchability, recruitment, population size) (Arnason and Schwarz 2002). The MRR results would then feed MetaConnect to perfrom the PVA analysis of *O. coerulescens* within the bog network.
2. Realizing a MRR analysis with a multistate model to estimate the individual flow between patches (Doligez et al. 2002; Lebreton and Pradel 2002). The results would then allow comparing flows estimates between the Narcettes and Cham de Chabreille for the 2007 and 2011 sessions to test the corridor functionnality and fed MetaConnect to perform the PVA analysis.

## 2 Descriptive results of the 2011 MRR campaign

In 37 days (30/05/2011 to 23/07/2011) of field work with a capture intensity proportional to the patch areas (Narcettes, Cham de Chabreille and Granges des Rouveyrettes) and with respect to the presence of dragonflies (no dragonflies in Cham du Jou and Vernède due to regular drought (Jullian and Coïc 2002)), the dragonfly population do not significantly differ from 2007 (Wilcoxon test, W=54, p=0.25) and 1196 *O. coerulescens* males were marked. As in 2007 and in another study in the Swiss Jura (Kery and Juillerat 2004), 75% were males (N=1116) and 25% females (N=377).

## 3 MRR analysis

### a. Selection of meteorological covariates

The meteorological station of Loubaresse (44°35’59.18” N, 4°03’01.32” E, alt.: 1200 m, 9.23 km from the study site) and Sablière (44°32’33.14” N, 4°24’31.60” E, alt.: 172 m, 32.06 km from the study site) provided us with maximum, minimum and mean temperature, rainfall, maximum and mean windspeed (respectively $T_{max}$, $T_{min}$, $T_{mean}$, $R$, $W_{max}$ and $W_{mean}$) for the 2007 field work. For 2011, only data from Sablière were available. Fortunately, the two meteorological station are close enough to provide consistant measures of the meteorological variables (Figure 27) and for the analysis, only meteorological reccords from Sablière were used.

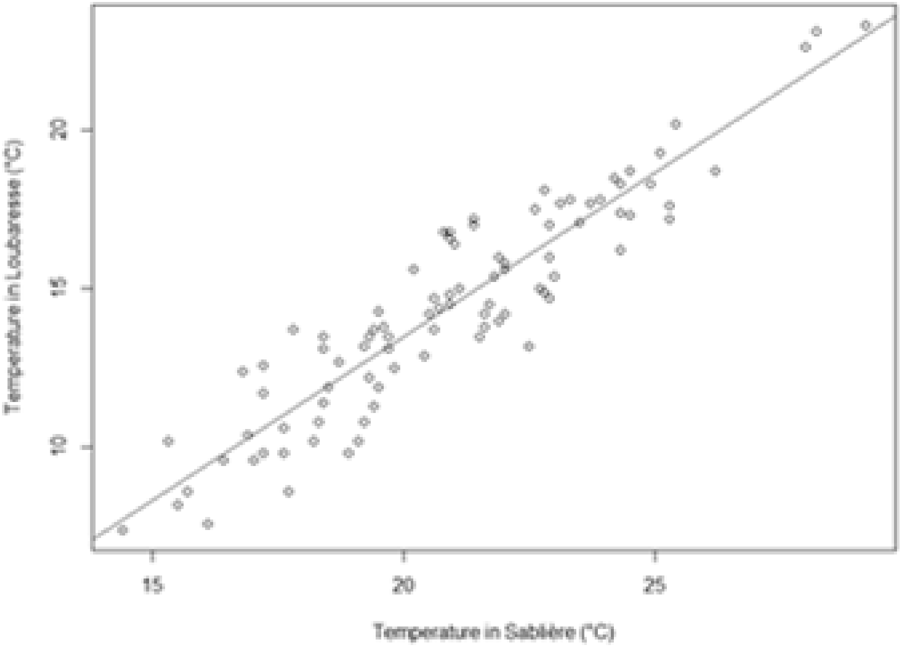


Figure 27: Mean daily temperature in Loubaresse as a function of the daily temperature in Sablière. The meteorological condition of both stations are closely related ($r^2 = 0.85$) so meteorological conditions measured in Sablière would be a consistant proxi for Loubaresse conditions.

I then performed a principal component analysis to select the most representative variables of the meteorological conditions (Figure 28). The two first components of the PCA explain 85% of the variance with the first axis describing temperature (44% variance) and the second axis describing windspeed which was negatively correlated to the rainfall (40% variance). For simplicity and biological consistancy in the results, I chose to keep $T_{mean}$ and $W_{max}$ as temporal covariates of the MRR analysis instead of the PCA. Indeed, as an ectothermic species, dragonfly activity is related to temperature. In addition, dragonflies are not active during rainy days. Finally, wind speed affects dragonfly dispersal (Angelibert and Giani 2003).

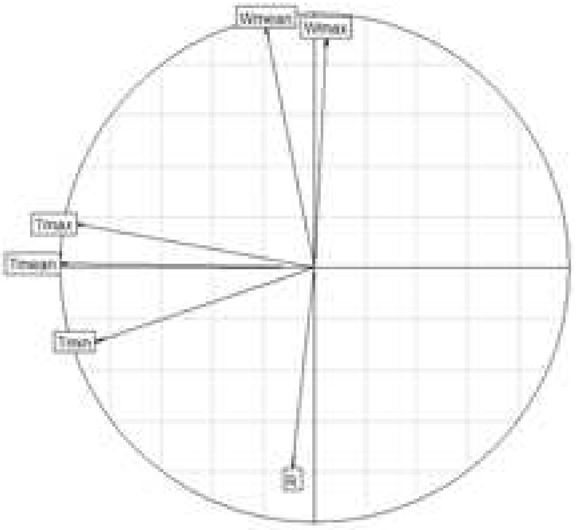


Figure 28: Correlation circle on the two first components of the principal component analysis of meteorological reccords in Sablière (France) during the field work of 2007 and 2011.

The MRR analysis were performed assumring that each week of field work is an independent capture session (9 weeks per year) where each individual is captured (1) or not (0) to constitute its capture history (White and Burnham 1999). The meteorological variables ($T_{mean}$ and $W_{max}$) and their interaction were defined as covariates of the capture sessions. In both the MRR analysis (POPAN and multistate), we tested the goodness of fit (GOF) to check for overdispersion in the data and then performed all the combinations of models and selected the best group of models using the Akaïke criterion (Akaike 1974) and its derivatives when necessary (AICc (White and Burnham 1999), QAIC (Richards 2008)). I consider two models significantly different when $\Delta AIC < 2$. The 2007 and 2011 datas were analysed separately.

### b. Multistate model analysis of *Orthetrum coerulescens* within the Montselgues' bog network

I performed the multistate analysis of capture histories of *Orthetrum coerulescens* within the Montselgues' bog network using, the patch ($pa$), the time ($t$), the mean temperature ($T_{mean}$), the maximum windspeed ($W_{max}$) and the interaction between $T_{mean}$ and $W_{max}$ ($T_{mean} \times W_{max}$)

as covariates of the dragonfly's survival ($\Phi$), capturability ($p$) and dispersal probabilities from patch $i$ to patch $j$ ($\Psi_{i,j}$). In addition, the euclidean distance ($D$) between patches (patch centers) and its interactions with $T_{mean}$ ($D \times T_{mean}$) and $W_{max}$ ($D \times W_{max}$) were considered as coviarates of $\Psi$. The goodness-of-fit was estimated using a 50 run bootstrap estimation of the $\hat{c}$ median and the correction for over-dispersion was applied in the model selection procedure (Richards 2008) ($\hat{c}_{2007} = 1.02$, $\hat{c}_{2011} = 1.3$, table 5).

<u>Table V:</u> Multistate model selection and model goodness of fit for capture histories of O. coerulescens in the bog network of the Montselgues plateau in 2007 and 2011.

| **Year** | **Model** | **AICc** | **ΔAICc** |
|---|---|---|---|
| 2007 | $\hat{c} = 1.02$ | | |
| | $\Phi(\sim T_{mean} \times W_{max})p(\sim pa + t + T_{mean} + W_{max})\Psi(\sim D \times T_{mean} + W_{max})$ | 1520.9 | 0 |
| | $\Phi(\sim T_{mean} \times W_{max})p(\sim pa + t + T_{mean} + W_{max})\Psi(\sim D \times W_{max} + T_{mean})$ | 1520.9 | 0.0 |
| | $\Phi(\sim T_{mean} + pa)p(\sim pa + t + T_{mean} + W_{max})\Psi(\sim D \times T_{mean} + W_{max})$ | 1521.4 | 0.5 |
| | $\Phi(\sim T_{mean} + pa)p(\sim pa + t + T_{mean} + W_{max})\Psi(\sim D \times W_{max} + T_{mean})$ | 1521.4 | 0.5 |
| | $\Phi(\sim T_{mean})p(\sim pa + t + T_{mean} + W_{max})\Psi(\sim D \times T_{mean} + W_{max})$ | 1521.5 | 0.6 |
| | $\Phi(\sim T_{mean})p(\sim pa + t + T_{mean} + W_{max})\Psi(\sim D \times W_{max} + T_{mean})$ | 1521.5 | 0.6 |
| | $\Phi(\sim T_{mean} \times W_{max} + pa)p(\sim pa + t + T_{mean} + W_{max})\Psi(\sim D \times T_{mean} + W_{max})$ | 1521.6 | 0.7 |
| | $\Phi(\sim T_{mean} \times W_{max} + pa)p(\sim pa + t + T_{mean} + W_{max})\Psi(\sim D \times W_{max} + T_{mean})$ | 1521.6 | 0.7 |
| | $\Phi(\sim T_{mean} \times W_{max})p(\sim pa + t + T_{mean} \times W_{max})\Psi(\sim D \times T_{mean} + W_{max})$ | 1522.2 | 1.4 |
| | $\Phi(\sim T_{mean} \times W_{max})p(\sim pa + t + T_{mean} \times W_{max})\Psi(\sim D \times W_{max} + T_{mean})$ | 1522.2 | 1.4 |
| | $\Phi(\sim T_{mean} \times W_{max} + t)p(\sim pa + t + T_{mean} + W_{max})\Psi(\sim D \times T_{mean} + W_{max})$ | 1522.4 | 1.5 |
| | $\Phi(\sim T_{mean} \times W_{max} + t)p(\sim pa + t + T_{mean} + W_{max})\Psi(\sim D \times W_{max} + T_{mean})$ | 1522.4 | 1.5 |
| | $\Phi(\sim T_{mean} \times W_{max})p(\sim pa + t + T_{mean} + W_{max})\Psi(\sim D \times T_{mean})$ | 1522.4 | 1.5 |
| | $\Phi(\sim T_{mean} \times W_{max})p(\sim pa + t + T_{mean})\Psi(\sim D \times T_{mean} + W_{max})$ | 1522.4 | 1.5 |
| | $\Phi(\sim T_{mean} \times W_{max})p(\sim pa + t + T_{mean})\Psi(\sim D \times W_{max} + T_{mean})$ | 1522.4 | 1.5 |
| | $\Phi(\sim T_{mean} \times W_{max} + pa + t)p(\sim pa + t + T_{mean} + W_{max})\Psi(\sim D \times T_{mean} + W_{max})$ | 1522.7 | 1.8 |
| | $\Phi(\sim T_{mean} \times W_{max} + pa + t)p(\sim pa + t + T_{mean} + W_{max})\Psi(\sim D \times W_{max} + T_{mean})$ | 1522.7 | 1.8 |

| | | | |
|---|---|---|---|
| | $\Phi(\sim T_{mean} \times W_{max})p(\sim pa + t + T_{mean} + W_{max})\Psi(\sim T_{mean})$ | 1522.7 | 1.8 |
| | $\Phi(\sim T_{mean} + pa + t)p(\sim pa + t + T_{mean} + W_{max})\Psi(\sim D \times T_{mean} + W_{max})$ | 1522.8 | 1.9 |
| | $\Phi(\sim T_{mean} + pa + t)p(\sim pa + t + T_{mean} + W_{max})\Psi(\sim D \times W_{max} + T_{mean})$ | 1522.8 | 1.9 |
| 2011 | $\hat{c} = 1.3$ (QAICc) | | |
| | $\Phi(\sim T_{mean} \times W_{max} + t)p(\sim pa + t + T_{mean})\Psi(\sim D)$ | 2289.5 | 0 |
| | $\Phi(\sim T_{mean} \times W_{max} + t)p(\sim pa + t + T_{mean})\Psi(\sim D + W_{max})$ | 2290.2 | 0.7 |
| | $\Phi(\sim T_{mean} \times W_{max})p(\sim pa + t + T_{mean})\Psi(\sim D)$ | 2290.8 | 1.2 |
| | $\Phi(\sim T_{mean} \times W_{max} + t)p(\sim pa + t + T_{mean})\Psi(\sim D + t)$ | 2290.8 | 1.3 |
| | $\Phi(\sim T_{mean} \times W_{max} + pa)p(\sim pa + t + T_{mean})\Psi(\sim D)$ | 2291.4 | 1.8 |
| | $\Phi(\sim T_{mean} \times W_{max})p(\sim pa + t + T_{mean})\Psi(\sim D + W_{max})$ | 2291.5 | 1.9 |
| | $\Phi(\sim T_{mean} \times W_{max} + t)p(\sim pa + t + T_{mean} + W_{max})\Psi(\sim D)$ | 2291.5 | 2.0 |

### c. POPAN analysis and *Orthetrum coerulescens* population size

The complete model I used to perform the POPAN analysis is:

$$\Phi(\sim t + T_{mean} \times W_{max})p(\sim t + T_{mean} \times W_{max})pent(\sim t + T_{mean} \times W_{max})N(\sim t + T_{mean} \times W_{max})$$

Where $\Phi$ is the survival probability between 2 capture events (i.e. 2 weeks), $p$ the capture probability, $pent$ the recruitment probability (immigration and local recruitment (Arnason et al. 1979)), $N$ the population size and $t$ the time. After checking for GOF with RELEASE (White and Burnham 1999), I performed the model selection (table 4) and a model averaging on the selected models for each patch and for the total population (all patches pulled) for the 2007 and 2011 datasets (Figure 29.

## 4 MRR results

Results are consistent between 2007 and 2011 (table 5) and between models when comparisons are possible (survival, catchability). However, the 2011 results are of higher quality so I use them to describe the covariate effects on demographic parameter estimation.

- Survival ($\Phi$) is mainly affected by the seasonality of *O. coerulescens* adult persistence. $T_{mean}$ possitively affect *O. coerulescens* survival and $W_{max}$ negatively. The $T_{mean} \times W_{max}$ interaction shows that *O. coerulescens* survival decreases under bad weather conditions (low $T_{mean}$ and high $W_{max}$).
- Catchability ($p$) is weather-dependent. As an ectothermic species, *O. coerulescens* is not active when temperatures are cool or under windy conditions. Because the patches present different landcovers (openess gradient), I found a patch effect. Dragonflies are easier to catch in the open fields. Finaly, the catchability increases with student experience (time effect).

Table VI: POPAN model selection and model goodness of fit for capture histories of O. coerulescens in the bog network of the Montselgues plateau in 2007 and 2011.

| Site | Model | AICc | ΔAICc |
|---|---|---|---|
| **2007** | | | |
| **Global** | $\hat{c} = 0.3$ | | |
| | $\Phi(\sim T_{mean} \times W_{max})p(\sim t + T_{mean})pent(\sim t + T_{mean} \times W_{max})N(\sim 1)$ | 1660.4 | 0 |
| | $\Phi(\sim T_{mean} \times W_{max})p(\sim t + T_{mean} \times W_{max})pent(\sim t + T_{mean} \times W_{max})N(\sim 1)$ | 1661.6 | 1.2 |
| | $\Phi(\sim t + T_{mean} \times W_{max})p(\sim t + T_{mean} \times W_{max})pent(\sim t + T_{mean} \times W_{max})N(\sim 1)$ | 1661.7 | 1.3 |
| | $\Phi(\sim t + T_{mean} \times W_{max})p(\sim t + T_{mean})pent(\sim t + T_{mean} \times W_{max})N(\sim 1)$ | 1661.7 | 1.3 |
| | $\Phi(\sim T_{mean} \times W_{max})p(\sim t + T_{mean} + W_{max})pent(\sim t + T_{mean} \times W_{max})N(\sim 1)$ | 1661.8 | 1.4 |
| **Narcette** | $\hat{c} = 0.9$ | | |
| | $\Phi(\sim T_{mean} + W_{max})p(\sim W_{max})pent(\sim t + T_{mean} \times W_{max})N(\sim 1)$ | 471.5 | 0 |
| | $\Phi(\sim T_{mean} + W_{max})p(\sim T_{mean} + W_{max})pent(\sim t + T_{mean} \times W_{max})N(\sim 1)$ | 472.8 | 1.2 |
| | $\Phi(\sim T_{mean} \times W_{max})p(\sim W_{max})pent(\sim t + T_{mean} \times W_{max})N(\sim 1)$ | 473.2 | 1.7 |
| | $\Phi(\sim t + T_{mean} + W_{max})p(\sim W_{max})pent(\sim t + T_{mean} \times W_{max})N(\sim 1)$ | 473.3 | 1.7 |
| | $\Phi(\sim t + W_{max})p(\sim T_{mean} + W_{max})pent(\sim t + T_{mean} \times W_{max})N(\sim 1)$ | 473.3 | 1.8 |
| | $\Phi(\sim 1)p(\sim t + T_{mean} \times W_{max})pent(\sim t + T_{mean} \times W_{max})N(\sim 1)$ | 473.5 | 1.9 |
| **Chabreille** | $\hat{c} = 0.3$ | | |
| | $\Phi(\sim 1)p(\sim t + T_{mean} \times W_{max})pent(\sim t + T_{mean} \times W_{max})N(\sim 1)$ | 435.5 | 0 |
| | $\Phi(\sim T_{mean} \times W_{max})p(\sim W_{max})pent(\sim t + T_{mean} \times W_{max})N(\sim 1)$ | 435.7 | 0.2 |
| | $\Phi(\sim T_{mean} + W_{max})p(\sim W_{max})pent(\sim t + T_{mean} \times W_{max})N(\sim 1)$ | 437.1 | 1.6 |
| **Rouveyrettes** | $\hat{c} = 0.4$ | | |
| | $\Phi(\sim W_{max})p(\sim t + T_{mean} \times W_{max})pent(\sim t + T_{mean} \times W_{max})N(\sim 1)$ | 750.3 | 0 |
| | $\Phi(\sim T_{mean} + W_{max})p(\sim t + T_{mean} \times W_{max})pent(\sim t + T_{mean} \times W_{max})N(\sim 1)$ | 751.6 | 1.3 |
| **2011** | | | |
| **Global** | $\hat{c} = 1.3$ (QAICc) | | |
| | $\Phi(\sim t + T_{mean} \times W_{max})p(\sim t + T_{mean})pent(\sim t + T_{mean})N(\sim 1)$ | 2136.5 | 0 |
| | $\Phi(\sim t + T_{mean} \times W_{max})p(\sim t + T_{mean} \times W_{max})pent(\sim t + T_{mean})N(\sim 1)$ | 2136.6 | 0.2 |
| | $\Phi(\sim t + T_{mean} \times W_{max})p(\sim t + T_{mean} + W_{max})pent(\sim t + T_{mean})N(\sim 1)$ | 2137.5 | 1.0 |
| | $\Phi(\sim T_{mean} \times W_{max})p(\sim t + T_{mean} \times W_{max})pent(\sim t + T_{mean})N(\sim 1)$ | 2137.9 | 1.4 |
| **Narcettes** | $\hat{c} = 0.9$ | | |
| | $\Phi(\sim T_{mean} \times W_{max})p(\sim 1)pent(\sim t + T_{mean})N(\sim 1)$ | 926.6 | 0 |
| | $\Phi(\sim t + T_{mean} \times W_{max})p(\sim t + W_{max})pent(\sim t + T_{mean})N(\sim 1)$ | 927.6 | 1.0 |

| | | | |
|---|---|---|---|
| | $\Phi(\sim t + T_{mean} \times W_{max}) p(\sim t) pent(\sim t + T_{mean}) N(\sim 1)$ | 927.7 | 1.0 |
| | $\Phi(\sim t + T_{mean} \times W_{max}) p(\sim 1) pent(\sim t + T_{mean}) N(\sim 1)$ | 928.4 | 1.7 |
| | $\Phi(\sim T_{mean} \times W_{max}) p(\sim 1) pent(\sim T_{mean} + W_{max}) N(\sim 1)$ | 928.5 | 1.9 |
| | $\Phi(\sim T_{mean} \times W_{max}) p(\sim T_{mean}) pent(\sim t + T_{mean}) N(\sim 1)$ | 928.5 | 1.9 |
| **Chabreille** | $\hat{c} = 0.8$ | | |
| | $\Phi(\sim t) p(\sim t + T_{mean} \times W_{max}) pent(\sim t) N(\sim 1)$ | 749.6 | 0 |
| | $\Phi(\sim t + W_{max}) p(\sim t + T_{mean} \times W_{max}) pent(\sim t + T_{mean}) N(\sim 1)$ | 749.7 | 0.1 |
| | $\Phi(\sim t) p(\sim t + T_{mean} \times W_{max}) pent(\sim t + T_{mean}) N(\sim 1)$ | 749.8 | 0.2 |
| | $\Phi(\sim t + W_{max}) p(\sim t + T_{mean} + W_{max}) pent(\sim t) N(\sim 1)$ | 749.9 | 0.3 |
| | $\Phi(\sim t + W_{max}) p(\sim t + T_{mean} + W_{max}) pent(\sim T_{mean}) N(\sim 1)$ | 749.9 | 0.3 |
| | $\Phi(\sim t + W_{max}) p(\sim t + T_{mean} + W_{max}) pent(\sim T_{mean} \times W_{max}) N(\sim 1)$ | 750.5 | 0.9 |
| | $\Phi(\sim t) p(\sim t + T_{mean} + W_{max}) pent(\sim T_{mean} \times W_{max}) N(\sim 1)$ | 750.8 | 1.2 |
| | $\Phi(\sim t + W_{max}) p(\sim t + T_{mean} + W_{max}) pent(\sim t + T_{mean} + W_{max}) N(\sim 1)$ | 751.2 | 1.6 |
| | $\Phi(\sim t + W_{max}) p(\sim t + T_{mean} + W_{max}) pent(\sim t + T_{mean} \times W_{max}) N(\sim 1)$ | 751.5 | 2.0 |
| **Rouveyrettes** | $\hat{c} = 0.9$ | | |
| | $\Phi(\sim W_{max}) p(\sim T_{mean} + W_{max}) pent(\sim W_{max}) N(\sim 1)$ | 1096.0 | 0 |
| | $\Phi(\sim t + W_{max}) p(\sim T_{mean} + W_{max}) pent(\sim W_{max}) N(\sim 1)$ | 1096.9 | 0.9 |
| | $\Phi(\sim W_{max}) p(\sim T_{mean} + W_{max}) pent(\sim t + W_{max}) N(\sim 1)$ | 1096.9 | 0.9 |
| | $\Phi(\sim W_{max}) p(\sim t + T_{mean} + W_{max}) pent(\sim t) N(\sim 1)$ | 1096.9 | 0.9 |
| | $\Phi(\sim t + W_{max}) p(\sim T_{mean} + W_{max}) pent(\sim t) N(\sim 1)$ | 1097.7 | 1.8 |
| | $\Phi(\sim t + W_{max}) p(\sim T_{mean} + W_{max}) pent(\sim t + W_{max}) N(\sim 1)$ | 1097.9 | 1.9 |
| | $\Phi(\sim t + W_{max}) p(\sim t + T_{mean} + W_{max}) pent(\sim W_{max}) N(\sim 1)$ | 1097.9 | 2 |
| | $\Phi(\sim t + W_{max}) p(\sim T_{mean} + W_{max}) pent(\sim W_{max}) N(\sim 1)$ | 1097.9 | 2 |

- Dispersal ($\Psi$) depends on inter-patch distances. The longer the distance, the less dispersal events occur. When temperature is high and wind speed low, dispersal increases.
- Recruitment ($pent$) as a combination of dispersal and local emmergence of individual is linked to weather conditions. In addition, the time effect reflects the adult emmergence events patterns across time (few new adults at the beginning and end of the season).
- Population size ($N$) seems to be constant regardless of the year, the site or weather conditions.

### *Population viability analysis*

The 2011 MRR surveys of *Orthetrum coerulescens* permits to setup MetaConnect with localy estimated demographic parameters (Figure 29).

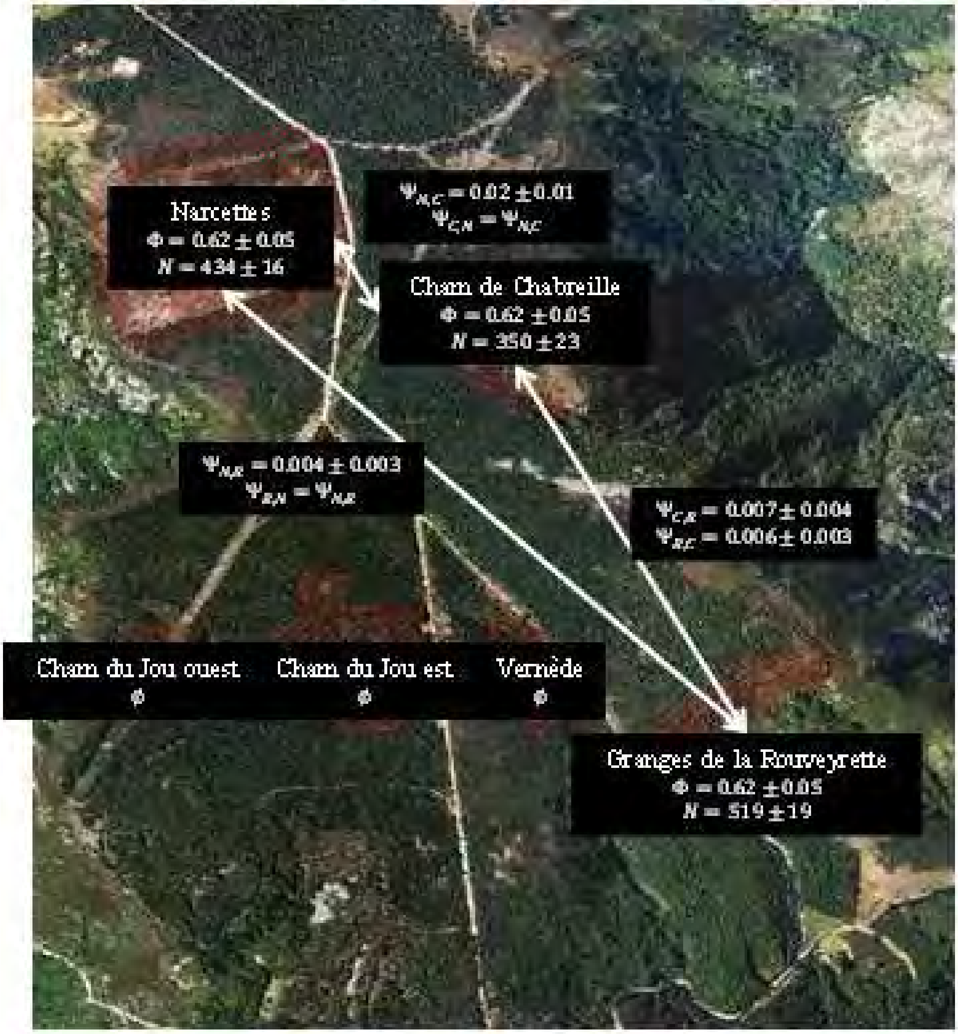


Figure 29: Keeled skimmer survival ($\Phi$), population size ($N$) and dispersal probability ($\Psi_{i,j}$) within the Montselgues' bog network in 2011. In Cham du Jou and Vernède, dragonflies rarely occurred due to regular droughts and no demographic parameters could be estimated for those sites using MRR models. The parameter estimations are presented as mean ± SE and only the mean value was used to setup MetaConnect to perform the PVA of *Orthetrum coerulescens* within the bog network.

I performed PVAs with (1) the entire bog network and (2) considering separately each of the patches (Narcettes, Chabreille and Rouveyrette) as independant. Results show that the species is not threatened by extinction (all $p_e = 0$) either at the network scale or at the individual patch scale, (Figure 30). In addition, the flow of individuals prevents genetic structure within the bog network (Figure 30).

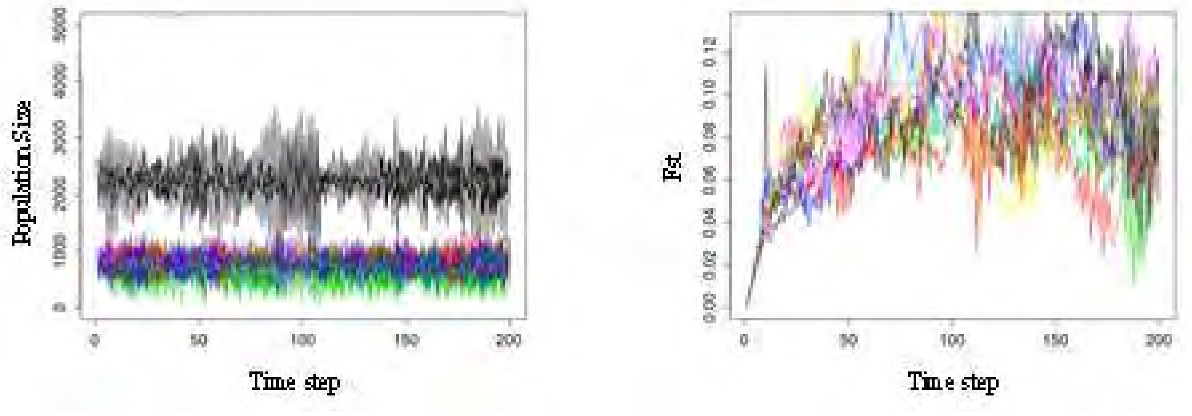


Figure 30: Examples (10 runs show) of individual population dynamics (left panel) and genetic structure (Fst, right panel) of *Orthetrum coerulescens* within the bog network of the Montselgues plateau in France simulated with MetaConnect. At the global scale (black line) and for each bog of the network separately (Narcettes: red, Chabreille: green and Rouveyrettes: blue), the keeled skimmer population is viable and stable over the 200 time step simulations. In addition, the low asymptotic value of Fst (0.1), shows that the actual landscape would probably have the ability create population genetic structure with lower gene flow.

Although the bog network is viable, simulations examining each bog independently show that while the population size is stable over the 200 time step, the heterozygosity ($H_o$) level is declining in Chabreille (Figure 31) whereas Narcettes and Rouveyrettes are not threatened by inbreeding. This result suggests that the smallest bog by its area and population size should be protected from isolation.

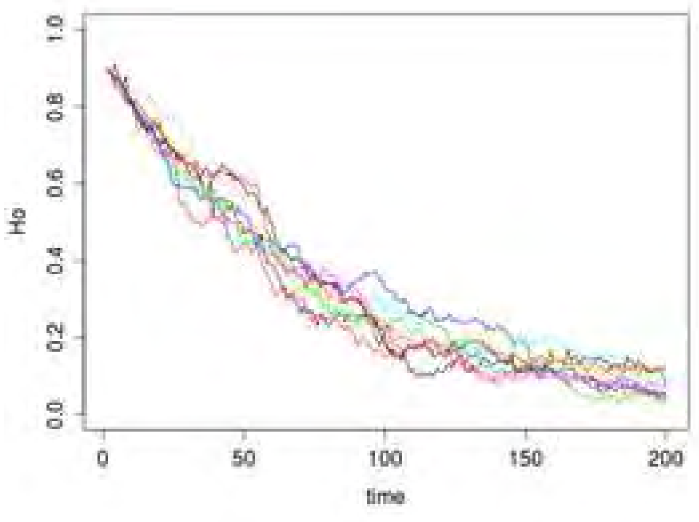


Figure 31: Simulated (10 runs show) heterozygosity in the Cham de Chabreille over 200 time steps for *Orthetrum coerulescens*. The inbreeding depression in Chabreille tends to homogenize the population genotypes.

### *Corridor functionality for dragonflies*

The site conservation managers would like to know whether the corridor they built was functional. The multistate MRR analysis of the 2007 datas ($\Psi_{i,j}$ estimates) were compared to those obtained in 2011 and I found that all the dispersal probabilities are greater in 2011 (Wilcoxon after Box-Cox transformation, all $p = 0.016$). Unfortunately, this difference is not specific to the Narcettes-Chabreille pair and it can be explained by the data quality difference between years. Indeed, the better experimental design in 2011 allowed me estimating correctly the dispersal probabilities. However, the uncertainty of the 2007 estimates lead to overlap in the estimates ± confidence interval of 2011 data preventing any definitive conclusions concerning the efficiency of the corridor (Figure 32).

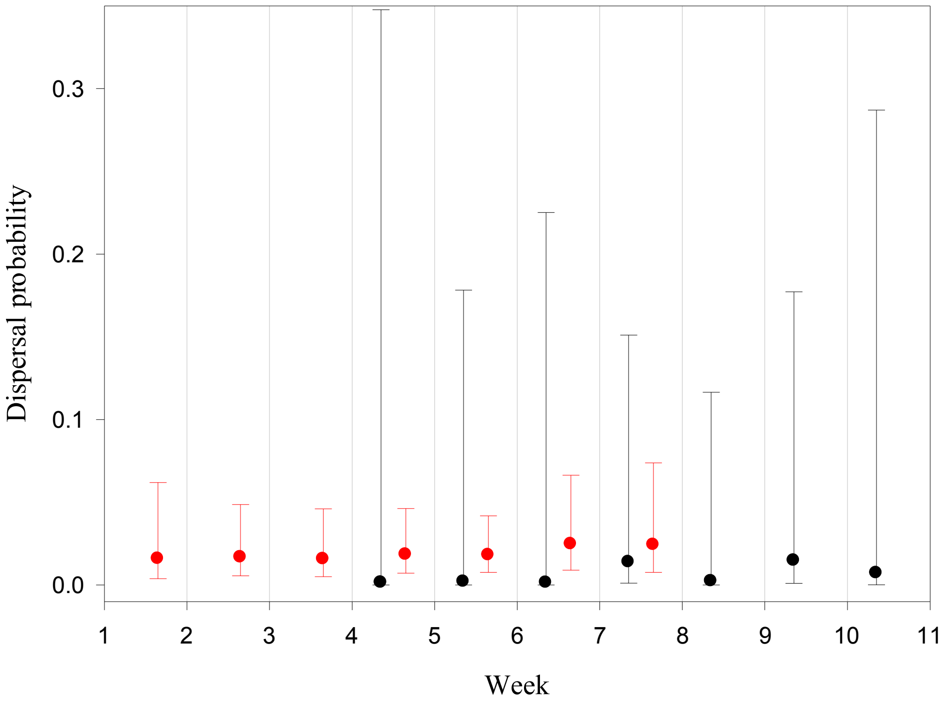


Figure 32: Dispersal probability (± confidence interval) between Narcettes and Cham de Chabreille where the PNR MA and the CEN RA built a corridor to enhance movement of *O. coerulescens* across patches. Even though, dispersal probabilities in 2011 (red) are statisticaly higher than in 2007 (black) (see text), interpretation is limited by the uncertainty of the 2007 dispersal probability estimates.

## Phengaris alcon conservation planning (adapted from Cornuau and Moulherat 2013; Moulherat and Cornuau 2013)

This part corresponds to an non-research applied study I conducted for the Parc Naturel Régional des Monts d'Ardèche in 2013 and clearly illustrates how MetaConnect can be used for conservation planning.

### *Study context and MetaConnect parameterization*

In 2013, the PNR MA and CEN RA wanted to evaluate *Phengaris alcon* metapopulation functioning within the Montselgues bog network. In this respect, we used MetaConnect to estimate the metapopulation viability and functioning. Demographic characteristics of *P. alcon* are well documented (Thomas et al. 1998; Als et al. 2001; WallisDeVries 2004; Mouquet et al. 2005; Habel et al. 2007; Nowicki et al. 2007; Dupont 2010) especially on the study site (Lhonoré 1996; Rozier 1999; Merlet and Dupont 2012), but the interaction between the land cover and *P. alcon* dispersal abilities remains unclear. To determine *P. alcon* locomotor performances and behavior in a new habitat, I adapted the protocol proposed by Turlure et al. (2011) and determined the rugosity coefficient for *P. alcon* associated with each of the different land covers it could meet within the bog network (see related reports).

For MetaConnect simulations, the *P. alcon* population dynamic was assumed to follow the life cycle described Figure 33 and demographic parameters were parameterized using values extracted from the literature. Dispersal characteristics (dispersal kernel, rugosity coefficients...) were derived from a field study designed based on Turlure's and coauthor's (2011) experimental design (see related report X for details of MetaConnect parameterizaton). Suitable habitat patches (Figure 33.A) were defined as buffers of 10 meters (Figure 33.B) around each geolocalized *Gentiana pneumonanthes* which is the host plant of *P. alcon*.

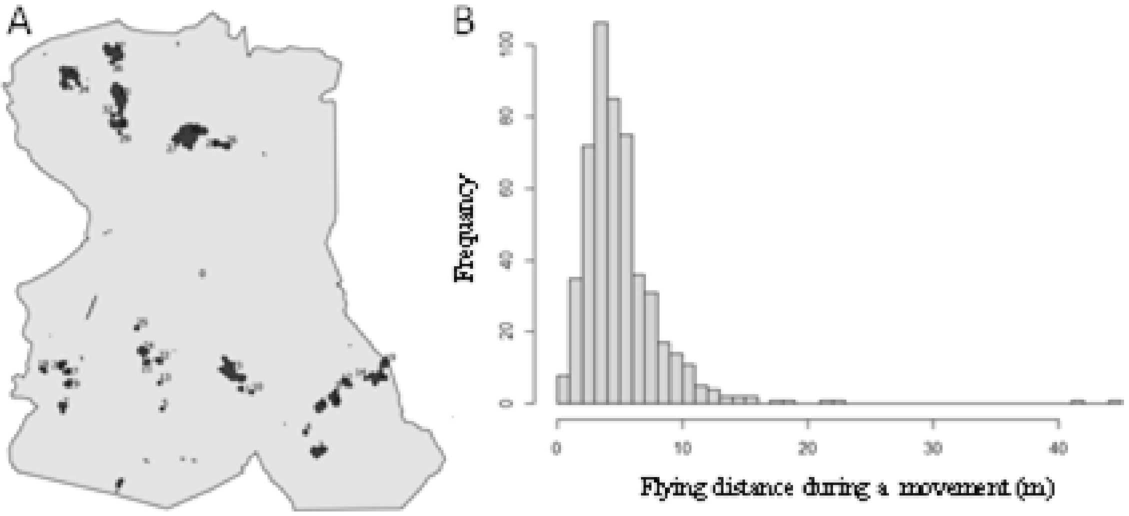


Figure 33: Suitable habitat patches for *P. alcon* within the bog network of the Montselgues plateau in France (A). Dark green corresponds to patchs positions. B shows the frequency distribution of the distance travel by *P. alcon* between two stops during foraging.

### *Simulation results*

After simulations of 100 years, results (Figure 34) show that:

1) The metapopulation would persist
2) South-western populations cannot persist
3) There is no dispersal between northern and southern populations
4) Genetic differentiation would be very high between populations (a genetic cluster per population)
5) The artificial corridor between Narcettes and Chabreille favored dispersal but not sufficiently to avoid genetic differentiation.

More detailed results are available in the related report (see related report).

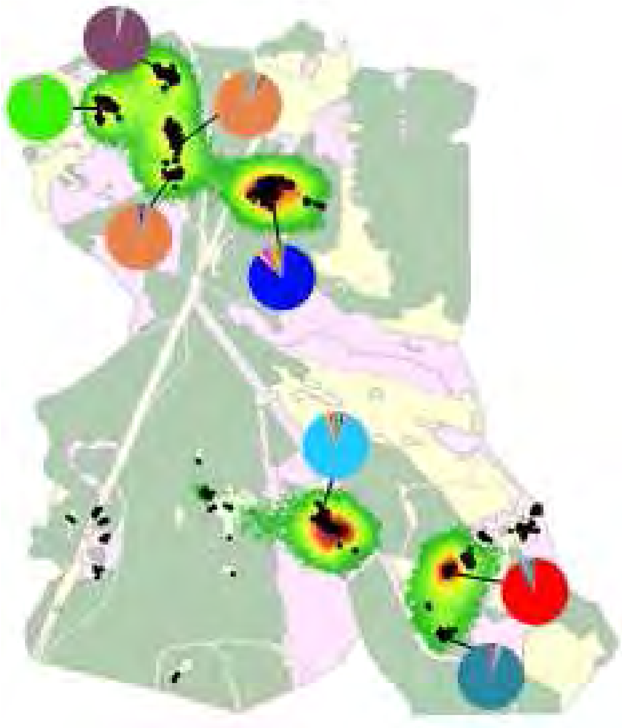


Figure 34: MetaConnect simulation results for *P. alcon* within the Montselgues bog network. Suitable habitat patches defined as 10m buffers around existing *G. pneumonanthes* are in black. Pies correspond to population assignation to a genetic cluster performed with STRUCTURE (Pritchard et al. 2000) and analyzed following the Evanno method (Evanno et al. 2005). Landscape occupation during functional dispersal events follows a gradient from green (rare) to red (often).

### *Conservation planning and a management proposal*

Figure 35 shows the action that may reinforce *P. alcon* metapopulation persistence. These actions are currently under evaluation by the CEN RA for feasibility and prioritization. The CEN RA will then provide maps including the proposed new landscape element they would create (corridors, habitat quality improvement...) to evaluate their efficiency through a new set of MetaConnect simulations.

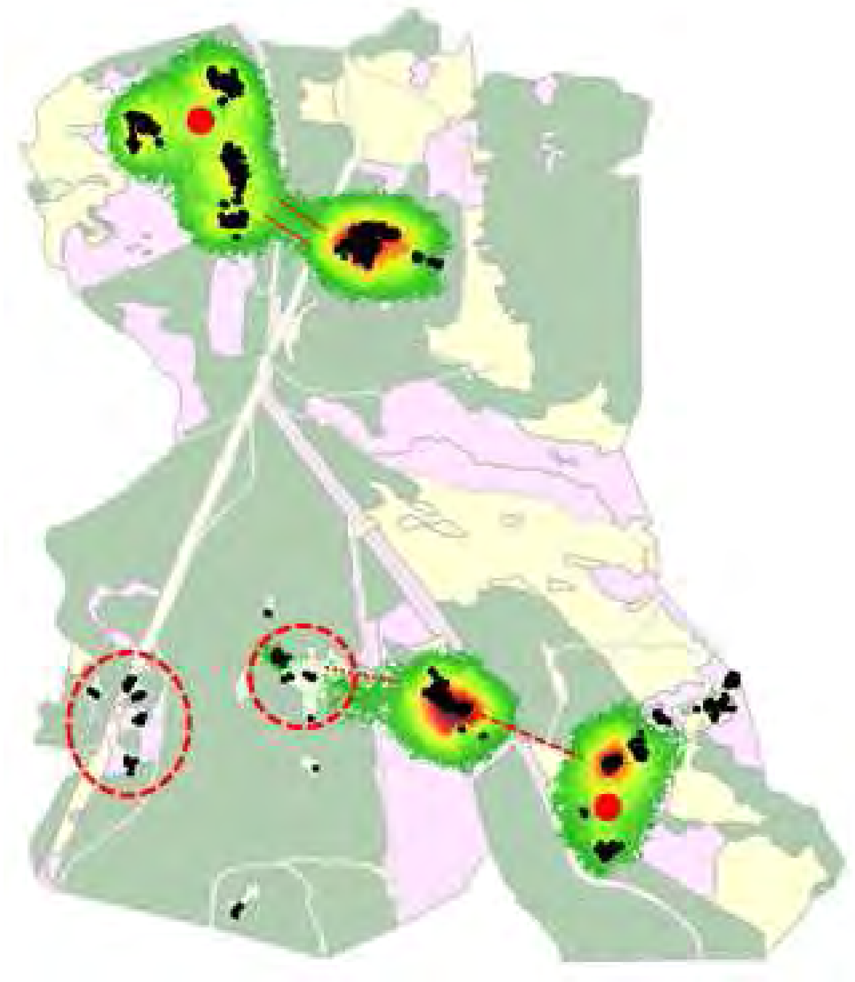


Figure 35: Management proposal to improve viability and connectivity of *P. alcon* within the Montselgues bog network. The existing corridor is delimited by red lines. Red points correspond to areas for which the *G. peumonanthes* development should be favored (pasture exclusion) to relax the genetic differentiation between close populations. Red dashed lines correspond to new corridors which may improve connectivity between bogs. Red dashed circles correspond to current areas of very low quality but presenting *G. pneumonanthes* and for which habitat quality must be highly improved to sustain viable populations of *P. alcon*.

## IV. MetaConnect in regulatory studies: potential landscape functional connectivity for the natterjack toad (Epidalea calamita) and spatial planning (adapted from Moulherat 2013a; Moulherat et al. submitted-a)

We used a model designed with MetaConnect to determine the potential impact of the development of an industrial area and terrestrial transport infrastructure (high-speed railway), both alone and together (Art. L122-1 modified by Loi n° 2010-788 du 12 juillet 2010 – art. 230 of the French environmental legislation), on the population viability of *Epidalea calamita* populations in south-western France close to Agen (44°11’36”N, 0°31’14”E) (Figure 36).

The philosophy of this analysis was not to determine the absolute functional connectivity for *E. calamita* but rather to estimate the landscape potentialities in regard to *E. calamita* functional connectivity (i.e. Does the current landscape presents barriers and corridors for *E. calamita*?).

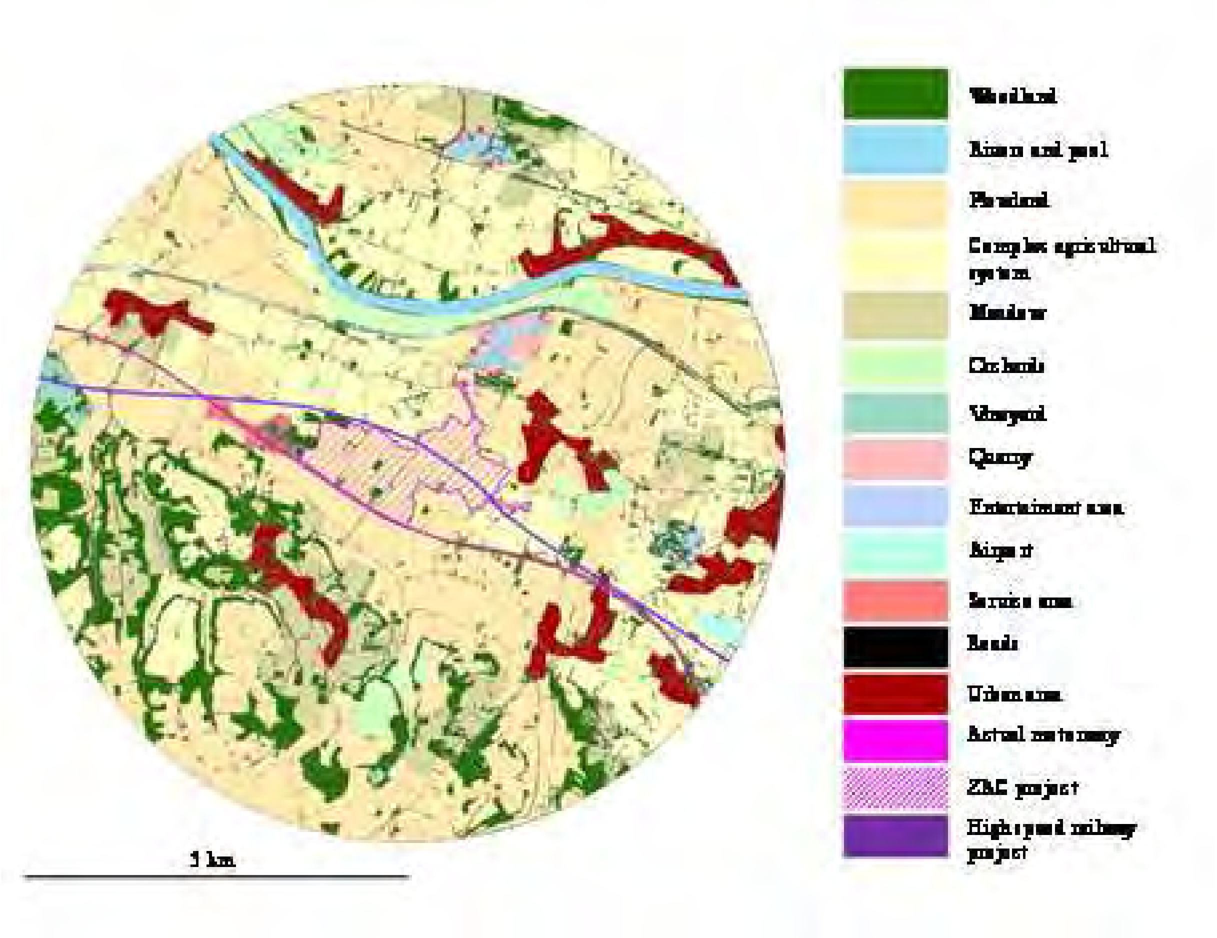


Figure 36: Study site land cover with actual position of the motorway and the projects of the incoming industrial area and high-speed railway.

## MetaConnect parameterization

Habitat patches were determined *a priori* based on expert assessment (Figure 37). Preferences for landscape elements were derived from those experimentally determined by Stevens et al. (2006b). *B. calamita* was assumed to disperse following the stochastic-movement-simulator (SMS) algorithm (Palmer et al. 2011). The rational for this choice was that, even though Stevens et al. (2006) found that least-cost-path movement was consistent with the population genetic structure of *E. calamita* in their study site, Coulon et al. (in prep) recently improved the fit between simulated dispersal of *E. calamita* and the observed population genetic structure using the SMS model. The model derived from MetaConnect was parameterized with published values of life history traits (Stevens and Baguette 2008) (appendix A of related paper).

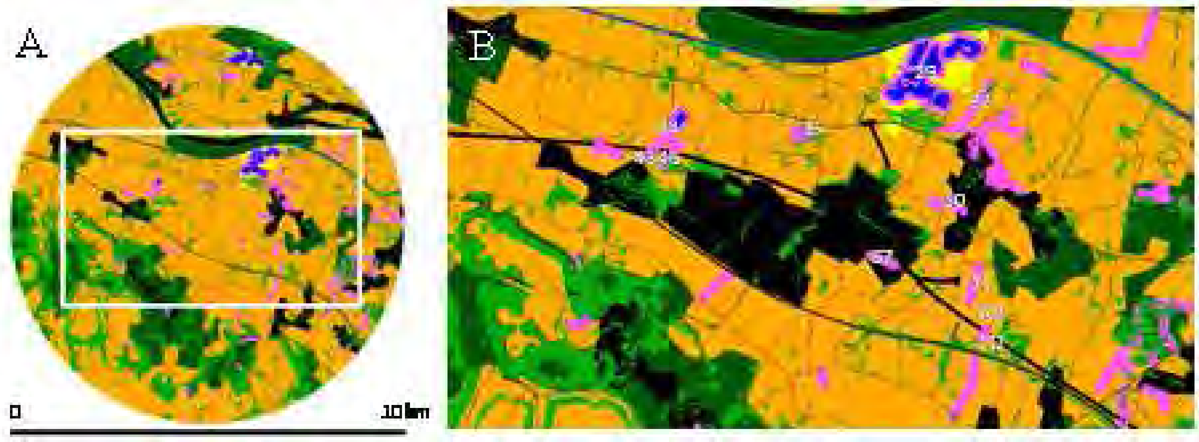


Figure 37: Site of industrial and transport development in south western France close to Agen. The A panel presents the complete study site used for MetaConnect simulations, rasterized to a grid of 10m × 10m cells. The B panel shows the future development of an industrial area and of a high-speed railway. Potential habitat patches are in pink and the other colors correspond to the land cover and its associated habitat preference values derived from Stevens et al. (2004) and used in MetaConnect (SMS module) to perform dispersal simulations. Our analysis is focused on the patches for which a number is indicated in white. Patch 67 will be destroyed by development of the industrial area. In addition, patches 45, 46 and 112 currently do not currently exist, but will appear with the building of the high-speed railway as a result of the fragmentation of patches 34 (split into 34, 45 and 46) and 103 (split into 103 and 112).

Table VII: Rugosity coefficient for Bufo calamita derived from Stevens et al. (2006) use in MetaConnect.

| Land cover | Rugosity coefficients |
|---|---|
| Urban area/motorway/service area/large river (Garonne) | 20000 |
| Orchards/Meadow | 85.74 |
| Woodland/Airport | 84.41 |
| Plowland/Complex agricultural system/Vineyard | 79.62 |
| Rivers and pools | 58.2 |
| Roads | 57.05 |
| Career | 49.38 |

We analyzed MetaConnect genetic outputs for all the patches containing more than 10 individuals at the end of each MetaConnect run. This analysis was performed using STRUCURE (Pritchard et al. 2000) with an admixture model assuming that the origin population of an individual is known and the allele frequencies are independent. STRUCTURE runs were performed for a variable number of clusters between 1 and 7, and with 10 iterations of 100000 steps (50000 burning steps and 50000 analysis steps to ensure model convergence). This procedure was reproduced for each iteration of the *B. calamita* simulation of a given scenario (10 times). We determined the best number of genetic clusters following the Evanno method (see Evanno et al. 2005) using STRUCTURE HARVESTER (Earl and Vonholdt 2012). Robustness of patch assignation to a cluster was tested performing a $\chi^2$ test. Contingencies of patches assignation were summed among MetaConnect simulations, and STRUCURE results for the best number of genetic clusters.

Current metapopulation functioning

Within the study site, (10 km around the industrial development area), the *E. calamita* population is not threatened (extinction probability $p_e = 0$) and we observed two main dispersal corridors. The major corridor joins the north (quarry, patch 29) to the south (motorway, patch 103) with the motorway acting as the boundary at its southern end and the Garonne river at its northern end. A minor corridor joins the quarry (east) to a pond (patch 34, west) (Figure 38.A). Our focal area covers these two corridors (Figure 38). Both corridors are made from stepping stone sites (patches 50 and 67 for the north-south corridor and 36 for the east-west one) (Figure 38.A). Within the focal area all the patches have a very low extinction probability (all close to 0, see Figure 38), and except for patch 103, all patches are able to exchange individuals with each of the others. Indeed, only patch 103 is enable to provide individuals to patch 34, 36, 29 and 32 or to receive individuals from patch 34 and 36.

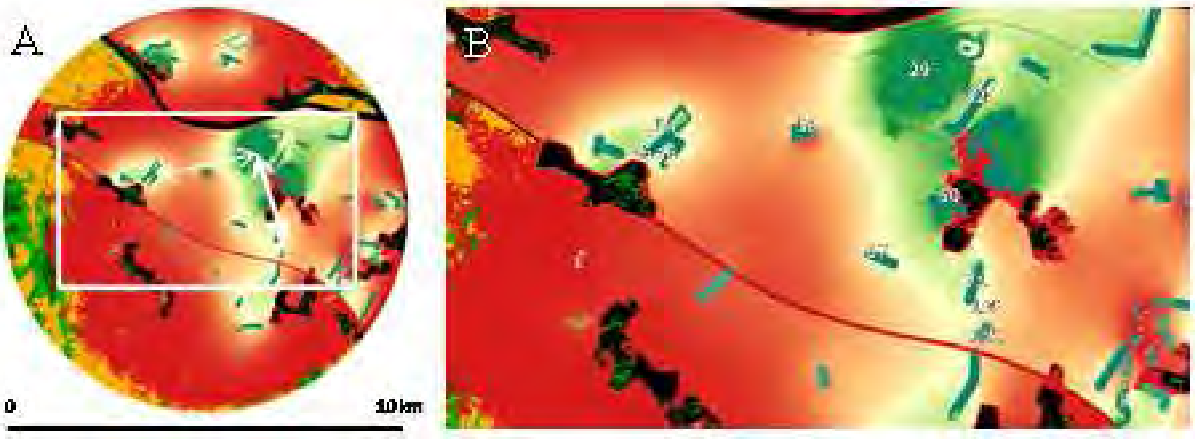


Figure 38: MetaConnect output layers of patch extinction probabilities (from 1 brown to 0 green) and mean cell frequentation during efficient dispersal events (from 1 individual/100 years in red to more than 10/year in green). At the global scale, dispersing individuals follow 2 main corridors (white arrows in panel A): a major corridor along the north-south axis and a minor one along the east-west axis (see text).

The analysis of the genetic output suggests that currently the study site is divided into three separate clusters (all p.values<0.05). The first one is situated to the north of the Garonne, the second between the Garonne and the motorway and the third to the south of the motorway which underlies the structuring role of the river and the motorway on the population genetics of *E. calamita* (Figure 37.A).

Expected consequences of development of the industrial area and construction of the high-speed railway

Development of the industrial area and the associated destruction of patch 67 leads to a reduction of dispersal between patches to the north of the industrial area and patches to the south in addition to a population size reduction within the southern patches (Figure 38.B). Such a modification would not threaten *E. calamita* persistence in the study site (all $p_e = 0$, Figure 38.D). However, dispersal would be more concentrated between patches 29, 32 and 50 due to the repellent effect of the industrial area for *E. calamita* (Figure 38.D). In addition, the reduction of dispersal along the north-south axis should lead to a fourth genetic cluster in the western part of the study sites (Figure 37.B).

The high-speed railway project will fragment the study site landscape. This would lead to the loss of patches 34 and 103 (since none of the newly created patches 45, 46 nor 112 could shelter a population (Respectively $p_e = 0.71, 0.71, 0.61$, Figure 38.E) and inducing the reduction of their sheltered population sizes (Figure 38.C). In addition, dispersal along the north-south corridor would be reduced to the south of patch 50 (Figure 38.C). Moreover, the connectivity along the north-south corridor would also decrease due to the population size reduction of patches 71 and 103. The reduction of patch population sizes and the change in the connectivity along the north-south axis change the clustering outcome of these last simulations. While the Evanno method suggests that 2 clusters can be identified, the $\chi^2$ test shows that the assignment to a second cluster does not differ significantly from random, suggesting that only a single cluster exists or that cluster 1 (north of the Garonne) is poorly differentiated from cluster 2 between the Garonne and the high-speed railway (Figure 37.C).

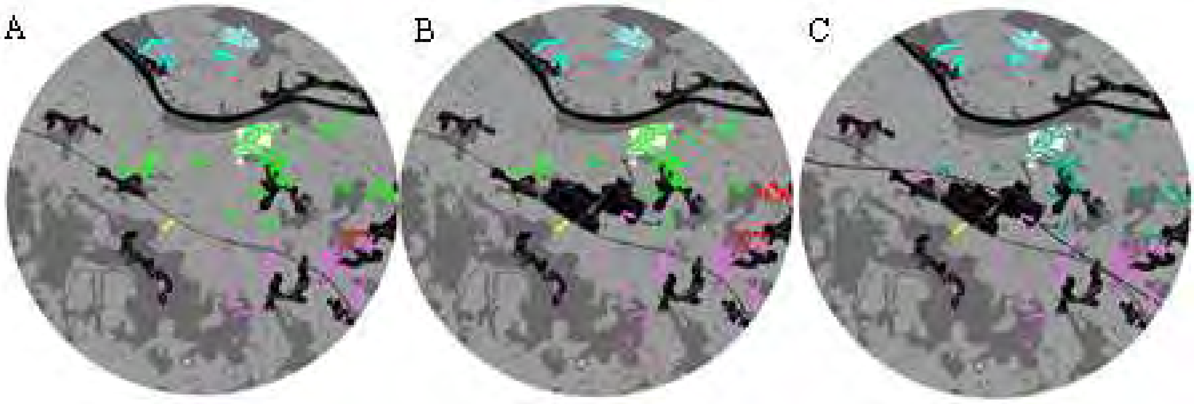


Figure 39: Genetic clustering of suitable patches for *E. calamita*. Pink patches correspond to patches containing fewer than 10 individuals at the end of the MetaConnect simulation, which were removed from the STRUCTURE analysis (Pritchard et al. 2000). The development of the industrial area (B) does not change the genetic structure of the *E. calamita* population from the current situation (A). Four clusters are identified (cyan, light green, red and yellow clusters of patches) and separated by the Garonne (isolating the cyan and light green clusters), a forest (barrier between the light green and the red cluster) and the motorway (separating the light green cluster from the yellow). The addition of the high-speed railway (C) would modify the genetic exchanges and two clusters would be kept (cyan and yellow). The dark green patches are non-significantly linked to the yellow or the cyan cluster (all *p* values non-significant) due to the reduction of the number of individuals per patch and the change in inter-patch genetic exchange (see text).

Although development of the industrial area and the building of high-speed railway do not directly threaten *E. calamita* survival in the study site (Figure 38.F), they will restrain the species' displacement capabilities and reduce the global population size. In addition, Figure 38.F highlights the central role of patch 50 as a stepping-stone patch, which allows the maintenance of connectivity between the north (patches 29 and 32) and the south (patches 71 and 103). Such a map constitutes a powerful decision-making tool in the new ecological context of compensatory measures which will be taken in the context of large landscape planning (Lanius et al. 2013; Regnery et al. 2013).

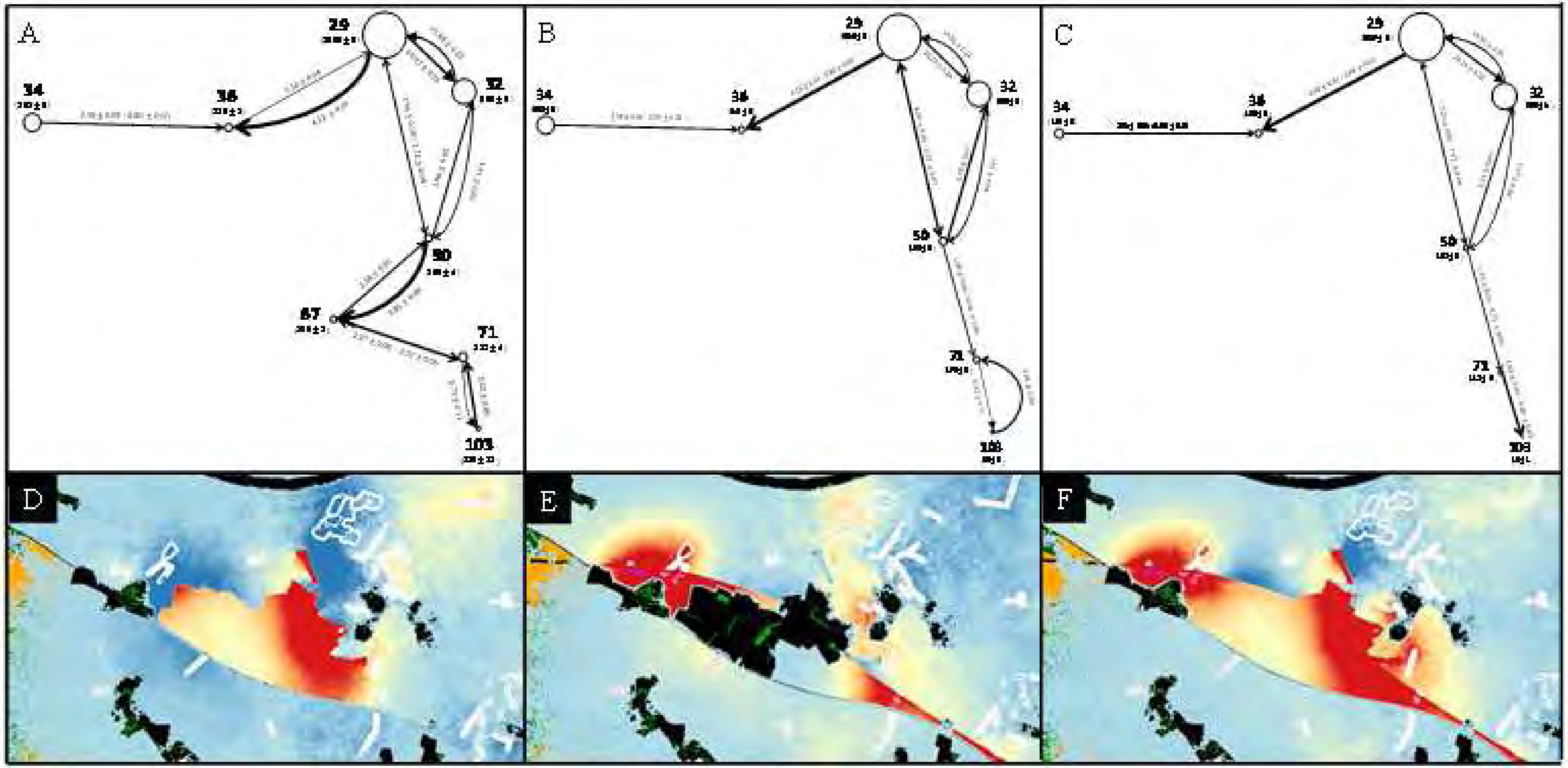

A
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Figure 40: Graphical representation of metapopulation functioning of the focal area derived from MetaConnect outputs for the current situation (A) with the addition of the industrial area (B) and with both the industrial area and the high-speed railway added (C). Node sizes are proportional to patch population sizes (mean ± SE). Arrows represent dispersal intensity (mean ± SE) and direction (bidirectional arrow values correspond to symmetrical dispersal). For clarity, only dispersal superior to 1 individual a year has been represented and dispersal superior to 5 individuals a year has a width reduced by 10 and shown as a plain arrow. Maps represent the variation of extinction probabilities per patch where variation = patch extinction probability in scenario B – patch extinction probability in scenario A (from brown for an extinction probability variation of -1 to purple for a variation of 1 where white is used if no differences are recorded) and of cell occupancy during efficient dispersal (from red for a loss superior to 3 individuals a year to dark blue for a gain of 1 where a constant number of individual per year corresponds to light blue). The D scenario corresponds to change in the metapopulation functioning after the development of the industrial area. The E scenario presents the change between post-development of the industrial area and after the construction of the high-speed railway. Finally, the F scenario summarizes the differences between the current metapopulation functioning and the expected functioning after the cumulative development of the industrial area and the high-speed railway.

# V. **MetaConnect sensitive input acquisition** (adapted from Lagarde et al. 2012; Stevens et al. 2013; Moulherat et al. 2014; Grimm et al. in prep; Legrand et al. submitted; Trochet et al. submitted)

During the previous chapters, we saw that most of the MetaConnect inputs linked to demography are well known life-history traits easy to find in the literature (fecundity, mating system, survival, sex-ratio…). In contrast, life-history-traits related to dispersal are difficult to find in the literature (Stevens et al. 2012; Grimm et al. in prep; Trochet et al. submitted) and greatly affect PVA output (Ruckelshaus et al. 1997; Mennechez et al. 2004). Ruckelshaus et al. (1997) found that models of dispersal are sensitive to (1) the dispersal mortality, (2) the maximum distance an individual can move and (3) the rugosity coefficients of the different landscape features (behavior/landscape interaction). During my PhD, I was led to work on points (2) and (3) by my own or through different collaborations. I will show in this chapter the work I did to experimentally determine the maximum distance an individual can do during dispersal events. I also contribute to a study that aimed at ranking the causes of dispersal which play a role in point (3) but just present the paper in the annex below since I contributed to the statistical analysis and interpretation and therefore played a more minor role in the experimental design (Legrand et al. submitted).

## Experimentally estimated maximum distance of movement for the Moorish totrtoise (Testudo graeca soussensis) (adapted from Lagarde et al. 2012; Moulherat et al. 2014)

Terrestrial chelonians are threatened worldwide by habitat destruction and illegal harvesting. Urbanisation, overgrazing, desertification and associated habitat fragmentation worsen the situation. Indeed, tortoises are slow moving animals susceptible to dehydration and overheating during movements in open habitats. Many species inhabit arid steppes where the availability of thermally buffered refuges (e.g. burrows) is a limiting factor. Determining the maximal distance between refuges that individuals can safely traverse during the active season is thus essential. We examined the relationship between body temperature variation and movement patterns in adult *Testudo graeca* in the arid steppes of Morocco.

*Maximum distance traveling under physiological constraints.*

First I determined the maximum distance a Moorish tortoise can travel assuming that the only constraint is physiological and due to the overheating risk (Gans and Dawson 1976). I then measured internal temperature of physical models that mimic the thermal dynamic of live individuals exposed to solar radiation at different moments of a journey (8:30, 10:00, 11:30, 14:00, 15:30, and 17:00) (Vitt and Sartorius 1999; O'Connor et al. 2000; Shine and Kearney 2001; Lelievre et al. 2011; Lagarde et al. 2012). I measured locomotor performances of 14 adult tortoises and linked these performances to tortoises' body temperature (Huey and Stevenson 1979; Bennett 1980; Hertz et al. 1982; Hertz et al. 1993; Angiletta et al. 2002). I then built a predictive model of tortoise velocity as a function of their internal temperature and size (shell length) and applied this model to measured temperatures of the physical models (Figure 40).

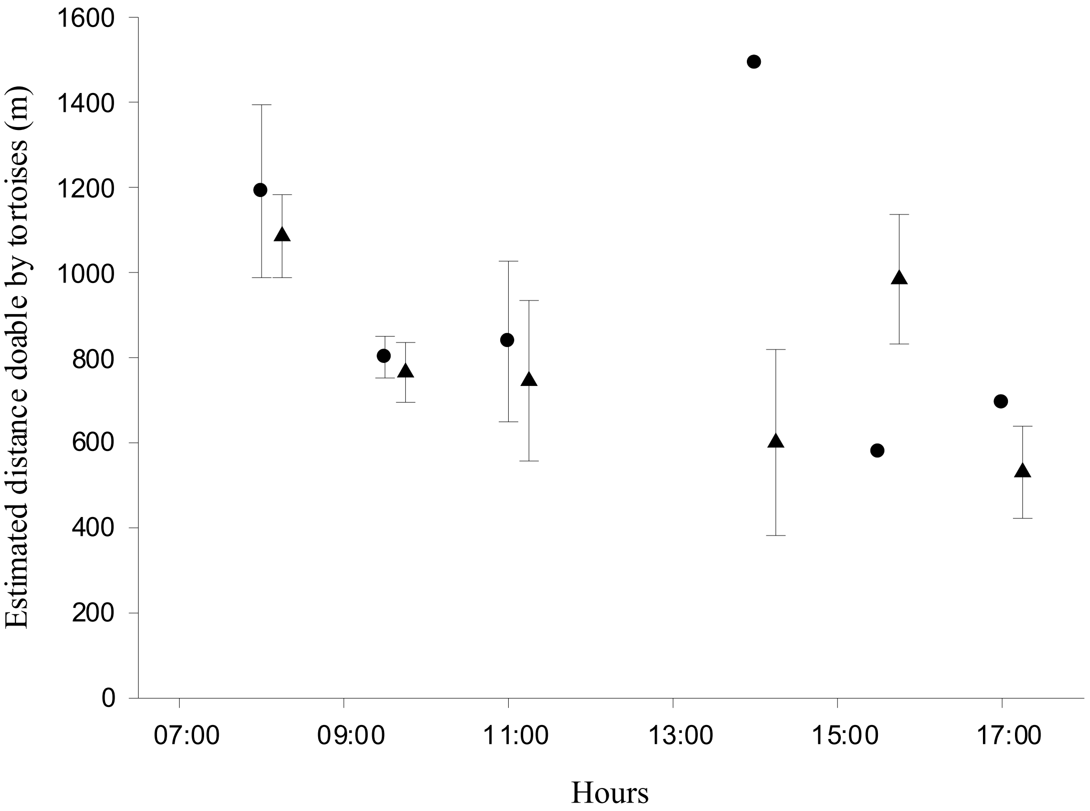

Figure 41: Estimated cumulative distances achievable by Moorish tortoises (Y axis) during sunny days. Individuals are diurnal, active during sunny days, and they are in the open during

movement. Solar radiation can entail overheating risks; the tortoises must cease movements and shelter into a refuge. The mean estimated maximal distances for females (circles) and for males (triangles) were derived from the temperature measured in physical models, locomotor performances measured in the field on tortoises and from the change in body temperature over time.

### *Maximum distance traveled by free ranging individuals*

In parallel to the estimation of the maximum distance tortoises can travel before overheating, I radio-tracked 13 adult tortoises (6 males, 7 females) fitted with a temperature data logger (Figure 41). I localized the tortoises 3 times a day during 12 days. The temperature data logger fitted on the tortoises' shell permitted me to estimate their internal body temperature using a simple mathematical heat diffusion model (Box C).

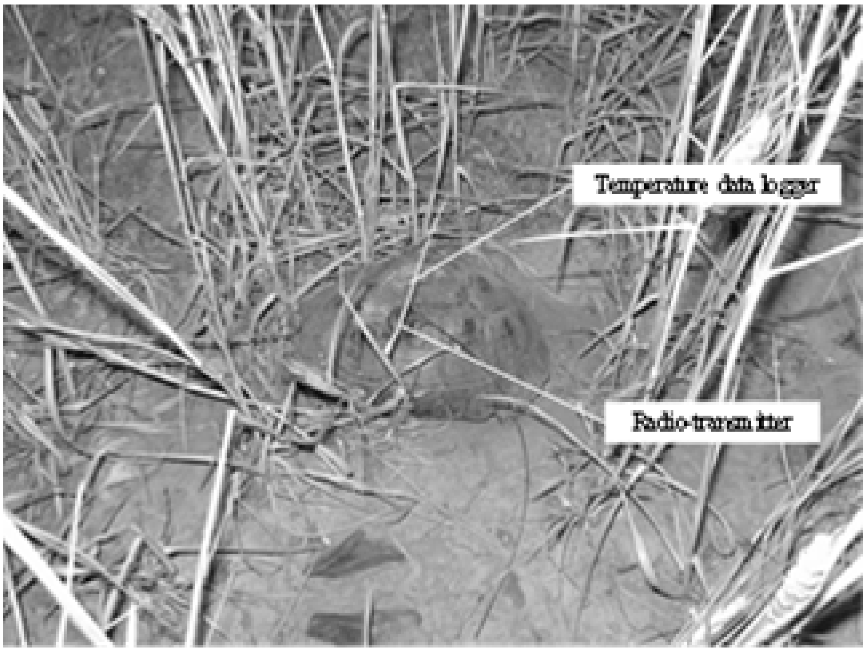

Figure 42: Female Moorish tortoise (*Testudo graeca soussensis*) equipped with a temperature data logger and a radio-transmitter for the radio-tracking.

Box C: Model of heat diffusion

Numerous models of heat diffusion have been developed by physisists and thermal ecologists to deal with thermal ecology of ectotherms (Porter et al. 1973; Spotila et al. 1973; O'Connor 1999; O'Connor 2000; O'Connor et al. 2000; Christian et al. 2006; Sinervo et al. 2010). However, these models require a huge amount of data from environmental parameters that may be very hard to measure on the field (i.e. wind direction on the tortoise's shell, solar radiation ...). Here, I developed a simple model derived from Delmas et al. (2008) to be used as a surrogate of temperature data logger surgical implantation (Edwards 2005):

$$Tb_t = Tb_{t-1} + (Tb_{t-1} - Td_t)\left(e^{\alpha m^{\beta} \Delta t} - 1\right)$$

Where $Tb$ is the body temperature at $t$, $\alpha$ and $\beta$, the heating and cooling conductivity coefficients (Porter et al. 1973; O'Connor 1999; O'Connor et al. 2000; Christian et al. 2006) and $m$ the individual mass as suggested by Christian and Tracy (2006). Both coefficients $\alpha$ and $\beta$ were fitted using maximum likelihood for heating and cooling patterns derived from measurements obtained on 16 physical models (Lagarde et al. 2012). I previously showed in Lagarde et al. (2012) that temperatures measured in physical models, underestimate body (cloacal) temperatures recorded in living individuals by approximately 2°C (comparisons between internal temperatures of 6 pairs of tortoises *versus* physical models of similar size: mean Pearson $r^2 = 0.79 \pm 0.2$, range 0.52-0.98), regardless of ambient temperature conditions. Therefore, we considered that an internal physical model temperature of 38° C corresponded to a critical body temperature of 40° C for a living specimen (Hutchison et al. 1966).

For $\Delta t = 5$ min, $\alpha$ and $\beta$ were fitted to - 0.0217 and - 0.8804 for the heating phase and -0.0490 and -0.9980 during cooling phases respectively (Figure C.1). As expected, the linear regression between measured (physical model) and estimated *Tb* (mathematical model) suggested that the model provided accurate estimates ($r^2 = 0.993$; slope = 0.996). The mean difference between estimated and observed values was -0.24 ± 0.02° C (mean ± SD), 95% of the differences were contained in a small interval (-3.14° C to 3.84° C), and they were normally distributed. The strongest differences usually occurred at the early beginning of the cooling phases were values generated by mathematical model were influenced by greater thermal inertia than physical models (Figure C.1).

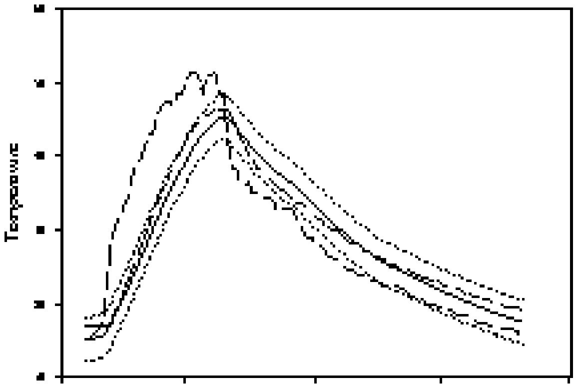

Figure C.1: Estimated central body temperature (*Tb*) of a physical model of *Testudo graeca soussensis* using a mathematical model (solid grey line) during a single day with 95% confidence interval (dotted lines). The estimated *Tb* was obtained using shell surface temperature (*Td*) recorded with loggers glued on the back of the physical models (long dashes). The actual central body temperature of the models (using implanted loggers) is represented with the dashed grey line. All recorded central temperatures are comprised in the 95% confidence interval (see text).

The main determinants of the distance travelled by the tortoises are sex and date. Importantly, both males and females remained motionless during cool days and were located moving only during warmer periods (date effect). During hot days, although the occurrence of movement was not different between sexes (W=66.2, p=0.77), females travelled longer distances (79.95 ± 10.98 m, max=570.71 m) than males (38.80 ± 6.91 m, max=154.48 m, W=1557, p=0.0036; Figure 42) and walked during longer periods (W=16038.5, p=0.01).

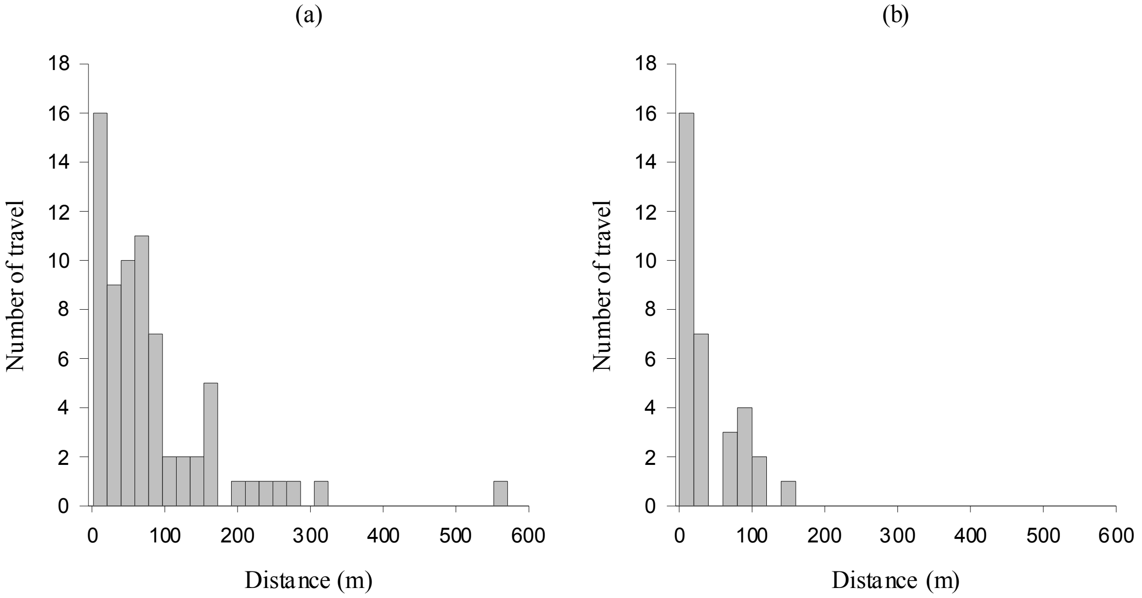

Figure 43: Distribution of the daily distances travelled by thirteen radio-tracked Moorish tortoises (six males and seven females) in the Jbilet central (Morocco) monitored during twelve days in April 2008. Females (a) move more often and farther than males (b).

The maximal *Tb* of the free-ranging tortoises estimated by our model was 34.7°C for males and 33.6°C for females. In both cases these high values were recorded in tortoises walking in the open. The mean maximal body temperature was 32.09 ± 0.56°C (all individuals, hence sexes, pooled). Males exhibited higher maximum *Tb* than females (33.06 ± 0.84°C versus 31.26 ± 0.69°C; $W = 34$, $p = 0.037$). Maximal estimated *Tb* were recorded between 16:00 and 19:36. Broadly, half (N = 6) of the maximal estimated *Tb* were obtained from tortoises that undertook relatively short trips (43.93 m - 320.16 m) and that broke up movements with episodes sheltered in refuges.

### *Conclusion*

In arid landscapes, individuals navigate between refuges (e.g. thick shrubs for the Moorish tortoise, burrows for the gopher tortoise) to escape lethal conditions. Thermal characteristics of available microhabitats suggest that only large bushes are safe (Lagarde et al. 2012). The difference between the theoretical maximal distance that can be safely traversed by adult tortoises (~1 km) and the actual shorter values (~0.5 km) recorded in free-ranging tortoises suggests that individuals keep a safety margin. Clearly, the tortoises carefully avoided body temperatures greater than 35° C (by sheltering under large bushes), a value markedly below the 40°C threshold, by limiting trip duration and distance. Such prudent behaviour must be integrated in conservation management as it considerably reduces the appropriate distance that must be retained between refuges. Interestingly, Lagarde et al. (2012) reached a similar conclusion based on a population survey in 12 different sites that varied in terms of bush size and density. This strong congruence between physiological constraint assessments (current study) and population surveys (Lagarde et al. 2012) strengthens our conclusions. The sex difference we observed, with the body size advantage for large females, in limiting overheating was expected as larger body mass confers thermal buffering (O'Connor et al. 2000). We acknowledge that we did not study the most vulnerable age cohorts: the neonates and the juveniles. Incorporating the high thermal sensitivity and low velocity of such small individuals would automatically suggest that a 0.5 km distance between refuges is already excessive. Little is known about dispersal in tortoises, but field evidence suggests that it may be ensured by females through nest site selection (unpublished data). Indeed, during the egg laying period, females can traverse long distances (> 5 km.week$^{-1}$, unpublished radio-tracking data). Thus, some connectivity between patches could be maintained by the females and to a lesser extent by the males (Lagarde et al. 2002, 2008). Overall, field managers should maintain a maximum distance shorter than 0.5 km between refuges to maintain the connectivity between populations (e.g. using corridors of bushes). To our knowledge, such a precise field recommendation has never been proposed to promote the conservation of reptiles living in superficially homogeneous arid zones. Our simple albeit precise approach might usefully contribute to habitat protection/restoration of a wide range of organisms that face strong overheating constraints during movements.

## Estimating maximum dispersal distance through meta-analysis and data base development (Stevens et al. 2013; Grimm et al. in prep; Trochet et al. submitted)

I actively participated in the data collection (Grimm et al. in prep; Trochet et al. submitted) and method development of the predictive models used in the lab (Stevens et al. 2012; Stevens et al. 2013; Trochet et al. submitted). In addition, these data and methods could be useful in order to set up MetaConnect's dispersal module and thus related to my own thesis. That is why I chose to devote a short part of my thesis to this methodology.

### *Theoretical framework*

Maximum distance that an individual can disperse is of prime importance when dealing with functional connectivity (Hanski and Gilpin 1997; Ruckelshaus et al. 1997; Clobert et al. 2001b; Doebeli and Dieckmann 2003). In addition, we already showed that spatially explicit models are very sensitive to this parameter (Ruckelshaus et al. 1997). Previous studies derived species dispersal abilities from morphological traits (Beck and Kitching 2007; Dawideit et al. 2009; Thompson et al. 2010; Turlure et al. 2010; Sekar 2012; Stevens et al. 2012). However, the predictive power is low and seems to be of limited use to predict dispersal abilities (Dawideit et al. 2009; Sekar 2012; Stevens et al. 2012). The lack of fit can be partially attributed in some cases to local adaptation which could lead to different behavior within a species exhibiting similar morphological traits within its habitat range (Baguette et al. 2000; Turlure et al. 2011). In contrast, we believe that dispersal abilities may be related to a larger range of life-history-traits and influenced by phylogenetic relationships and are part of an individual strategy (behavioral syndrome) (Ducatez et al. 2012; Ronce and Clobert 2012; Sih et al. 2012).

### *Models*

To predict dispersal abilities of species using life-history-traits, we used available databases of butterflies (Stevens et al. 2010; Stevens et al. 2012; Stevens et al. 2013) and amphibians (Trochet et al. submitted) separately and performed linear predictions (Remm 2004; Han and Kamber 2006; Cornuejol and Miclet 2013; Moulherat et al. In Press) of the maximum distance that species can disperse in relation with their morphological characteristics, life-history-traits and phylogeny (Stevens et al. 2012; Stevens et al. 2013; Trochet et al. submitted). The general design of the analysis consists of:

1. Perform a principal component analysis (PCA) to summarize the morphological traits and their relation by their orthogonal projection on the retained PCA axis.
2. Perform a backward selection of the most relevant linear models that explain maximum distance dispersal variance as a function of the summarized morphological traits and other non-collinear relevant life history traits using the training dataset (Burnham and Anderson 2004).

2'. If more than one model is retained by the model selection, then perform model averaging to build a final averaged model.

3. Partition the data base into a training dataset and a test dataset (Han and Kamber 2006; Cornuejol and Miclet 2013).
4. Fit the final model on the training dataset and then predict the maximum dispersal distance on the test dataset.
5. Bootstrap steps (3) and (4) (KNN or leave-one-out cross-validation (Han and Kamber 2006)) to estimate the model predictive capacities and accuracy (Figure 43, 44).

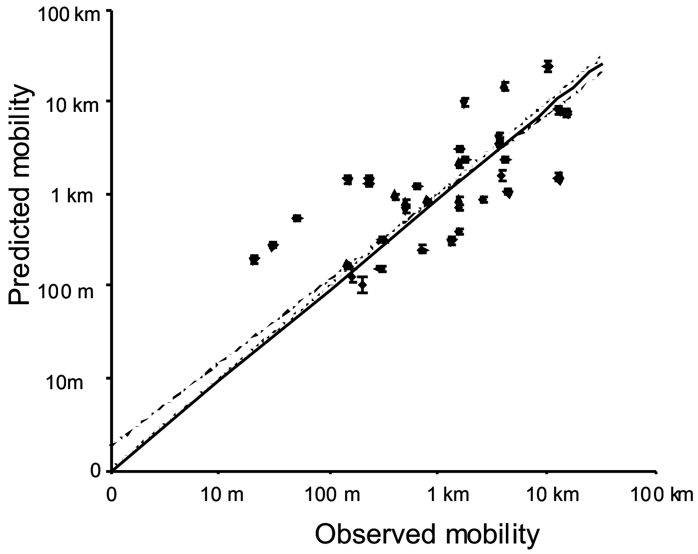

Figure 44: Cross-validations for predictions of amphibian mobility, obtained using a linear model with fecundity, total length and sampling scale as independent explanatory variables of amphibian mobility.

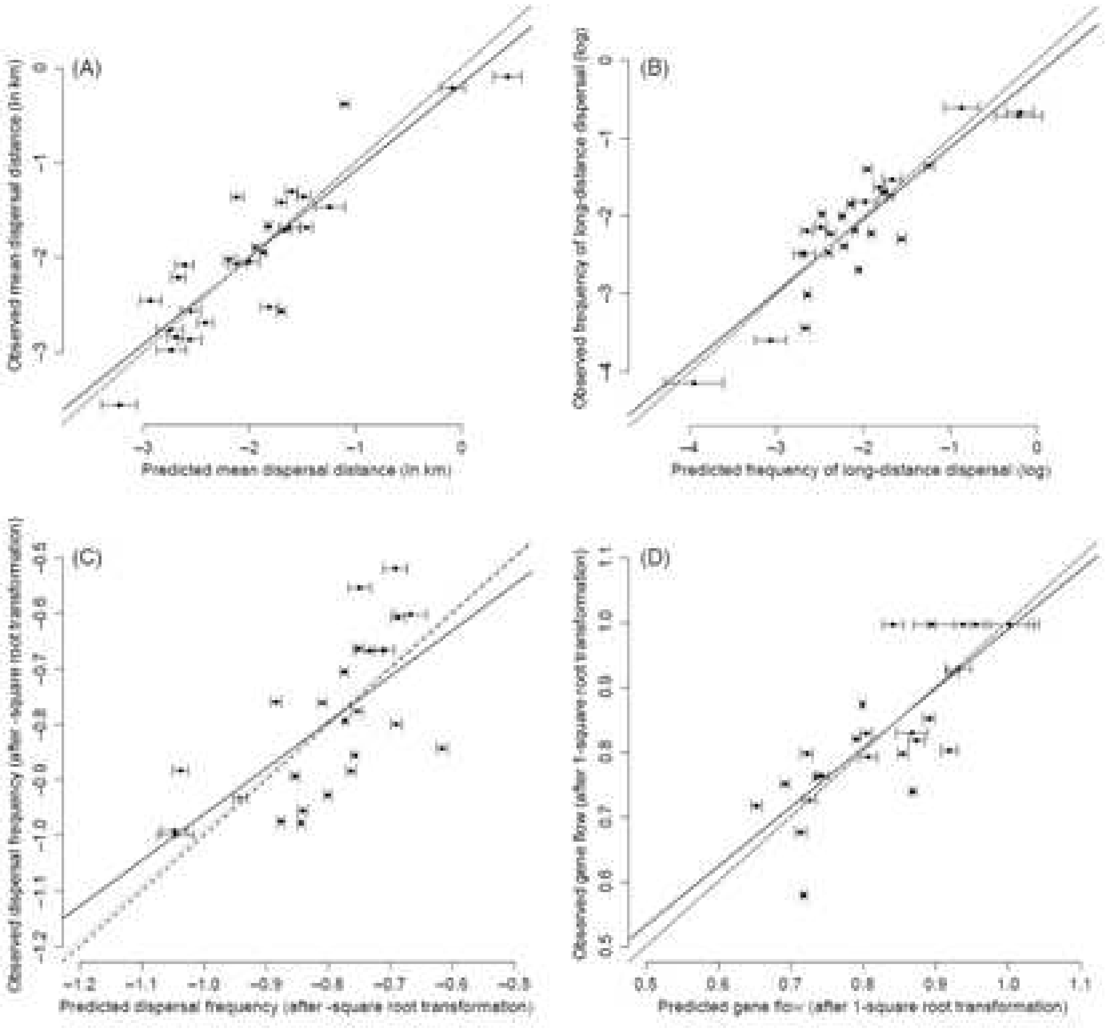

Figure 45: Cross-validations of predictive models for butterfly dispersal: predictions obtained from information on multiple life-history traits, together with wing size (B) or not (panels A, C, D) (see Stevens et al. 2013 for details). A: mean dispersal distance; B: frequency of long-distance dispersal; C: dispersal propensity; D: intensity of gene flow, observed for 25-30 butterfly species, all plotted against the mean predicted values and their respective 95% CI (obtained after 100 random partitions). Black lines show the linear regressions; for comparison, dotted lines show the slope 1:1, and gray line show the regression with a 0:0 intercept (from Stevens et al. 2013).

*Applicability*

In both of these case studis, even though the addition of various life-history traits to morphological traits greatly improves predictive models efficiency, the errors made in prediction are often important and poorly informative in conservation planning. Stevens et al. (2013), suggested that several improvements could improve this efficiency based on the quality of input data. However, the model structure by itself could be a limit to its predictive power. Indeed, in these cases, we used linear models, so we implicitly assume that the relationships between traits we used are linear when in fact some traits may have non-linear and complex links (Zuur et al. 2009). To integrate this complexity in the relationships between traits, the used of other modeling technics such as data mining and artificial intelligence like Generalized-Additive-Models (GAM) (Zuur et al. 2009) or artificial neural networks (Lek et al. 1996; Lek and Guegan 1999) which could outperform generalized linear models in their predictive abilities (Han and Kamber 2006; Cornuejol and Miclet 2013).

## Conclusion

Dispersal characteristics are difficult to measure and spatially explicit models are sensitive to the quality of this input variable (Ruckelshaus et al. 1997). Here I developed two simple ways to estimates dispersal characteristics. The first one is empirical and highly realistic but is time and data consuming which limits the applicability of this procedure to a single species. In addition, the procedure can only be applied to traceable species. The second one is based on the design of a life-history trait database and its exploitation through statistical modeling. This second framework requires a large set of data (time consuming) but permits the estimation of dispersal parameters for multiple species within a single analysis. However, even if the models presented here perform better than previous morphological based models (Sekar 2012), it still has limited predictive power in many cases and their applicability would be of limited interest in conservation planning. I imagine additional two ways to improve the reliability of dispersal characteristics and life-history traits beyond those provided by Stevens et al. (2013): (1) improving the model structure using more powerful predictive tools (Han and Kamber 2006; Cornuejol and Miclet 2013). (2) Integrating landscape/species interaction correlations which could participate to local adaptation that can mask some parts of the dispersal syndromes (Baguette et al. 2000; Gunton et al. 2013).

# Conclusion - Future of MetaConnect: TerrOïko's activity

MetaConnect offers a large range of opportunities in pure research and applied fields. In addition, during my PhD I had the opportunity to develop various and complementary skills and tools useful for decision makers and planning managers. Actors of urban and conservation planning expressed their difficulties to make the link between their management needs and the scientific tools and methods available (Barbrault 2005; Loreau 2010; Besnard et al. 2013; ITTECOP 2013). Based on stackeholder needs and my skills, I built my own enterprise (TerrOïko) in collaboration with Catherine Boreau de Roincé with the objectives of:

1. Assisting stakeholders of landscape development to integrate biodiversity in their plans
2. Developing/adapting user-friendly tools for urban and conservation managers to help them access high quality methods to improve consistency and efficiency of their studies.
3. Participating to the technological transfer from research labs to operative actors.
4. Participating to the applied ecology research field.

Through TerrOïko, we aim at assisting conservation managers and landscape planners providing them tools and methods coming from the scientific world. In the TerrOïko rapport above, I provide an example of our activity. This example shows how we provided a Parc Naturel Régional a protocol to experimentally estimate *Phengaris alcon* rugosity coefficients based on Turlure's et al. (2011) recommendations and then uses these results to perform a PVA for the species in order to support conservation planning.

More details on my current activity can be found on www.terroiko.fr.

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# Related papers

# 1. MetaConnect, a new platform for population modeling to assist decision makers in conservation and urban planning

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Submitted in Environmental Modelling & Software

*Résumé en français*

Dans un contexte de changements globaux, scientifiques et législateurs requièrent des outils pour endiguer la perte de biodiversité. L'analyse de viabilité des populations (PVA) est historiquement le principal outil pour traiter cette question. Cependant les outils développés pendant les années 90 n'intègrent que peu ou prou les récentes avancées scientifiques concernant la génétique du paysage ou la dispersion. Nous avons développé une plateforme de modélisation flexible et modulaire qui permet de palier à la plupart des lacunes des modèles existants. MetaConnect est une plateforme de modélisation individu centrée, processus centrée et dérivée de modèle de PVA qui peut être utilisée comme un outil de recherche ou un outil d'aide à la décision. Dans cet article, nous présentons le cœur de fonctionnement de MetaConnect et sa validation. Dans un second temps, nous illustrons à travers un cas d'études les capacités de la plateforme à permettre la réalisation de PVA intégrées qui comprennent l'estimation des probabilités d'extinction, de la différentiation génétique et l'analyse de la connectivité fonctionnelle. Nous avons utilisé MetaConnect pour évaluer l'impact du développement d'une zone d'activité concertée et d'une ligne TGV sur le fonctionnement de la metapopulation de crapaud calamite.

Abstract:

In a context of global change, scientists and policy-makers require tools to address the issue of biodiversity loss. Population viability analysis (PVA) has been the main tool to deal with this problem. However, the tools developed during the 90s poorly integrate recent scientific advances in landscape genetics and dispersal. We developed a flexible and modular modelling platform for PVA that addresses many of the limitations of existing software. MetaConnect is an individual-based, process-based and PVA-based modelling platform which could be used as a research or a decision-making tool. Here, we present the core base modelling of MetaConnect, and its validation. We then illustrate its potential use through a case study illustrating the platform's capability for performing integrated PVA including extinction probability estimation, genetic differentiation and landscape connectivity analysis. We used MetaConnect to assess the impact of infrastructure works (industrial area and high-speed railway connections) on the natterjack toad metapopulation functioning.

Keywords:

Modelling platform, individual-based model, process-based model, decision-making tool, dispersal, landscape genetics, landscape planning

Introduction:

In a context of rapid global change, habitat loss and habitat fragmentation have become the major threats to biodiversity (IUCN 2013). Over the last centuries, the conversion of natural and semi-natural ecosystems into agricultural, industrialized or urbanized areas has a major impact. This conversion has accelerated over the past decades, with tropical forest being destroyed at an annual rate of 13 million ha per year (FAO 2006). Habitat loss and fragmentation have been considered for some time as the major cause of species extinction (Dobson et al. 1997; Millenium Ecosystem Assessment 2005).

Habitat destruction and fragmentation have consequences from the ecosystem (Fahrig 2003; Cardinale et al. 2012; de Mazancourt et al. 2013) to the genetic scale (Ingvarsson 2001; Baguette et al. 2013a). Habitat loss and fragmentation modify landscape patterns in a four-step process: reduction in amount of habitat, increase in number of habitat patches, decrease in size of habitat patches and increase in isolation of patches (Fahrig 2003). This alteration of landscape patterns has diverse effects on population dynamics (e.g. Baguette et al. 2013a). As patches become smaller, the size of the population supported decreases; this can increase stochastic risk of extinction from demographic processes (Legendre et al. 1999; Reed et al. 2002), but also from genetic stochasticity: small populations are more subjected to risk of inbreeding and consanguinity depression (Brook et al. 2002b), loss of genetic diversity and mutational accumulation (Rowe and Beebee 2003), leading to the extinction vortex (Gilpin and Soulé 1986; Fagan and Holmes 2006). Moreover, by increasing the distances between patches and therefore dispersal cost, habitat loss and fragmentation decreases the probability of individuals moving from one population to another. This can hinder the recolonization or the demographic rescue of patches where sub-populations have become extinct, or declining, potentially leading to stochastic extinction of metapopulations (Fahrig 2003). Furthermore, by reducing gene flow between populations, isolation can lead to genetic differentiation of sub-populations and impede genetic rescue of populations with high levels of inbreeding (Ingvarsson 2001; Keller and Waller 2002; Tallmon et al. 2004).

Historically, an ecological tool much-used by scientists and conservation managers was population viability analysis (PVA), which aims at estimating extinction or quasi-extinction probability of a species (Boyce 1992; Legendre and Clobert 1995; Beissinger and Westphal 1998) and which was used to inform conservation programs (Southgate and Possingham 1995; Ferriere et al. 1996; Letcher et al. 1998; Schtickzelle and Baguette 2004; Radchuk et al. 2013). Most of the PVA models focused on species population dynamics (Lindenmayer et al. 1995; Brook et al. 1999; Legendre et al. 1999), and offered limited flexibility regarding population genetics (but see Lacy et al. 2009) or metapopulation functioning (Reed et al. 2002; Pe'er et al. 2013). PVA is focused on demographic processes involved in species persistence but other processes, such as genetic or individual movement behavior can have a strong influence on species persistence (Hanski and Gilpin 1997; Clobert et al. 2001a; Ingvarsson 2001; Keller and Waller 2002; Fahrig 2003; Tallmon et al. 2004). Population genetics has made major advances during recent decades, and it is now possible to (1) identify at generation *g* those individuals that are offspring of individuals immigrating at generation *g-1*; and (2) assign these immigrants to their population of origin (Beerli and Felsenstein 2001). Besides, the rise of landscape genetics allows the assessment and quantification of how landscape elements affect gene flow in a metapopulation (Manel et al. 2003; Manel and Holderegger 2013). It should be highly valuable to integrate both population and landscape genetics approaches in models of metapopulation functioning to support conservation managers' and policy makers' plans (Baguette et al. 2013a; Pe'er et al. 2013).

Initially, matrix-based models were adapted to implement generic PVA software (Lindenmayer et al. 1995; Legendre et al. 1999; Akcakaya 2001; DeAngelis and Mooij 2005). Nowadays, the development of computational power favors the development of individual-based-models (IBM) and allows building of highly realistic models (DeAngelis and Mooij 2005; Grimm et al. 2006; Pe'er et al. 2013) that can better satisfy scientific, regulatory and decision-making needs. The growth of a community of ecological modelers using IBMs rather than the mathematical approaches that until the late 90s had completely dominated the ecological modelling field, has led to the production of a huge number of models (DeAngelis and Mooij 2005) and metrics (Moilanen and Nieminen 2002; Calabrese and Fagan 2004). In addition, most of these models were developed to answer specific questions, which renders comparison between outputs difficult, if not impossible (DeAngelis and Mooij 2005; Kindlmann and Burel 2008; Pe'er et al. 2013) and their application inefficient out of the narrow context for which each was typically developed (Grimm et al. 2004; Kindlmann and Burel 2008). Generic PVA modelling platforms built from IBM and process-based modelling are lacking (but see Grimm et al. 2004; Lacy et al. 2009; Bocedi et al. 2014) or do not permit a sufficient level of flexibility to deal with a large spectrum of conceptual framework and ecological themes (DeAngelis and Mooij 2005; Evans et al. 2012; Evans et al. 2013; Purves et al. 2013).

Because PVA is a relevant basic decision-making tool (Brook et al. 2000; Brook et al. 2002a; Pe'er et al. 2013), we developed MetaConnect, a generic PVA-based IBM following the Beissinger & Westphal (1998) framework, which allows the design of a range of models from very simple to highly detailed and which integrates demography and genetics in a spatially-explicit context (Baguette et al. 2013a). MetaConnect not only aims at performing traditional PVAs based on demographic data, but also integrates the recent development of population and landscape genetics, which allows the assessment of functional connectivity (sensu Taylor et al. 2006). We expect that this integrated modelling platform will be a useful tool for scientists, conservation managers and policy makers. Here we present the core base modelling of MetaConnect, and its validation by comparison with previous models outputs. In addition, we present a case study showcasing the use of MetaConnect to assess the impact of infrastructure works (roads, industrial area and railways) on the population viability, genetic differentiation and functional connectivity of existing populations of the endangered natterjack toad. This example illustrates how with very basic knowledge of species' life-history-traits and landscape structure, we can perform a consistent and integrated predictive scenario of *E. calamita* future in a study with MetaConnect.

## Model

### Model design

MetaConnect simulates metapopulation dynamics and genetics using the species life cycle and life history traits, the landscape characteristics and their interactions. The simulations allow inferring of local and global extinction probabilities, individual dispersal within the meta-population, local and global genetic diversity and local and global genetic differentiation (from classical Fst analyses or as input files for the Structure software (Pritchard et al. 2000)).

MetaConnect is an individual and process-based-model which means that:

(1) all individuals in the model are independent and behave in respect to their phenotype (individual-based model)

(2) patterns emerging in the different outputs of the model are the products of flexible and adjustable rules implemented in the model (process-based-model).

## Model structure

Table 1: Nomenclature of MetaConnect's main parameters and variables.

| Parameters and variables | Description | Distribution law of random variables |
|---|---|---|
| ***Demographic characteristics*** | | |
| $K$ | Carrying capacity* | |
| $k$ | Total competition coefficient | |
| $s_i$ | Survival of individual from class $i$ | Bernoulli |
| $F$ | Fecundity | Poisson |
| $\sigma$ | Primary sex ratio | Bernoulli |
| ***Mating system*** | | |
| Mating system | Mating system assumptions | |
| $H_m$ | Male harem size | Poisson |
| $H_f$ | Female harem size | Poisson |
| ***Genetics*** | | |
| $L$ | Number of locus | |
| $A$ | Number of alleles per loci | |
| $\mu$ | Mutation rate | |
| ***Dispersal*** | | |
| $p_d$ | Dispersal probability | Bernoulli |
| Dispersal rule | Dispersal algorithm | |
| ***Initialization and model parameterization*** | | |
| $N_0$ | Initial number of individuals | |
| $\sigma_0$ | Initial sex-ratio | |
| $f_a$ | Initial class structure | |
| $T$ | Time steps | |
| $MC_l$ | Number of landscape random generations | |
| $MC_d$ | Number of population dynamic simulations per landscape | |

* The carrying capacity $K$ is derived from the competition coefficient and the competition assumption.

## Landscape

The modelling platform MetaConnect requires three layers describing the landscape:

(3) Patches: locates suitable habitats for the focal species. Each patch is identified by its name and is constituted by all the adjacent cells having the same name.

(4) Carrying capacities: provides the carrying capacity for each cell of the layer. The patch carrying capacity then corresponds to the sum of the values of its constitutive cell's carrying capacities.

(5) Costs: provides a coefficient representing preferences for each cell of the map

The landscape layers can be imported from GIS software as raster files.

### Demography

The population dynamics is represented by a succession of individual states linked by transitions. The user can himself build the species life-cycle by assembling “bubbles” representing the individual state and “arrows” representing transition rules between individual states (Figure 1, 2). The “bubbles” correspond to what we will call class within this manuscript and can correspond to age classes or sex or anything that can be defined as a group of individuals with the same demographic characteristics. Density dependence can be scramble or contest and can be designed as a part of a transition. The mating system can be chosen from monogamy, polygamy, polyandry and/or polygyny (Legendre et al. 1999). The demographic parameters (Table 1) can be patch-specific. Environmental stochasticity has been included as random processes inducing normal variation around the patch’s mean value of demographic parameters. As an example, the fecundity parameter follows a Poisson distribution (demographic stochasticity) with parameter λ equal to the average fecundity ($F \rightsquigarrow \mathcal{P}(\bar{F})$). The average fecundity can vary from one patch to another and within simulation time steps following a Gaussian distribution (Table 1).

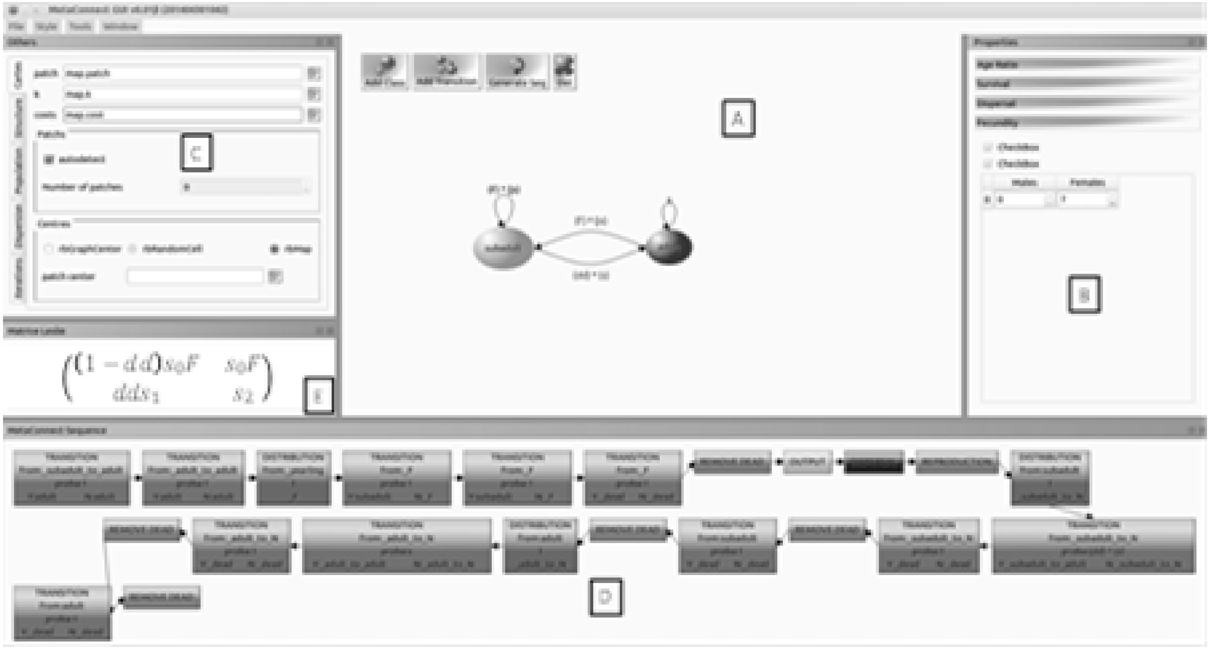

Figure 1: Example of the Lengendre et al. (1999) passerine life cycle with two age-class and two sexes that can be modeled with MetaConnect. On this MetaConnect screenshot, in the A section, “bubbles” correspond to reproductive status of individuals (subadult or adult) and *dd* is the density-dependent recruitment probability that depends on the competition assumption (contest or scramble competition respectively equations 1a and 1b). The user defines the species life-cycle as a combination of settable “bubbles” (add class) and “arrows” (add transition) (A). The species life history traits are set up in the B section and the run setting is defined in the C section. Then, the MetaConnect workflow (D) and the Leslie matrix (E) are generated automatically. The Leslie matrix and the MetaConnect workflow can be changed by the user which automatically adjusts the life-cycle graph.

Dispersal decision $p_d$ is implemented by setting a proportion of individuals (reproductive and/or non-reproductive) leaving a patch. The density-dependent recruitment probabilities $p_r$ is determined by equation 1 where $N_T$ can be a chosen combination of the number of individuals per class (i.e. $N_T$.could be the total population or just the reproductive individuals) (Caswell 2001).

Equation 1:

$$\text{If contest competition: } p_r = \frac{1}{1 + kN_T}$$

$$\text{If scramble competition: } p_r = e^{-kN_t}$$

Dispersal is age- and sex-dependent, and the process by which individuals disperse can be chosen from three families of movement rules:

(6) The first family of dispersal rules does not take account of the preference coefficients. Dispersal between patches is modeled by a probability for an individual to reach another patch. The probability of reaching a patch can be equal between patches, or depend on the Euclidean distance between patches' centers, or can be set, manually.

(7) The second family represents the interaction between individuals and their environment through the use of preference coefficients. This family comprises the random-walk dispersal rule (RW) and a correlated random-walk rule (CRW). The CRW assumes a degree of directional persistence, (i.e., the movement direction at time *t+1* depends on the direction taken at time *t*) and not solely an environmental based one.

(8) The last family of rules assumes that individuals have knowledge of their environment. They are able to reach the other patches passing by the easiest way (Least-Cost-Path, LCP). From a focal patch, the LCP algorithm usually assumes that a single patch can be reached (Botea et al. ; Adriaensen et al. 2003; Pe'er and Kramer-Schadt 2008; Barraquand et al. 2009). Such an assumption is unrealistic, and to relax it we implemented a multiple LCP movement rule, in which we calculated all possible combinations of LCPs between the focal patch and all the other patches (Urban et al. 2009; Foltete et al. 2012). Then, for the reachable patches (i.e. LCP length less than the maximum dispersal distance), the probability to reach a patch is weighted by the LCP length (number of map cells crossed) or cumulative cost (total cost of all map cells crossed). We also adapted the Stochastic Movement Simulator (SMS) (Palmer et al. 2011; Aben et al. 2014; Palmer et al. 2014; Coulon et al. in prep), which relaxes the assumption of omniscience inherent in the LCP approach. With the SMS rule, individuals make movement decisions based on the environment within a limited perceptual range and a tendency to directional persistence similar to that in a CRW. At each movement time step, the SMS algorithm calculates a movement probability for each cell surrounding the individual's current cell. The calculation process

evaluates the displacement cost for each surrounding cell based on the coefficient of rugosity of the cells in the neighborhood and its distance to the current place (see Palmer et al. 2011 for details).

The dispersal event ends when the individual dies or reach a patch different from his original patch regardless of the arrival patch quality.

### Genetics

Individuals are genetically tagged using neutral polymorphic loci. The number of loci and number of alleles per locus can be specified by the user. A single mutation rate (probability of creating a new allele without possibility of reverse mutation) implemented in the model allows the production of new alleles at each locus during simulations, and can be specified by the user. Gene transmission is assumed to be Mendeleian and siblings are assumed to have the same father (randomly chosen from the female harem for polyandrous cases).

### Model outputs

The model provides many forms of outputs based on focal species life history traits and landscape maps, which are adaptable to various theoretical and applied contexts. The outputs report the results at three levels at a frequency specified by the user, allowing dynamic visualization of the simulations:

(9) Demography: the model provides population size that can be split into the different classes implemented in the MetaConnect set up. The model also derives extinction probability, colonization probability and time before extinction and colonization. These indicators are calculated at the local (patch) and global (metapopulation) scales.

(10) Dispersal: for each pair of patches, the model provides the number of individuals that reach a new patch or die during the dispersal process. In addition, maps of cell occupancy are drawn from successful dispersal events (number of individual who visited the map cells during the whole run).

(11) Genetics: the model provides the genetic diversity and differentiation (Fst, Fis, Fit, He and Ho) at the local and global scale respectively to the consistency of such statistics.

All these results can be directly plotted using the MetaConnect results viewer (figure 2) or extracted as text files. In addition R (R Development Core Team 2005) has been incorporated to the viewer, which allows direct analysis of the MetaConnect outputs.

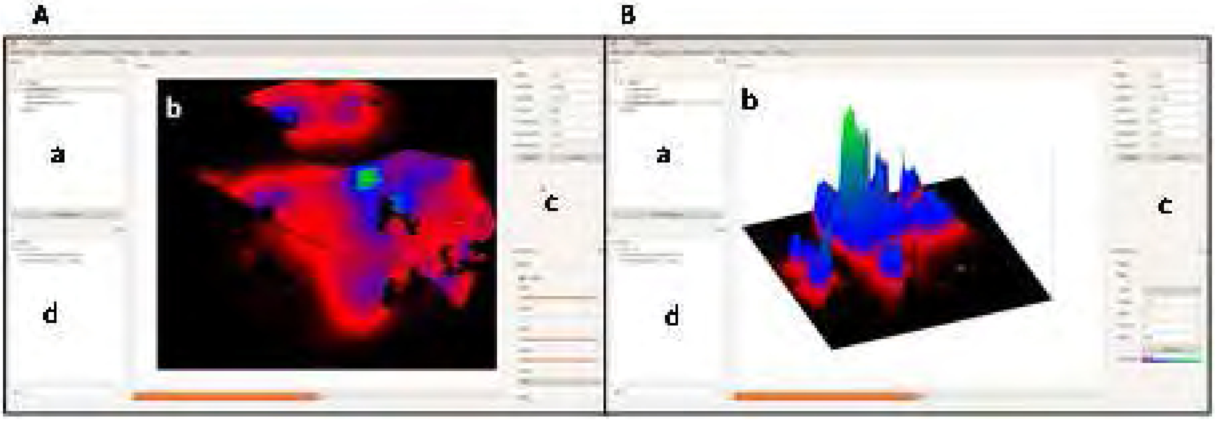

Figure 2: Screenshot of an example of MetaConnect results. Data to be display or analyzed are selected in the a section and displayed in the b in two (A) or three (B) dimensions. Section c allows setting of the display options, and the d section is an R console to perform simple analysis and plotting of the MetaConnect outputs. A R package and a specific section of MetaConnect are under development for a complete integration of R into MetaConnect. The example in these figures illustrates the *E. calamita* landscape use during dispersal expressed in mean number of individual per year visiting the map cell (from 1 per 10 years in red to more than 1 per year in green passing by 1 per 5 five years in blue).

### Model validation:

As suggested by Pe'er et al. (2013), we validated components of MetaConnect by comparing its results when possible with results provided by equivalent analytical models and IBMs. Results from MetaConnect and previous models matched well, and we present here only the most important comparisons that pertain to MetaConnect PVA core modelling, namely the validation of (1) the demographic core modelling, (2) the mating system toolbox and (3) the genetic module.

### Validation of the demographic core modelling

Brook et al. (1997) compared the ability of 5 PVA packages (INMAT, GAPPS, RAMAS/age, RAMAS/metapop and VORTEX which is the only non-analytical model of this study) to predict the behavior of real populations by making a retrospective study on the Lord Howe Island woodhen *Gallirallus sylvestris*. This study provides us the opportunity to compare in a single context the convergence between MetaConnect and the most used PVA software (Lindenmayer et al. 1995; Brook et al. 1997; Brook et al. 1999; Pe'er et al. 2013) as well as the ability of MetaConnect to estimate the behavior of a real species' population dynamics.

MetaConnect was set up based on the VORTEX system provided in Brook et al. (1997); a stable age structure was determined manually by calculating the eigenvector associated to the first eigenvalue of the Leslie matrix (Caswell 2001) provided for the RAMAS/metapop system and the competition type was assumed to be contest (Appendix A).

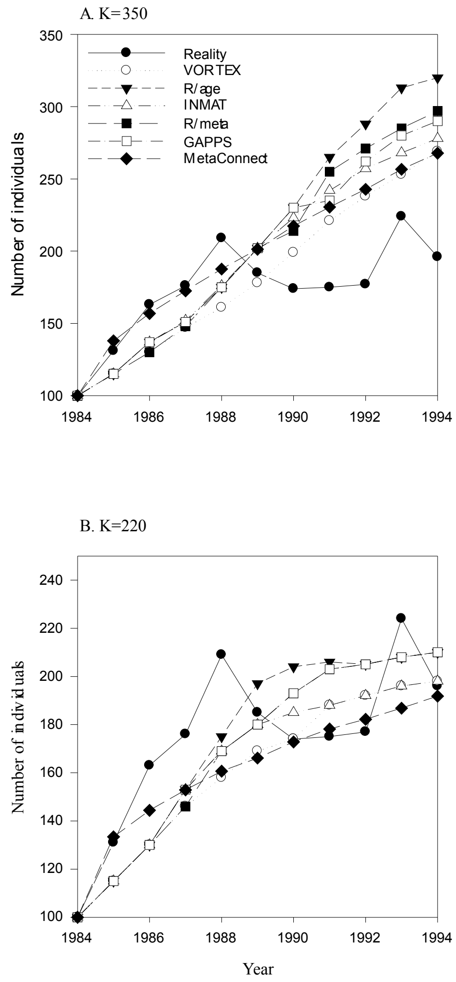

Figure 3: Simulated projections of the Lord Howe Island woodhen for the PVA software packages MetaConnect, GAPPS, INMAT, RAMAS/age, RAMAS/metapop and VORTEX under the effect of demographic and environmental stochasticity (derived from Brook et al.

1997). Initial population size set at stable age distribution of 100 individuals. Population history of the woodhen (1984-1994) is included for comparison. Models of density dependence were contest for MetaConnect and ceiling for models from Brook (1997) with carrying capacities set to K=350 (A) and K=220 (B).

Regardless of the carrying capacity, MetaConnect population dynamics predictions agreed with those provided by the other softwares (Figure 3) and the predicted population size after 10 years was similar to the size predicted by the other software (difference in mean number of individuals (± SD) between MetaConnect and the 5 PVA models for *K*=350: 21.8 ± 22.2, for *K*=220: 9.2 ± 6.8, Table 2). Moreover, MetaConnect better captured the initial (1984-1988) strong growth of the population (Figure 3).

Table 2: Estimates of final population size (after 10 years) predicted by software GAPPS, INMAT, RAMAS/age, RAMAS/metapop, VORTEX (Brook et al. 1997) and MetaConnect PVA core modelling. Mean ± standard deviation (as reported by Brook et al. 1997) are given for *K*=350 and *K*=220.

| Scenario | GAPPS | INMAT | R/age | R/metapop | VORTEX | MetaConnect |
|---|---|---|---|---|---|---|
| *K*=350 | 325±57 | 277±43 | 283±78 | 296±82 | 268±83 | 268±48 |
| *K*=220 | 211±28 | 199±20 | 194±49 | 205±40 | 197±37 | 192±35 |

### Validation of the mating system toolbox

Population dynamics model outputs are highly sensitive to the mating system (Doebeli and Koella 1994; Lindstrom and Kokko 1998; Legendre et al. 1999; Calsbeek et al. 2002). Legendre et al. (1999) related the colonization success of invasive passerine birds in New Zealand to their mating system (monogynous *vs* polygynous). To validate the mating system toolbox of MetaConnect core modelling, we compared outputs of MetaConnect population dynamics to those obtained by Legendre et al. (1999).

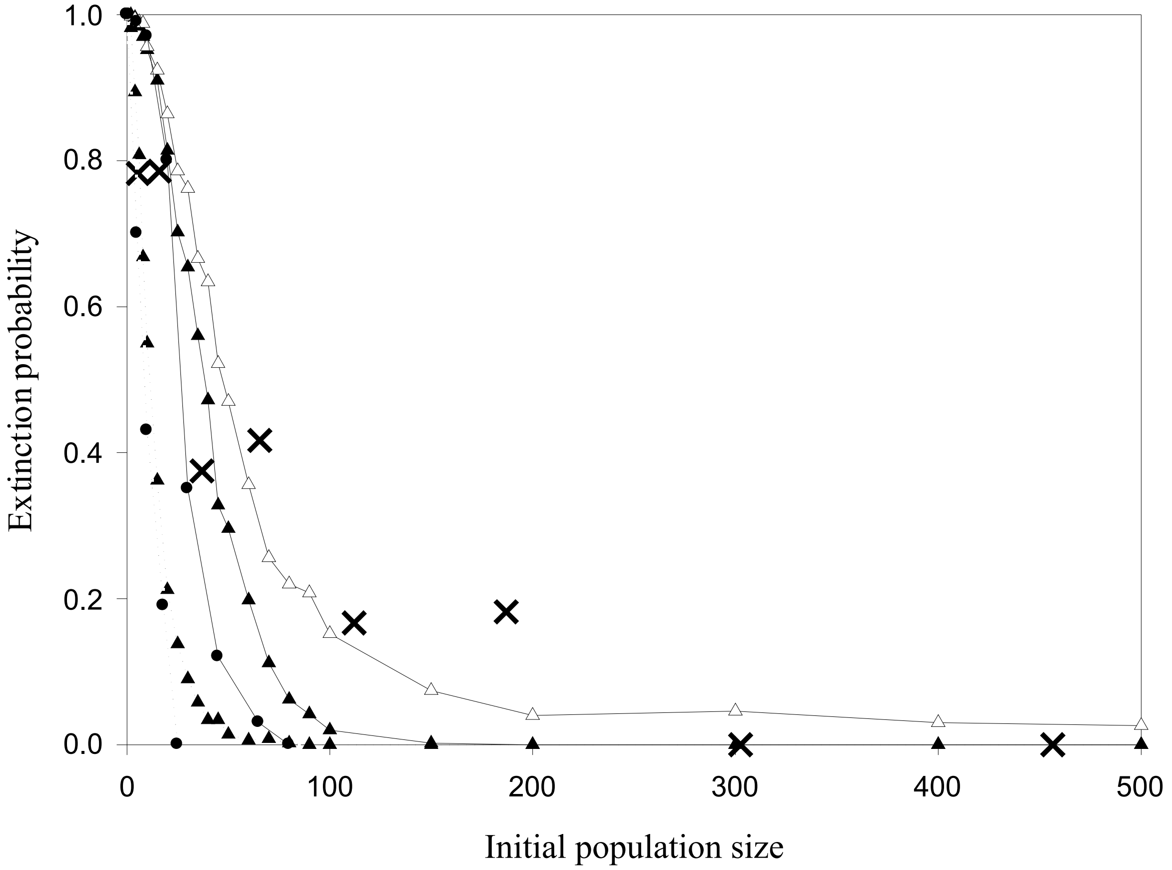

Figure 4: Predicted extinction probability of passerine bird species as a function of the mating system and initial population size. Filled symbols (triangles and circles) correspond to the predictions made by the model used by Legendre et al. (1999) and crosses correspond to natural observations of passerine invasion success in New-Zealand. Triangles are extinction probability estimates provided by MetaConnect. Plain lines correspond to a monogamous mating system and dotted lines to polygynous mating system. Black circles are estimates assuming high carrying capacities (*K*=500) and empty triangles correspond to low carrying capacities (*K*=250).

MetaConnect provides results consistent with those provided by Legendre et al. (1999) (Figure 4). The main difference between the models comes from the assumption of the recruitment function. Indeed, Legendre et al. (1999) assume an infinite Malthusian growth function, which is not currently implemented in MetaConnect. Rather, we used a contest recruitment function with *K* set at 1000 (results not shown), and decreased it to 500 and 250 (Figure 4) to examine the role of setting a constraint on the Malthusian growth rate. Unsurprisingly, for *K*=1000 results from MetaConnect converged with those obtained by

Legendre et al. (1999) under an infinite Malthusian growth function. As the carrying capacity was reduced, extinction probability increased and tended more closely to the pattern observed in the wild. Legendre et al. (1999) suggested that the difference observed between their modelling outputs and the natural observations were probably explained by environmental stochasticity. However, our results suggest that, even though environmental stochasticity may explain a significant part of the discrepancy, the carrying capacity of the population must play a major role in the observed pattern of invasive species extinctions (Figure 4). This is matching another study of spider extinctions on island where carrying capacity was also found to play a significant role on extinction even in presence of environmental stochasticity (Schoener et al. 2003).

### Validation of the genetic module

MetaConnect assumes panmixia in local populations (i.e. in patches). Regardless of initial conditions and model assumptions, MetaConnect provides estimates of Fis close to 0. Moreover, simulations considering two patches without dispersal generate a strong genetic structure at the metapopulation scale (Fst≈1) but no structure within local populations (Fis≈0). These two results provide initial confidence that the genetic core base of the platform behaves properly.

In addition, we validated the genetic part by comparing the MetaConnect genetic outputs to predictions of the CDPOP model (Landguth and Cushman 2010) in comparable situations. Landguth and Cushman (2010) explored with CDPOP the genetic outcome of three scenarios of landscape structures. The first corresponds to a panmictic population, the second assumes that two patches are isolated by an impassable barrier and the third assumes a simple diffusion model weighted by landscape preference coefficient. Figure 5 shows that results patterns from MetaConnect converged with those obtained by Landguth and Cushman (2010).

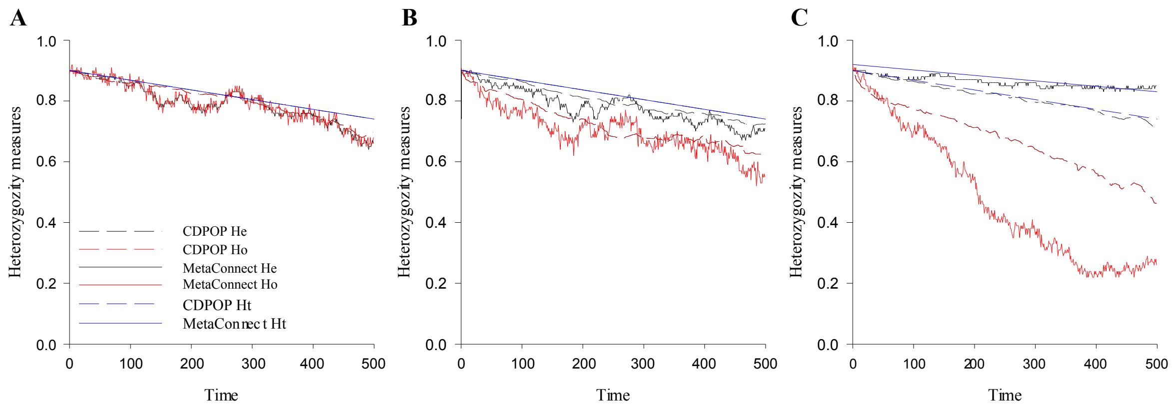

Figure 5: Heterozygosity measures derived from Landguth and Cushman (2010) (long dash) or estimated with MetaConnect (solid lines) under similar dispersal scenarios described in Landguth and Cushman (2010) (A. Panmictic scenario, B. patches are separated by impassable barrier, C. Individuals navigate between patches in a displacement matrix where rugosity coefficients are comprised between 1 and 63, and dispersers are dispersing following the random walk dispersal rule). For CDPOP and MetaConnect runs, $H_e$ and $H_o$, the expected and observed heterozygosity can be compared with curves of decay of heterozygosity produced according to the equation 1. The differentiation patterns are similar and variations between MetaConnect results and CDPOP can be attributed to variability between runs and differences in the displacement matrix map used in scenario B and C:

$$H_t = \left(1 - \frac{1}{2N_e + 1}\right)^t H_o$$

Where:

$$N_e = \frac{4N_M N_F}{N_M + N_F}$$

$H_o$ is the observed heterozygosity and $N_M$, $N_F$ and $N_e$ are respectively the number of males, females and the effective population size.

## Model sensitivity:

MetaConnect is a highly flexible and modular IBM, which means that dozens of variables can be specified in various modelling contexts, rendering complete sensitivity analyses impossible to run (Cross and Beissinger 2001; Naujokaitis-Lewis et al. 2009; Pe'er et al. 2013). A thematic sensitivity analysis will be presented in the MetaConnect user manual, in which sensitivity of extinction probabilities, genetic structure and connectivity metrics will be analyzed in relation to the appropriate model parameters and their relative contribution to the sensitivity estimate (Cross and Beissinger 2001).

### Case study: Changes in the metapopulation functioning of an existing natterjack toad population under scenarios of economic development.

#### MetaConnect parameterization

We used a model designed with MetaConnect to determine the potential impact of the development of an industrial area and a terrestrial transport infrastructure (high-speed railway), both alone and together, on the population viability of *Bufo calamita* populations in south-western France close to Agen (44°11’36”N, 0°31’14”E) (figure 5).

Habitat patches were determined *a priori* based on expert assessment (figure 5). Preferences for landscape elements were derived from those experimentally determined by Stevens et al. (2006b). *B. calamita* was assumed to disperse following the stochastic-movement-simulator (SMS) assumptions (Palmer et al. 2011). The rational for this choice was that, even though Stevens et al. (2006) found that least-cost-path assumption was consistent with the population genetic structure of *E. calamita* in their study site, Coulon et al. (in prep) recently substantially improved the fit between simulated dispersal of *E. calamita* and the observed population genetic structure using the SMS model. The model derived from MetaConnect was parameterized with published values of life history traits (Stevens and Baguette 2008) (Appendix A).

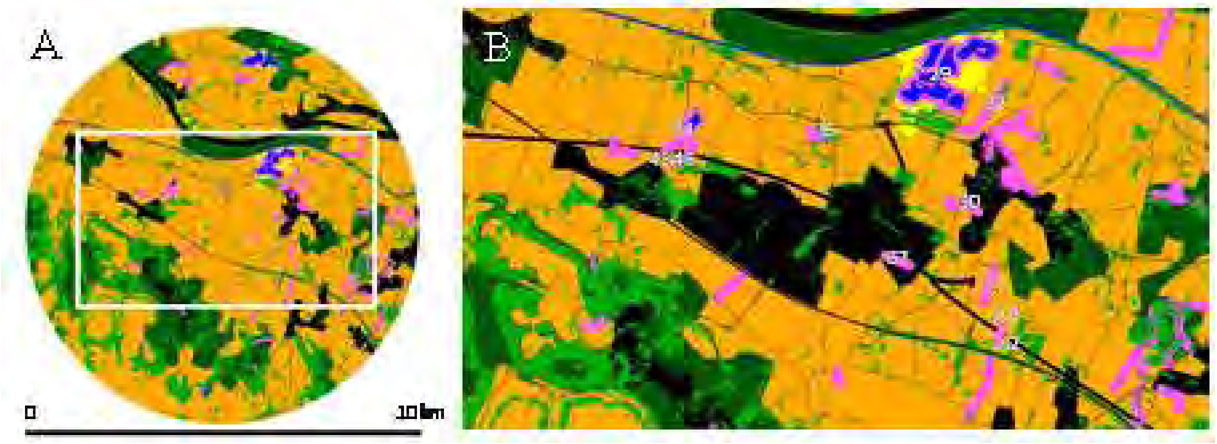

Figure 5: Site of industrial and transport development in south western France close to Agen. The A panel presents the complete study site used for MetaConnect simulations, rasterized to a grid of 10m × 10m cells. The B panel shows enlarged the future development of an industrial area and of a high-speed railway. Potential habitat patches are in pink and the other colors correspond to the land cover and its associated habitat preference values derived from Stevens et al. (2004) and used in MetaConnect (SMS part) to perform dispersal simulations. Our analysis is focused on the patches for which the number is indicated in white. The patch 67 will be destroyed by the industrial area development. In addition, currently patches 45, 46 and 112 do not currently exist, but will appear with the building of the high-speed railway as a result of the fragmentation of respectively patches 34 (split into 34, 45 and 46) and 103 (split into 103 and 112).

We analyzed MetaConnect genetic outputs for all the patches containing more than 10 individuals at the end of each MetaConnect run. This analysis was performed using STRUCURE (Pritchard et al. 2000) with an admixture model assuming that the origin population of an individual is known and the allele frequencies are independent. STRUCTURE runs were performed for a variable number of clusters between 1 and 7, and with 10 iterations of 100000 steps (50000 burning steps and 50000 analysis steps to ensure model convergence). This procedure was reproduced for each iteration of the *B. calamita* simulation of a given scenario (10 times). We determined the best number of genetic clusters following the Evanno method (see Evanno et al. 2005) using STRUCTURE HARVESTER (Earl and Vonholdt 2012). For each STRUCTURE analysis iteration of the best number of cluster of each simulation of *E. calamita* metapopulation functioning, we counted the assignation of a patch to a cluster and test the clustering robustness by performing a $\chi^2$ test per patch.

Within the study site, (10 km around the industrial area development), the *B. calamita* population is not threatened (extinction probability $p_e = 0$) and we observed two main dispersal corridors. The major corridor joins the north (quarry, patch 29) to the south (motorway, patch 103) and is stopped by the motorway in its southern part and by the Garonne river at its northern part. The minor corridor joins the quarry (east) to a pond (patch 34, west) (figure 6.A). Our focal area covers these two corridors (figure 5). Both corridors are constituted by stepping stone sites (patches 50 and 67 for the north-south corridor and 36 for the east-west one) (figure 7.A). Within the focal area all the patches have a very low extinction probability (all close to 0, see figure 6), and except for patch 103, all patches are able to exchange individuals with all the others. Indeed, only patch 103 is unable to provide individuals to patches 34, 36, 29 and 32 or to receive individuals from patches 34 and 36.

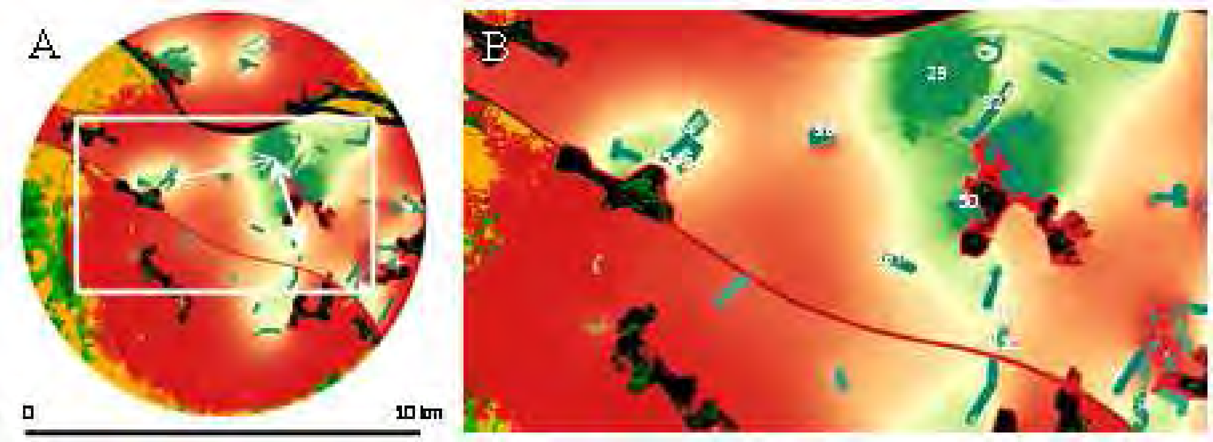

Figure 6: MetaConnect output layers of patch extinction probabilities (from 1 brown to 0 green) and mean cell frequentation during efficient dispersal events (from 1 individual each 100 years in red to more than 10 a year in green). At the global scale, dispersing individuals follow 2 main corridors (white arrows in panel A): a major one along the north-south axis and a minor one along the east-west axis (see text).

The analysis of the genetic output suggests that currently the study site is divided into three separate clusters (all p.values<0.05). The first one is situated on the north of the Garonne, the second between the Garonne and the motorway and the third on the south of the motorway underlying the structuring role of the river and the motorway on the population genetic of *E. calamita* (figure 7.A).

### Expected consequences of the industrial area development and of the high-speed railway building

The industrial area development and the associated destruction of patch 67 leads to a reduction of individual flow between patches to the north of the industrial area and patches to the south in addition to a population size reduction within the southern patches (figure 8.B). Such a modification would not threaten *E. calamita* persistence in the study site (all $p_e = 0$, figure 8.D). However, dispersal would be more concentrated between patches 29, 32 and 50 due to the repellent effect of the industrial area for *B. calamita* (figure 8.D). In addition, the reduction of dispersal along the north-south axis should lead to a fourth genetic cluster in the western part of the study sites (Figure 7.B).

The high-speed railway project will fragment the study site landscape. This would lead to the reduction of patches 34 and 103 areas (since none of the newly created patches 45, 46 nor 112 could shelter a population (Respectively $p_e = 0.71, 0.71, 0.61$, figure 8.E)) inducing the reduction of their sheltered population sizes (figure 8.C). In addition, dispersal along the north-south corridor is reduced to the south of the patch 50 (figure 8.C). Moreover, the connectivity along the north-south corridor is also decreased due to the population size reduction of patches 71 and 103. The reduction of the patch population size and the change in the connectivity along the north-south axis change the clustering outcome of these last simulations. Indeed, if the Evanno method suggests that 2 clusters can be identified, the $\chi^2$ test shows that the assignation to a second cluster does not differ significantly from a random assignation, suggesting that only a single cluster exists or that the cluster 1 (north of the Garonne) is poorly differentiated from cluster 2 between the Garonne and the high-speed railway (Figure 7.C).

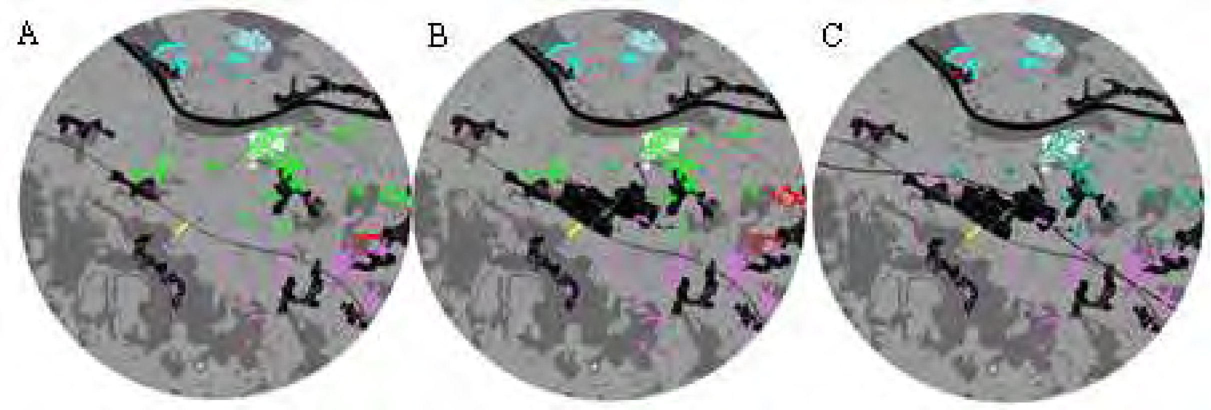

Figure 7: Genetic clustering of the suitable patches for *E. calamita*. Pink patches correspond to patches containing fewer than 10 individuals by the end of the MetaConnect simulation, which were removed from the STRUCTURE analysis (Pritchard et al. 2000). The development of the industrial area (B) does not change the genetic structure of the *E. calamita* population from the current situation (A). 4 clusters are identified (cyan, light green, red and yellow clusters of patches) and separated by the Garonne (isolating the cyan and light green clusters), a forest (barrier between the light green and the red cluster) and the motorway (separating the light green cluster from the yellow). The addition of the high-speed railway (C) would modify the genetic exchanges and two clusters would be kept (cyan and yellow). The dark green patches are non-significantly aggregated to the yellow or the cyan cluster (all p.values NS) due to the reduction of the number of individual per patches and the change in the inter-patches genetic exchanges (see text).

Although the development of the industrial area and the building of the high-speed railway do not directly threaten the *E. calamita* survival in the study site (figure 8.F), it will restrain the species' displacement capabilities and reduce the global population size. In addition, figure 8.F highlights the central role of patch 50 as a stepping-stone patch, which allows the maintenance of connectivity between the north (patches 29 and 32) and the south (patches 71 and 103). Such a map constitutes a powerful decision-making tool in the new ecological context of compensatory measures which will be taken in the context of large landscape planning (Lanius et al. 2013; Regnery et al. 2013).

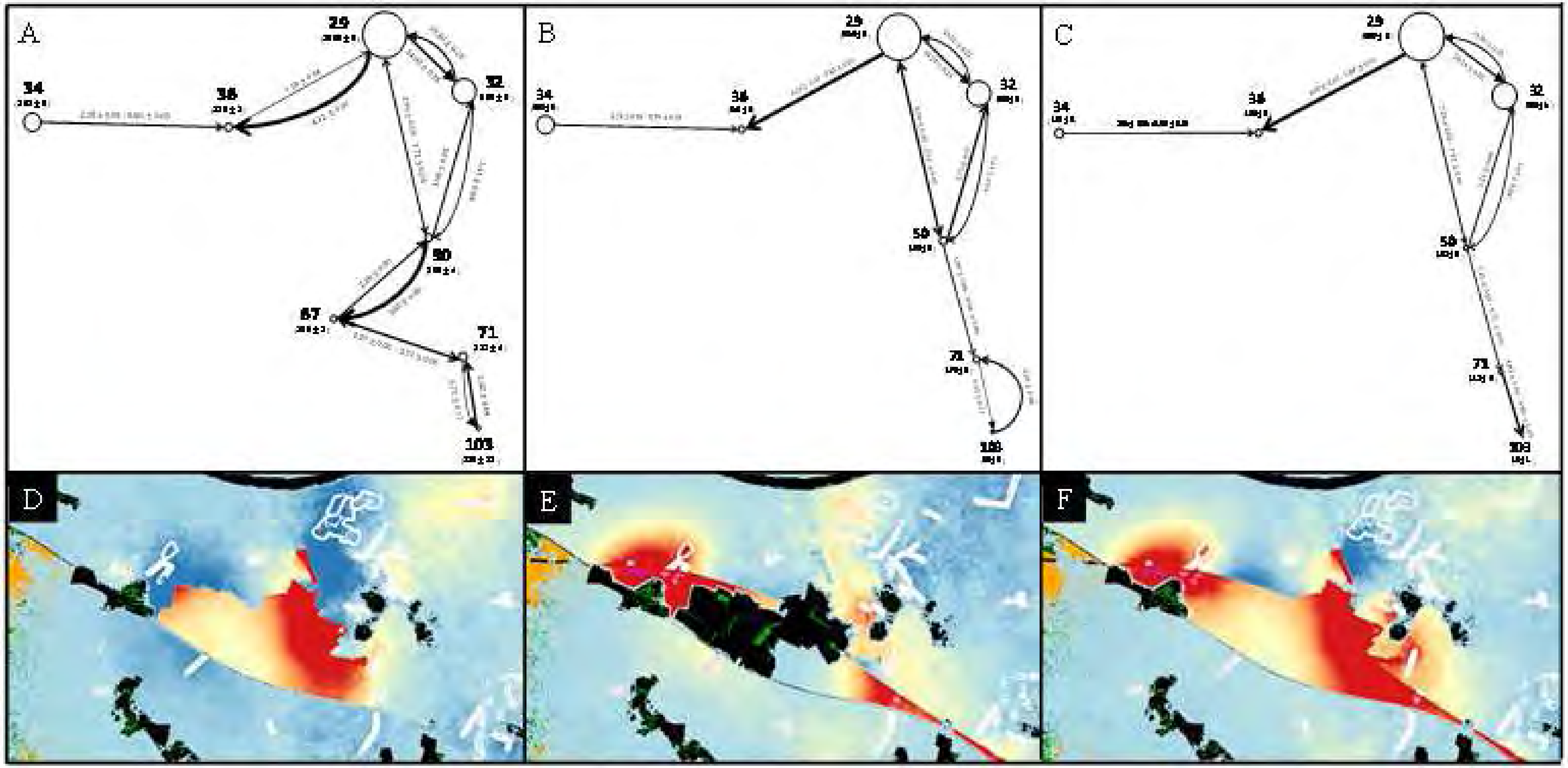
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Figure 8: Graphical representation of the focal area metapopulation functioning derived from MetaConnect outputs for the current situation (A) with the industrial area (B) and with both the industrial area and the high-speed railway (C). Node sizes are proportional to patch population sizes (mean ± SE). Arrows represent dispersal intensity (mean ± SE) and direction (bidirectional arrows values correspond to smaller patch number to larger patch number flow: larger patch number to smaller). For clarity, only flows superior to 1 individual a year have been represented and flows superior to 5 individuals a year have their width reduced by 10 and symbolized by a plain arrow. Maps represent the variations of extinction probabilities per patch where variation = patch extinction probability in scenario B – patch extinction probability in scenario A (from brown for an extinction probability variation of -1 to purple for a variation of 1 where white is used if no differences are recorded) and of cell occupancy during efficient dispersal (from red for a loss superior to 3 individuals a year to dark blue for a gain of 1 where a constant number of individual per year corresponds to light blue). The D scenario corresponds to the metapopulation functioning variations after the development of the industrial area. The E scenario presents the variation between the situations after the industrial area development and after the high-speed railway building. Finally, the F scenario summarizes the differences from the current metapopulation functioning to the expected functioning after the cumulative development of the industrial area and the high-speed railway.

## Model limitations:

By its structure, MetaConnect allows the user to take into account most of the requirements necessary for a complete and flexible PVA and decision-making tool (i.e. metapopulation dynamics and genetics) (Grimm et al. 2004; Baguette et al. 2013a; Pe'er et al. 2013).

Currently, its main limitations come from the landscape representation. In MetaConnect the landscape is represented using the patch-matrix approach, in which a pixel is a suitable habitat or not. This approach will not be fully unrealistic for many species (Clobert et al. 2001a; Urban et al. 2009; Pe'er et al. 2011). Furthermore, reproduction in the suitable habitat is assumed to be panmictic which is usually not true because (1) patch shape and structure isolate or aggregate individuals within a patch and (2) individual behavior (territoriality, mating system, cooperation,…) may aggregate or isolate individuals within a patch (Doebeli and Koella 1994; Clobert et al. 2001a; Sinervo and Clobert 2003; Snyder and Chesson 2003; Ylonen et al. 2003; Nonacs and Kapheim 2007). Further development of MetaConnect toolboxes would allow tackling this limitation of intra-patch spatial structuring by splitting individual use of space into daily movements and dispersal events (Mueller and Fagan 2008; Roshier et al. 2008; Pe'er et al. 2013).

A second limitation is the way genetic mutation is modeled. Currently, the mutation model is very simple, just assuming that a new allele can occur at a given constant rate and that no reverse mutations are possible. Further toolbox development would allow various methods for modelling mutations to be incorporated (Willi et al. 2006; Neher 2013; Wray 2013) and in addition would permit simulation of the action of the genotypes on the individual phenotype (Montalvo et al. 1997; Baskett 2012; Mouquet et al. 2012; Chauvenet et al. 2013; Clobert et al. 2013; Moulherat et al. submitted-b).

## Conclusion: model application and perspectives

European regulation requires spatial planners to evaluate precisely the impacts of developments on ecological network functioning. Baguette et al. (2013) recommended a robust workflow in that direction. The procedure comprises performing an analysis of metapopulation dynamics and dispersal over a landscape for each affected species to design sound ecological network functioning. MetaConnect provides an important tool that moves in this direction. Indeed, the user can easily build consensus networks for several species within the same study site and under the same framework with standardized and comparable outputs. However, such an approach does not yet incorporate the inter-specific interactions that could greatly change population dynamics and dispersal (Caswell 2001; Clobert et al. 2013). The next generation of MetaConnect (MetaSystem) will integrate basic inter-specific interactions such as competition, predation and parasitism.

Application of graph-theoretic connectivity is increasing at an exponential rate in ecology and conservation (Kindlmann and Burel 2008; Kadoya 2009; Urban et al. 2009; Moilanen 2011). In this framework, graph nodes represent habitat patches and graph edges represent the connectivity between patches (Urban and Keitt 2001; Taylor et al. 2006; Kindlmann and Burel 2008; Urban et al. 2009; Foltete et al. 2012). Whilst the mathematical background of graph theory is promising to deal efficiently with the ecological connectivity (Urban and Keitt

2001), the simplification made by modelling a metapopulation with a graph (Urban et al. 2009; Moilanen 2011) has the potential to lead to limited interpretation and operational efficiency (Kadoya 2009; Moilanen 2011). As an example, Kadoya (2009) concluded that graph modelling of metapopulations provides little congruence with connectivity inferred from population genetic structure. Moilanen (2011) listed the limits of graph-theoric connectivity in spatial ecology. With MetaConnect, as shown in our case study (figure 8.A-C), we provide an efficient tool to define habitat patches (nodes of a graph) with predictions of the demographic module and dispersal functionality between patches (graph's edges) with dispersal assessed from dispersal simulation or derived from genetic estimates applicable from local to national scales and grid-based data. In such an application of graph-theoretic connectivity, the graph does not model the metapopulation functioning as such, but is used as a powerful analytical tool preventing the graph-theoretic connectivity analysis from biases described by Moilanen (2011). Bergerot et al. (2013) obtain relevant assessments of landscape functional connectivity for a butterfly with a similar framework.

We conclude by highlighting the recent call for a new generation of models that begin to provide predictive systems ecology (Evans et al. 2012; Evans et al. 2013). This call argued that while in a few sub-disciplines such as dynamic vegetation modelling and climate change modelling we have already developed a capacity for simulating complex systems, typically we lack such a predictive modelling capability elsewhere in ecological and evolutionary disciplines (Moorcroft et al. 2001; Evans et al. 2012; Hoban et al. 2012; Purves et al. 2013; Bocedi et al. 2014). The climate modelling community and the dynamic vegetation modelling communities both possess several models with which they can conduct inter-model comparisons, conduct cross-validations, etc (Evans et al. 2012; Pe'er et al. 2013). This is also now true of the population genetics community, which has several software packages available (reviewed in Hoban et al. 2012). We believe that MetaConnect, together with the recently published software, RangeShifter (Bocedi et al. 2014), begin to address this challenge to develop complex systems models that can be used to inform policy in the sphere of spatial and landscape ecology. Our hope is that further modelling platforms will be developed that have similar philosophy and that over the next few years a strong community may grow involving multiple models and a broad range of user communities and stakeholders.

## Where to find MetaConnect

MetaConnect (IDDN.FR.001.430001.000.S.C.2012.000.20600) is developed in C++ by SM and MM and runs exclusively in cloud-computing under UNIX. A Graphical User Interface (GUI) developed in OpenGL insures the access to MetaConnect from multiple platforms (currently available for windows and Linux centos, debian and ubuntu). MetaConnect's GUI is available for academic purposes through the ANAEE service modelling platform (www.ecoex-moulis.cnrs.fr) and the TerrOïko website (www.terroiko.fr) and by contacting the authors (SM or JC).

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Appendix A: MetaConnect parameterization for its validation and for the *B. calamita* case study.

Table I: Species life history traits values used to validate MetaConnect and run the *E. calamita* case study

| **Parameters and variables** | **Passerine (Legendre et al., 1999)** | **Tricholimnas sylvestris (Brook et al., 1997)** | **Passerine like species for genetic pattern comparison with Landguth and Cushman (2010) results** | ***B. calamita* case study** |
|---|---|---|---|---|
| ***Demographic characteristics*** | | | | |
| $K$ | 1000-500-250 | 350-220 | 500 | 8 ind.ha-1 (Trochet et al., submitted) |
| $s_2$ | 0.5 | 0.75 | 0.5 | Male: 0.535; Female: 0.735 (Trochet et al., submitted) |
| $s_1$ | 0.35 | 0.35 | 0.3 | 0.22 (Trochet et al., submitted) |
| $s_3$ | | | | 0.269 (Trochet et al., submitted) |
| $s_0$ | 0.2 | 1 | 0.2 | 0.007 |
| $F$ | 7 | 2.5 | 14 | 3000 (Arnold and Ovenden, 2004; Stevens et al., 2003) |
| $\sigma$ | 0.5 | 0.5 | 0.5 | 0.5 (Beebee, 2011) |
| ***Mating system*** | | | | |
| Mating system | Monogamous/ Polgynous | | | |
| $H_m$ | 8 | Monogamous | Monogamous | Monogamous |
| $H_f$ | 0 | | | |
| ***Genetics*** | | | | |
| $L$ | 10 | - | 1 | 10 |
| $A$ | 10 | - | 10 | 10 |
| $\mu$ | 0.00001 | - | 0.00001 | 0.00001 |
| ***Dispersal*** | | | | |
| $p_d$ | - | - | 0-0.2 | 0.05 (Arnold and Ovenden, 2004) |
| Dispersal rule | - | - | Random Walk | SMS (Coulon et al., Submitted) |
| ***Initialization and model parameterization*** | | | | |
| $N_0$ | 0 - 500 | 100 | 500 | 5000 |
| $\sigma_0$ | 0.5 | 0.5 | 0.5 | 0.5 |
| $T$ | 100 | 10 | 500 | 100 |
| $MC_l$ | 1 | 1 | 10 | 1 |
| $MC_d$ | 1000 | 500 | 100 | 10 |

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# 2. What genetic mechanisms underlying mating strategy expression maintain trimorphism in a rock-paper-scissors game?

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*Résumé en français*

Bien que la sélection naturelle ait tendance à réduire la variabilité, le polymorphisme est commun dans le milieu naturel même lorsque les traits polymorphiques sont sous forte pression de sélection. Les polymorphismes discrets de stratégie de reproduction sont courants et offrent une bonne opportunité de comprendre les processus génétiques qui permettent le maintien du polymorphisme dans des contextes relativement simples. Dans cette étude, nous avons exploré les capacités de différents processus génétiques sous tendant l'expression des stratégies de reproduction discrètes du jeu pierre-feuille-ciseaux (RPS). Les hétérozygotes supportent l'information pour des stratégies différentes cependant, jusqu'à présent, peu d'études se sont intéressées aux mécanismes d'expression phénotypique des hétérozygotes dans le cadre des stratégies alternatives de reproduction. Nous avons exploré la possibilité de maintien du polymorphisme sous les hypothèses de 1) dominance génétique ou 2) plasticité phénotypique comme mécanismes dirigeant l'expression phénotypiques des stratégies de reproductions alternatives chez les mâles et les femelles. Nous avons construit un model individu centré stochastique et analysé les résultats de simulations à l'aide d'analyse de séries temporelles. Nous résultats montrent que les deux mécanismes sont susceptibles de maintenir le polymorphisme mais que les conditions de maintien sont liées aux stratégies démographiques des espèces. Si les deux sexes présentent des stratégies de reproductions alternatives, les possibilités de maintien sont différentes en fonction du mécanisme considéré. L'analyse de série temporelle montre que le jeu RPS est principalement dirigé par la stratégie

polygyne et que sa prépondérance dans le jeu a de multiples conséquences sur les possibilités de maintien du polymorphisme en fonction du mécanisme d'expression phénotypique chez les hétérozygotes.

Abstract:

Although natural selection is expected to reduce variability, polymorphism is common in nature even when traits are under strong selection. Discrete polymorphism of mating strategies are widespread and offer a good opportunity to understand the genetic processes that allow the maintenance of polymorphism in relatively simple systems. In this study we aim at exploring the genetic mechanism underling the expression of discrete mating strategies in the rock-paper-scissors (RPS) game. Heterozygotes carry the information for different strategies, yet little attention has been devoted to the mechanisms underlying heterozygote phenotype. We explored the maintenance of polymorphism under 1) genetic dominance or 2) plasticity as mechanisms driving the expression of alternative strategies in males and females. We built a stochastic individual-based-model and analyze the output using time series analyses. Our results show that both genetic mechanisms can sustain polymorphism but that this depends on demography. Coupling male and female alternative strategies has a different effect on the maintenance of polymorphism for the two genetic mechanisms. The time series analysis shows that the RPS game is mostly driven by the polygynous strategy and that this disequilibrium has different impacts for the maintenance of polymorphism according to the genetic mechanism underlying reproductive strategy expression in heterozygotes.

## Introduction:

Evolutionary mechanisms such as natural selection and genetic drift are expected to reduce genetic variation within populations, yet polymorphism is surprisingly widespread in animal and plant populations. To explain this paradox, a number of mechanisms have been proposed, such as heterosis (Cook 2007), niche selection (Snyder and Chesson 2003; Bach and Kelly 2007) or negative frequency dependent selection (Gross 1996; Sinervo and Lively 1996; Punzalan et al. 2005; Bond 2007) which are all known to allow the stable maintenance of polymorphism in natural populations (Berry et al. 1991; Danchin et al. 2005). Discrete polymorphism such as trimorphism in mating strategies, which have been reported over the last two decades (reviewed in Sinervo and Calsbeek 2006) are particularly interesting in this respect since they offer the opportunity to study the maintenance of polymorphism in a very simple context. Theory suggests that trimorphism can be maintained by negative frequency dependent selection in a system as long as (1) each strategy is not an evolutionarily stable strategy (ESS) (Gross 1996) and (2) strategy pay-offs form a rock-paper-scissors game (RPS game) with the other two strategies (Maynard-Smith 1982; Gross 1996; Sinervo and Lively 1996). In the RPS game, each strategy beats another one but is beaten by the third one leading to an equilibrium of strategy frequencies. Theoretically, such a game can lead to a stable equilibrium of strategy frequencies or oscillations over time (Maynard-Smith 1982; Sinervo and Lively 1996).

The first empirical observation of a RPS game in the field was in the side blotched lizard *Uta stansburiana* (Sinervo and Lively 1996). In this species, males show three alternative reproductive strategies associated with trimorphism in throat-color. The orange males ('O') are aggressive with high stamina (Sinervo et al. 2000*a*), large harem size (Zamudio and Sinervo 2000) and home range (Calsbeek and Sinervo 2002). Orange males are able to steal females from blue males ('B'). Yellow males ('Y') are female mimics and are very successful in invading and reproducing in the harems of orange males (Sinervo and Lively 1996; Zamudio and Sinervo 2000). Blue males are mate guarders (Sinervo and Lively 1996), and more monogamous than the other two males types (Zamudio and Sinervo 2000) with small home ranges (Sinervo 2001*b*) and cooperative behavior among neighboring blue males (Sinervo et al. 2006). The mate guarding behavior allows them to prevent cuckoldry from yellow sneaker males. In the side blotched lizard, the strategies are heriTable based on laboratory crosses (Sinervo et al. 2001), field pedigree (Sinervo and Zamudio 2001) and gene mapping (Sinervo et al. 2006). Each strategy is thought to be driven by a single allele ('*o*', '*b*', '*y*') (Sinervo and Lively 1996; Sinervo 2001a; Sinervo et al. 2001; Kerr et al. 2002). Since 1996, the RPS game has been described in various taxa such as bacteria, lizards, damselflies, isopods and plants (Kerr et al. 2002; Sinervo and Calsbeek 2006).

The RPS game has been extensively modeled assuming clonal reproduction and pure phenotypic strategies (Maynard-Smith 1982; Sinervo and Lively 1996; Sinervo 2001a; Kerr et al. 2002) as well as diploid models (Sinervo 2001a; Svensson et al. 2005). Although theoretical models show long term maintenance of the RPS game, some discrepancies, such as strategy frequencies at equilibrium or strategy frequency oscillatory dynamics, remain between theoretical model predictions and field observations (Sinervo and Lively 1996; Sinervo 2001a; Kerr et al. 2002; Sinervo and Calsbeek 2006). For instance, while evidence from the field suggests a pattern of cycles of strategy frequencies with no attenuation (Sinervo and Calsbeek 2006), models predict the stability of strategy frequencies as cycles attenuate over time (Maynard-Smith 1982; Sinervo and Lively 1996; Sinervo 2001a). Differences between theoretical and empirical observations may have various origins. First, previous models of the RPS game rarely integrate demographic stochasticity (Sinervo and Lively 1996; Sinervo 2001a; Svensson et al. 2005); however, see (Calsbeek et al. 2002). But demographic stochasticity occurs in most biological systems and has an important impact on population dynamics (Legendre et al. 1999; Caswell 2001) and game stability (Maynard-Smith 1982; Caswell 2001). Secondly, interactions between the sexes are also known to influence game stability and population dynamics(Maynard-Smith 1982; Lindstrom and Kokko 1998; Alonzo and Sinervo 2001; Svensson et al. 2005; Alonzo and Sinervo 2007). In some lizards, including *Uta* females also express alternative reproductive strategies (Sinervo et al. 2000b; Sinervo et al. 2007). Female color types express an r/K-strategy set expressed as a trade-off between clutch size that is inversely correlated with offspring size and survival (Sinervo et al. 1992; Sinervo et al. 2000b; Svensson et al. 2005; Vercken et al. 2010).

Only a few studies have attempted to explore the genetic mechanisms underlying mating strategy games, usually assessing that a dominance relationship exists between the three alleles (Sinervo and Lively 1996; Sinervo 2001a; Svensson et al. 2005; Sinervo et al. 2008). Although there are some arguments for dominance (Cordero 1990; Sinervo et al. 2001; Svensson et al. 2005), field evidence such as specific morph expression in heterozygote (Sinervo and Lively 1996; Sinervo et al. 2007) or switches in individual reproductive strategies (Sinervo 2001a) suggest that a plastic mechanism may underlie the RPS game. Maynard-Smith (1982) showed that phenotypic plasticity could generate infinite cycles of strategy frequencies. Other than this work by Maynard-Smith (1982), very little theoretical attention has been devoted to phenotypic plasticity as a mechanism underlying expression of reproductive strategies in an RPS game (Sinervo 2001a; Sinervo and Calsbeek 2006), despite empirical evidence that suggests an endocrine basis to plasticity in male strategies (Sinervo et al. 2000a; Mills et al. 2008). Field evidence also suggests social-context dependant phenotypic plasticity could underlie the RPS game (Sinervo and Lively 1996; Sinervo 2001b; Sinervo et al. 2001; Svensson et al. 2005; Sinervo and Calsbeek 2006). Heterozygotes carry genetic information for at least two strategies and thus would be the most likely candidates for expressing mating strategy plasticity. For both sexes, plasticity could be advantageous if the alleles coding the different strategies have no interactions or are synergetic (Sinervo 2001a). However, this information can be antagonistic and reduce a heterozygote's performance compared to homozygotes. For example, an individual with a territorial polygyny allele and a non-territorial sneaker allele could not express both strategies. Such a cost could impact the stability of the RPS game (Maynard-Smith 1982). Consequently, heterozygosity and social environment should be taken into account to describe and model the RPS game.

The first goal of this study is to explore how different genetic mechanisms leading to the expression of alternative reproductive strategies and demographic stochasticity could influence the maintenance of trimorphism in RPS games. In this regard, we performed population dynamic simulations of a virtual species playing a generic RPS game using an individual-based-model. The individual-based-model allows us to simulate effects of individual genetic information (i.e. are individuals homozygotes or heterozygotes for the gene coding reproductive strategy?), social environment and demographic stochasticity on individual expression of reproductive strategies. In this framework, we explored the maintenance of polymorphism under two hypotheses on the mechanisms leading to individual phenotype:

- Genetic dominance: we assessed genetic dominance of allele *o* on *y* and *y* on *b* (*o*>*y*>*b*) (Sinervo 2001a; Svensson et al. 2005; Sinervo et al. 2007). Past diploid models explicitly ignored over-dominance and only considered co-dominance to dominance (Sinervo 2001a).

- Plasticity: heterozygotes are plastic and able to apply the best strategy given their alleles for the current social context thereby providing an advantage to heterozygotes (Sinervo 2001*a*; Sinervo 2001*b*).

The second goal is to explore the impact on the maintenance of polymorphism of the genetic structure and life history traits under each genetic hypothesis, given different assumptions about the strategies: (1) alternative reproductive strategies in either one or both sexes and (2) costs linked to heterozygosity. Finally we tested our general model on a case study using parameters from *Uta stansburiana* to determine which processes most likely explain the maintenance of trimorphism in this species and predict strategy frequencies emerging from an RPS game.

## Models and their analysis:

The goal of our model is to evaluate the ability of different genetic mechanisms underlying an RPS game to maintain alternative mating. The model evaluates the extinction probability of alleles associated with reproductive strategies under chosen sets of life history strategies. Even though the RPS game occurs in various ways (Sinervo and Calsbeek 2006), we have chosen to implement the RPS game as male alternative strategies as described in *Uta stansburiana* since it is the best documented case study of a RPS game.

### *Model structure*

The model is based on a simple demographic model of two sexes and two age classes coupled with a genetic model assuming a Mendelian transmission of genes supporting the expression of alternative reproductive strategies.

### Demography

Our model is a single population individual based model with two age classes (juvenile and adult) and two sexes as is the case with many species that play a RPS game

(Sinervo and Calsbeek 2006). We assumed reproductive competition between individuals to be a scramble (equation 1) and that our population ($N$) is growing at an asymptotic rate $\lambda$~1.1 (see Table 1). The competition coefficient $k$ was fitted (Table 1) to correspond to populations with carrying capacities ($K$) of 500, 1000 and 2000 individuals. Competition is assumed to be the same both within and between age classes (following Sinervo and Lively 1996).

$$N_{(t+1)} = \lambda N_{(t)} e^{(-kN_{(t)})} \quad (1)$$

In our model, the demographic process is divided in four main phases (see online appendix C):

1. Juvenile recruitment: juveniles are recruited randomly into the adult population depending on population density. The non-recruited juveniles survive depending on the juvenile survival probability ($s_j$).
2. Mating: males and females are associated through male RPS game rules depending on the neighborhood composition and mating strategies.
3. Reproduction: new juveniles are produced according to the genotype of their parents
4. Adult death: adults survive according to the adult survival probability $s_a$.

During each phase demographic stochasticity is implemented for all individuals as a random process according to the appropriate distribution probability law underpinning each demographic parameter (see Table 1). For example, each female has her own fecundity ($F$), such that the realized fecundity is randomly chosen using a Poisson distribution with parameter $\bar{F}$.

## Individual genetic and phenotypic consequences on reproductive strategies

The genetic mechanism controlling reproductive strategies is implemented as an individual characteristic acquired by transmission of genes from parents to offspring. The number of loci involved in the different strategies has rarely been clearly determined and different genetic structures have been proposed (Maynard-Smith 1982; Sinervo 2001a). The simplest one assumes that a single locus with three alleles drives the strategies (Maynard-Smith 1982; Sinervo and Lively 1996; Svensson et al. 2005; Sinervo et al. 2006). Other structures such as two alleles on two loci cannot be definitely excluded (Sinervo et al. 2001). In our model we chose to implement the simplest genetic structure using a single locus with three alleles (*p* = polygynous, *m* = monogamous, *s* = sneaker) leading to six genotypes ('*pp*', '*mm*', '*ss*', '*pm*', '*ps*', '*ms*') since this structure is consistent with *U. stansburiana* inheritance patterns (Sinervo et al. 2001; Sinervo et al. 2006) and is the one described for the damselfly *Ceriagrion tenellum* after crossing experiments (Cordero 1990).

When juveniles are recruited into the adult population, their reproductive strategies are determined as Polygynous ('P'), Monogamous ('M') or Sneaker ('S') depending on the genetic rule underlying the social strategy:

- For models with allelic dominance ('*p*'>'*s*'>'*m*'), all genotypes containing a '*p*' ('*pp*', '*pm*', '*ps*') allele lead to a P strategy, the M strategy exists when individuals have '*mm*' genotypes and all other genotypes ('*ss*', '*ms*') lead to a S strategy.
- Under the plasticity hypothesis, heterozygotes have the possibility to apply both strategies provided for by their alleles. The strategy applied will depend in this case on the frequency of each strategy in the population. New adults will adopt the rarest strategy in the population given their alleles and carry that strategy for the rest of their life (i.e. irreversible developmental plasticity, discussed in Sinervo 2001a).

Male reproductive strategy phenotype and the RPS game

The mating system is largely driven by male social strategies as described in Sinervo and Lively (1996). Fitness depends on the capacity of any given male to monopolize a harem. A male's ability to win the RPS game against his neighbors is summarized in the dyadic pay-off matrix $w_0$ (Table 1). A male's reproductive success is thus altered by the spatial structure of neighboring genotypes which is closely linked to the average frequency of each phenotype. For example, if we consider a sneaker male playing against a polygynous one, the sneaker male will probably win. However, if a polygynous male is surrounded by monogamous males, the sneaker male will have a lower probability to copulate with the female of the polygynous harem due to the presence of blue males able to exclude the sneaker from the area (Sinervo 2001b). We used equation 2 (derived from Sinervo and Lively 1996), where $W_i$ is the relative average gain of the strategy $i$, $f$ the frequencies of strategies $i$ and $k$ and $w_1$ the relative dyadic payoff matrix, to estimate at each time step the relative average fitness of each strategy playing against the beaten strategy (i.e. P *vs* M, M *vs* S, S *vs* P).

$$W_i = f_i \sum_{1}^{k} f_k w_{1(i,k)} \quad (2)$$

The mating process is implemented in three steps. First, a male's neighborhood is determined. The neighborhood size is assumed to follow a Poisson distribution and neighbors are randomly allocated among other males regardless on their strategy. Then males of each strategy constitute their harem. Harem size also follows a Poisson distribution and females are allocated randomly among non-mated females. The constitution of harems begins with polygynous males and ends with sneakers. Next, the contests between male starts with S playing against P neighbors, then M playing against S neighbors and finally P playing against M neighbors. In this game phase, each pair of males plays the game where the winning strategy, has a probability of acquiring each female of the loosing male's harem according to a Bernouilli event with $p = 0.5 + W_i$ where $W_i$ is calculated using equation 2.

## Female reproductive investment as a gradient of r-K strategies

Female mating strategies might have some impact on the maintenance of male strategies. We implemented female alternative reproductive strategies as an r-K gradient (Sinervo 2001a; Sinervo et al. 2007). We assumed that the alleles supporting female strategies are the same as in males (i.e. Polygynous, monogamous and sneaker male strategies are coding by genes leading to the expression respectively of r, intermediate and K reproductive strategies in females) following the empirical example in *Uta stansburiana* (Sinervo 2001a; Sinervo et al. 2007). We also assume that the genetic mechanisms leading to female phenotypic strategies are the same as for males (allelic dominance or plasticity).

Offspring number and recruitment probability for intermediate females did not change from the model without the female game. However, the mean number of offspring ($F$) produced by K strategy females was 15% higher than for intermediate females, and 15% lower for r strategy females compared with intermediate females. Accordingly to an r-K gradient of strategies, the recruitment probability of offspring from r strategy females was reduced by a factor of 0.015 ($\Delta_s$) while offspring from an K strategy female had a recruitment probability increased by a factor $\Delta_s$.

### Cost of heterozygosity

Heterozygotes might be less efficient than homozygotes in applying their strategy to reproductive competition. Indeed, in the well-known example of human sickle-cell anemia, even though heterozygotes have better global fitness than homozygotes when malaria is present, their hemoglobin is less efficient than that of homozygotes (Fleming et al. 1979). Using a similar effect in our model, an aggressive homozygote polygynous male may be more aggressive than a heterozygote leading to a larger harem size than for the heterozygote individual. To determine the impact of this possible effect on the maintenance of trimorphism we implemented a cost (*c*) to heterozygotes in both male and female games. For males, we considered that a game between heterozygotes is equivalent to a game between pure strategists but that pure strategist had an advantage playing against heterozygotes which was implemented as an increased probability to win by a factor *c*. For heterozygotes, we assumed that the initial mean harem size was reduced by a factor *c* as was the probability of winning in the RPS game against a pure strategist. In the female game, heterozygotes had their differential fecundity from pure intermediate strategy reduced by a factor *c* and their offspring survival difference from intermediate female offspring was also reduced by a factor *c*.

### Case study, *Uta stansburiana*

The side blotched lizard has both a RPS game in males and an r-K game in females. We parameterized our model of the male and female games with *Uta*'s values of life history traits and pay-off matrices (see Table 1). In this specific case, nothing is known about a potential cost of heterozygosity so we perform runs with and without a cost.

### Runs

Under each genetic mechanism, we examined a range of possible life history trait values provided in Table 1. For each combination we considered several initial conditions of allele frequencies.

4. We aimed to determine if the genetic mechanism allowed maintenance of reproductive strategy trimorphism. Consequently, we assumed that all alleles were initially equally represented in the population.
5. We performed runs with only two alleles in the population to determine how long it took for each strategy to outcompete the other.
6. We simulated the "appearance" of a third allele (and therefore a third strategy) in a two allele population. Appearance of the new allele is implemented by exchanging 20 heterozygote individuals (carrying alleles for the two initial strategies) into 20 heterozygotes who carry the new allele and the allele coding the strategy that is currently losing. For example, in a population with only '*p*' and '*m*' alleles, we removed 20 '*pm*' individuals and replaced them by 20 '*ms*'.

Simulations were run 300 time steps which usually allowed enough time to reach stability of the population dynamics and genetic structure. All runs were repeated 200 times. In the specific case of time series analysis, simulations were run 2050 time steps.

### *Time series analysis*

To check the consistency of our population dynamic results with theoretical and empirical studies of the RPS game, we performed time series analysis. When trimorphism was not maintained after 2050 time steps, we performed the time series analysis on the maximum length available (minimum 1050 time steps). No time series analyses were performed for simulations exploring costs of plasticity for the heterozygote phenotypes due to the impossibility of maintaining a trimorphism during enough time steps. RPS games are likely to show cyclical dynamics due to the interactions between strategies (Sinervo and Calsbeek 2006). To identify and characterize the temporal structure emerging from the RPS game, we performed a wavelet analysis using "Morlet" wavelet (Soubies et al. 2005; Cazelles et al. 2008). Wavelet analysis detects when information in a general sense (Shannon 1948) is present and how this information "travels" across temporal scales (Meyer and Roques 1993; Mari et al. 1997; Cazelles et al. 2008). Wavelet analysis allows us to identify if a cyclical structure exists in the time series and to determine its periodicity. We then performed multi-resolution analysis to identify the time scale of patterns emerging from the RPS game (Box and Jenkins 1976). Multi-resolution analysis assumes that the global signal is the result of the addition of multiple signals at different temporal scales and thus separates the global signal into its different components (Jenkins and Watts 1968; Box and Jenkins 1976). Multi-resolution analysis allows us to analyze interactions between different time series signals and we used this analysis to identify interactions between time series emerging from the RPS game due to different strategies. Finally we used cross-correlational analysis (Platt and Denman 1975; Andreo et al. 2006; Cazelles et al. 2008) between strategies to identify the causal nature of changes in allelic frequency (see appendix A for more details on these statistics). In other words, did some strategies drive the cycles of others or are cycles due to some other effect.

Modeling and time series analyses were performed under the MATLAB® environment and time series analyses were performed using the Wavelab toolbox (Donoho et al. 1999).

## Results:

### *Stability of two strategy populations*

Simulations with populations initially composed by individuals presenting only two of the three alleles leads to the fixation of the strongest strategy in the RPS game (i.e. P wins against M, M wins against S, and S wins against P). However, the time necessary for allele extinction depends on the genetic mechanism underlying the strategies. Indeed, the monogamous allele disappears faster than the polygynous and sneaker alleles under the genetic dominance hypothesis. In contrast, this same allele disappears more slowly under the plasticity hypothesis (see online appendix D).

The female game seems to have no major influence on the time of allele extinction (see online appendix D.

Under the genetic dominance mechanism, a cost to heterozygote genotypes does not significantly change the probability of or time before extinction of alleles. In contrast, under the plasticity hypothesis, the cost of being a heterozygote strongly influences the extinction speed of the *p* allele when P plays against S. When both sexes have alternative reproductive strategies and heterozygosity is costly, patterns of allele extinction become very similar across alleles and the speed at which alleles disappear is slightly increased (see online appendix D).

Whilst all simulations lead to mono-strategic populations, the speed at which this occurs presents high variation among strategies and is closely linked to population carrying capacity and demographic traits (i.e. adult and juvenile survival). Indeed, regardless of the losing strategy the global pattern is the same for a given set of parameters for carrying capacity and fecundity. The time to monomorphism increases with carrying capacity and decreases with fecundity (see Figure D3 in online appendix D).

### *Maintenance of trimorphism*

The underlying genetic mechanism has a strong effect on the maintenance of polymorphism when only the male game is modeled. Even though, the two genetic scenarios we modeled can maintain polymorphism, alternative reproductive strategies are more easily maintained under the hypothesis of plasticity than under the genetic dominance hypothesis (maintenance probabilities equal to 1 and 0.4 respectively) (Figures 1, 2).

Coupling male and female games have different effects depending on the genetic mechanism underlying the reproductive strategies. The female game increases the ability of the three morphs to coexist under the genetic dominance hypothesis. In our simulations, when a male strategy is rare, it is also rare for females and this means that the allele for this strategy will be favored in both sexes at the same time, thereby reinforcing the fitness advantages arising from negative frequency dependence. In contrast, when plasticity underlies the strategies, the female game reduces the maintenance probability of trimorphism (figure 1). Time series analysis shows that the female game destabilizes the male game through an increase of the effect of environmental stochasticity (social interactions).

Not surprisingly, when heterozygosity has a cost, the maintenance probability of trimorphism decreases. This reduction is low regardless of the genetic mechanism underlying reproductive strategies when only males are playing (Figure 2). However, if both sexes present alternative reproductive strategies, the cost of heterozygosity drastically reduce the maintenance probability of trimorphism and does so regardless of the genetic mechanism underlying strategies (Figure 2). Under the dominance hypothesis, the reduced probability of maintaining the trimorphism when heterozygosity is costly seems to be explained by a change in average strategy frequencies. The polygynous strategy frequency is similar to other scenarios but the sneaking strategy frequencies fell from 0.4 to 0.3 thus increasing the risk of stochastic extinction. Under, the plasticity hypothesis, the amplitude of strategy frequency oscillations was increased, thereby increasing occurrences of extinctions due to stochastic events at low frequency phases of a rare allele.

The trimorphism is more easily preserved when individual lifespan and carrying capacity increase, as one would expected given a reduction of stochastic extinction events as these parameters increase (Figures 1, 2). The life cycle also influences the probability that the trimorphism is maintained. Long lived species have an enhanced chance to produce rare offspring genotypes providing them with an advantage for their own reproduction. Indeed, when offspring are long lived, they have a possibility to "wait" for their genotype to become rare and therefore to enjoy a high selective advantage, hence reducing the extinction probability of their alleles compared to short lived species.

### *Time series analysis of trimorphism frequency cycles*

Regardless of the specific model configuration, wavelet analyses and multi-resolution analyses show that strategy frequency time series are structured on different time scales. Time scales of the different processes described above increase with increasing lifespan. For short-lived species ($S_a = 0.2$ and $S_j = 0.2$), the interpretable emerging structures are situated between 4 and 32 time steps (Figure 3). The fastest structure (a 4 time step period) is generated by density-dependence of recruitment. Indeed, these structures are independent of the alternative strategy interactions (Figure 3) and appear in the analysis of the population dynamic (result not show). The structures emerging from the RPS game occur at time scales between 16 and 32 time steps corresponding to the maximum correlation between pairs of strategy frequencies (Figure 3). Moreover, strategies do not influence each of the other strategies at the same time scale. For example, the polygynous strategy dynamic influences the monogamous and the sneaker strategies at a 16 time step scale whereas monogamous and sneaker strategies influence each other and the polygynous strategy at a 32 time step scale (Figure 3). Wavelet power spectra of the strategy frequency dynamic show that cyclical structures are detected at the 16 and 32 time scales (Figure 3). The multi-resolution spliting confirms that these structures that make up the overall dynamic are cyclical (Figure 3) and that the relative frequency of strategies exhibit a consistent pattern with those expected for the RPS game (see appendix B for a detailed view of the pattern). A third category of structure exists at the 8 time step scale due to the link between the mating system and the density-dependence of the recruitment (Figure 3).

The cross-correlation functions (see appendix A) between frequencies of pairs of strategies show that the polygynous strategy has a negative effect on the monogamous strategy and a positive effect on sneakers. Surprisingly, the frequency of the monogamous strategy does not influence the sneaker strategy frequency suggesting that the game is largely driven by the polygynous strategy (Figure 4).

The gain functions (Box and Jenkins 1976) between the number of individuals applying a strategy and total population size show that cyclical structures emerging from the RPS game are attenuated by demographic stochasticity (all gains < 1). The attenuation is more important under the plasticity hypothesis suggesting that the global demography is less constrained by the RPS game demographic outcome when strategies are plastic.

Under the genetic dominance hypothesis, the addition of the female game sustains RPS cycles acting against the effect of demographic stochasticity and stabilizing cyclical structures. In contrast, the female game has a synergetic effect with demographic stochasticity when heterozygotes can adopt the best phenotype (are plastic) and contributes to attenuate RPS game cycles. The two previous results show that, even though polymorphism is easily maintained, oscillations of strategy frequency emerging from the RPS game are most likely to disappear under the plasticity hypothesis.

### *Invasion of Rare Strategies*

When rare, strategies differ in their probability of extinction. Indeed, under the genetic dominance hypothesis, S and M are unable to persist when rare regardless of the presence of the female game, heterozygosity costs, carrying capacity or lifespan. In contrast, when the *p* allele is initially rare, the P strategy can invade and the probability of maintenance of the trimorphism converges to the same pattern as if all alleles would have been equally represented initially.

Under the plasticity hypothesis, both M and P strategies are able to reach high enough frequencies to allow for the maintenance of trimorphism while S cannot be maintained when initially rare. As under the genetic dominance mechanism, the pattern observed when *p* or *m* alleles are initially rare are similar to the pattern observed when the three alleles are equally represented. In this case, trimorphism maintenance probabilities are lower than in the case of equal frequency of all alleles (respectively reduced by 0.1 ± 0.01 and 0.3 ± 0.02) due to the enhancement of stochastic extinction of alleles.

The female game has a stabilizing effect under the dominance hypothesis, and increases the probability that trimorphism is maintained when *p* is initially rare but does not allow any other allele to invade into a two strategy populations. In contrast, under the plasticity hypothesis, the female game reduces the probability that trimorphism is maintained when *p* or *m* are initially rare. The cost of plasticity reduces even more the ability of a rare allele to establish itself, but only when male and female games are considered.

### *A case study:* Uta stansburiana

Because empirical pay-off matrices differ from the generic RPS game, we also tested the whole set of scenarios on the specific case of *Uta stansburiana*. When alleles are initially at the same frequency, the dominance hypothesis leads to monomorphic populations for $K<2000$. Trimorphism is easily maintained under the allelic dominance hypothesis when $K\geq2000$ or under the plasticity hypothesis. Under the plasticity hypothesis, the trimorphism is easily maintained even in small populations (Figure 5).

Cross-correlation function analyses show that the RPS game generates cyclical structures with periods of 24 time steps for the male game under the dominance hypothesis and 51 time steps when considering both the male and female games under the plasticity hypothesis.

As in the general case, the female game has a stabilizing effect on cyclical structures emerging from the RPS game under the dominance hypothesis and a destabilizing one under the plasticity hypothesis. The result is an increase of the probability of maintenance of trimorphism under the dominance mechanism and a reduction of this probability under the plasticity hypothesis.

The cost of heterozygosity has little effect on the allele extinction probability when considering only the male game but increases this probability when the female game is added suggesting that in the wild the cost of heterozygosity may be low or non-existent as the trimorphism seems to be stable.

When a given allele is initially rare, no allele is able to invade the population when allelic dominance underlies the strategies. In contrast, *p* and *m* alleles but not *s* can invade when rare under the plasticity mechanism thereby leading to the maintenance of trimorphism. This suggests that the evolution of this trimorphism could only have occurred under the plasticity hypothesis in very large population sizes, or if the payoff matrix has evolved.

## Discussion

### *Genetic mechanism underlying frequency dependant selection and demographic stochasticity in trimorphic populations*

As in previous studies (Sinervo and Calsbeek 2006; Bond 2007; Dijkstra et al. 2008), we found that discrete polymorphism can be maintained by negative frequency dependent selection (Maynard-Smith 1982). However, our results suggest that the underlying genetic control of the mating strategy phenotype strongly affects the probability that polymorphism is maintained. Previous genetic models studying the properties of the RPS game assumed that a dominance relationship existed in alleles coding for the three strategies (Gross 1996; Svensson et al. 2005; Sinervo and Calsbeek 2006). However, even though trimorphism can be maintained under the allelic dominance hypothesis, the conditions for its maintenance are restrictive when compared to the plasticity hypothesis. Indeed, inclusion of demographic stochasticity under the genetic dominance hypothesis will likely lead to a loss of one of the three alleles underlying the RPS game, especially when compared to the plasticity hypothesis. This would be especially important since evolution of a third strategy will most likely require some length of time where the two other strategies are sTable. From our results, we should expect that the trimorphism will be maintained by dominance and negative frequency dependent selection only in large populations or in long-lived species with high dispersal rates, where these trimorphisms are apparently rare (Sinervo and Calsbeek 2006) or in small populations of a metapopulation as suggested by Corl et al. (Corl et al.).

In contrast, when heterozygotes are able to adopt the best strategy with respect to their social environment, polymorphism can be maintained regardless of the population size or the species life cycle. The plasticity hypothesis (or an advantage to the heterozygote) seems to allow the maintenance of polymorphism for a wider range of species life strategies and environments; and accordingly we might expect the plasticity mechanism to be more widespread in trimorphic species and by extension in simpler mating systems such as r-K gradient of strategies (Vercken et al. 2010). In this respect, previous empirical as well as theoretical studies have shown that the advantage to the heterozygote often underpins the maintenance of polymorphism of reproductive strategies in natural populations (Fleming et al. 1979; Sinervo and Zamudio 2001; Sinervo 2001a; Cook 2007; Vercken 2007; Vercken et al. 2010).

### *Time series analysis and cyclical system functioning*

Theory says that in the RPS game, all strategies must have equivalent global fitness over long time scales to constitute an ESS (Maynard-Smith 1982; Gross 1996). This would imply that the maintenance of the cyclical structures generated by the RPS game may be equally sensitive to frequency variation of the three strategies. Our time series analyses have shown that, in the general case, the genetic dynamic emerging from the RPS game is driven by a single strategy the polygynous strategy. This suggests that even though all the strategies have equivalent global fitness, the system will be more sensitive to perturbation of the polygynous strategy and that trimorphism is closely linked to the frequency dynamic of the polygynous strategy. While most empirical systems do not have a sufficiently long time series to investigate the influence of each strategy on population frequency dynamics, the time series analysis techniques used here suggest that social game payoff structure may not reflect the actual importance of each social strategy when demographic stochasticity is present.

### *Two sex model and stability of the RPS game*

Literature about two sex models predicts that sexual reproduction can have various impacts on population dynamics (Doebeli and de Jong 1999) sometime stabilizing the system and reducing extinction probabilities (Doebeli and Koella 1994; Ruxton 1995; Doebeli and de Jong 1999) and sometime destabilizing the dynamic and increasing the extinction probability (Lindstrom and Kokko 1998; Doebeli and de Jong 1999; Legendre et al. 1999). In our case, the addition of a female game had a mixed effect depending on the genetic mechanism underpinning the male strategies. In the case of allelic dominance, the female game increased the trimorphism maintenance probability whereas maintenance decreased under the plasticity hypothesis. The genetic dominance hypothesis in our model is equivalent to an asexual model without phenotypic variability. Doebli and Koella (1994) and Ruxton (1995) have shown that in this case, two sex models reduce the propensity for chaotic behavior of global population dynamic favoring therefore in our case the maintenance of polymorphism by reducing extinction risk. Moreover, Doebli and de Jong (1999) have shown that polymorphism in the mating system with over-dominance, as in our model, tends to favor system stabilization by decreasing the extinction probability relative to monomorphic populations used by Legendre et al. (1999) where polymorphism increases extinction probability. On the other hand, the plasticity hypothesis allows some phenotypic variability and, under such conditions, Lindström and Kokko (1998) and Doebli and de Jong (1999) have shown that two sex models can reduce system stability.

The loss of stability in the case of alternative reproductive strategies may have two consequences. First it may increase the extinction probability of an allele (by increasing the extinction of the rare allele phases). Secondly, in the case of the RPS game with cyclical structures of strategy frequencies, cycles may disappear and the dynamic may tend to behave chaotically (Doebeli and Koella 1994; Lindstrom and Kokko 1998; Caswell 2001). Thus the genetic mechanism that underlies trimorphic alternative mating strategies in natural systems will greatly depend on whether these strategies are sex limited or found in both sexes. In our model, strategies had similar pay-off structures in both sexes, but increasing empirical evidence for sexual conflict (Arnqvist and Rowe 2005) and sexual antagonistic genes (Morrow et al. 2008) could alter the predictions made here.

### *RPS game in* Uta stansburiana

In *Uta stansburiana*, theoretical models and field observations present important discrepancies with regard to frequency cycles which could be due to insufficient information on genetic mechanisms underlying male strategies. Usually, average phenotype frequencies predicted in models were different from the frequencies observed in the field (Sinervo and Lively 1996; Sinervo 2001a; Sinervo and Calsbeek 2006) and/or oscillations of strategy frequencies observed on the field cannot be modeled for longer periods of time (Sinervo and Lively 1996; Sinervo 2001a). In this study, we took into account the ability of heterozygote individuals to adopt a plastic strategy i.e. the strategy corresponding to the two specific alleles they carry (Maynard-Smith 1982; Doebeli and de Jong 1999). We also considered in a two sex model that males and females were playing different games because we were expecting that sex and games relative to sex will influence the maintenance of alternative reproductive strategies (Lindstrom and Kokko 1998; Sinervo 2001a). Unlike previous models, the combination of two sex games in a single model allowed us to predict similar oscillation patterns of strategy frequencies as those observed in the field (Figure 10) (Sinervo and Lively 1996; Sinervo 2001a; Sinervo and Calsbeek 2006).

In the *Uta stansburiana* case, our model results did not fully allow us to reject one of the two genetic mechanisms underlying the RPS game that we simulated. While the trimorphism maintenance probabilities for *Uta stansburiana* estimated by our model (but also by some empirical results) provide support for the plasticity hypothesis, the analysis of the population dynamic (the cyclical periodicity) arising from this hypothesis does not match the observed cycles, since our model predicted cycles with a 33-51 time steps cycle versus a 5-6 time steps cycle in nature (Sinervo and Lively 1996; Sinervo 2001a; Sinervo and Calsbeek 2006). The inclusion of the two sex scenario provided support for the dominance hypothesis since it is gives both a high probability of maintaining the game over long periods of time and a shorter time period for the cycle (16-32 time steps), which is closer to the periodicity seen in nature.

Regardless of the genetic mechanism underlying the RPS game, our results still do not provide an exact match with field observations of the cyclical periodicity. This discrepancy could have many explanations. Alonzo and Sinervo (2001), show that females may adopt a mating strategy targeting males with rare phenotypes. Implemented in our model, such a process would increases significantly the role of the RPS game into the global population dynamic. It would most likely, enhance maintenance probabilities of trimorphism and accelerate the oscillating dynamic of the RPS game. Another possible explanation of the discrepancy between observed and predicted patterns in our case can be computational. Indeed, for memory allocation and computation time optimization we set *U. stansburiana* fecundity to 14 instead of 50 (Zamudio and Sinervo 2000) and adapt sub-adult survival (0.8 instead of 1 (Sinervo and Lively 1996)) to keep the asymptotic growth rate constant at 0.1 since natural populations do not appear to be increasing. With real demographic parameters of *Uta stansburiana*, generation time would be shorter and cyclical periodicity of strategy frequencies should reduce with increasing fecundity so we might reach a cyclical period consistent with those observed on the field. However these parameters would only apply in a rapidly increasing population.

Field observation of strategy frequencies in the side blotched lizard do not extend for a sufficiently long time period to perform time series analysis to calculate the period of cycles analytically rather than graphically (Sinervo 2001a; Sinervo and Calsbeek 2006). If we graphically measure the time period of cycles, we obtain similar results as observed in the field (~4 year cycles regardless on the genetic mechanism scenario). Time series analysis is very powerful even with noisy data (Box and Jenkins 1976; Abarbanel 1996; Abry 1997) and for this time period, simulation outcomes are explained by population dynamic density-dependence rather than the frequency dependent mating game. This suggests that the periods usually measured graphically (Sinervo and Lively 1996; Sinervo 2001a; Svensson et al. 2005; Sinervo and Calsbeek 2006; Sinervo et al. 2006) are likely due to demographic density-dependence rather than induced by the RPS game and that cycles observed in the field might not correspond to cycles emerging from the RPS game per se.

*Evolutionary sequence of the RPS game initiation in the side blotched lizard*

In the case of *Uta stansburiana*, we have shown that when rare, the $p$ allele is the most able to invade a population constituted of the two other morphs and that the other morphs have little chance to persist under similar conditions. In addition, monogamous and sneaker strategies only have weak effects on each other whereas the polygynous strategy drives the overall trimorphic game dynamic. The weak interaction between the monogamous and sneaky strategies and further simulations with reinforcements of sneaker fitness ($\overline{h_s} = 1.5$) under plasticity mechanism leads easily to dimorphic monogamous-sneaker sTable oscillations (results not showed) and suggests that initially, these two strategies might have coexisted in an ancestral dimorphic population. Then, a polygynous $p$-like allele appeared and monogamous and sneaker like strategists have evolved under pressure from the new polygynous-like strategy leading to the RPS game we actually observe. Other scenarios are also possible if we relax the assumption that strategies have been sTable over time. Invasion of a new strategy could impose strong selection on the existing strategies and cause evolution of the existing strategies. If this is true, then many more evolutionary scenarios are possible, but current game payoff matrices do not help us understand this process. Further adaptive dynamic modeling would help to determine how the RPS game has evolved in *Uta stansburiana*. Regardless of these scenarios, plasticity plays a major role in game maintenance, and thus could have been ancestrally important in leading from the evolution of a plastically determined M to S types, and subsequent invasion of P. Subsequent canalization of behavioral strategy genes and dominance between alleles could the occur after initial invasion of a third strategy.

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| Parameters, variables and their distributions | Value(s) of parameter for general model | Value(s) for *Uta stansburiana* | Description | References for *Uta stansburiana* |
|---|---|---|---|---|
| **Demography** | | | | |
| $\sigma \rightsquigarrow \mathcal{B}(p)$ | 0.5 | 0.5 | sex ratio | (Sinervo 2001a) |
| $s_j \rightsquigarrow \mathcal{B}(p)$ | 0.2, 0.5 | 0.2 | juvenile survival | (derived from Sinervo and Lively 1996) |
| $s_a \rightsquigarrow \mathcal{B}(p)$ | 0.2, 0.5 | 0.2 | adult survival | (derived from Sinervo and Lively 1996) |
| k | 0.004, 0.002, 0.001 | 0.004, 0.002, 0.001 | competition coefficient | see text |
| $F \rightsquigarrow \mathcal{P}(\bar{F})$ | 14, 9.2, 6.5, 4.4 | 14 | fecundity | (derived from Sinervo and Lively 1996) |
| **Life history traits** | | | | |
| *Males* | | | | |
| $w_0$ | $\begin{array}{cccc} & \mathbf{P} & \mathbf{M} & \mathbf{S} \\ \mathbf{P} & 1 & 2 & 0.50 \\ \mathbf{M} & 0.5 & 1 & 2 \\ \mathbf{S} & 2 & 0.5 & 1 \end{array}$ | $\begin{array}{cccc} & \mathbf{P} & \mathbf{M} & \mathbf{S} \\ \mathbf{P} & 1 & 1.32 & 0.35 \\ \mathbf{M} & 0.34 & 1 & 2.3 \\ \mathbf{S} & 3 & 0.69 & 1 \end{array}$ | RPS game pay-off matrix<br>*(Mean number of females won by a focal male of a given strategy (by row: orange (O), blue (B) or yellow (Y)) when play against 3 other males of the same strategy (by columns))* | (Sinervo 2001b) |
| $h_p \rightsquigarrow \mathcal{P}(\overline{h_p})$ | 3 | 3 | Orange harem size | (Zamudio & Sinervo 2000) |
| $h_m \rightsquigarrow \mathcal{P}(\overline{h_m})$ | 1 | 1 | Blue harem size | (Zamudio & Sinervo 2000) |
| $h_s \rightsquigarrow \mathcal{P}(\bar{\bar{h}}_s)$ | 0.5 | 0.5 | Yellow harem size | (Zamudio & Sinervo 2000) |
| $n_p \rightsquigarrow \mathcal{P}(\overline{n_p})$ | 3 | 2.95 | Number of orange male neighbors | (Sinervo 2001b) |
| $n_m \rightsquigarrow \mathcal{P}(\overline{n_m})$ | 3 | 2.35 | Number of blue male neighbors | (Sinervo 2001b) |
| $n_s \rightsquigarrow \mathcal{P}(\overline{n_s})$ | 3 | 5.05 | Number of yellow male neighbors | (Sinervo 2001b) |
| *Females* | | | | |
| ΔF | ± 0.15 | ± 0.15 | Deviation from mean fecundity for r and K strategists | see text |
| Δs | ± 0.015 | ± 0.015 | Deviation from mean recruitment for juveniles from r or K | see text |
| *Heterozygosity cost* | | | | |
| c | 0.2 | 0.2 | Cost factor | see text |

Table 1: Nomenclature and default parameter value used in the different models

Figure caption:

**Figure 1:** Trimorphism maintenance probability when males express alternative reproductive strategies or males and females express alternative reproductive strategies under the allelic dominance or phenotypic plasticity hypotheses. The maintenance of the trimorphism is more easily maintained when individuals are plastic than if the phenotype depends on an allelic dominance relationship. The female game sustains the maintenance of the trimorphism (see text).

**Figure 2:** Trimorphism maintenance probability when males express alternative reproductive strategies or males and females express alternative reproductive strategies under allelic dominance or phenotypic plasticity hypotheses when heterozygote phenotypes are less efficient than homozygotes (cost of heterozygosity). The trimorphism is more easily maintained if individuals are plastic. The heterozygosity cost induces a strong decrease of probability of maintenance of trimiorphism when females are affected by the cost (see text).

**Figure 3:** Wavelet ("Morlet" wavelet) and multi-resolution analyses (see suplementary material for a description of the analysis) of strategy frequencies dynamics. From top to bottom, results are provided for focal time series of respectively polygynous, monogamous and sneaker strategy frequency dynamics. The time series are outputs from a simulation with allelic dominance, both the males and females exhibit alternative reproductive strategies and heterozygosity is not costly. Left panels correspond to the correlation between the non focal time series of strategy frequencies and the multi-resolution decomposition of the focal time series (see appendix A). Dark blue, black and red lines represent the effect of each of the two non-focal time series on the component of the focal time series listed on the left of the graph and grey, orange and light blue lines are the effects of the focal time series on each of the non-focal time series. Non-focal time series are red, blue or black for polygynous, monogamous and sneaker strategy frequency dynamics respectively and are orange, light blue and grey respectively when considering the effect of focal time series on non-focal time series. Central panels are the power spectra analysis of the strategy frequencies. The colors code for power values from relative low values (dark blue) to relative high values (dark red). The nature

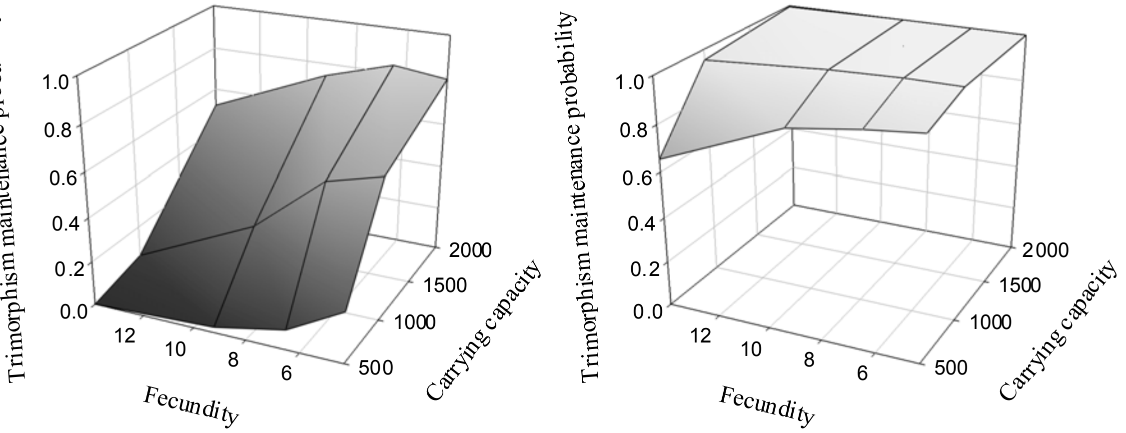
(period, stationarity) of the cycles detected in the time series are determined using their specific signatures of alternation between red and blue regions (see appendix A for details of the signatures). Right panels are the multi-resolution decomposition of the strategy frequencies which correspond to the time series decomposition at each time scale. The analyzed time series is the sum of the multi-resolution decomposition.

**Figure 4:** The influence that two time series impose on each other is detected by cross-corelation plots. If no causal relation exist between them, the cross-correlation function is symetric and centered on 0. The existence of a causal relation between the two series generates an asymmetry in the cross-correlation function (Box and Jenkins 1976). Moreover, the direction of the deviation along the ordinate axis provides the direction of the correlation (see appendix A for more details). Here, asymetry and the direction of deviation in the cross-correlation function between the polygynous strategy and the monogamous strategy as well as between the polygynous strategy and the sneaker strategy, means that the polygynous strategy has a negative effect on monogamous individuals and a positive effect on sneakers. In contrast, the cross-correlation function between the monogamous strategy and the sneaker shows that the monogamous strategy does not affect the allele frequency dynamic of the sneaker strategy. At the global scale of the RPS game, the polygynous strategy appears to drive the game.

Figure 1:

**Allelic dominance** **Phenotypic plasticity**

Male game Male game



Male and female games Male and female games

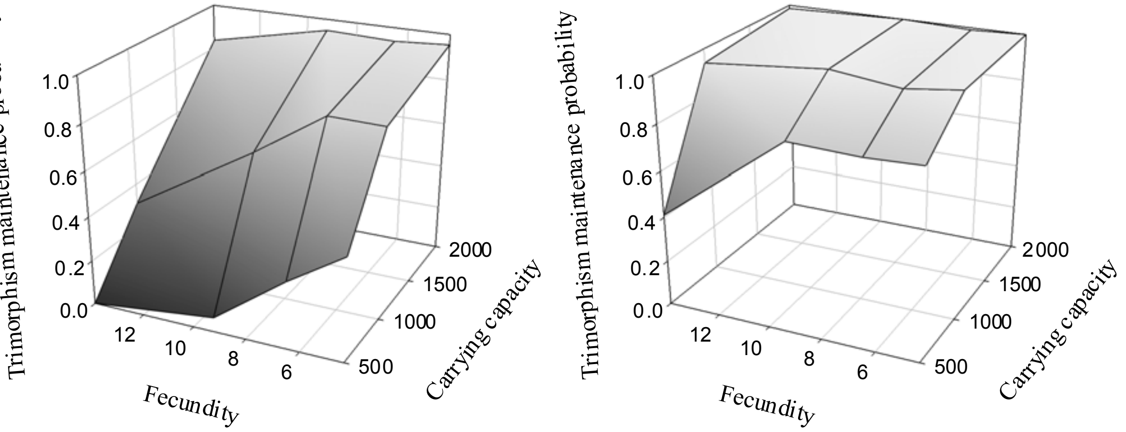
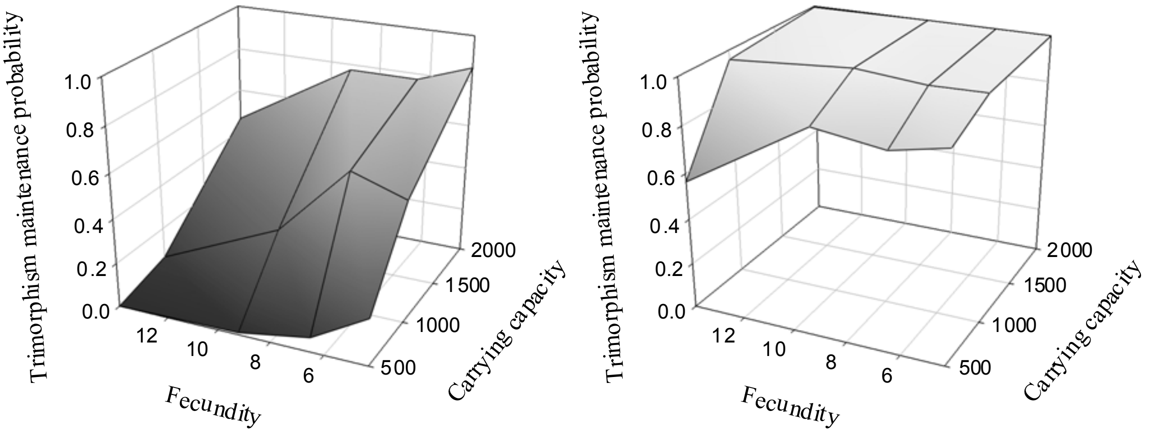


Figure 2:

**Allelic dominance** — Male game with cost to heterozygosity

**Phenotypic plasticity** — Male game with cost to heterozygosity



Male and female games with cost to heterozygosity

Male and female games with cost to heterozygosity

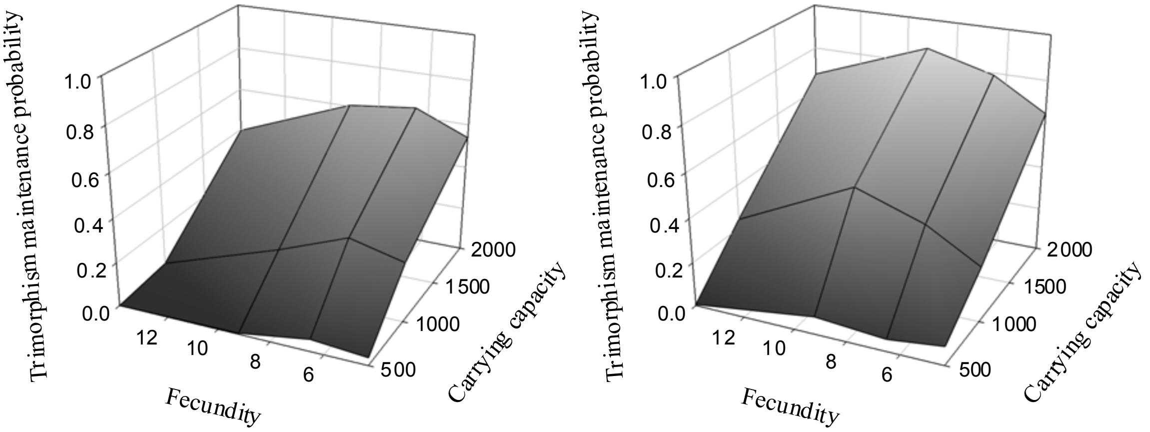


Figure 3:

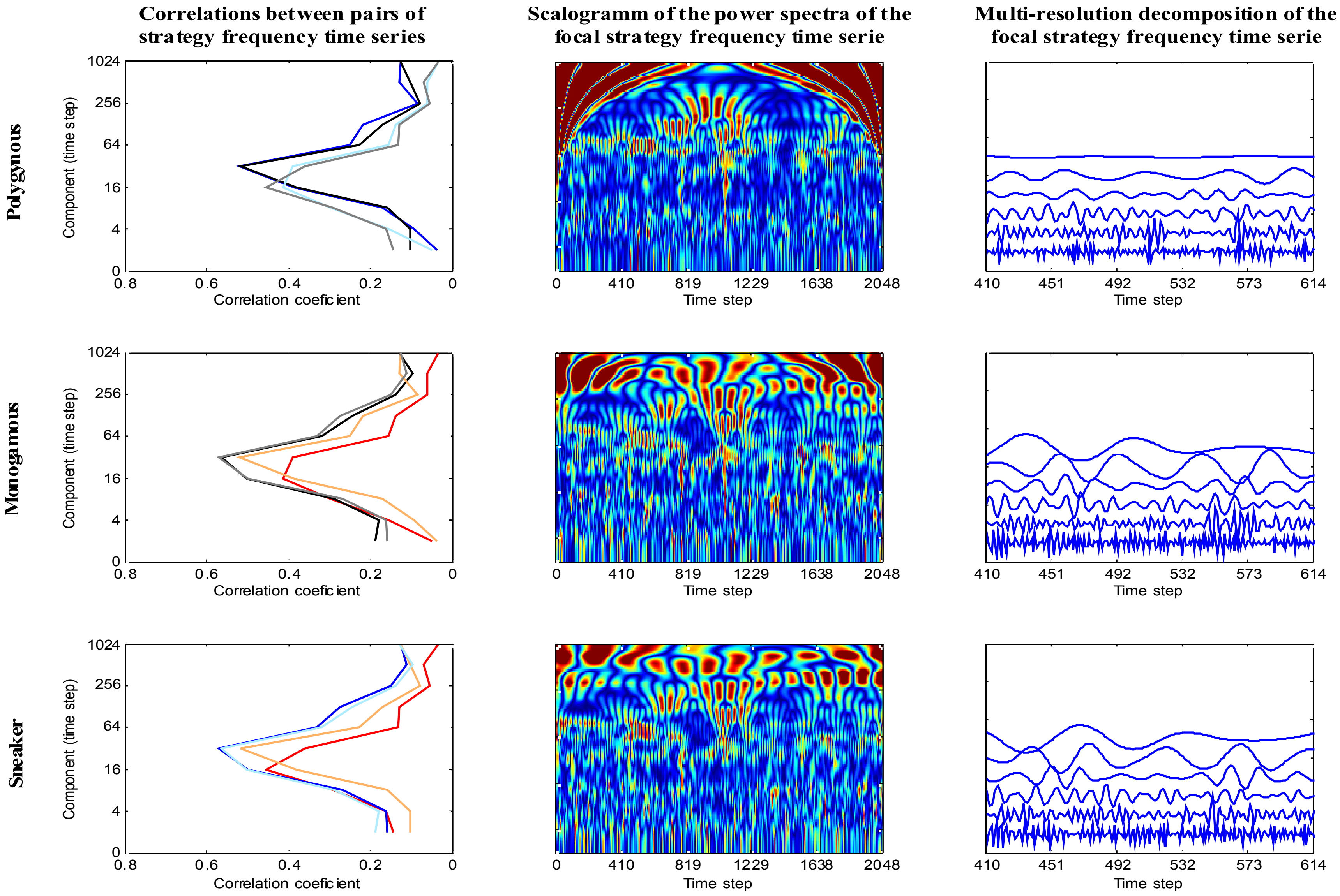

Correlations between pairs of strategy frequency time series
Scalogramm of the power spectra of the focal strategy frequency time serie
Multi-resolution decomposition of the focal strategy frequency time serie
Polygynous
Monogamous
Sneaker
Component (time step)
1024
256
64
16
4
0
0.8
0.6
0.4
0.2
0
Correlation coeficient
0
410
819
1229
1638
2048
Time step
410
451
492
532
573
614
Time step

Figure 4:

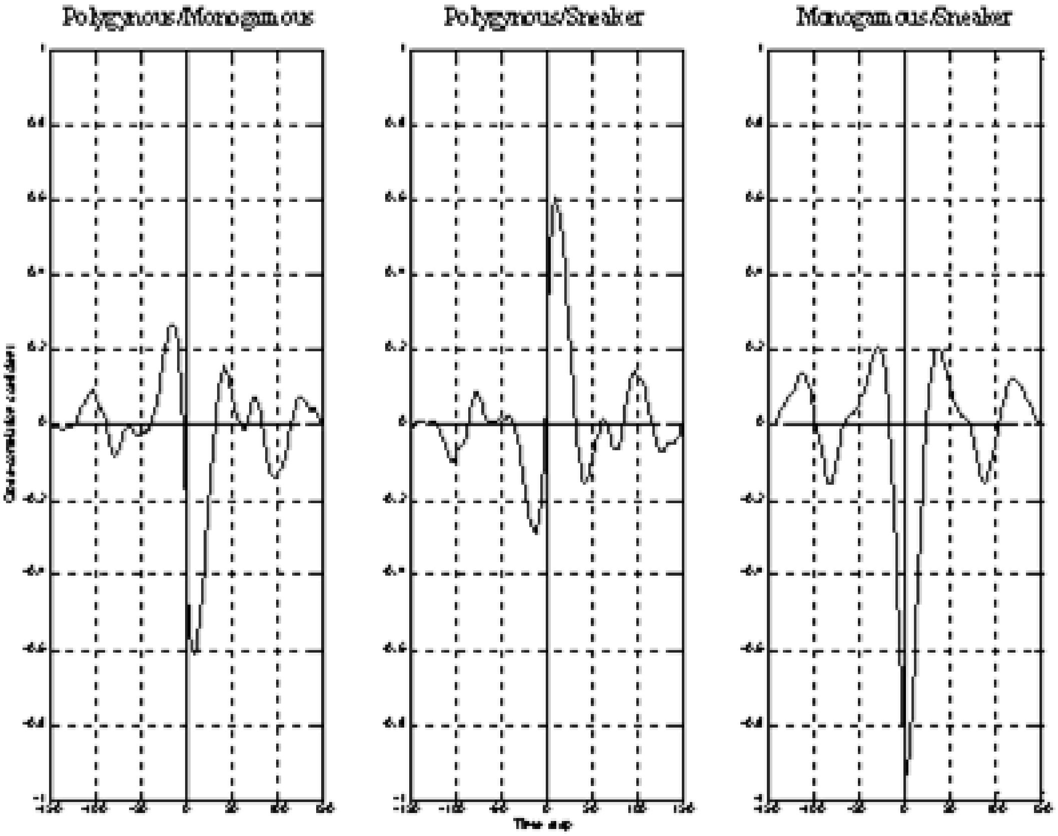
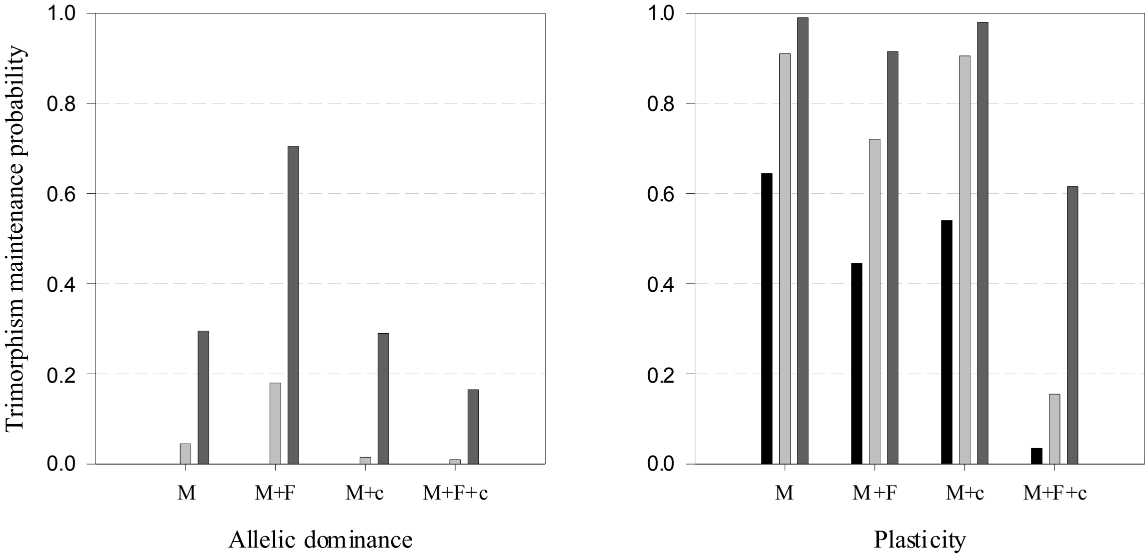

Polygynous/Monogamous
Polygynous/Sneaker
Monogamous/Sneaker
Cross-correlation coefficient
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-150
-100
-50
0
50
100
150
Time lag

Figure 5:


Trimorphism maintenance probability
1.0
0.8
0.6
0.4
0.2
0.0
M
M+F
M+c
M+F+c
Allelic dominance
1.0
0.8
0.6
0.4
0.2
0.0
M
M+F
M+c
M+F+c
Plasticity

## Appendix A: Elements of time series analysis

The goal of this supplement is to explain the major concepts underlying wavelet and multi-resolution analysis rather than all the mathematics and statistics which are well established (see Jenkins and Watts 1968; Box and Jenkins 1976; Meyer and Roques 1993; Abarbanel 1996; Mandelbrot 1997; Mari et al. 1997). In essence, we provide a mini-primer on these analyses which are less familiar to evolutionary ecologists.

### *Wavelet and single signal multi-resolution analyses*

Here we examine wavelet analysis and multi-resolution signature of a simple sinusoidal function. We built three simple sinusoidal functions ($f_1$, $f_2$ and $f_3$) made of 10400 points. Function $f_1$ (equation A1) presents an abrupt change in oscillation frequency, $f_2$ (equation A2) is composed of two sinusoidal functions of different amplitude and period and $f_3$ (equation A3) presents a slow-down in its oscillations (Figure A1):

$$\begin{cases} f_1(t) = A_1 \sin\left(\frac{2\pi t}{p_1}\right) & if\ t < t_t \\ f_1(t) = A_1 \sin\left(\frac{2\pi t}{p_2}\right) & if\ t \geq t_t \end{cases} \quad \text{(equation A1)}$$

$$f_2(t) = A_1 \sin\left(\frac{2\pi t}{p_1}\right) + A_2 \sin\left(\frac{2\pi t}{p_2}\right) \quad \text{(equation A2)}$$

$$\begin{cases} f_3(t) = A_1 \sin\left(\frac{2\pi t}{p_1}\right) & if\ t < t_t \\ f_3(t) = A_1 \sin\left(\frac{2\pi t}{p(t)}\right) & if\ t \geq t_t \end{cases} \quad \text{(equation A3)}$$

Where $A_1$=2, $A_2$=1, $p_1$=$\pi$/60, $p_2$=$\pi$/12, $t_t$=5000 and $p(t) = p_1 + (p_2 - p_1)\left(\frac{t - at_t}{bt_t}\right)$ with $a$=0.9 and $b$=0.2.

We then perform a wavelet analysis using the "Morlet" wavelet (Cazelles et al. 2008) and a multi-resolution analysis on the modeled signals (Figure A1). The wavelet analysis determines the temporal localization of information carried by the signal. This analysis detects when information (Shannon 1948) is present and how this information "travels" across temporal scales(Meyer and Roques 1993; Mari et al. 1997; Cazelles et al. 2008). Multi-resolution analyses then assumes that the global signal is the result of the addition of the signal carried by the different temporal scales and separates the global signal into its different components(Jenkins and Watts 1968; Box and Jenkins 1976).

Figure A1 shows the wavelet and the multi-resolution signatures of the three functions:

- $f_1$: The wavelet power spectrum shows that a high power spectrum (in dark red) is moving-down from low frequency oscillations (period ≈ 20 temporal unit or t.u.) to medium frequency oscillations (period ≈ 8 t.u.). The multi-resolution analysis shows that the signal is composed of a single signals at period = 16 t.u. until $t$ = 5000 t.u. where it switches to another single signal at period = 8 t.u.
- $f_2$: The wavelet power spectrum shows that high power of spectrum is carried by two different temporal scales (period ≈ 20 t.u. and period ≈ 8 t.u.) all along the signal. The multi-resolution analysis shows that the signal is composed of a mixture of two signals with at period = 16 t.u. and period = 8 t.u.
- $f_3$: The wavelet power spectrum shows that a high power spectrum is located around the 8 t.u. time scale until the signal shows an amplified oscillation. Then the high power spectra moves-up to lower frequencies of oscillations. The multi-resolution analysis shows that the signal is initially composed of a single signals at period = 8 t.u.. Then, the global signal becomes a combination of signals from period = 4 and sliding to 16.

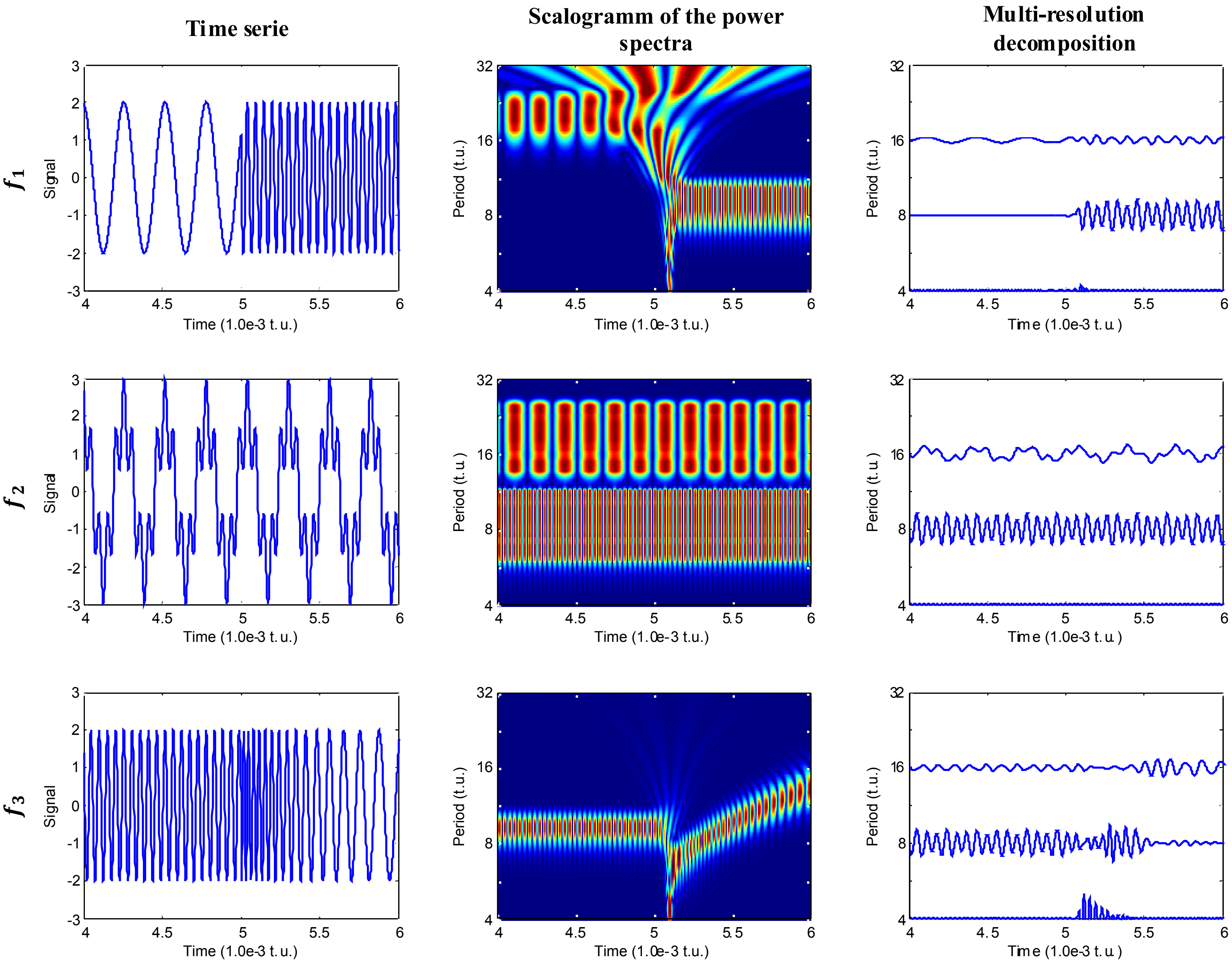

Time serie
Scalogramm of the power spectra
Multi-resolution decomposition
f1
f2
f3
Signal
3
2
1
0
-1
-2
-3
Period (t.u.)
32
16
8
4
4
4.5
5
5.5
6
Time (1.0e-3 t.u.)

**Figure A1:** Wavelet analyses and multi-resolution analyses of three simple sinusoidal functions (left panels) with an abrupt change in oscillation period ($f_1$), a function ($f_2$) composed of two sinusoidal functions and a function ($f_3$) beginning regularly and at mid-time accelerating and then slowing-down. Central panels are the wavelet power spectra of the different signals and represent the specific signature (see text). The multi-resolution (right panels) presents the specific signatures of the different functions in a discrete environment. Signals are split into time scale specific sub-signals. For readability, only temporal windows between 4000 t.u. and 6000 t.u., temporal components of oscillations between 4 t.u. and 32 t.u. are represented and the multi-resolution analysis have been normalized.

### *Multiple signal analyses*

In this section, the main idea is to determine if a signal ($S_1$) influences another signal ($S_2$) and if so, to determine the temporal scale of this influence. The cross-correlation function between $S_1$ and $S_2$ allows us to determine if a causal relation exists between $S_1$ and $S_2$ (see Box and Jenkins 1976 for details). If no causal relation exists, the cross-correlation function is symmetrical whereas it is asymmetrical if a causal relationship exists. When the function is asymmetrical, if both the signals are in phase, the function is centered on 0, otherwise the function is centered on the phase difference. The direction of the deviation from 0 reflects the direction of the relationship. If $S_1$ is positive and induces a positive response of $S_2$, the deviation will be also positive. Finally, the cross-correlation function presents at the same time the effect of $S_1$ on $S_2$ ($x>0$) and the feed-back effects of $S_2$ on $S_1$ ($x<0$) (Box and Jenkins 1976).

With complex signals, it can be interesting to identify the time scale at which one signal, $S_1$ or its components influences $S_2$ (Figure A2). We have seen before that multi-resolution tools can split the signal into its different components and we are able to perform correlations between $S_1$ or its components and $S_2$'s components to detect the time scale of interactions between $S_1$ (or its components) and $S_2$ (or its components). The intensity of these correlations corresponds to the intensity of the transfer of information from $S_1$ to $S_2$ (Box and Jenkins 1976).

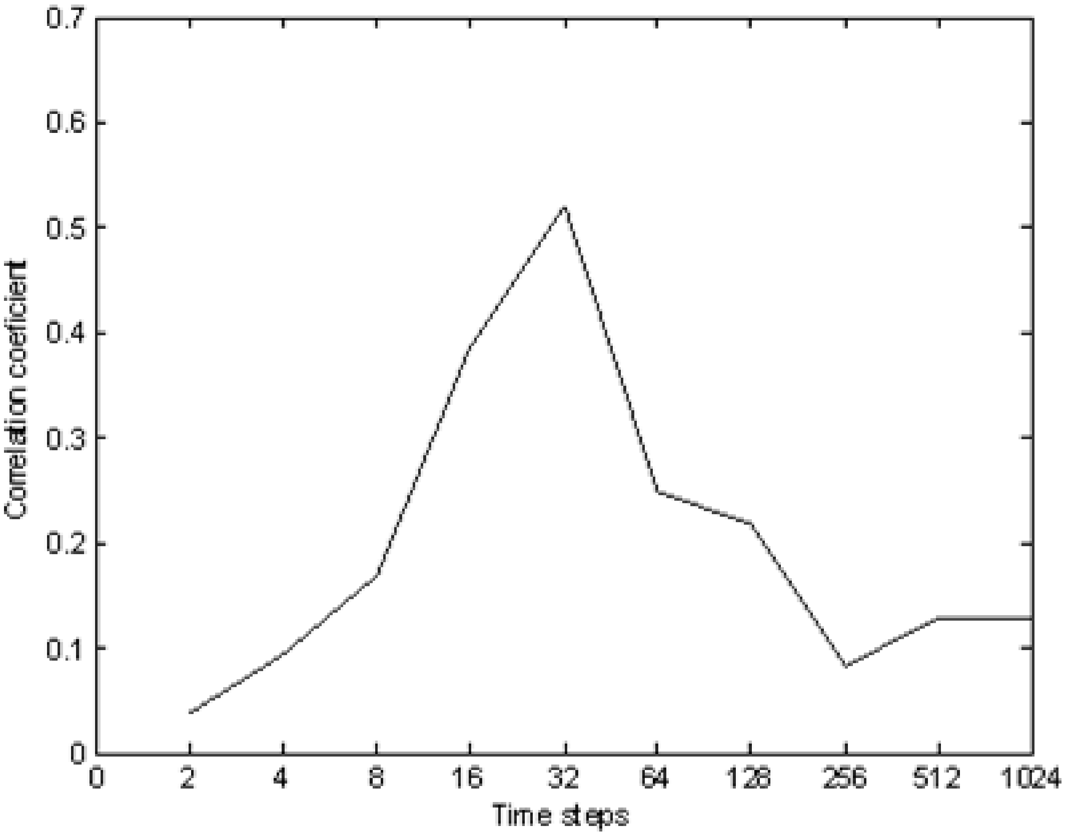


**Figure A2:** Correlation between a signal $S_1$ and the components of a signal $S_2$. $S_1$ is the frequency of the M strategy and $S_2$ is the frequency of P strategy under the genetic dominance mechanism without cost of heterozygosity and both the sexes exhibiting alternative reproductive strategy. The maximum of information transfer from $S_1$ to $S_2$ occurs at the 32 time steps scale and corresponds to the maximum correlation between $S_1$ and each components of $S_2$.

# Appendix B: Multi-resolution building of phenotype frequencies of the RPS game

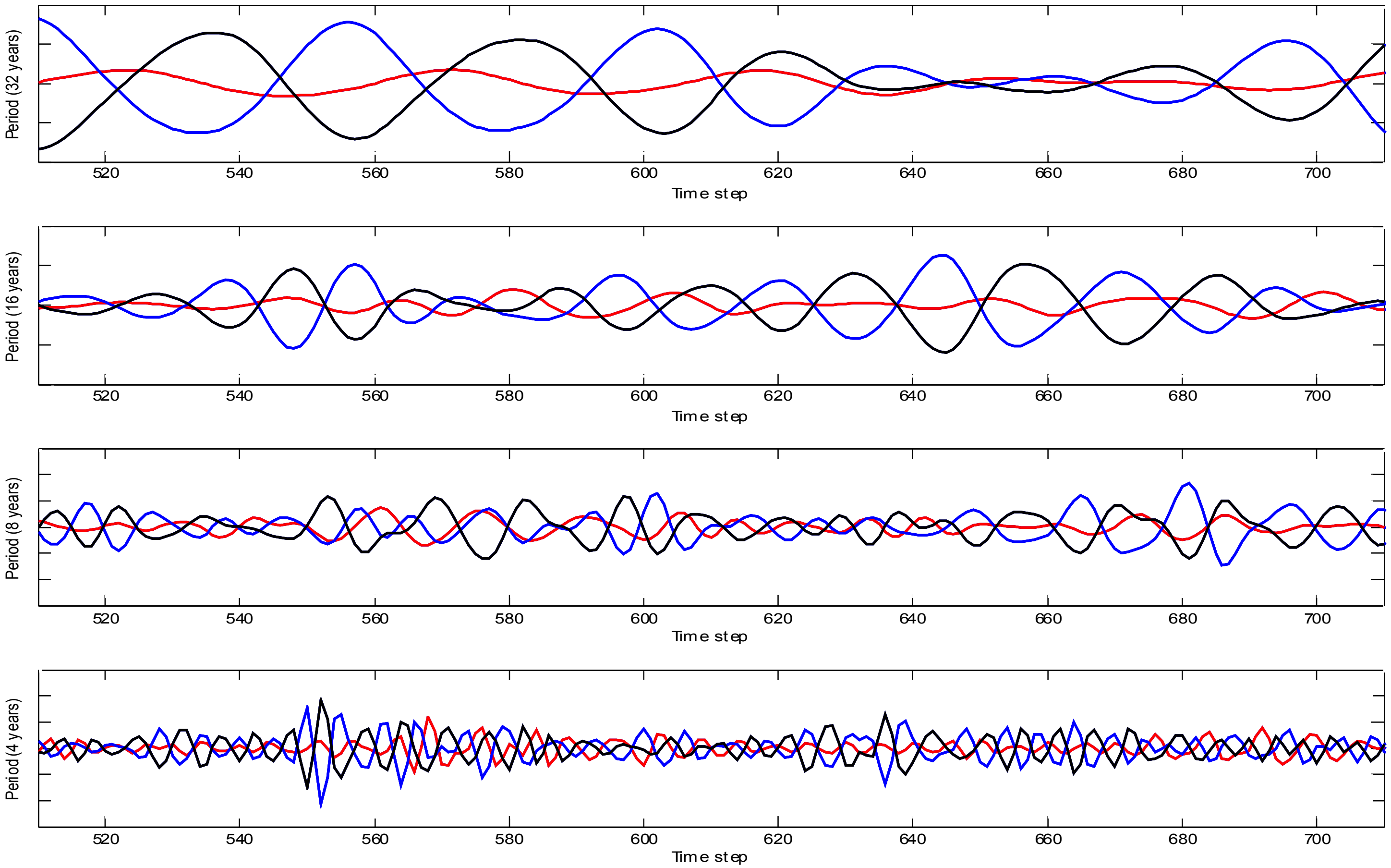


**Figure B1:** Construction by multi-resolution analysis of the three strategy frequencies in the RPS game context at time scales for which we detected cyclical structures (4 time step to 32 time step scale). Polygynous strategy corresponds to the red lines, monogamous corresponds to the blue lines and sneaker corresponds to the black lines. The pattern exhibited by strategy frequencies between 16 and 32 time steps scale is consistent with the pattern expected for an RPS game (Maynard-Smith 1982; Sinervo and Lively 1996; Sinervo and Calsbeek 2006). Structures exhibited at the 4 time step scale corresponding to density-dependence of population dynamics and the structures of the 8 time step time scale is a mixture of density-dependence and the RPS game.

Online Appendix C: Model structure.

The model is a basic demographic model of two sexes and two age classes. The demographic process is divided in four main phases:

1. Juvenile recruitment: juveniles are randomly recruited into the adult population depending on population density. The non-recruited juveniles survive depending on the juvenile survival probability ($s_j$).
2. Mating: males and females are associated through male RPS game rules depending on the neighborhood composition and mating strategies.
3. Reproduction: new juveniles are produced according to the genotype of their parents
4. Adult death: adults survive according to the adult survival probability $s_a$.

The mating phase is composed by four sub-phases allowing us to implement the effect of social environment on reproductive strategy phenotype and the RPS game (Figure C1).

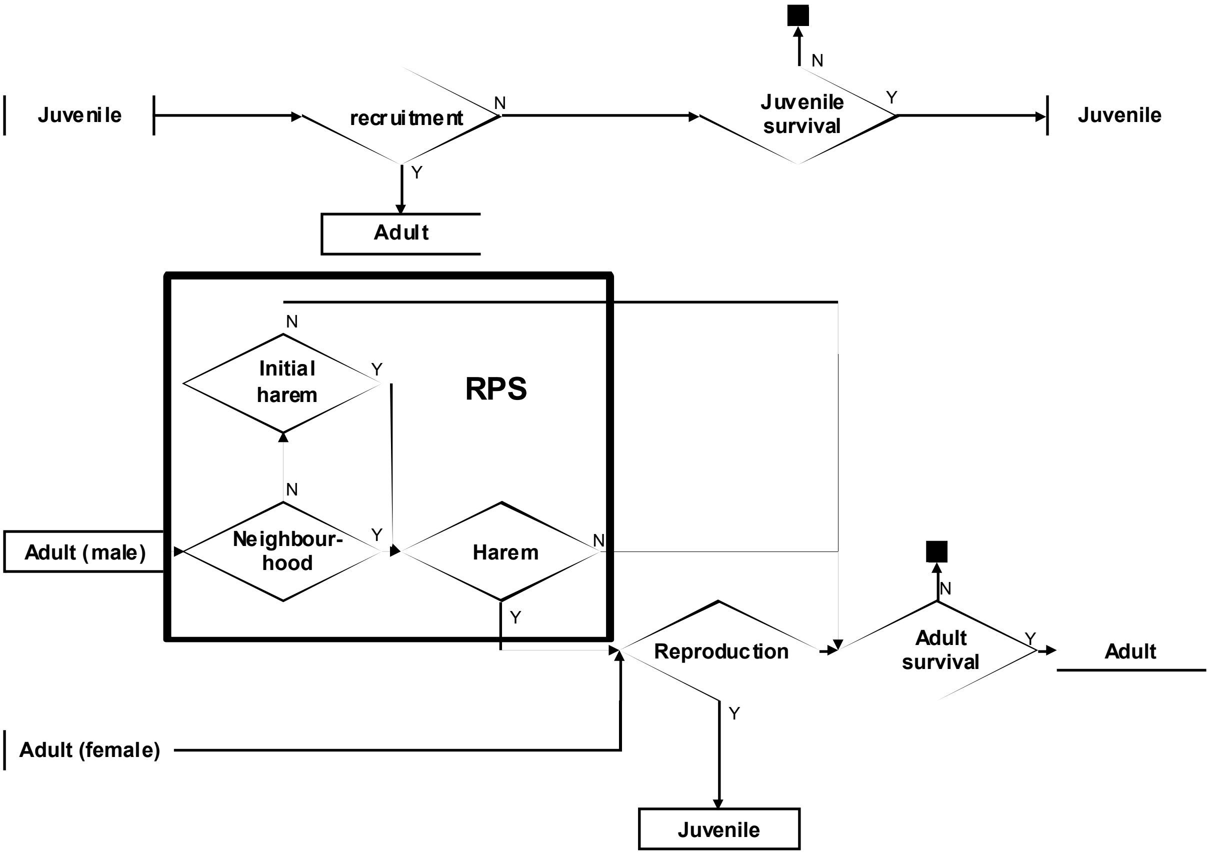


**Figure C1:** Life history of the model during a single time step showing the transitions between age classes (rectangles) and decisional rules (rhombus) regulating these transitions. For example for a juvenile, if in the recruitment phase (recruitment rhombus) the result is 'yes' (Y), it becomes an adult and the individual enters the cycle for adults and then begin the mating phase. If the result is 'no' (N), it stays in the juvenile cycle and will be tested for its survival. The black squares correspond to death of an individual die that is removed from the simulation. The mating phase is composed by the three processes in the bold rectangle and includes the implementation of the RPS game.

Online Appendix D: Extinction probability and strategy extinction time in two phenotype populations

Simulations with populations initially composed of individuals presenting only two of the three alleles leads to the fixation of the strongest strategy in the RPS game context (i.e. P wins against M, M wins against S and S wins against P) (Figure D1).

Under the genetic dominance hypothesis, the loss of monogamous alleles is higher compared to other alleles and the monogamous allele persists roughly three times longer than the sneaker and polygynous alleles do (Table D1). This difference between the monogamous allele and the others alleles can be explained by the fact that in a polygynous/monogamous population, heterozygotes carry the *m* allele but express the winning P phenotype whereas in the other heterozygous cases, individuals express the loosing strategy (i.e. *ps* and *ms* individuals express respectively loosing phenotypes P and S) increasing allele extinction speed.

Under the plasticity hypothesis, the trend is reversed and the monogamous allele disappears faster than the polygynous and sneaker alleles (Table D1). Loss times for *p* and *s* alleles were similar to *m* under the genetic dominance assumption due to the ability of heterozygotes to express the winning strategy. As expected, extinction time of the *m* allele decreased under the plasticity hypothesis because *pm* individuals can adopt a strategy of low selective value. In addition, the losing M strategy can be sometime chosen whilst genetic dominance leads to the P strategy winning in all cases. However, this decrease may also be explained by the asymmetry of mating systems between polygynous and monogamous strategists (Legendre et al. 1999). Such asymmetry may accelerate the extinction speed of *m* allele compared to others.

The female game seems to have no major influence on the time of alleles extinction (Table I).

Under the genetic dominance hypothesis, the heterozygote cost does not significantly change the probability or time before extinction of alleles (Table D1). Under the plasticity mechanism, the heterozygote cost only influences the extinction speed of the *p* allele when P plays against S (Table D1). Under this hypothesis, *p* and *m* alleles have similar extinction patterns explained by the matting system asymmetry. Indeed, in these cases, *ps* individuals have reduced harem size compared to *pp* and *ss* individuals. When both sexes present alternative reproductive strategies and heterozygosity is costly, patterns of allele loss become very similar and the speed at which alleles disappear are slightly increased (Table D1). Under genetic dominance, *pm* individuals lose their advantage compared to *ps* and *ms* under similar conditions due to the reduction of *pm* offspring survival. In a similar way, under the plasticity hypothesis, heterozygote juvenile survival costs increased the extinction speed of alleles by reinforcing the strength of the winning strategy.

**Table D1:** Under the RPS game assumptions, populations composed of only two phenotypes leads to the fixation of a single phenotype according to the RPS game rules. In this case, P wins against M, M wins against S and S wins against P. The Table reports the number of time steps before extinction of the disappearing strategy in a population with two phenotypes (i.e. extinction of *p* allele in a population of P and S strategists). Alleles are assumed to be carried by a single locus and $p > s > m$ (Sinervo et al. 2001).

| **model hypothesis** | **genetic dominance** | | | **plasticity** | | |
|---|---|---|---|---|---|---|
| **allele** | *p* | *m* | *s* | *p* | *m* | *s* |
| **mean extinction time** | 27 | 130.4 | 34.9 | 157.7 | 69.7 | 154.5 |
| **SE extinction time** | 1.8 | 11.6 | 2.5 | 10.8 | 5.2 | 12.1 |
| **heterozygosity cost** | | | | | | |
| **mean extinction time** | 27.3 | 127.2 | 32.5 | 79.9 | 70.3 | 152.3 |
| **SE extinction time** | 1.9 | 11.4 | 2.3 | 5.2 | 5.5 | 12.5 |
| **female game** | | | | | | |
| **mean extinction time** | 40.3 | 126.9 | 30.2 | 144.7 | 80 | 163 |
| **SE extinction time** | 2.8 | 11.7 | 2.1 | 11.8 | 5.7 | 12.1 |
| **Female game and heterozygosity cost** | | | | | | |
| **mean extinction time** | 33.2 | 62.4 | 27.5 | 45.8 | 50.4 | 67.9 |
| **SE extinction time** | 1.8 | 4 | 1.7 | 2.7 | 2.9 | 4.1 |

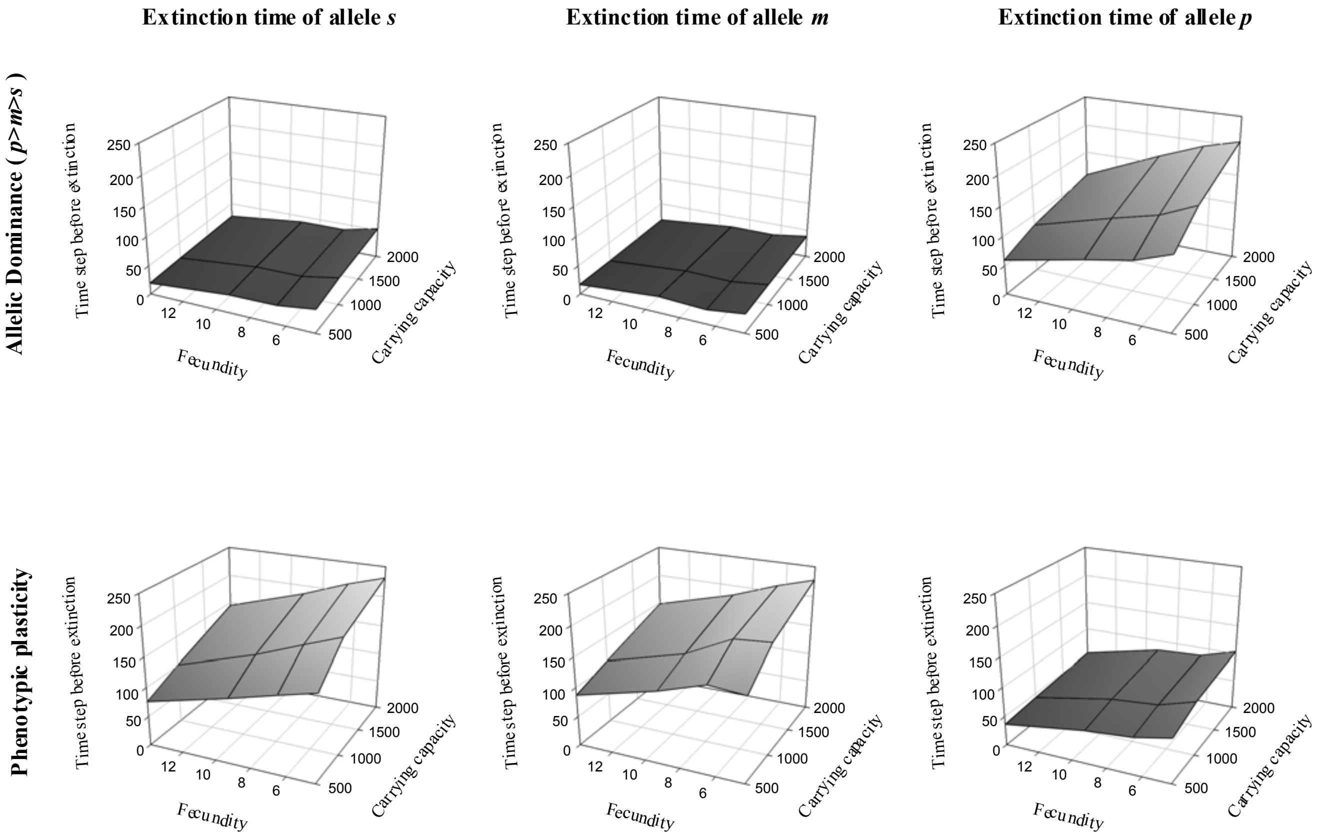


**Figure D1:** Under the RPS game assumptions, populations composed of only two phenotypes leads to the fixation of a single phenotype according to the RPS game rules. In this case, P wins against M, M wins against S and S wins against P. The graphs represents the number of time steps leading to the extinction of the loosing strategy assuming allelic dominance $p>s>m$ (upper panels) and that heterozygotes express the best strategy (lower panels). From left to right, eviction time of *s* allele when playing against M strategists, loss time of *m* allele in a P/M population and eviction time of *p* allele when the P strategy is sneaked by S strategists.

# 3. Bushes protect tortoises from lethal overheating in arid areas of Morocco

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*Résumé en français :*

Les régions arides méditerranéennes qui hébergent une biodiversité unique sont menacées par l'augmentation des pressions anthropiques telles que l'urbanisation, le surpâturage et le changement climatique. Au Maroc, l'une des espèces arbustive des zones arides, le jujubier (*Ziziphus lotus*), souffre de programmes de destruction gouvernementaux. La tortue mauresque (*Testudo graeca soussensis*) est menacée par cette destruction de son habitat naturel. Comme la plupart des animaux, la tortue mauresque doit naviguer entre microhabitats pour se nourrir et réguler sa température. Cette étude combine le suivi télémétrique d'individus sauvages avec des suivis de températures dans les microhabitats et la caractérisation des différents microhabitats. Les tortues sont trouvées dans des secteurs à relativement forte densités de jujubiers de grande taille qui leurs permettent de se protéger d'une surchauffe létale. Ainsi, (1) l'actuel encouragement officiel pour la destruction des buissons de jujubiers devrait être réévalué ; (2) la restauration de l'habitat basée sur la plantation de jujubiers devrait être encouragée ; et (3) le suivi à large échelle des densités critiques de jujubiers pourrait être utilisée pour améliorer l'organisation (protection et/ou restauration des milieux) des plans de conservation de la tortue mauresque.

## Abstract

Arid Mediterranean regions that shelter unique biodiversity are threatened by increasing anthropogenic pressures: urbanization, overgrazing, climatic changes. In Morocco, one of the main bush species in arid areas, the jujube (*Ziziphus lotus*), suffers from officially promoted programs of removal. The Moorish tortoise (*Testudo graeca soussensis*) is threatened by such habitat loss. Like most animals, the Moorish tortoise must navigate between microhabitats to find essential feeding resources and thermal refuges. We combined radio-tracking of free-ranging individuals, microhabitat temperature monitoring and transects in a range of habitats. Our results (home ranges, habitat use, displacements, and thermal characteristics of different micro-habitats) show that tortoises depend on the occurrence of large and relatively abundant bushes to escape lethal overheating. These results have strong conservation applications: 1) current official encouragements for the destruction of the jujube bushes should be reappraised; 2) habitat restoration relying on replanting jujube bush should be encouraged; 3) wide scale monitoring of the critical bush densities should be used to better organize conservation plans (protection and/or restoration of areas) of the Moorish tortoise.

**Keywords:** arid region, habitat quality, home range, lethal temperature, refuge, thermoregulation, tortoise, reptile

## Introduction

Habitat destruction is one of the major threats for biodiversity and one of the main causes of species extinction (Pimm and Raven 2000). Habitat loss involves the reduction of natural areas and is often worsened by various other perturbations such as pollution, invasions by exogenous species, or fragmentation for instance. Environmental impacts vary with the intensity and the type of anthropogenic assaults, but they also depend on the quality, the size and the characteristics of the concerned areas (Scott et al. 2006). On average, arid zones are far more fragile compared to temperate and tropical areas, and irreversible desertification is rapidly increasing in the dry regions of the planet (Geist and Lambin 2004; Kéfi et al. 2007). Indeed, for most types of organisms, low water availability severely restricts individual growth, reproduction and population regeneration rates.

Mediterranean ecosystems are considered as major biodiversity hotspots (Médail and Quézel 1999; Myers et al. 2000). Unfortunately, many arid areas of the Mediterranean region suffer from an intensification of human pressures due to rapid population increase and from global warming; rapid erosion of natural habitats entail environmental damages (Milchunas and Lauenroth 1993; Taïbi and El Hannani 2004; McGregor et al. 2009). At numerous locations throughout the Maghreb (western North Africa), urbanization, extension of the road network and intensification of agriculture occur at a very fast rate (McGregor et al. 2009; Pleguezuelos et al. 2010). It is therefore of prime importance to identify key characteristics of critical habitats that allow the maintenance of important species. Such an assessment is essential to retain crucial elements of the ecosystems, for instance to select optimal sites for natural reserves, to improve specific protection status, or to promote effective habitat restoration programs.

In the current study the relationship between habitat quality and the occurrence of a vertebrate species was investigated. We focused on one of the main bush species of the arid zones of Morocco, the jujube bush (*Ziziphus lotus*, a spiny plant species), and on one endemic threatened vertebrate species, the Moorish tortoise (*Testudo graeca soussensis*). These two species provide a clear illustration of the current conservation situation of many ecosystems of the Maghreb in general. Spiny shrubs and ephemeral grasses represent the food source for a vast assemblage of species (Tripathi et al. 2007; Bliege-Bird et al. 2008). Thorns, spines, and prickles of the main bushes provide effective defenses against large herbivores (Cooper and Owen-Smith 1986). The bushes also offer crucial shelters for many other animal species (insects, arachnids, rodents…); they reduce soil temperature during hot periods, but they also increase temperature during cooler periods (e.g., night), they preserve soil moisture, and enrich soil fertility (Castro et al. 2004). As expected, mammals, birds and reptile populations decline when shelter availability is reduced (Webb and Shine, 1997; Grillet et al. 2010). However jujube bushes compete with several agricultural land practices, particularly for space and nutrients and therefore are considered as pests (Milchunas and Lauenroth 1993; Fleischner 1994; McGregor et al. 2009). Consequently, shepherds and farmers often destroy spiny bushes; in addition the wood is harvested as firewood or to build fences (Slimani et al. 2006). Moreover, official government agricultural authorities encourage the destruction of jujube bushes and have developed specific programs and techniques to eradicate this plant, notably by chemical means (Regehr and El Brahli 1995; Rsaissi and Bouhache 2002; Programme National de Transfert de Technologie en Agriculture 2002). Although jujube bush is particularly resistant to drought (Mader 2003), and despite the fact that this species was considered as one the most important species in terms of abundance and for its ecological services in the past decades (Mader 2003), the intensification of eradication programs has led to the rarefaction of this bush (Mamva 1996). The Moorish tortoise is a typical inhabitant of the jujube bush steppes. Due to habitat changes and to over-collecting for the tourist trade, the once flourishing tortoise populations of Morocco have also drastically declined (Bayley and Highfield 1996; Slimani et al., 2001, 2006; El Mouden et al., 2006).

The importance of bushes for the survival of the Moorish tortoises (along with many other animal species) is intuitive, but precise field or experimental data are lacking. Bushes can provide food and shelter, but what are the critical densities and characteristics (e.g. dimensions) of bushes to offer suitable micro-habitats? Answering such questions is fundamental to setting up cost-efficient conservation programs, notably to provide information and tools to select the most appropriate areas and/or to propose specific measures (e.g. replanting actions).

Many animals can survive food or water shortage, at least in the short term, but none can afford even short term overheating (Vickers et al. 2011). This notion strongly applies in arid areas of Maghreb that are characterized by strong elevations in ambient temperatures during extensive periods of the year. In most places, jujube bushes represent the only shelter available; Moorish tortoises cannot dig deeply into the rocky substrate. Therefore we explored the relationship between the main characteristics of the spiny bushes, the thermal conditions within and outside the bushes, and the critical thermal limits of the tortoises. We emphasize that this study does not examine seasonal and daily patterns of thermoregulation of the Moorish tortoise, but instead assesses the physiological limits imposed by different types of micro-habitats on their survival. The aim of this study is to evaluate the importance of density and quality of the main spiny bush species (*Ziziphus lotus*) on the occurrence of an endemic tortoise (*Testudo graeca soussensis*) in a dry and overgrazed environment. Therefore we also performed surveys in a range of zones representative of strongly to less degraded habitats.

## Methods

### Study sites

The general study site is located in the Jbilet mountains, in the north of Marrakech (central Western Morocco) towards the edge of the Haouz plain. The study site (550 m to 600 m above sea level) is spread over 22 km, from 5 km from the Tansift River to the village of Sidi Bou Othamne along the road to Casablanca (31°42’ to 31°55' N, 7°54’ to 7°59’ W; Figure 1). The substrate is essentially rocky and consists of shale mixed with granite with very limited arable soil. Climatic data were obtained from the meteorological station of Sidi Bou Othmane and from the MétéoMaroc website (http://www.meteomaroc.com). The vegetation of the Jbilets is typical of the arid steppes that cover important surfaces of Morocco. It is characterized by the presence of spiny shrubs, mainly jujube (*Ziziphus lotus*) and acacia bushes (*Acacia gummifera*), with retams (*Retama monosperma*) representing the main non-spiny bushes (Slimani et al. 2006; El Mouden et al. 2006; Louzizi 2006). An ephemeral herbaceous layer occurs following late winter and spring rainfalls, essentially under the spiny bushes where the impact of overgrazing is attenuated and where localized patches of soil persist. This herbaceous layer disappears quickly in late spring due to the summer drought. As in many parts of Morocco, our study site is subjected to the combination of overgrazing (sheep and goats) and use of the bushes for firewood and to build enclosures; the vegetation is consequently degraded (Rsaissi and Bouhache 2002; El Mouden et al. 2004).

Within the general study site, we selected 12 study areas (1 km$^2$) to cover a range of situations from highly degraded to relatively preserved habitats (Figure 1). To estimate the number of bushes, each study area was randomly divided into three non-adjacent subareas (300 m x 300 m, total number of subareas = 36). Within each subarea we randomly selected four quadrats (100 m x 100 m, total number of quadrats = 144). As the jujube bushes are the most important bushes (both in quantity and size, they represent more than 90% of the bushes), we focused on this species to characterize the quality of the habitat. We counted all the jujube bushes within each quadrate. The dimension (maximal length L, width W and height H) of the jujube bushes was measured using a measuring tape, the surface occupied on the substrate was determined using the ellipsoid equation ($S = \pi*\text{Length}/2*\text{Width}/2$), and the volume was estimated. In practice, these measurements were all highly correlated (tall bushes always have a large substrate surface) and our main results were not affected by the use of one parameter in isolation *versus* various combinations of them. For simplicity we essentially characterized the bush using their height because a tall bush provides more shade than a short one whilst the remains of a recently cut bush can occupy an important surface on the substrate without providing much shade. Based on field observations we defined three main classes of bushes. Small jujube bushes corresponded to highly degraded plants: maximal height was less than 1.0m, the foliage was very sparse and thus the soil was always visible through the bush. Medium bushes were characterized by a height between 1.0 m and 1.5 m, with at least part of the soil hidden by the foliage. Large bushes were taller than 1.5 m and the foliage was dense enough to provide permanent shade on most of the surface covered by the bush. Evidences of recent wood cutting were noted. The position of each jujube bush was determined using a GPS (Garmin III; Chicago USA).

### Thermal characterization of the micro-habitats

Open rocky habitats and spiny bushes represented the two major types of microhabitats available for adult tortoises. Thermal characteristics of microhabitats were assessed from October 2008 to September 2009 in a zone situated in the center of the general study site (study area #1, Figure 1). For that we deployed temperature data loggers (iButton, Thermochron®, Maxim, Dallas Semiconductor, USA, accuracy ± 0.5 degrees C°) as follows: two were placed in the open, two were placed in small jujube bushes, two in medium size jujube bushes, and two in large jujube bushes.

Moorish tortoises spend almost all their time sheltered under bushes (El Mouden et al. 2006), therefore we recorded the thermal characteristics of models representing adult tortoises resting under bushes. For that we used six models fitted with two temperature data loggers. One logger was glued on the shell, a second logger was inserted inside the model in a central position. The models were made with empty (but intact) adult tortoise shells collected in the study site. The shells were consolidated using small amounts of resin. Each model was then filled with a hydro-gel (agar-agar) poured into a plastic bag positioned inside the shell. The mass of each model was adjusted using the regression between body mass against body size (shell length) obtained on more than 200 tortoises. The internal temperature logger was placed after the solidification of the gel. The model was then carefully sealed.

We examined the accuracy of the models in estimating the internal temperature of living tortoises. To do so, we generated 6 pairs, with one model associated to one living tortoise. For each pair, the deviations between the physical model *versus* living animal were low in terms of shell mass (mean deviation 2.7 ± 7.3% [±SD], N = 6 pairs) and in terms of shell length (5.6 ± 4.1%). The pairs were stored in a climatic chamber at 20°C for at least 12h and then exposed to the sun in a 10 m x 25 m outdoor enclosure without shelter. The internal body temperature of the living tortoises was recorded every 10 min using a probe thermometer inserted into the cloaca (5 cm). We stopped the experiment when body temperature reached 38°C, or before, if we detected any preliminary sign of overheating such as salivation (Hailey and Coulson 1996). The comparison of the temperature recorded showed that the models provided values very similar to those recorded in the tortoise. Internal body temperature measured in the tortoises and in the models were indeed highly correlated (mean $r^2$ values [Pearson correlations] = 0.79 ± 0.2, range 0.52 - 0.98). In practice, the models slightly, albeit systematically, underestimated the internal temperature of the associated living individual (t-test comparisons between tortoises and physicals models, all $p<0.0001$); the mean difference was of 2.12°C ± 0.31 (SE). Such underestimation was probably caused by the physiological capacity of the tortoises to limit over-heating (Galli et al. 2004). Consequently, for the current study, we fixed the upper threshold temperature at 38°C for body temperatures, hence at 40°C for the models to take into account underestimation.

Three models were placed into small jujube bushes (less than 1 m height); three others were placed into a large jujube bushes (>2 m height). The small jujube bushes did not provide a strong shade whilst the large jujube bushes had thick foliage. We also placed eight temperature data loggers in open habitats (directly on the rocky substrate) and within other jujube bushes of various dimensions (from small to large bushes). The data loggers were programmed to record one temperature value every 60 minutes (hence they were regularly downloaded).

## Habitat use by the tortoises

Morphological and ecological characteristics of *Testudo graeca soussensis* in the Jbilet mountains have been previously assessed (Ben Kaddour 2005; El Mouden et al. 2006; Slimani et al. 2001; Lagarde et al. 2008; Sereau et al. 2010). This species is diurnal, exhibits a peak of activity in spring (main period of sexual activity), and forages on various ephemeron herbaceous plant species that develop following late winter and early spring rainfalls.

In one study area (#1, Figure 1), we fitted 6 adult tortoises (3 males and 3 females) with long life (>1 year) radio transmitters (AVM Instrument Company, Colfax, CA, USA). The electronic devices were glued on the shell using very small amounts of resin; the overall mass of the equipment was always less than 4% of tortoise body mass. This load was well tolerated by the tortoises and did not cause any detectable trouble to the animals (Lagarde et al. 2003a, b, 2008). The tortoises were located on a weekly basis during one year (from early November 2008 until late October 2009) with a 4-element Yagi antenna connected to a LA12Q-AVM receiver and their position was noted using GPS. Each individual was located on an average of 50 occasions (mean = 49.7 ± 2.4, range 45 - 51). The broad activity of each tortoise was then recorded (sheltered, active, feeding) along with the microhabitat (open habitat, under a bush). The current study did not aim to precisely record behaviors (Lagarde et al. 2008), only the instantaneous activity of individuals was recorded (e.g. a tortoise firstly observed walking and feeding later was considered as walking only). At the end of the study we removed the equipment by gently pushing the blade of a knife between the shell and the synthetic resin (Lagarde et al. 2008). None of the tortoises presented any damage on the shell.

## Transects: number and dimensions of jujube bush *versus* tortoise occurrence

We assessed the occurrence of tortoises in each of the 36 subareas (hence in the 12 selected study areas) using visual transects. During one to two hours, the surface of each area was intensively searched by one person (TL) during the estimated main activity period of the tortoises (Lagarde et al. 2008). Each tortoise observed was counted only once owing to the low velocity of the species. Because tortoises are often sheltered, and hence not easily detected in the field, we performed three transects in almost all subareas, except for the subarea 7 where only 2 transects were performed (Total N = 101 transects, 8.5 transects per study area on average).

### Analyses

Prior to analyses, thermal data were visually inspected to remove outliers. Our objective was not to describe and analyze the thermal profiles in the course of the year; instead we focused on potentially lethal temperature episodes. The precise lethal body temperatures (critical thermal minima and maxima) of the studied species are not known, and we did not attempt to measure them for ethical reasons. Based on studies performed on reptile species including chelonians (Hutchison et al. 1966; Webb and Witten 1973; reviews in Bartholomew 1982; Lillywhite 1987; Peterson et al. 1993) we confidently estimated that exposure to prolonged periods (> 1 hour) of ambient temperatures at 40°C are unsafe and that a prolonged central body temperature above 38°C precipitates dehydration and can be eventually lethal (there is no record of reptiles surviving to an elevation of body temperature above 44°C). Most reptiles can easily tolerate ambient temperature close to 0°C for prolonged time periods, critical lower body temperatures are generally markedly negative (Bartholomew 1982). We emphasize that we distinguish central (internal) body temperature, from external (cutaneous) body temperature that can momentarily reach values above 40°C or below -5°C without visible damage, especially in relatively large species such as terrestrial chelonians (McGinnis and Voigt 1971; Webb and Witten 1973). The main consequence of focusing on critical minima and maxima of body temperatures is the straightforwardness of the analyses: no individual can survive a single episode of prolonged overheating (e.g. > 45°C during one hour). Therefore the detection *versus* the lack of detection of potential overheating episodes is a sufficient criterion to assess the compatibility of micro-habitats in terms of tortoise survival, and a visual inspection of the thermal profiles is a suitable method for this. Home ranges were calculated by the method of the minimum convex polygon (MCP). Homes ranges and movements were assessed using ArcGIS 9.3 (Esri, New York, USA). Statistical tests were performed using STATISTICA 7.1 (Statsoft, Tulsa, USA).

## Results

### Climate

Mean monthly temperature and precipitation values, along with monthly extreme temperatures show that the region of the study zone is arid and hot: scarce and irregular rainfalls occurred essentially between November and May whilst the summer period (June to

September) was characterized by very low rainfall and high ambient temperatures. Although maximal ambient temperatures recorded under shelters regularly exceeded 40°C in summer, minimal temperatures always remained above 0°C in winter.

## Thermal characteristics of micro-habitats

As expected, the temperatures recorded by the data loggers positioned in the field (N>74,000 temperature readings) during the study period were strongly influenced by seasons, time of the day, and the type of microhabitat, and they followed the average temperature fluctuations recorded over longer time periods. Because the current study focuses on extreme (potentially lethal) temperatures, we examined exclusively relevant patterns, notably those recorded during the coolest and hottest periods. The minimal and maximal ambient temperatures recorded in the open habitat (i.e. 1.5°C *versus* 48.0°C) were obtained respectively in winter (January) and summer (August); extreme temperatures recorded within small and medium sized bushes were relatively similar (Table 1). However, a closer inspection of the data revealed significant differences: for instance selecting the temperatures below 3.5 °C the means were 3.46 ± 0.25 (N=20) versus 3.98 ± 0.32 °C (N=15) in the small and medium sized bushes respectively (T-test P<0.01). Importantly, although statistically significant such differences remained weak in terms of mean values. In comparison within large bushes, considerably buffered temperature values were recorded (Table 1, *a fortiori* further comparisons provided highly significant values, not reported here for conciseness). Models provided higher maximal values, not lower however, with marked differences between the temperatures recorded on the dorsal surface of the shell (hottest) *versus* inside the model (more buffered, Table 1). The number of values ≥40°C varied markedly between habitats, and was always larger using models rather than using the data loggers alone (Table 1). The only data loggers that never recorded temperatures above 40°C were inside the models situated into the large bushes.

Inspection of the whole data set enabled us to discard low temperatures as a potentially lethal factor. Indeed, we never recorded any temperature below 0°C. Consequently we focused on the patterns recorded during the hot periods. For that, we pooled the 16 hottest days when ambient temperature exceeded 40°C for more than 5 hours. During the hottest periods, the mean ambient temperatures recorded in open habitat, small bushes and medium bushes regularly exceeded 40°C. By contrast, temperature remained systematically below

40°C under the large bushes.

Compared to the data loggers directly positioned in the field, the models accumulated heat at a greater rate when exposed to solar radiation (Figure 2). We found marked differences between external (on the shell) and internal (within the model) temperatures. External temperatures fluctuated over a larger range of values, peaking during the day and cooling at night and they exhibited faster variations over time, especially at sunrise and sunset. Importantly, the internal temperature of the physical models situated into the large bushes never reached 40°C (max 38.5°C).

In order to assess the duration of the potentially lethal overheating episodes, we visually inspected the hottest daily patterns. Figure 3 illustrates that during sun-scorching periods, the only micro-habitat where estimated internal temperatures of models remained under 40°C was represented by large bushes. In all other micro-habitats, hot episodes lasting several hours with ambient temperatures or estimated external and internal body temperatures markedly above 40°C (sometimes above 45°C) were observed; the models placed in these micro-habitats presented particularly long periods with "body" temperatures (internal estimate) above 50°C.

### Habitat use by the tortoises

During the hottest and driest periods (late May to early September) 100% of the radio-tracked tortoises were found sheltered under large bushes, they did not exhibit any detectable movement, and they were located at exactly the same spot every week suggesting that they remained immobile between observations. During mild periods, notably spring and autumn, the tortoises spent most of their time under the bushes but regular movements were detected (Table 2 and 3). Three individuals visited a small wheat field situated in the study area where they spent an important amount of time (31% to 50% of the fixes). More than 80% of the jujube bushes used by the radio-tracked tortoises were large, exceeding three meters in height. The few oleanders (*Nerium oleander*) also used by the tortoises were taller than two meters. The tortoises were observed in the open on less than 10% of occasions.

Behavioral observations provided complementary results (Table 3). On most occasions (89%) the tortoises located under shelter (essentially jujube bushes and wheat field) were immobile and often partly buried. Although our displacement data (see below) show that none of the monitored individuals remained under a single refuge, the tortoises were rarely observed moving in the open, suggesting that movements between shelters were relatively rapidly achieved.

Minimum convex polygon (MCP) home ranges represented less than 1/3 of a hectare on average (mean ± SD = 2,708 ± 3,365 $m^2$, ranging from 360 $m^2$ to 8,877 $m^2$, N = 6). The tortoises travelled 2.5 km on average (mean ± SD = 2,529 ± 920 m, ranging from 1,310 m to 3,877 m). The distance travelled and home range were highly correlated (Spearman rank correlation: $r_s = 0.94$, N = 6, $P<0.05$). Males tended to travel over longer total distances compared with females (3,178 ± 731m *versus* 1,880 ± 564 m) and to exhibit larger home ranges (4,742 ± 3,956 $m^2$ *versus* 674 ± 495 $m^2$) (small sample size precluded robust statistics on these data).

Number and dimensions of jujube bush *versus* tortoise occurrence

During the habitat assessment in the 12 selected study areas (1 $km^2$ each), a total of 1,635 jujube bushes were counted, providing an average density of 1.4 per hectare. Two of the 36 subareas did not contain any jujube bush. Strong differences in the estimated number of jujube bushes were observed among the 12 areas (comparing the actual distribution against a theoretical homogenous distribution, $\chi^2 = 300.8$, df = 11, $P < 0.001$; Table 4). The size (height) of the jujube bushes also varied greatly among study areas (ANOVA with jujube bush height as the dependent variable, the study area as the factor, and subarea as a random factor: $F_{11, 22} = 10.40$, $P < 0.001$). The number of bushes counted per area did not correlate with the mean size (height) of jujube bushes ($r = 0.42$, $F_{1, 10} = 2.09$, $P = 0.179$); this result held true using subareas (N = 34 with bushes) instead of study areas (N = 12) to increase the power of the analysis ($r = 0.28$, $F_{1, 32} = 2.80$, $P = 0.104$; Figure 4). For example the study area#4 contained approximately the same number of otherwise smaller bushes than the study area#6 (Figure 4). Using other characteristics of the bushes (volume for instance) led to similar results.

The probability of finding a tortoise increases with bush size (logistic regression with mean bush height as the independent variable and the presence/absence of tortoise as the dependent variable: $\chi^2 = 10.95$, df = 1, $P = 0.001$) and with bush number ($\chi^2 = 3.73$, df = 1, $P = 0.053$). The combination of these two independent variables (height and number) did not significantly improve the model however (both independent factors included: $\chi^2 = 12.46$, df = 2, P = 0.001), suggesting that bush quality (i.e. height) was the main factor involved. The mean height of the bushes positively influenced the number of tortoises detected in the field (Spearman rank correlation: r=0.638, N=34, P<0.05). A closer inspection of the data suggested the existence of a bush-height threshold of 1.25 m for the occurrence of the tortoises (Figure 5).

## Discussion

Our results provide strong evidence that during hot and dry periods that characterize arid zones of Morocco, the sole microhabitat that can provide safe shelters to adult Moorish tortoises are bushes taller than 1.25 m (i.e. 'large bushes' in our study). In the absence of such relatively large bushes the tortoises are under serious threat of lethal overheating, indeed ambient and estimated body temperatures largely exceeded 40°C for prolonged time periods in all other microhabitats. Therefore, the short term (hours in summer) survival of the tortoises tightly depends on the presence of relatively large bushes; all individuals use this specific microhabitat intensively all year round, and exclusively in summer. Surveys in the 12 different study areas with contrasted vegetation cover provided complementary information: the probability to detect a tortoise was very low (close to zero) in the study areas where the bushes were smaller than 1.25 m, and this probability markedly increased in the study areas with taller bushes. The number of tortoises detected was also positively correlated with the mean height of the bushes. The importance of shelters, notably provided by plants and burrows, as a means to control for temperature fluctuations experienced in an extreme environment has been documented in desert tortoises (Bulova 2002; McMaster and Downs 2006) and in other reptiles (Beck and Jennings 2003; Bonnet et al. 2009; Grillet et al. 2010; Lelièvre et al. 2010), however our study is the first to report information demonstrating the vital importance of precise characteristics of the microhabitat.

Our results have strong conservation implications. The short term maintenance of tortoise populations requires the retention of jujube bushes rather than their removal. In our 12 study areas we counted 102 jujube bushes that had been cut to the ground and all of them were situated in the study areas where large bushes and tortoises occur (there is no utility to harvest small bushes). Unfortunately in many places the growth rate of the jujube bushes is very slow (using a crude method of annually measuring the circumference of a subset of bushes, the growth was undetectable over 3 years, unpublished data) due to the combination of regular droughts and overgrazing (goats are fond of jujube green leaves). Consequently, current official encouragements to destroy the jujube bushes should be seriously reconsidered and changed. Ideally, bush destruction, such as total cutting, should be banned whilst the use of alternative sources of energy (e.g. solar accumulators) should be promoted, at least in the richest areas in terms of biodiversity. Our results may prove valuable in the set up of surveys using satellite image analyses as large bushes are clearly visible (Pringle et al. 2009; Figure 1). Potentially important populations of tortoises could then be more rapidly localized and thus monitored in the long term. Replanting programs could be better organized to reinforce and/or (re)connect disjointed populations.

On a positive note we observed that even small areas (1 km²) sheltered tortoises and thus setting up appropriate small natural reserves (e.g. several square kms) is likely sufficient, at least in the short term, to retain functioning populations. The mean home ranges of the individuals we monitored were indeed relatively small (4,742 m² for males and 831 m² for females). Thus, urgent practical actions can be imagined with limited funding. The other side of the coin is that species exhibiting small home ranges, and with limited travelling capacities, are particularly vulnerable to habitat destruction and fragmentation (Pimm and Raven 2000). Thus we emphasize that small sized natural reserves cannot be considered as a panacea, but rather as an immediate efficient option to target important areas. Our main study areas (e.g. #1) used for long term population monitoring, where approximately 30 hectares are suitable to the tortoises, provides a typical example. This small area does not benefit from any conservation status and it is thus vulnerable, but a modification is conceivable (small surface means fewer conflicts for the use of the land) and would be efficient (the resident tortoise population is important; Slimani et al. 2006). Practically, our data suggest that to provide suitable microhabitats the total surface covered by large bushes in the selected areas must be greater than 800 $m^2$ per square kilometer whilst the mean distance between large bushes should be maintained below 400 m (unpublished).

Examination of the potential caveats of the current study suggests that the analyses were conservative. Firstly, we focused on a particular region of Morocco, but global climate projections predict a rapid elevation of the mean temperatures in most regions of the Maghreb (IPCC; http://www.ipcc.ch/) reinforcing the relevance of our analyses based on ambient temperatures. Secondly, one may argue that our surveys were not sufficiently efficient to accurately assess presence *versus* absence of secretive and often hidden animals. However, in our study areas tortoises can hardly dig into the rocky substrate, except the soft soil present at the feet of large bushes and in the cultivated field (e.g. recently managed wheat field), and thus they are often forced to stay at the surface where their shiny shell is easily detected. By contrast, the tortoises sheltered into large jujube bushes where they can bury themselves remain invisible most of the time, requiring intensive effort to discern them. Therefore, the probability to detect a given individual was far greater in open areas compared to bushy areas. Consequently, our counting markedly underestimated the abundance of tortoises in the high quality habitat, but likely not in the bare soil study areas. Thirdly, our thermal profiles were obtained on immobile objects, thereby missing the fact that living individuals can navigate between shelters. However, our data show that remaining sheltered under a poor quality bush (<1 m) was insufficient to escape lethal overheating, and moving in between shelters would worsen the situation because the tortoises would have then accumulated heat at a greater rate and suffered from dehydration, precipitating death. In a parallel study (unpublished) we evaluated to 400 m the maximal distance that an adult tortoise can travel before overheating under various situations. During hot periods, individuals must move between large bushes separated by less than 400 m in a straight line and without stopping. Low quality habitat areas do not offer such a possibility. In addition, we did not measure dehydration, energy budget and we neglected small and more vulnerable individuals; all these factors add further constraints on the thermal biology of tortoises. The fact that during summer all the radio-tracked individuals remained well sheltered under large bushes supports the notion that in natural conditions they must not simply avoid lethal overheating but likely save water and energy. Finally, we observed females laying their eggs exclusively in the soft and well protected soil within large bushes. Overall, all the available information indicates the crucial role of the large bushes.

Finally, we did not directly measure lethal body temperatures; for instance by forcing individuals to stay in the open during hot days. Instead we relied on physical models that exhibited strong overheating patterns. The rapid heat accumulation observed in the models compared to the data-loggers alone is a well known process: relatively large objects with high water content indeed accumulate heat as solar accumulators (Porter et al. 1973; Fasulo et al. 2001). The tight correlation and the strong similarities we observed between the thermal patterns of the living tortoises *versus* models exposed to the sun during the testing phase mean that although living tortoises possess physiological means to control body temperature (salivation, modification of the internal blood circulation; Stevenson 1985; Bradshaw 1997), such capacities are limited. The patterns we provided are thus realistic: a tortoise exposed to the sun during a hot day probably accumulates heat and rapidly reaches lethal temperatures.

In the Jbilets Centrales, the jujube bushes are still abundant (Louzizi 2006). The importance of these bushes as shelter, although only assessed so far for *Testudo graeca soussens*is, is very likely for different (many?) species of animals living in this arid region. Jujube bushes may also help to control the rate of desertification and soil erosion in desert areas (Depommier, 1988; Rsaissi and Bouhache 2002). Overall, various arguments can be invoked to convince authorities to better protect this bush. Fortunately jujube bushes are extremely vigorous (Mader 2003), and they have a strong colonization potential. Usually, locals harvest the parts above the surface while the root system remains intact, which can penetrate 50 to 80 cm into the soil. This allows the bushes to regenerate (Regehr and El Brahli, 1995). The species also spreads by fruits, fragmentation of roots and other plant parts (Long, 1954; Mader 2003).

In conclusion, by targeting a single bush species a series of practical options is conceivable: a) modification of official agricultural and educational programs, b) encouragements to change bush overharvesting (e.g. promoting the use of other sources for cooking, and fence building), c) setting up small natural reserves, reinforcing and connecting populations via replanting actions. A better protection of the jujube bushes could rapidly considerably improve the habitats and the status of the biodiversity in various arid Mediterranean areas.

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Table 1: extreme temperatures recorded in the four main microhabitats used by the tortoises (source). The temperatures were recorded using data loggers (one record per hour) alone (= ambient temperature of the microhabitat) or using physical models (=estimate of the body temperature of a tortoise in the microhabitat). The month of the record is indicated along with the number of data points exceeding a threshold of 40°C. “External” refers to data logger placed on the shell of the tortoise model; “internal” refers to data loggers placed inside the model.

| Source | Minimal | Month | Maximal | Month | N>40°C |
|---|---|---|---|---|---|
| Open habitat | 1.5 | Jan | 48.0 | Aug | 83 |
| Small bush | 2.0 | Dec | 50.5 | Aug | 110 |
| Medium size bush | 2.0 | Dec/Jan | 47.5 | Aug | 65 |
| Large bush | 5.0 | Jan | 42.5 | Aug | 19 |
| Model inside small bush, external | 0.5 | Jan | 73.5 | Aug | 393 |
| Model inside small bush, internal | 4.8 | Jan | 59.0 | Aug | 222 |
| Model inside large bush, external | 4.5 | Jan | 55.0 | Aug | 105 |
| Model inside large bush, internal | 5.5 | Jan | 38.5 | Aug | 0 |

Table 2: mean proportion of time spent in the main microhabitats used by 6 females and 6 males monitored in the field during one year (2008-2009). Each individual was briefly observed once per week. Data are expressed as mean percentages of the total of observations (N=298). The tortoises were localized 71.5% of the time under bushes.

| Microhabitat | Mean % ± SD |
|---|---|
| Acacia bush | 1.1±2.4 |
| Jujube bush | 60.2±22.0 |
| Oleander | 9.1±19.0 |
| Retam bush | 1.1±2.5 |
| Wheat field | 16.9±12.3 |
| In the open | 7.8±2.9 |
| Other | 3.87 |

Table 3: Main activities recorded in 6 females and 6 males monitored in the field during one year (2008-2009). Each individual was briefly observed once a week. Data are expressed as mean percentages of all observations (N=298).

| Attitude | Mean % ± SD |
|---|---|
| Basking in the sun | 8.0±3.1 |
| Buried and partly visible under shelter | 26.2±12.6 |
| Immobile under shelter | 38.6±12.3 |
| Invisible within shelter | 24.2±18.7 |
| Sexual activity | 0.3±0.8 |
| Walking | 2.7±2.9 |

Table 4: number and surface area of the jujube bushes, and number of tortoises observed during transects in 12 areas monitored.

| Selected study areas | Number of jujubes | Surface occupied ($m^2$) | Avg. surface of jujubes | Number of tortoises |
|---|---|---|---|---|
| Area 1 | 213 | 5468.2 | 25.7 | 26 |
| Area 2 | 176 | 1401.5 | 8.0 | 11 |
| Area 3 | 134 | 2016.4 | 15.0 | 8 |
| Area 4 | 166 | 331.8 | 2.0 | 0 |
| Area 5 | 180 | 1517.6 | 8.4 | 5 |
| Area 6 | 192 | 5934.3 | 30.9 | 5 |
| Area 7 | 38 | 668.2 | 17.6 | 0 |
| Area 8 | 195 | 1788.6 | 9.2 | 0 |
| Area 9 | 38 | 43.1 | 1.3 | 0 |
| Area 10 | 84 | 254.6 | 3.1 | 0 |
| Area 11 | 127 | 1752.9 | 13.8 | 2 |
| Area 12 | 92 | 871.6 | 9.7 | 1 |

## Captions to figures

Figure 1: The 12 study sites (1 km² square each) are indicated (A, North of Marrakech, Morocco). The vegetation of the different study sites covers a range of highly degraded habitats (B, e.g. study area # 4) where most of the bushes have been destroyed to less degraded habitats (C, e.g. study area # 1) where large jujube bushes can be observed. D: enlargement of the 1 km² study area #1; large jujube bushes are easily identified especially in the small valleys (eucalyptus trees are visible along the road).

Figure 2: Comparison of a) ambient temperatures recorded using data loggers alone (top graph) where line pattern indicates the different microhabitats (see legend box), the grey/black color pattern indicates the mean value (black) and the upper 95% interval limit (grey); and b) the mean estimated body temperatures (± SD) using data loggers glued on the back of the shell (i.e. “on the shell”) or implanted into (i.e. “inside the shell”) models placed in two microhabitats (large versus small bushes) (lower graph).

Figure 3: temperature fluctuations in different sized bush during three typical hot days (mid august). Temperatures were recorded using data logger alone (ambient), glued on the top of the shell of physical models (on the shell), and inside the physical model (inside the shell). For clarity, only one temperature profile is presented for the medium sized-bush microhabitat. The horizontal lines indicate a dangerous threshold (40 °C, continuous line) and a lethal threshold (45 °C, dashed line) respectively.

Figure 4: absence of relationship between the mean height (mean ± SE) and the number of jujube bushes in 34 areas (300 m² each) sampled. The grey circles indicated the three subareas sampled in the study area # 6, the three open circles indicates the three subareas sampled in the study area # 4. The dashed lines indicates the limits between small bushes (< 1.0 m), medium sized bushes (1.0 - 1.5 m) and large bushes (> 1.5 m).

Figure 5: relationship between mean bush height and the number of tortoises detected in the

34 subareas that contained jujube bushes. Note that no tortoise was observed in the 2 other subareas without bushes (hence not presented here).

FIGURE 1

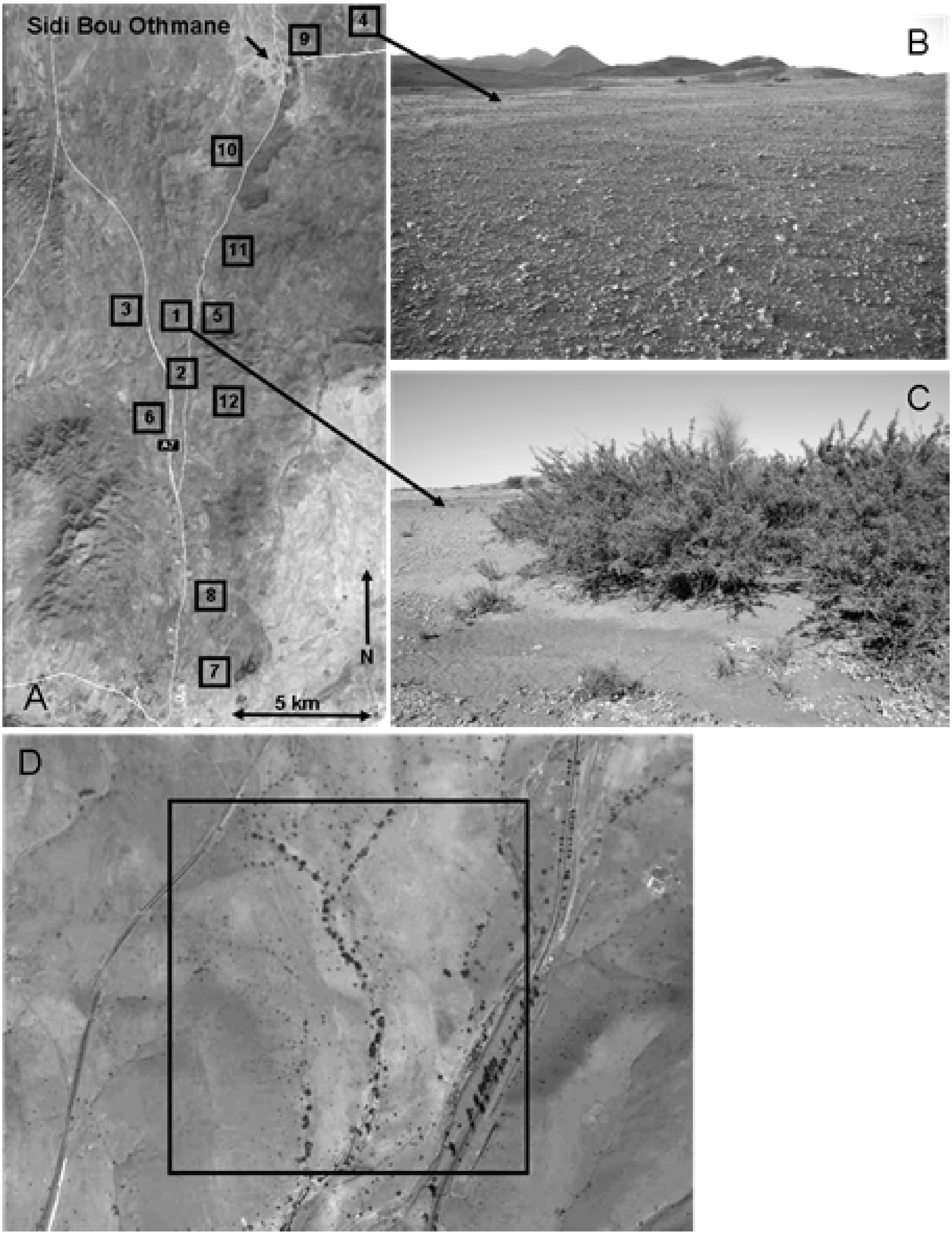


FIGURE 2

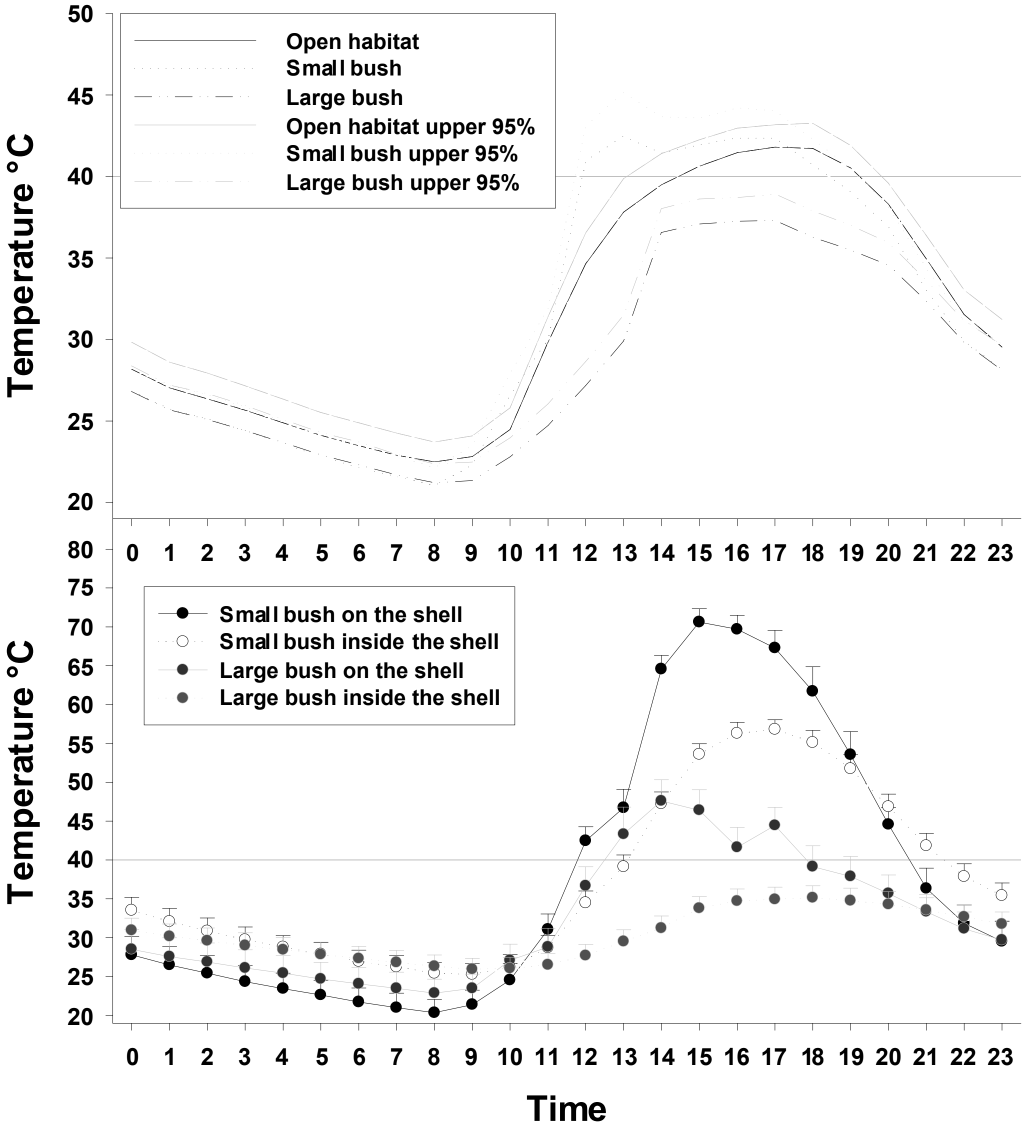

Open habitat
Small bush
Large bush
Open habitat upper 95%
Small bush upper 95%
Large bush upper 95%
Temperature °C
50
45
40
35
30
25
20
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Small bush on the shell
Small bush inside the shell
Large bush on the shell
Large bush inside the shell
Temperature °C
80
75
70
65
60
55
50
45
40
35
30
25
20
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Time

FIGURE 3

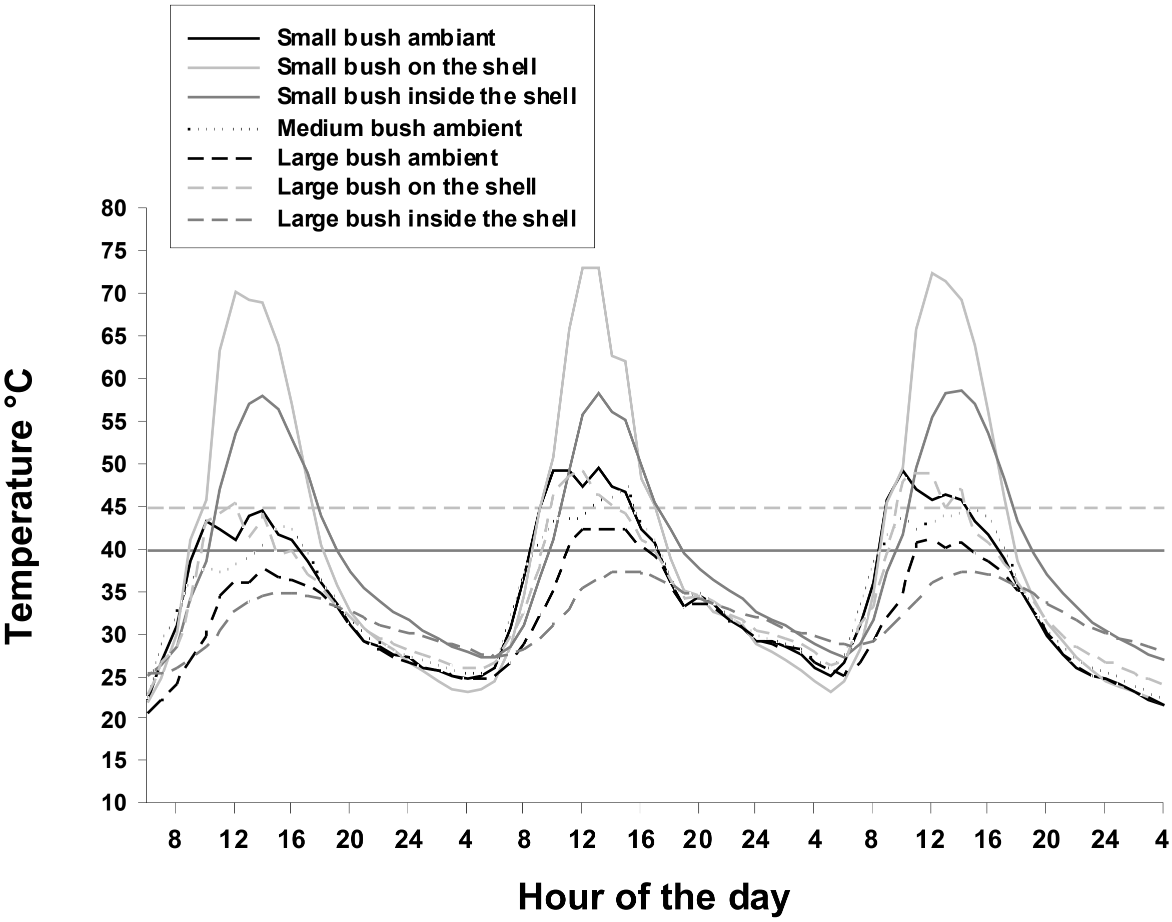

Small bush ambiant
Small bush on the shell
Small bush inside the shell
Medium bush ambient
Large bush ambient
Large bush on the shell
Large bush inside the shell
Temperature °C
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
8
12
16
20
24
4
8
12
16
20
24
4
8
12
16
20
24
4
Hour of the day

FIGURE 4

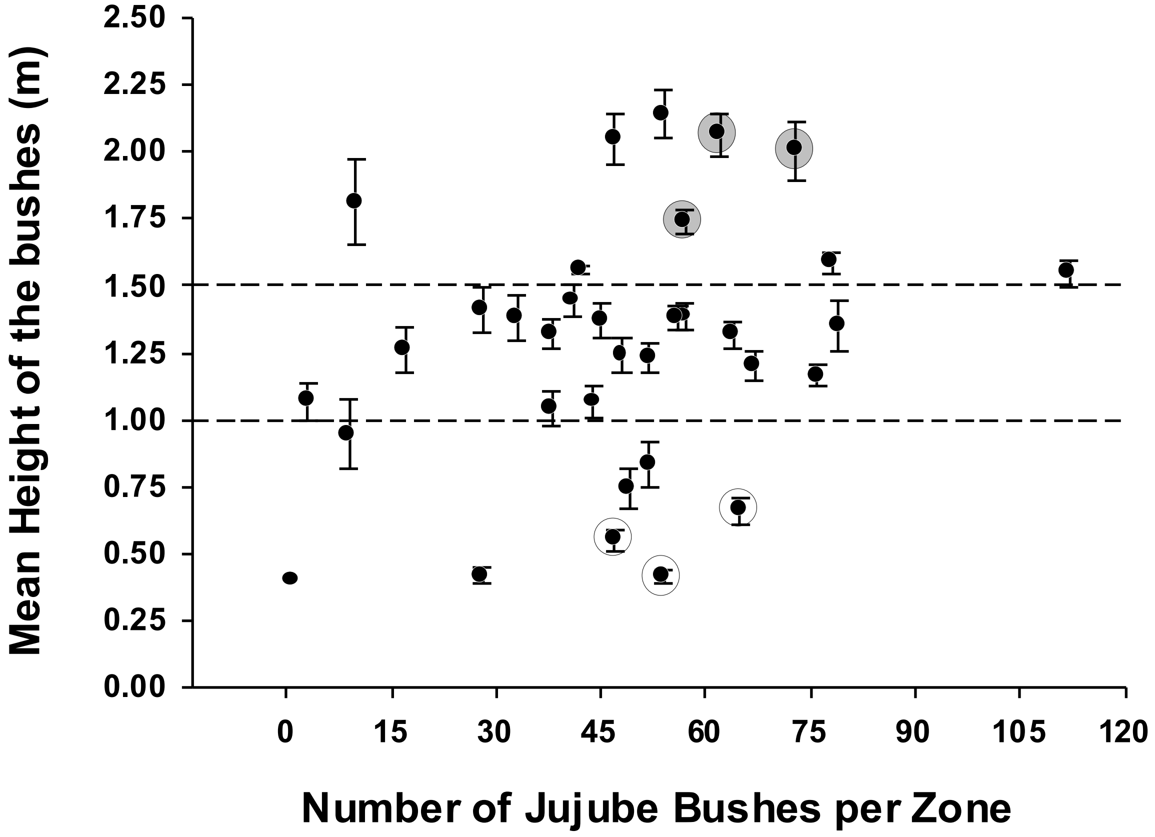
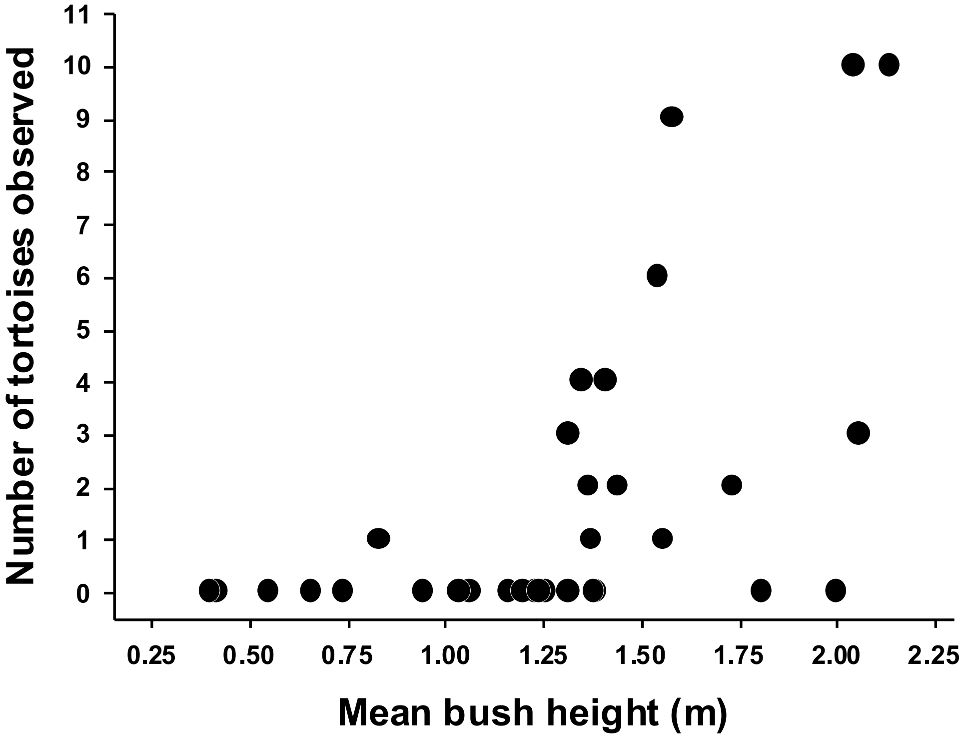

Mean Height of the bushes (m)
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
15
30
45
60
75
90
105
120
Number of Jujube Bushes per Zone

FIGURE 5


Number of tortoises observed
11
10
9
8
7
6
5
4
3
2
1
0
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
2.25
Mean bush height (m)

# 4. How far can a tortoise walk in open habitat before overheating? Implications for conservation

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*Résumé en français :*

Les chéloniens terrestres sont menacés à travers le monde par la destruction de leur habitat et le commerce illégal. Les tortues sont des animaux à déplacements lents susceptible de se déshydrater ou de surchauffer lors de leurs déplacements en milieu ouvert. De nombreuses espèces de vivent dans les steppes arides où les refuges présentant des températures tempérées (e.g. terriers) sont une ressource limitante. La détermination des capacités maximales de déplacement des individus sans prise de risque pendant leur période d'activité est donc déterminante. Nous avons étudié, les relations entre les variations de températures corporelles et les patterns de déplacement d'adultes de *Testudo graeca* dans les steppes arides marocaines. A l'aide de modèles physiques et mathématiques, nos résultats, suggèrent que durant la période d'activité, les adultes ne peuvent parcourir plus d'1km sans sérieux risque de surchauffe. Cependant, les nos résultats de télémétrie suggèrent que les individus libres ont des comportements qui limitent leurs déplacements à 500m entre deux refuges. Globalement, le maintien d'une distance de 500m entre deux refuges (e.g. à travers la plantation de buissons) est essentiel pour limiter la fragmentation de l'habitat et conserver des populations saines. Cette restauration bénéficierait à d'autres espèces dépendantes des refuges que constituent les buissons.

## Abstract

Terrestrial chelonians are threatened worldwide, notably by the destruction of their habitat (e.g. logging) and illegal harvesting. Urbanization, global change, overgrazing, desertification, and habitat fragmentation worsen the situation. Indeed, many tortoise species inhabit arid steppes where the availability of thermally buffered refuges (e.g. burrows) is a limiting factor. Slow moving animals susceptible to dehydration and overheating during displacements in open habitats are especially vulnerable to loss of thermal refuges. Determining the maximum distance between refuges that individuals can safely traverse during the active season is thus essential. We examined the relationship between body temperature variations and displacement patterns in adult *Testudo graeca* in the arid steppes of Morocco. Using physical and mathematical models, our results suggest that during the active season adults cannot travel more than 1 km without serious risk of overheating. However, radio-tracking suggests that free-ranging individuals are behaviorally limited to 0.5 km trips between refuges. Overall, maintaining a distance less than 0.5 km between refuges (e.g. through bush planting) is essential to limit fragmentation and to retain healthy populations. This restoration would also benefit ectothermic vertebrate species.

Keywords:

body temperature, ecophysiology, refuges, tortoises, functional connectivity, conservation management

## Introduction

Monitoring the impact of habitat destruction and fragmentation on animal populations is important (Workman and Bos 2007). However, accurately assessing the population consequences of habitat changes can be an arduous task in the field. For instance, the impact of fragmentation on populations depends on the biological traits of the species (e.g. reproductive mode), on the dynamics of the population (e.g., number of individuals, age structure) and various environmental factors (Caswell 2001; Legendre et al. 2008). Moreover, the effects of fragmentation *per se* are often related with other threats (e.g. resource depletion, invasive species) further complicating analysis. In practice, fragmentation is often described in terms of connectivity between patches whereas habitat loss is characterized through the reduction of resources (e.g. food, shelter). For example the rapid extension of the road network and urbanized areas effectively splits habitats and populations into more or less interconnected discrete functioning units (patches); but within patches, high quality habitats can be nonetheless retained (Votsi et al. 2012).

The degradation of habitat can provoke strong fragmentation of populations even when the landscape is not intersected by a network of barriers (e.g. roads, dams) (Pe'er et al. 2005). For example, many animal species depends on refuges to escape biotic (e.g. predation) and abiotic (e.g. dehydration) threats (Ultsch and Anderson 1986; Schwarzkopf and Alford 1996; Roper et al. 2001; Bulova 2002; Berryman and Hawkins 2006). Consequently, even in an apparently homogenous landscape, a rarefaction of appropriate refuges (e.g., thick bushes, logs, rocks) can generate inconspicuous barriers that isolate individuals or sub-populations (Pe'er et al. 2005). Although not immediately visible (i.e. compared to roads) decreasing refuge density can be highly detrimental. Organisms with limited dispersal abilities are dependent on the quality and density of appropriate refuges (Lagarde et al. 2012). The effects of fragmentation should be submissive to a refuge density thresholds where individuals cannot cross the invisible barriers represented by the distance between refuges.

Terrestrial chelonians provide typical examples of poorly mobile species that depend heavily on the availability of suitable refuges (Bulova 2002). Tortoises are declining worldwide, and many species live in arid steppe habitats where the landscape is superficially homogenous (Buhlmann et al. 2009). During the active season individuals navigate between thermally buffered refuges to escape overheating and unsurprisingly the density of these refuges is a major determinant for the presence of the tortoises (Lagarde et al. 2012).

Assessing the degree of connectivity among populations often requires considerable research effort in the field (e.g. using large scale mark-release-recapture studies or large scale genetic studies) (Hailey 1989; Hailey and Coulson 1996a; Stevens et al. 2004). Measuring the distance that individuals can safely traverse between refuges (or patches) provides a direct means to address this issue and to propose accurate practical actions to improve habitat quality.

We investigated this question in the Moorish tortoise (*Testudo graeca soussensis*) in the arid steppes of central Morocco where tortoises population viability is highly linked to shrub density (Lagarde et al. 2012), threatened by illegal trade (Znari et al. 2005) and where thermal refuges are destroyed by farmers through land use practices (Ben Kaddour 2005), overgrazing (El Mouden et al. 2006) and firewood production (Ben Kaddour 2005; El Mouden et al. 2006). Overheating represents the main threat for individuals during movements due to the open nature of the steppe habitat; the tortoises are directly exposed to solar radiation and they rapidly accumulate heat (Spotila et al. 1973; Bailey and Highfield 1996; Lagarde et al. 2012). Lethal overheating can theoretically occur within a few hours if the tortoise cannot reach a thermally buffered refuge (Gans and Dawson 1976). We estimated the maximum distance that a tortoise can move at different periods of the day using physical models (Vitt and Sartorius 1999; O'Connor et al. 2000; Shine and Kearney 2001; Lelievre et al. 2011) to estimate how long tortoises could be exposed to solar radiation and coupled these results to tortoise's locomotors performances as a function of their internal temperature. We then compared estimated distance with distance done by free ranging animals for which we estimated internal body temperature using a simple mathematical model we developed.

## Methods

### Study site

Fieldwork was conducted in April 2008 in the central Jbilet Mountains, 25 km north of Marrakech in Morocco (31°37' N, 8°02' W, 580 m a.s.l.). The region is arid (mean rainfall 240 mm) with precipitations falling essentially between September and February (Le Houérou 2001; Ben Kaddour 2005; Znari et al. 2005). In our study area, high (summer) and low (winter) ambient temperatures reduce tortoise activity (Lagarde et al. 2008). In spring, the main active season, air temperatures are often >35°C and the ground surface can reach much higher (> 60° C). Although the tortoises are not entirely in contact with the ground during movements (only the soles of their feet), they are exposed to the hot air layer that forms above ground. The evergreen vegetation where the tortoises can shelter is mainly composed jujube shrubs (*Ziziphus lotus*), retams (*Retama monosperma*), acacia (*Acacia gummifera*) and oleanders (*Nerium oleander*) (El Mouden et al. 2006). Due to overgrazing and over-harvesting of fire-wood, many bushes are destroyed and refuge availability is limited in most places (Lagarde et al. 2012). The substrate consists of a hard schistose surface that remains bare for the majority of the year. Following rainfall, an ephemeral herbaceous layer is present for only a few weeks but the hard schistose substrate remains bare for the majority of the year (El Mouden & al. 2006).

### Study species

The Moorish tortoise (*Testudo graeca soussensis*) is an endemic species threatened by illegal trade (Dodd and Franz 1993) but also by habitat destruction and fragmentation (Bailey and Highfield 1996; Lagarde et al. 2003; Lagarde et al. 2012). In the central Jbilets, tortoise are surveyed since 2000 and their occurrence is closely associated with jujube shrub density (Lagarde et al. 2012). In contrast to several other terrestrial tortoises, *Testudo graeca* does not dig burrows but shelters (sometimes half-burried) in thick vegetation (Cloudsley-Thompson 1970; Hailey and Coulson 1996b; Lagarde et al. 2002; Ben Kaddour et al. 2006; Nussear et al. 2007; Lagarde et al. 2012) and jujube bushes protect the herbaceous vegetation from overgrazing by livestock and thus provide food (Bailey and Highfield 1996; Ben Kaddour 2005; El Mouden et al. 2006). In addition, jujube shrubs retain a soft soil where the tortoises can lay their eggs (Bailey and Highfield 1996; Lagarde et al. 2012). The Moorish tortoise is diurnal (Lagarde et al. 2008) and activity is concentrated during spring and autumn when climatic conditions are relatively temperate (Lagarde et al. 2012). The spring activity is maximal in April (our study period) due to the mating (male activity) and egg-laying (female activity) season (Ben Kaddour 2005; Lagarde et al. 2008; Lagarde et al. 2012).

### Body temperature estimate

To limit behavioral and physiological perturbation due to surgery (which could falsify our results), we decided not to implant thermal data loggers into the body cavity of the subjects

(Edwards 2005). To estimate central body temperature (*Tb*) we built a simple mathematical model based on the easily recordable dorsal temperature (*Td*) and the initial internal temperature. We used the approach previously developed by Delmas et al. (2008) to determine nest temperature of turtles. We notably implemented distinct heating (α) and cooling (*β*) conductivity coefficients for the heating and cooling phases (Porter et al. 1973; O'Connor 1999; O'Connor 2000; Christian et al. 2006). Tortoises rapidly accumulate heat during sun basking episodes (active thermoregulation) but they cool down slowly when they retreat into their refuge (Porter et al. 1973; Lagarde et al. 2012). We also integrated individual body mass (*m*) as suggested by Christian et al. (2006) in equation 1 where $\Delta t$ is the time step.

$$Tb_t = Tb_{t-1} + (Tb_{t-1} - Td_t)(e^{\alpha m^{\beta} \Delta t} - 1) \quad \text{(eq.1)}$$

Both coefficients α and β were fitted using maximum likelihood for heating and cooling patterns derived from measurements obtained on physical model (see below).

Lagarde et al. (2012) showed that temperature measured in physical models as described below, underestimated the body (cloacal) temperatures recorded in living individuals by approximately 2°C (mean $r^2$ values (Pearson correlations) = 0.79 ± 0.2, range 0.52-0.98, for comparisons between 6 pairs of tortoises and physical models of similar size.), regardless of temperature conditions (see Lagarde et al. 2012 for details). Likely because (1) physical models have never the exactly the same shape et size as alive individuals they are compared with ($r^2 = 0.52$ for the most different pair in shape and size, $r^2 = 0.98$ for the most similar (Lagarde et al. 2012)), (2) hydrogel do not have the same thermal behavior as living tissues and (3) physical models cannot reproduce physiological regulations (O'Connor 1999; Vitt and Sartorius 1999; Angiletta et al. 2002). Therefore, we considered that an internal physical model temperature of 38° C corresponded to a critical body temperature of 40° C for a living specimen (Hutchison et al. 1966).

We tested the accuracy of the mathematical model by comparing internal physical model temperature and estimated *Tb* (mathematical model) using 16 physical models fitted with both external and internal data loggers exposed to alternating of shade and solar radiation over 6 days under various climatic conditions (cloudy and sunny days in combination with presence or absence of wind). We used empty shells (consolidated with small amounts of resin) collected in the study site. The physical models were filled with a hydro-gel poured into a plastic bag placed inside the shell. The mass was adjusted according to the linear regression between body mass and shell length (both log-transformed) of free ranging tortoises captured in the study site other the last 8 years (N>200; see Lagarde et al. 2012). We glued 3 temperature data loggers (thermochron iButtons, Dallas Semiconductor): one on the top of the shell, one under the shell (ventral position) and one inserted in the gel at the center of the model. The model were then stored during at least 12h before temperature records. For $\Delta t = 5$ min, $\alpha$ and $\beta$ were fitted to - 0.0217 and - 0.8804 for the heating phase and -0.0490 and -0.9980 during cooling phases respectively (Fig. 1). As expected, the linear regression between measured (physical model) and estimated *Tb* (mathematical model) suggested that the model provided accurate estimates ($r^2 = 0.993$; slope = 0.996). The differences between estimated and observed values were - 0.24 ± 0.02° C (mean ± SD), 95% of the values were contained in a short interval (- 3.14° C - 3.84° C), and they were normally distributed. The strongest differences usually occurs at the early beginning of the cooling phases were the mathematical model models more thermal inertia than physical models (Fig. 1).

## Locomotors performances

We measured walking speeds in the field around midday (10:00 – 15:00) during sunny days in spring when the tortoises are naturally the most active (Sereau et al. 2010). We captured 6 adult females and 8 adult males. The tortoises were tested in a flat familiar environment. The tortoises were kept in the shade (in large bushes) at least 3 hours before the trials. For each trial we released the tortoise in a flat open area 5 m from a jujube bush, the head oriented toward the bush. Following varying latency periods (0 – 10 min) of immobility, the tortoises walked away to escape the observer in search of a refuge. We then recorded the time required to reach the bush. The tortoises systematically moved in a straight line. We also recorded both cloacal temperature and air temperature 5 cm above ground. To monitor short term variations of *Tb* we repeatedly tested individuals: the tortoise was then replaced at the start point and the trial was repeated until body temperature stabilized or reached 37.5° C (2 to 37 consecutive trials performed/individual depending on weather conditions). The 37.5° C threshold corresponds to the maximum central body temperature measured in free-ranging individuals (Bailey and Highfield 1996) beyond which overheating can occur (Lagarde et al. 2012). This test provided standard measurements of walking speed in changes in $T_b$ when a tortoise leaves a refuge and decides to undertake a movement in the open. As the observer threatened the tortoise, we assumed that the walking speed would correspond to an elevated value.

### Environmental thermal conditions

In the main microhabitats used by tortoises (see Lagarde et al. 2012 for details), we placed 8 data loggers to record ambient temperatures and 8 physical models to estimate tortoise's *Tb*. We placed 2 physical models and 2 data loggers in the open, into small jujube bushes (height < 50 cm high), medium jujube bushes (height 50 cm-2 m) and large jujube bushes (height > 2 m). The models were partially buried (half model shell's height) to mimic tortoise's behaviour when resting (Lagarde et al. 2008; Lagarde et al. 2012).

### Estimated body temperatures of tortoises leaving their refuge

Our central question was to determine the distance a tortoise can traverse in the open after having left a refuge. Individuals utilize shelters between movements in buffered micro-habitats. To answer to this question we half buried (half shell's height) 14 physical models under large jujube bushes for one night, thereby mimicking the natural resting behaviour of the tortoises. The following day, we retrieved the models from the bush and placed them into the open to mimic a typical resting/activity behavioural sequence (Lagarde et al. 2008). We randomly tested various emerging times: 8:30, 10:00, 11:30, 14:00, 15:30, 17:00, to explore the natural activity range of the species (Lagarde et al. 2008). When the dorsal temperature of the model exceeded 45° C for 30 min we stopped recording and the model was replaced into the bush. All models were left in the bushes between 19:00 and 8:30 to mimic the nocturnal activity of the species (Lagarde et al. 2008). We measured the temperature of these physical models over 12 days (3 April 2008 - 15 April 2008).

### Radio-tracking

In addition to the long term survey started in 2000 for which all found individuals are captured, measured, weighted and marked, we fitted 13 adult (shell length > 100 mm)

Moorish tortoises (6 males, 7 females) with a radio transmitter (see Sereau et al. 2010 for details) and with a temperature data logger glued to the shell. Individuals were maintained 3 to 6 hours in the shade before release to ensure that internal temperature and shell temperature are initially the same. Individuals were located 3 times a day over 12 days (see Lagarde et al. 2003; Lagarde et al. 2008). For each location GPS coordinates, micro-habitat type and behaviour were recorded.

### Statistical analysis

When necessary, data were normalised using a log transformation. To analyse the walking speed recorded during the locomotors performance tests, we used body size (straight shell length) and *Tb* as covariates in a linear mixed model (body size, *Tb* and the first order interactions as fixed factors and individual identity as a random factor). The distance travelled by free-ranging (radio-tracked) tortoises was analysed with a linear mixed model with the sex, date, broad period of the day (morning versus afternoon) as fixed explanatory variables and individual identity as a random factor. Model selection was based on Akaike criterion (AIC), we considered two models as significantly different when $\Delta$AIC > 2. This study does not aim to examine the thermoregulatory behaviour of the tortoises; instead we focused on one central question: how far can tortoises travel in the open without overheating? Consequently we do not present all the thermal readings recorded by the data loggers, we rather oriented the temperature analyses around the critical thresholds of 40° C (critical threshold measured in captivity) corresponding to 38° C inside physical models as mentioned earlier. Unless specified, the results are presented as means ± SE. Statistical analyses were performed using R software (R Development Core Team 2005) with lme4 package (Bates and Maechler 2004) for generalized linear mixed model analysis.

## Results

### Environmental temperatures, body mass and overheating risk

During the study the mean ambient temperature averaged 22.7° C and ranged between 4° C and 50° C. We considered two day types: cool/cloudy and hot/sunny days. During cool/cloudy days, both the air temperature and the *Tb* of the models suggested that the tortoises do not reach a *Tb* of 40° C. During sunny days, the tortoises were exposed to potential overheating (i.e. Model *Tb* > 40° C with prolonged exposure to solar radiation) for at least an hour. Large bushes provided thermally buffered conditions while other habitats were potentially risky (Fig. 2). Females were observed to be less prone to overheating, presumably due to their larger size; mean body mass was 999.0 ± 34.4 g (N = 64) versus 463.3 ± 10.8 g (N = 92) in adult females and males respectively (2008 long term survey).

### Locomotor performance

We recorded a mean velocity of 6.88 ± 0.34 $m.min^{-1}$ (mean ± SD). The selection procedure conserved a single model (AIC = - 149.7 and all $\Delta AIC > 2$) with body size (LMC) and central body temperature as the main explanatory variables for the variations in velocity. As expected, tortoise speed was positively correlated with body size and *Tb* (slopes were $6.10^{-5}$ $m.s^{-1}.mm^{-1}$ and 0.16 $m.s^{-s}.°C^{-1}$, $r^2 = 0.42$ respectively). Therefore, we used *Tb* and shell size to predict tortoise speed. Taking these variables into account enabled us to accurately predict individual walking speed as assessed through a leave-one-out procedure and regressing predicted against actual values ($r^2 = 0.91$, slope is 0.91) (Remm 2004; Stevens et al. 2012).

### Temporal windows of activity and maximum distance

We focused on the periods characterized by a moderate overheating risk: when *Tb* of at least one physical model reached 38° C. For simplicity, we considered half-day periods, morning (8:00 – 13:00) versus afternoon (15:30 – 19:00).

We estimated that during hot periods, the tortoises could safely travel 882.1 ± 37.5 m in the open, and that this excursion required on average 178.5 ± 12.0 min. However, this mean value was variable and dependent on the time of emergence (Fig. 4). The estimated safe distance rapidly decreased from 8:00 to 13:00, and then tended to stabilize after 15:30; reflecting the reduced intensity of solar radiation in the late afternoon (very low overheating risk) combined with the proximity of dusk (individuals must shelter before night) with a strong buffer effect of size (entailing marked differences between sexes). In addition, in the afternoon the *Tb* of the sheltered tortoises was already elevated, thereby improving locomotors performance compared to individuals leaving their refuge in the morning (night temperatures were often low), but also accelerated overheating risk. Finally, tortoises leaving the bushes after 17:00 could not travel far as they had time constraint before night (activity stopped around 19:00 - 19:30). The large body size of the females provided a buffering advantage and they were able travel farther than males (Student test, $T = 1.588$, $p = 0.06$). For example, during the hottest periods (at 11:00), maximum distance that a tortoise can do estimated from physical model temperature suggest that most males could not travel more than 493.9 m, while females can walk 673.08 m.

Radio-tracking

The model selection suggested that the effects of sex and date were the main determinants of the distance travelled by the tortoises. Importantly, both males and females remained motionless during cool days and were located moving only during warmer periods (date effect). Indeed, although males and females movement occurrence doesn't present significant differences (W=66.2, p=0.77), females use to do longer distances (79.95 ± 10.98 m, max=570.71 m) than males (38.80 ± 6.91 m, max=154.48 m, W=1557, p=0.0036; Fig. 5) and longest movement happen during hot days (W=16038.5, p=0.01).

The maximal *Tb* of the free-ranging tortoises estimated by our model was 34.7° C for males and 33.6° C for females. In both cases these high values were recorded on tortoises walking in the open. The mean maximal body temperature was 32.09 ± 0.56°C (sexes pooled). Males exhibited higher maximum *Tb* than females (33.06 ± 0.84° C versus 31.26 ± 0.69° C; $W = 34$, $p = 0.037$). Maximal estimated *Tb* were recorded between 16:00 and 19:36. Broadly, half (N = 6) of the maximum estimated *Tb* were obtained from tortoises that undertook relatively short trips (43.93 m - 320.16 m) and that broke up displacements with sheltering periods in refuges.

## Discussion

The first outcome of this study is methodological. The simple model we developed and tested provides estimates of the central body temperature (Tb) of free-ranging ectothermic vertebrates such as tortoises for instance. The Tb estimates were derived from shell temperatures that are easily collected with miniaturised data loggers. Our model, notably, adequately smoothed the strong temperature elevations of the dorsum part of the shell exposed to sunrays during the sun basking periods (Fig. 1). This smoothing procedure was essential to correctly estimate the maximal body temperatures of free-ranging tortoises and to discard misleading values (i.e. false very high $T_b$). As our model also integrates body mass, it can be apply on other populations, species, and possibly on other taxa (e.g. large lizards). However both the α and the β coefficients should be re-estimated to encompass variations in body shape and possible yet untested allometric size effects (Vitt and Sartorius 1999; O'Connor et al. 2000; Christian et al. 2006). The maximum estimated Tb we calculated in free ranging tortoises fits particularly well with the current knowledge of the thermal biology of the tortoises and of reptiles in general (Cloudsley-Thompson 1970; Huey 1982; Hailey and Coulson 1996c; Nussear et al. 2007). This latter result reinforces the notion that the 40° C threshold should be considered as a critical value avoided by free ranging individuals without exposing living individuals to harmful and thus unacceptable tests. Accurately assessing body temperature variations is essential in ectotherms (Bennett 1980; Stevenson 1985; Hertz et al. 1993; Angiletta et al. 2002). The novel method we propose (coupling physical and mathematical modelling) represents a safe surrogate compared to the use of surgically implanted loggers, especially for threatened species.

The main limit of our approach is also methodological. We selected hot periods to assess overheating risks during movements. However, the tortoises may remain sheltered and wait for favorable thermal conditions to undertake movements; rendering our selection of sunny-days inappropriate. This might be true if the tortoises could navigate in an unchanging environment with respect to refuge availability, in familiar habitats and perfectly predictable thermal condition. If all these conditions were met, individuals could then safely travel several kilometers during a cool day, navigating precisely in their habitat to reach distant patches. This is very unlikely for several reasons, however. First, the tortoises are diurnal and active almost only during sunny days and remained motionless under cool conditions (Hailey and Coulson 1996b; Lagarde et al. 2003; Nussear et al. 2007; Lagarde et al. 2008). This temperature driven shift in the activity is likely explained by the link between physiological performance and body temperature (e.g. movements are precluded under cool conditions) (Hailey and Coulson 1996b; Angiletta et al. 2002; Lagarde et al. 2003; McMaster and Downs 2006; Lagarde et al. 2008). Second, the habitat is changing rapidly (e.g. across seasons), especially in the arid Mediterranean areas. Relying purely on previous experience, a tortoise may not find the expected shelters in the course of a long trip, a possible lethal mistake. Third, dispersal is a vital process for the persistence of populations; most dispersing individuals are supposed to be naïve, and thus strong selection to avoid overheating must favor prudency, especially in long-lived species (see below).

In arid landscape, individuals navigate between refuges (e.g. thick shrubs for the Moorish tortoise, burrows for the gopher tortoise) to escape lethal conditions. The thermal characteristics suggest that only large bushes are safe (Lagarde et al. 2012). The difference between the theoretical maximum distance that can be safely traversed by adult tortoises (~1 km) and the actual shorter values (~0.5 km) recorded in free-ranging tortoises suggests that individuals keep a safety margin. Clearly, the tortoises carefully avoided body temperatures greater than 35° C (by sheltering under large bushes), a value markedly below the 40°C threshold (Hutchison et al. 1966), by limiting trip duration and distance. Such prudent behavior must be integrated in conservation management as it considerably reduces the appropriate distance that must be retained between refuges. Interestingly, Lagarde et al. (2012) reached a similar conclusion based on population survey in 12 different sites that varied in terms of large bush density. This strong congruence between physiological constraint assessments (current study) and population surveys (Lagarde et al. 2012) strengthens our conclusions. The sex difference we observed, with the body size advantage for large females, in limiting overheating was expected as larger body mass confers thermal buffering (O'Connor et al. 2000). We acknowledge that we did not study the most vulnerable age cohorts: the neonates and the juveniles. Incorporating the high thermal sensitivity and low velocity of such small individuals would automatically suggest that a 0.5 km distance between refuges is already excessive. Little is known about dispersal in tortoises, but field evidence suggests that it may be ensured by females through nest site selection (unpublished data). Indeed, during the egg laying period, females can traverse long distances (> 5 km.week$^{-1}$, unpublished radio-tracking data). Thus, some connectivity between patches could be maintained by the female and to a lesser extent by the males (Lagarde et al. 2002, Lagarde et al. 2008). Overall, field managers should maintain a maximum distance shorter than 0.5 km between refuges to maintain the connectivity between populations (e.g. using corridors of bushes). To our knowledge, such a precise field recommendation has never been proposed to promote the conservation of reptiles living in superficially homogeneous arid zones. Our simple approach might usefully contribute to habitat protection/restoration of a wide range of organisms that face strong overheating constraints during movements.

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**Figure captions:**

Figure 1: Estimated central body temperature (*Tb*) of physical model of *Testudo graeca soussensis* using mathematical model (solid grey line) during a single day with 95% confidence interval (dotted lines). The estimated *Tb* was obtained using shell surface temperature (*Td*) recorded with loggers glued on the back of the physical models (long dashes). The actual central body temperature of the models (using implanted loggers) is represented with the dashed grey line. All recorded central temperatures are comprised in the 95% confidence interval (see text).

Figure 2: ambient temperatures recorded under small jujube shrubs (black circles), medium jujubes (light grey circles) and large jujubes (dark grey triangles) in the central Jbilets (Morocco) during sunny (a) and cloudy days (b) (mean + SD). The horizontal line indicates a high threshold (40° C).

Figure 3: Estimated central body temperature during two sunny days for adult male (a) and female (b) tortoises situated into a refuge. The females are larger and males (450 g for males and 1000 g for females). Three situations were considered: the tortoises could shelter under small (solid line), medium (black dashed line) or large jujube bushes (grey dashed line). Central body temperature was estimated using the temperatures recorded on the shell surface of physical models placed in the different refuge types. Confidence interval has not been drawn for readability. Jujubes bushes prevent from overheating during cloudy days. However, during hot days, only large shrubs are able to protect tortoises against overheating.

Figure 4: Estimated cumulative distances doable by Moorish tortoises (Y axis) during sunny days. Individuals are diurnal, active during sunny days, and they are in the open during displacements. Solar radiations can entail overheating risks; the tortoises must cease

displacements and shelter into a refuge. The mean estimated maximal distances for females (circles) and for males (triangles) were derived from the temperature measured in physical models, locomotors performances measured in the field on tortoises and from the change in body temperature over time (see text for details).

Figure 5: Distribution of the daily distances traveled by thirteen radio-tracked Moorish tortoises (six males and seven females) in the Jbilet central (Morocco) monitored during twelve days in April 2008. Females (a) move more often and farther than males (b).

Figure 1

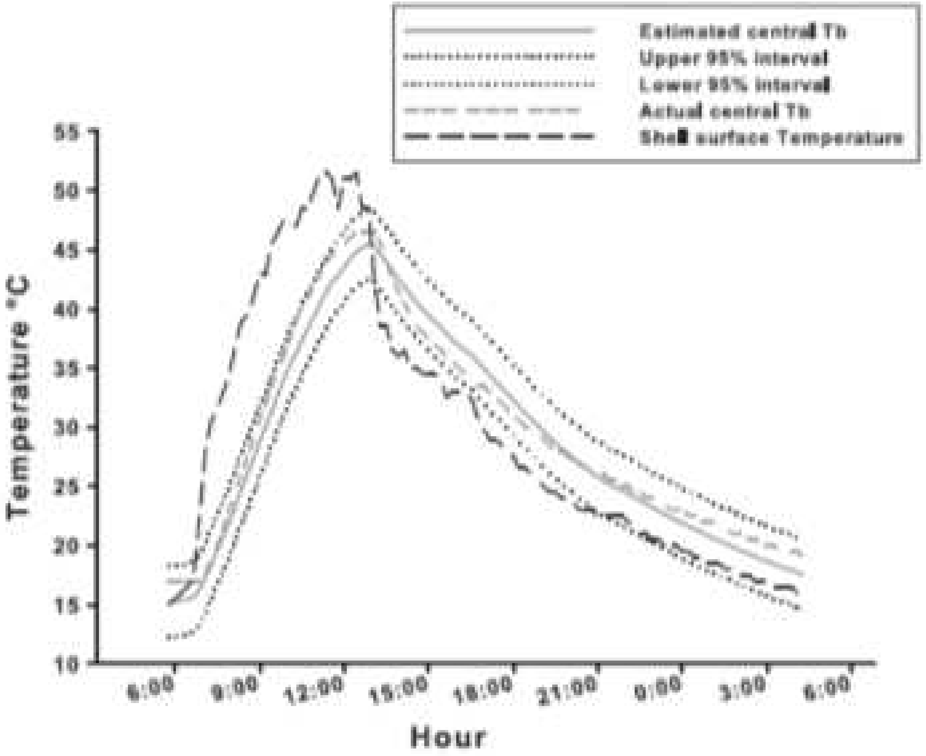


Figure 2

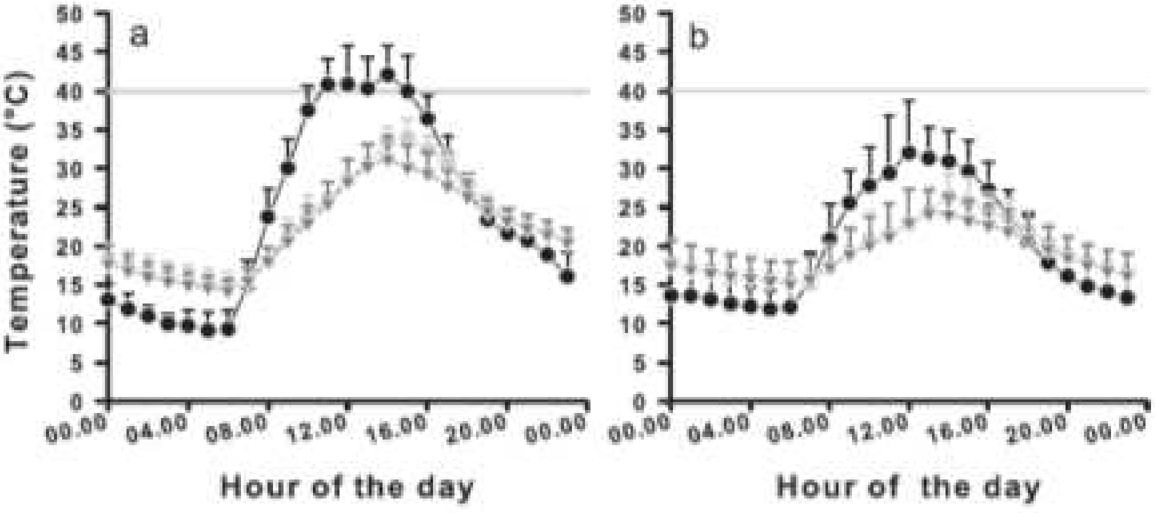


Figure 3

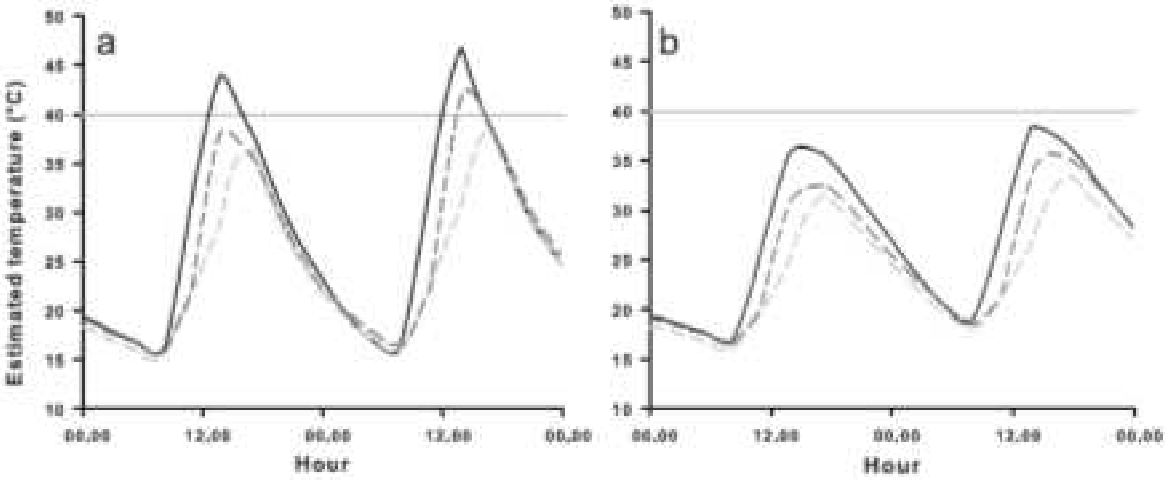


Figure 4

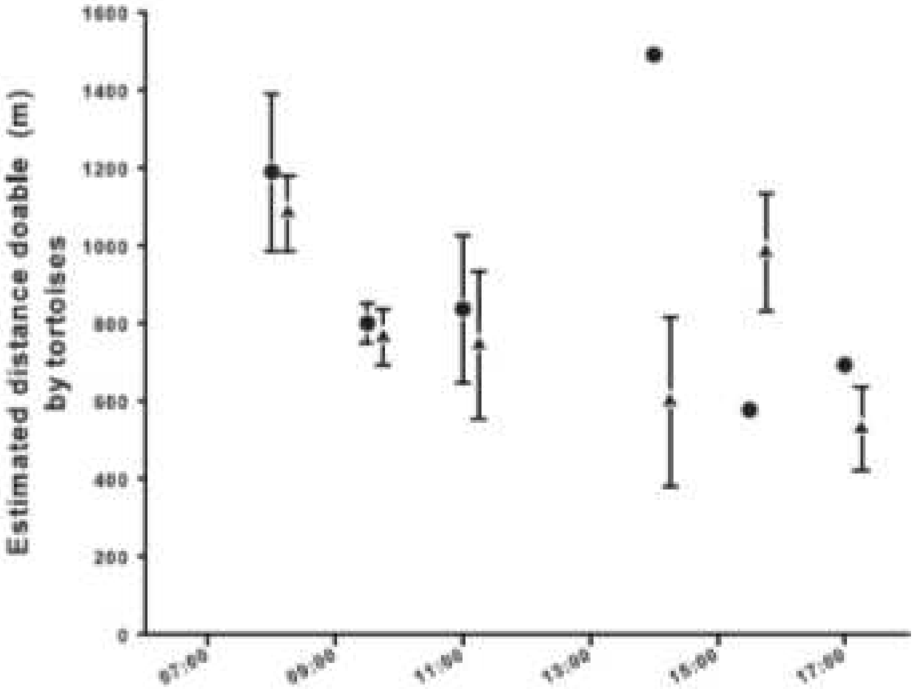
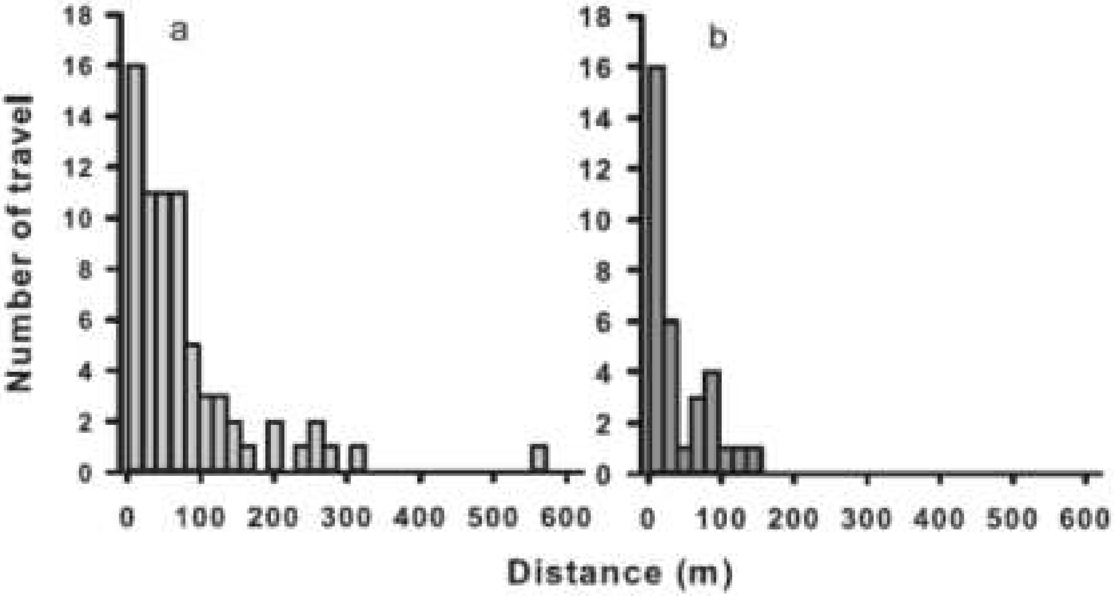


Figure 5


a
b
Number of travel
Distance (m)
0
2
4
6
8
10
12
14
16
18
0
100
200
300
400
500
600

# 5. Ranking the ecological causes of dispersal

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*Résumé en français*

La dispersion est un processus multicausal complexe qui permet aux espèces de fuir les conditions défavorables. De nombreux facteurs peuvent déclencher la dispersion, toutes liées au contexte social et/ou environnemental. De plus, il a été maintes fois démontré que des phénotypes présentant des capacités de dispersions contrastés peuvent coexister au sein des populations d'une même espèce. Le challenge actuel est de comprendre comment les facteurs sociaux et environnementaux vont influencer la décision de disperser chez des individus présentant des phénotypes distincts. Nous avons utilisé le Metatron, un mesocosme expérimental unique en son genre dédié à l'étude de la dispersion dans les environnements fragmentés, pour analyser l'importance relative de dix facteurs déclenchant de la dispersion à l'aide de metapopulations expérimentales de papillons de deux patches. Nous avons montré que le contexte environnemental (conditions climatiques et qualité de l'habitat) prévaut sur les facteurs sociaux et les phénotypes individuels dans la décision de disperser. Notre approche est applicable à toute espèces faisant face à des variations environnementales moyennes à fortes, et constitue une nouvelle méthode pour comprendre l'idiosyncrasie du processus de dispersion.

## ABSTRACT

Dispersal is a complex multicausal process allowing species to escape bad conditions. Many factors can trigger dispersal, all linked to the social and/or the environmental context. Moreover, it is now widely demonstrated that phenotypes with contrasted dispersal abilities coexist within populations of a same species. The current challenge is to elucidate how social and environmental factors will influence the dispersal decision of individuals with distinct phenotypes. We have used the Metatron, a unique experimental mesocosm dedicated to the study of dispersal within fragmented landscapes, to analyze the relative importance of ten potential dispersal triggers in experimental two-patch metapopulations of butterflies. We showed that the environmental context (weather conditions and habitat quality) prevail over social factors and individual phenotypes in the decision to disperse. Our approach is applicable to any species facing medium to strong environmental fluctuations, and constitutes a new way to master the idiosyncrasy of the dispersal process.

The response of species to global change is a subtle blend of three ingredients: tolerate new conditions or adapt to it, move to escape it, or decline locally. Dispersal (the movement of individuals resulting in gene flow between local populations[1-3]) is key in all these ingredients: it has a considerable role in the evolution of local adaptations[4], in the colonization of new areas to track suitable environmental conditions[5], and in the rescue of declining populations or in the recolonization of empty habitats after local extinction[6]. Reliable predictions of the potential for evolutionary adaptations, of distribution shifts under changing environmental conditions or of metapopulation dynamics require thus accurate information on dispersal. However, most models aiming at predicting where species are likely to be found under different scenarios of environmental changes are currently limited by a lack of, or incomplete treatment of dispersal[7]. Measuring dispersal is indeed challenging: it is the interaction between the individual phenotype and the particular social and environmental context that will influence why, when and where individuals disperse, which means that dispersal is most often unpredictable in space and time. Particularly, there have been very few attempts to determine the hierarchy of environmental, social and phenotypic factors influencing the decision to emigrate (the first step of the dispersal process)[8], with the exception of one theoretical model suggesting that environmental factors are more important than social factors[9]. Theory suggests, and experimental studies confirm that dispersal distances vary according to the dispersal cause and the disperser phenotype[10-13]. The identification of which factor drives the emigration can therefore substantially improve the precision of dispersal rate and distance predictions. Here we tackle this challenge with a different approach: we used a combination of experiments and statistical modeling to disentangle the relative effects of ten distinct dispersal triggers on the decision to emigrate. We showed the preeminence of environmental factors over intrinsic characteristics on emigration decisions in a model species. Because our approach do not rely on the initial assumptions inherent to theoretical modelling, it is applicable to any species facing medium to strong environmental fluctuations and constitutes a new way to master the idiosyncrasy of the dispersal process.

## RESULTS

Previously we designed the Metatron (Figure 1a), a unique experimental platform dedicated to the study of dispersal in terrestrial organisms that allows the manipulation of both spatial and climatic effects[14]. Butterflies have long been recognized as excellent model systems for the study of dispersal[15], for which experimental use in the Metatron have been validated. We created experimental, two-patch metapopulations of 20 individuals at maximum of the large white butterfly (*Pieris brassicae*) under these semi-natural conditions, in eight replicates along natural gradients (climatic conditions and habitat quality, Figure 1b, 1c), using butterflies previously phenotyped for five traits linked to dispersal[16-19]. During one month, we performed captured sessions to determine the position of each individual within each metapopulation (departure cage, corridor, arrival cage), and therefore obtained (i) the dispersal status of each individual, i.e. an individual always observed in a departure cage was resident and an individual observed at least once in a corridor or an arrival cage was disperser, (ii) the dispersal events at each time of individual's capture histories, i.e. the temporal succession of residency and dispersal events for each individual. We recorded 46 dispersers and 124 residents; this means that 27% of individuals were dispersers. In total, 77 dispersal events and 717 residency events were documented in the capture history of individuals. We then built three models to determine the relative importance among environmental context and individual phenotypes and between the two categories.

### Effects of environmental and social factors on emigration

Model 1 shows that weather conditions and habitat quality at departure had the strongest effect on emigration (Table 1). Emigration was positively related to daily air temperatures (Figure 2a) and luminosity, and negatively related to humidity. Moreover, results indicate that low-quality departure cages (i.e., low floristic richness and sparse vegetal cover, Figure 2b) encouraged dispersal. Individuals were also marginally encouraged to disperse by high-quality arrival cages. Marginal interactive effects between weather conditions and habitat quality of the departure and arrival cages were observed. Indeed, extreme air temperature led to increased emigration regardless of the quality of cages.

Interestingly, social factors were not important in emigration decisions. Indeed, population density was not retained in the best models and population sex ratio was kept only through a strong interaction with climate. Under moderate temperatures, we observed a general increase in emigration rate when a greater proportion of males was present. However, as air temperature increased and humidity decreased, emigration was independent of population sex ratio.

### Effects of phenotypes on emigration

Model 2 shows that sex had the strongest effect on emigration rate with a dispersal biased toward females (Table 1, Figure 3a). While age and thermoregulation ability had no effect on emigration, both the wing length and the flight performance had a significant effect ($p<0.01$ and $p<0.001$ respectively, Wilcoxon t-test; Figure 3b, Figure 3c). More mobile individuals as well as individuals with longer wings were more prone to leave the departure cage. Finally, we did not find evidence of significant interactive effects among phenotypic traits.

### Preeminence of environmental factors over phenotypes

Model 3 included all significant effects retained in Models 1 and 2. Weather conditions and habitat quality at departure had the strongest impact on emigration. Population sex ratio was kept only through the same interaction as described in Model 1 with climate. All other interactions retained by selection had marginal effects and included sex, which is the only phenotypic trait that was kept in Model 3. The tendency to emigrate from a male-biased cage was stronger for females, which were also more sensitive to warmer conditions than were males. However males were more sensitive to the quality of both departure and arrival cages, because they leaved more often departure poor-quality cages and avoided more often poor-quality arrival cages than females.

## DISCUSSION

Dispersal evolved as a common solution to multiple problems. Although numerous studies have demonstrated that the environmental and social context together with the phenotype of the individual concur to trigger dispersal, both in nature and under fully controlled conditions[20-22], multifactorial tests of the relative role of each of these factors are still lacking[9]. This is mainly because it is technically difficult to control for more than a few factors under natural conditions[14,23]. The pros and cons of the Metatron in studying dispersal have been lengthy discussed elsewhere; to sum up, this mesocosm provides an unrivaled opportunity to test the decision an individual will take to emigrate or not, which is the first step of the dispersal process[14,23]. Using the Metatron, here we provide the first ranking of ten different dispersal factors on a Metazoa at a previously unexplored spatial scale.

Weather conditions and habitat quality had the strongest impact on individual emigration decisions. Butterflies were more prone to emigrate from the departure cage when air temperatures were high and presumably far from optimal (*P. brassicae* distribution is limited southward by the summer isotherm of 28°C[24]), whereas high humidity rates impeded dispersal. In relation to their lifestyle, butterflies were directly encouraged to emigrate from low quality cages, i.e. from those with the lowest vegetal cover and species richness. *P.*

*brassicae*[24] and numerous butterfly species (review in [25]) indeed feed on a large panel of nectariferous flowers and hide on ground vegetation. Habitat quality in arrival cages had a marginal, yet important, role in dispersal (immigration was higher in arrival cages of better quality). Information about arrival cages might be provided by round trip after initial visits to low quality arrival cages, or by the acquisition of information about distant cages using olfaction or social interactions[26-27].

About social factors, population density was not retained in the model, and population sex ratio was only kept through a strong interaction with climatic factors. As individuals were confined in cages and were thus unable to escape harassment, we used density levels situated at the lower end of the range compared to wild population densities reported in the literature for Pierid-related species (20 individuals/100 m² at maximum in our experimental metapopulations, 5-800 individuals/100 m² in nature)[28]. Starting from equilibrated conditions, the sex-ratio changed according to the differential emigration of males and females. We report here increased emigration when a greater proportion of males was present, confirming our previous findings[29].

Under our experimental conditions, butterflies were more sensitive to environmental than to social factors. The interactive effects between weather conditions and habitat quality in one hand and between weather conditions and population sex ratio in the other hand (less important effect of departure cage quality and population sex ratio on emigration when temperatures were high) confirm the high sensitivity of butterflies to climate change. Under extreme temperature conditions, individuals will decide to disperse independently of other environmental cues.

Butterfly phenotypes strongly influenced their emigration decision. Sex had the strongest effect: like in many monoandrous butterflies, dispersal was female biased[16-29], independently of the density of egg batches in the departure cage (data not shown). The avoidance of male harassment has been proposed as the principal cause of female dispersal[e.g.29], particularly in monoandrous species such as *P. brassicae.* Flight performance and wing length had significant effects on the emigration decision, good performers and individuals with long wings being more often dispersers. Flight performance is part of the *mobility* syndrome described for *P. brassicae* and is associated with both exploration skills and consistency of flight orientation after emergence[30-31]. Thus, given that flight performance as well as wing length are related to emigration decision, this mobility syndrome is now extended to a real *dispersal* syndrome, in which disperser and resident phenotypes have distinct behavioral and morphological attributes[11]. We observed no significant interactions between any of the phenotypic traits. This suggests that the dispersal syndrome of *P. brassicae* results from simple co-variations between traits, which raises interesting question about its genetic architecture. This dispersal syndrome in *P. brassicae* indicates that dispersers did not constitute a random fraction of the released individuals. This result implies that models aiming at predicting the dynamics of spatially-structured populations should integrate inter-individual variability in order to correctly capture the dispersal process.

Environmental factors were more influential than phenotypic traits during emigration decisions. It might be interesting to assess the fitness of those individuals that stayed in poor environmental conditions, those that emigrated because of phenotypic predisposition (i.e. context-independent dispersers), and those that emigrated in response to poor environmental conditions (i.e. context-dependent dispersers). Besides, our data also revealed the existence of marginal interactions between sex and four environmental factors, suggesting that males and females would respond differently to environmental changes.

To conclude, here we showed that our approach combining experimental tests in the Metatron and statistical modeling provided a clear ranking of different dispersal causes in a terrestrial organism. Our results illustrate well how the life-style of the model species impacts its emigration decision, and how the degradation of environmental conditions may impact species' distributions through the initiation of dispersal movements. Being context- and condition-dependent, dispersal is inherently an idiosyncratic process that will vary across organisms, space and time. Our approach has thus the potential to create a real breakthrough in dispersal research, by providing to the scientific community the opportunity to test what are the main dispersal causes in a wide range of terrestrial and semi-terrestrial organisms. This should allow sounder predictions of dispersal rates, and hence improve the predictions of species distribution under global change.

# METHODS

## Butterfly breeding and characterization of phenotypic traits

In summer 2010, we collected 41 P. brassicae clutches originating from two 20km-distant sites in Ariège (France) and reared the caterpillars hatching from each clutch in separate plastic boxes. Boxes were placed in climatic chambers at 23°C ± 1°C under controlled photoperiod conditions (light:dark, 14:10 h) mimicking favorable season conditions (from April to November in P. brassicae) that allow continuous development. Fresh cabbage was provided ad libitum to caterpillars. After emergence, each butterfly was marked individually on the forewings. These individuals were then kept in the lab under common garden conditions similar to those of caterpillars (23°C ± 1°C and light:dark, 14:10 h) and fed with fresh flowers.

We studied five phenotypic traits related to morphology, physiology, and behavior in 212 butterflies. First, we determined the sex. Subsequently, one day after emergence, the following measurements were made on each butterfly:

- Wing length: one experimenter measured the left forewing length of each individual using a caliper (measures of the two wings are highly correlated in *P. brassicae*[31,32]).
- Flight performance: butterflies were subjected to a previously described flight performance test[30,33]. Briefly, each individual was placed in a 25x10x10-cm plastic chamber, which was perforated at its base and fixed to a rapid agitator (Vortex Genie 2, Scientific Industries). Experiments were performed at 23 ± 1°C, which is considered as the optimal temperature for *P. brassicae*[24]. After a one-minute habituation period, the vortex was used to strongly shake the chamber, impeding the butterfly from perching on the walls. The butterfly's behavior was then observed for a period of one minute. During the test, the butterflies either took flight or rested uncomfortably at the bottom of the agitating chamber. The time an individual spent flying was then recorded, with high values reflecting good flight performance.
- Thermoregulation ability: we measured the heating rate of butterflies based on a previously reported protocol[17]. Each individual was first cooled in a refrigerator (4°C, 10 min) and then warmed at 27°C for 180 seconds under a 300-W Ultra Vitalux solar lamp placed at a distance of 80 cm. During this warming procedure, the thorax temperature was monitored continuously with a TESTO845® infrared thermometer (emissivity $\varepsilon = 0.95$, one data point/second for 180 seconds). Heating rate was expressed as the slope of the thorax temperature plot versus the log-transformed time[34].

Finally, we determined the age of each individual at the beginning of the dispersal experiment. This was important because the emergence of butterflies was asynchronous, and they were kept in the lab until a sufficient number of individuals were available to perform the first release session in the Metatron.

## Experimental design and determination of habitat characteristics

We utilized 16 cages of the Metatron (each 200 $m^3$, 10x10x2 m and covered with insect-proof nets) with different weather and habitat quality conditions, which allowed us to create eight simple, two-patch metapopulations of butterflies living within natural environmental gradients (Figure 1). We connected each departure cage (in which butterflies were released) to an arrival cage using a corridor. Thus, the butterflies could either remain in the departure cage or freely cross a corridor into the arrival cage, with the possibility of returns to the departure cage. However, the narrow, S-shaped 19-meters long corridors were designed to be particularly challenging to cross (i.e., entries represented less than 2% of the total vertical surface of a cage fence) in order to allow discrimination between dispersal and resident individuals[14]. We also cut the vegetation to a very low height (i.e., without feeding flowers), ensuring unfavorable life conditions in the corridors. Within each departure and arrival cage, we placed feeding flowerpots and host plant pots in the same positions. We have previously shown that these conditions allowed the discrimination of dispersal events in *P. brassicae*[29]. Indeed, we introduced highly mobile butterflies within a similar two-patches configuration and identical corridor vegetation treatments, and observed that changes in population sex-ratio changed the dispersal decision of individuals. Because not all butterflies dispersed while they were all selected as highly mobile, and thus all had the physiological capacities to disperse in this previous experiment, then the Metatron platform successfully reveals individual dispersal decisions in *P. brassicae*.

We recorded weather conditions by monitoring temperature, humidity, and luminosity every ten minutes throughout the experiment via probes at the center of each cage. We excluded data recorded during nighttime because *P. brassicae* is inactive under dark conditions. These recordings were averaged between successive capture sessions (between *t* and *t-1*) to summarize weather conditions during each time interval. The three variables were summarized by a principal component analysis (PCA1, Supplementary Information), as they were highly correlated. Within each cage, weather conditions were then described by their scores along the first axis of PCA1 for those with eigenvalues > 1.0. These variables accounted for 83.13% of the total inertia of the climatic factors.

We determined the habitat quality within both the departure and arrival cages using a standardized protocol for vegetation sampling. We delimited 100 squares ($1m^2$ each) on the ground of each of the 100m2 cages and photographed the individual square from a height of 1.50m. This method allowed sufficient resolution for the identification of all plant species within these squares. Using ArGis 9.3 software, each of the 1,600 resulting pictures (100 squares, 16 cages) were geolocated to recreate a composite surface image for each cage. These pictures were then used to calculate four indexes of vegetation using Phytobase $8^{35}$:

- Cover rate: proportion of vegetal covering of the ground surfaces of the cages (Figure 1b, Figure 1c)
- Floristic richness: total number of species within a cage (Figure 1b)
- Vegetal community diversity: Shannon diversity index (*N'*) applied to phytosociology:

$$N'_q = \exp(-\sum_{i=1}^{q} S_i \ln(S_i))$$

where $S_i$ is the relative abundance of community *i* and *q* is the total number of communities within cage *s*. A community (synusia) is defined as the smallest group of ecologically similar species colonizing an area and growing together within the same strata during the same period.

- Number of vegetal communities: statistically homogenous groups, which are defined here by the spatial combination of communities.

Adults of *P. brassicae* use a large panel of nectariferous flowers and hides on ground vegetation[24]. The higher values of these four indexes will be, the greater habitat quality will be considered.

These habitat quality variables were highly correlated and summarized using two other PCAs: one for departure cages (PCA2) and one for arrival cages (PCA3). Habitat quality for each cage was described using its score along the first axes of these PCAs for eigenvalues > 1.0, accounting for 83.91% (PCA2) and 74.09% (PCA3) of the total inertia of the habitat quality variables in the departure and arrival cages, respectively (Supplementary Information).

### Capture–mark–recapture sessions and population structures

We initially introduced 120 butterflies, which were randomly chosen from among 41 clutches, into the eight departure cages. Four of the populations consisted of 20 butterflies, whereas the other four populations were comprised of 10 butterflies. These eight populations had balanced sex ratios at the beginning of the experiment. We subsequently performed capture sessions twice a day under good meteorological conditions (i.e., no captures on rainy days) in order to record the position of each individual until death (i.e., departure cage, corridor, or arrival cage). To maintain sufficient population densities, we replaced dead butterflies with individuals emerging from our stock of clutches for as long as possible (92 more butterflies, thus a total of 212 individuals was released during the experiment). In total, 29 capture sessions were conducted during the 28 days of the experiment, i.e. until all butterflies died. In addition, four extra release sessions were performed, introducing 35, 29, 9, and 19 butterflies, respectively. The lifetime capture histories of the butterflies were analyzed within the capture-mark-recapture framework[36]. We calculated the catchability, the number of individuals of each sex in each capture session, and the survival probability between capture sessions. These values were determined for the pairs of cages as well as for departure cages, employing the POPAN model using Mark software[37]. We included sex and flight performance as covariables, and we averaged the parameters of the best models (those for which the ΔAIC values were < 2) using the RMark package[38]. Parameter variation over time was not included in the models to avoid overparameterization. The daily survival rate and catchability were high and equivalent for all pairs of cages (on average: 0.8 and 0.83 for females, respectively, and 0.7 and 0.81 for males, respectively), which indicated that data from all replicates were informative. The POPAN results were used to determine departure cage population structures just before each emigration event. Sex ratio described the ratio of the number of males to females, whereas population density was considered to be the total number of individuals alive in cage *s* at time *t*.

### Ranking of dispersal causes

Statistical analyses were performed in three steps:

Model 1: To study the effects of environmental and social factors on dispersal, we defined dispersal as a binomial: dispersal or residency events, i.e. a specific event within the individual history of each butterfly. To do this, we extracted the capture histories for each individual at the end of the CMR sessions and separated them into two categories: a residency event corresponded to the capture of an individual in a departure cage at time *t-1* and *t*, and residency event corresponded to the capture of an individual in a corridor or in an arrival cage at time *t* and in a departure cage at time *t-1*. We excluded the events at time *t* for which butterflies had not been recaptured at *t-1* because we could not ascertain whether these events occurred during the preceding time interval. We determined the environmental conditions for each dispersal event at time *t-1* and recorded these values as explanatory variables, except for weather conditions that were averaged between *t* and *t-1*. To evaluate the effects of the five environmental factors on residency or dispersal events, we ran generalized linear mixed-effect models (GLMMs) using the lme4 package[39] in R 2.14.2[40]. Population density, sex ratio, weather conditions (summarized by the coordinates on the first axis of PCA1), habitat quality at departure (summarized by the coordinates on the first axis of PCA2), and habitat quality at arrival (summarized by the coordinates on the first axis of PCA3) were used as variables with fixed effects. Pairs of cages and individuals nested in their clutch of origin were included as factors with random intercepts. A full model that included all first-order interactions would have been overparameterized. Therefore, we refined the variable selection by starting with five different full models in which we retained all single effects, analyzing the interactions between one variable with all others. This selection process allowed us to retain the interaction effects that returned the lowest AIC values in a single model. We then compared the models nested within the full model to select the combination(s) of terms that returned the best AIC. We employed a model averaging procedure to determine the relative importance of each factor. The best models (i.e., those with ΔAIC values < 2) were averaged using the MuMIn package[41]. Two elements derived from this averaging procedure were used to assess the importance of each factor in butterfly dispersal: (i) the confidence interval of the averaged estimated slope of the selected term (i.e., strong effects had confidence intervals that did not contain zero, marginal effects were those with confidence intervals containing zero and shifted toward positive or negative values, negligible effects displayed confidence intervals roughly centered on zero); (ii) the relative weight of the term (i.e., the relative sum of Akaike's weights of the models, within the subset of models with ΔAIC<2 in which the term was included).

Model 2: To study the effects of phenotypic traits on dispersal, we used a binomial variable describing the dispersal status of each individual, i.e. the general "dispersotype" of an individual. We considered dispersers to be the butterflies that were captured either in a corridor or an arrival cage at least once, while residents were butterflies that stayed within departure cages throughout their lifetimes. Individuals that were never recaptured after their release (n=42 over the 212 released butterflies) were not included in the analyses. The same method of modeling and selection of terms used for the effects of environmental factors on dispersal events was used for evaluating phenotypic traits, using GLMMs and a model averaging procedure. Sex, age, flight performance, wing length, and thermoregulation ability were included as variables with fixed effects, whereas the pairs of cages and the individual's clutch of origin were included as factors with random intercepts. Interactions were tested following the same sequential procedure as described for Model 1.

Model 3: We built a third model to compare the relative weight of phenotypic traits and environmental factors in the dispersal decision. This model included factors that had a relative importance > 0.5 in Models 1 and 2 after model selection. Dispersal events were set as the response variable (environmental factors, as measured in our experiment, were not appropriate for use in models with the dispersal status as the response variable because the dispersal status is fixed over individual lifetime while environmental factors vary), with the pair of cages and the individual's identity nested in its clutch of origin as factors with random intercepts. Interactions were tested following the same sequential procedure as described for Model 1.

Before running the three models, we verified that the variance inflation factor was < 2 for all explanatory variables, using the car library in R[42], meaning that there was no multicollinearity among them. We also verified with a separate model that the orientation of the corridors had no effect on dispersal. Finally, we verified that each variable included in PCAs had individual effects corresponding to results obtained when models were run with summarized data. To do so, we ran successive models including only one of the variables summarized in each PCA (all combinations were tested) because full models were over-parameterized. As results were always concordant, we present models with PCAs in the Results section to gain in clarity, but we rather use individual variables in the Discussion section to simplify the interpretation of the data.

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## AUTHOR CONTRIBUTIONS

DL, VS, JC and MB conceived the study. DL, AT and OC performed the experiment. All authors participated to the statistical analyses at the exception of CMR analyses that were performed by DL and SM. DL and MB wrote the first draft of the manuscript and all authors contributed substantially to revisions.

## COMPETING FINANCIAL INTERESTS

The author declare no competing financial interests.

**FIGURES**

**Figure 1:** Experimental platform and design of the study

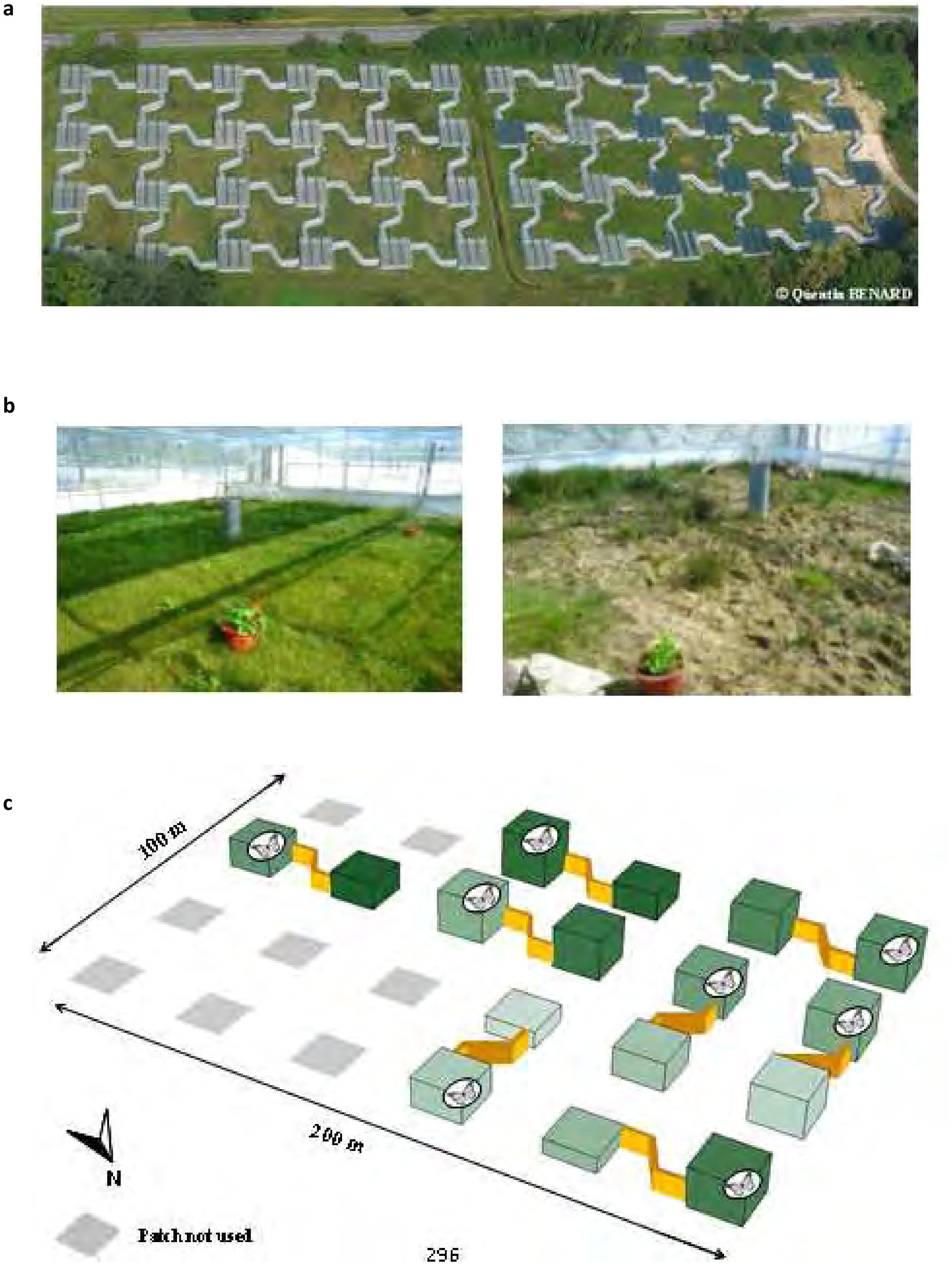
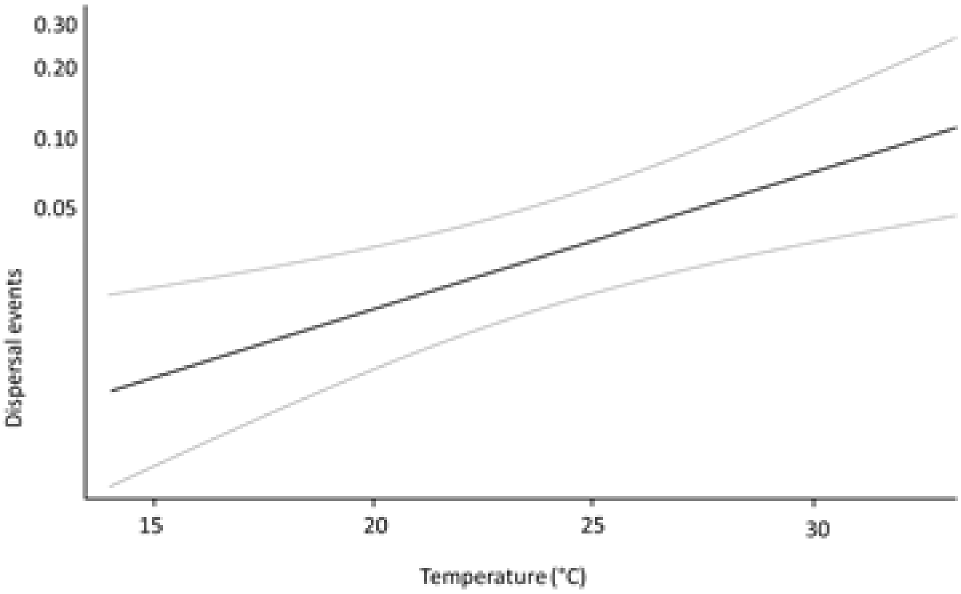


**Figure 2:** Relationships between emigration decisions and environmental factors

**a**



**b**

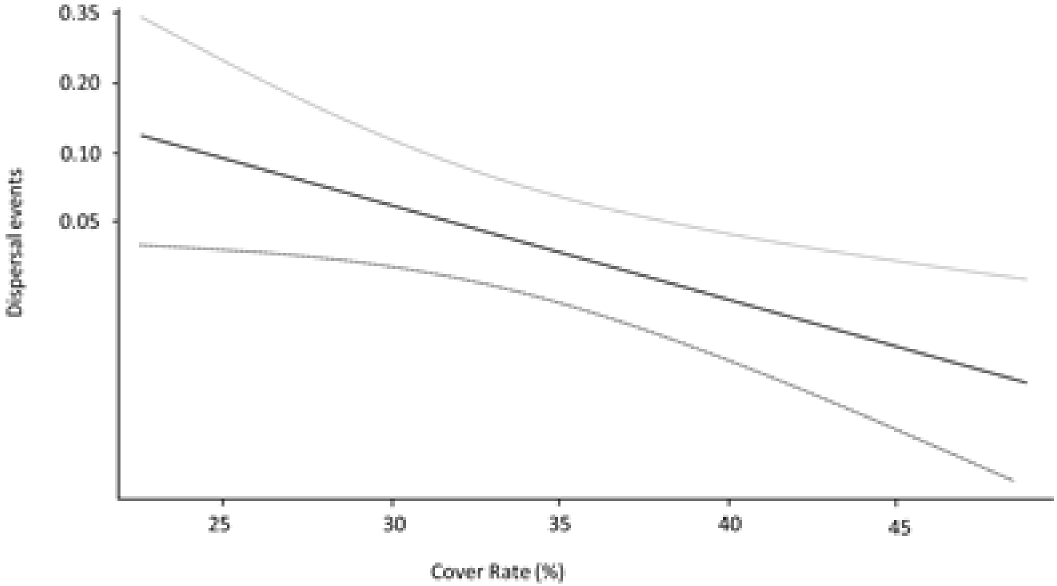
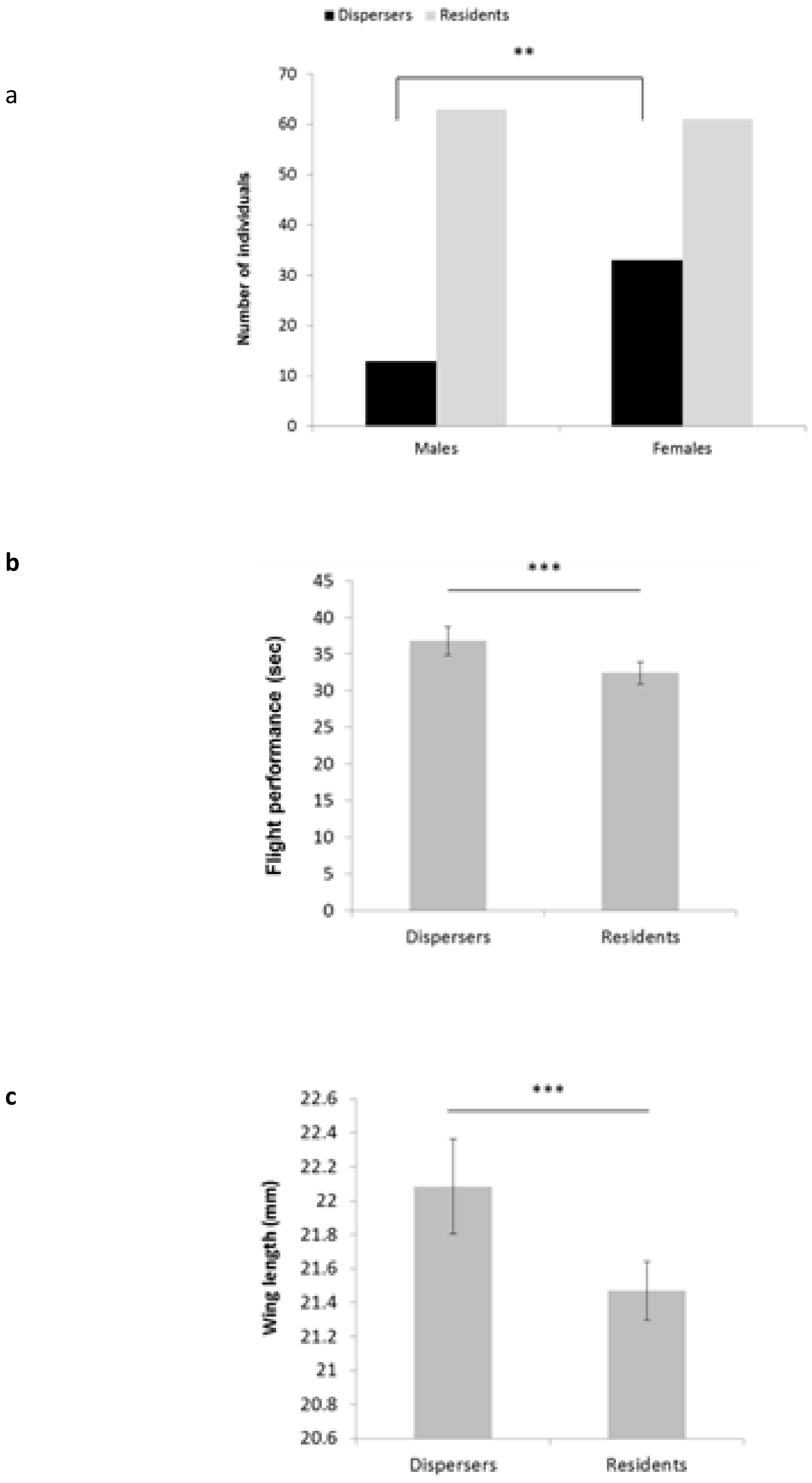


**Figure 3:** Relationships between emigration decisions and phenotypic attributes.



## FIGURE LEGENDS

**Figure 1:** (**a**) Aerial photograph of the Metatron. The basic units of the Metatron are cages of 10 m X 10 m, 2 m height fenced by tarps near the ground and a solid entomological net above, with herbaceous vegetation mowed twice a year. Cages are connected by 19 m, S-shaped narrow corridors. The present experiment was performed on the left hand cages of the photograph. Natural climatic and vegetation gradients are observed in cages and recorded by permanent climatic probes and regular vegetation samplings. (**b**) Photographs of a butterfly high-quality (dense vegetation cover) and a low-quality (sparse vegetation cover) cage. (**c**) 3D representation of the experimental design. We released 120 butterflies into eight departure cages (butterfly pictures on the scheme), each connected to an initially empty arrival cage. Before release, we measured on each butterfly five phenotypic traits related to morphology, physiology, and behavior: sex, wing length, thermoregulation ability, age of each individual at the beginning of the experiment, and flight performance using a standardized test that subsumes several personality traits. Besides, we monitored in each metapopulation three environmental factors (weather conditions and habitat quality in the departure and the arrival cage with dark green scale representing sparse to dense vegetation cover, and height of cages representing floristic richness), and two social factors (population density and sex-ratio). To maintain population densities, we replaced dead butterflies with individuals emerging from our breeding as long as possible. Sex-ratio was balanced at the beginning of the experiment.

**Figure 2:** **(a)** Plot displaying the proportion of emigration events as a function of the temperature in the best-fitted model. **(b)** Plot displaying the proportion of emigration events as a function of vegetal cover in departure cages in the best-fitted model.

**Figure 3:**

**(a)** Dispersers and residents were quantified based on sex. The data indicate a significant female-biased emigration (p<0.01, Wilcoxon t-test). **(b)** Average flight performance (and standard error) of dispersers and residents (p<0.001, Wilcoxon t-test). **(c)** Average wing length (and standard error) of dispersers and residents (p<0.001, Wilcoxon t-test).

**Table 1:** Ranking of dispersal causes

| | Model terms | | Estimate | 95% CI of estimate | Weight |
|---|---|---|---|---|---|
| **Model 1:environmental factors and their interactions** | | intercept | -3.91 | -4.64; -3.92 | |
| | Simple terms | climate | 0.82 *** | 0.35; 1.26 | 1 |
| | | habitat quality at departure | -0.75 * | -1.45; -0.05 | 1 |
| | | population sex ratio | 0.5 | -0.30; 1.29 | 1 |
| | | habitat quality at arrival | 0.45 | -0.25; 1.16 | 0.72 |
| | | population density | not retained by selection | | |
| | Interaction terms | climate:sex ratio | -0.53 * | -0.97; -0.09 | 1 |
| | | climate:habitat quality at departure | 0.2 | -0.18; 0.59 | 0.42 |
| | | climate:habitat quality at arrival | 0.25 | -0.18; 0.69 | 0.33 |
| | | other interactions | not retained by selection | | |
| **Model 2: phenotypic traits and their interactions** | | intercept | -0.71 | -1.39; -0.03 | |
| | Simple terms | sex | -1.02 *** | -1.81; -0.22 | 1 |
| | | flight performance | 0.28 | -0.14; 0.69 | 0.45 |
| | | wing length | 0.25 | -0.15; 0.65 | 0.42 |
| | | age | not retained by selection | | |
| | | thermoregulation ability | not retained by selection | | |
| | Interaction terms | all 2-ways interactions | not retained by selection | | |
| **Model 3: environmental vs phenotype and their interactions** | | intercept | -3.75 | -4.57; -2.93 | |
| | Simple terms | climate | 0.79*** | 0.33; 1.25 | 1 |
| | | habitat quality at departure | -0.56 | -1.23; 0.12 | 1 |
| | | population sex ratio | 0.83 | -0.09; 1.75 | 1 |
| | | sex | -0.1 | -1.40; 1.21 | 0.89 |
| | | habitat quality at arrival | 0.33 | -0.42; 1.08 | 0.67 |
| | Interaction terms | climate:sex-ratio | -0.68** | -1.11; -0.24 | 1 |
| | | sex:population sex-ratio | -1.31* | -2.56; -0.06 | 0.77 |
| | | sex:habitat quality at departure | -0.87 | -2.36; 0.63 | 0.43 |
| | | sex:climate | -0.49 | -1.32; 0.34 | 0.33 |
| | | sex:habitat quality at arrival | 0.83 | -0.49; 2.16 | 0.28 |

We used linear mixed-effect models to select environmental factors (Model 1) and phenotypic traits (Model 2) that best predict dispersal decisions. A full model incorporating all of the factors from both categories would have been overparameterized. Therefore, factors with the strongest effects in Models 1 and 2 (i.e., those with weights > 0.5) were selected for inclusion in Model 3, which compared the relative importance of environmental and phenotypic traits in the dispersal decision. Weights = relative Akaike weight of the top-ranked models ($\Delta$AIC<2) in which the term appeared. *** = $p<0.001$, **$p<0.01$, *$p<0.05$. Weather conditions and habitat quality are quantified using PCAs (see Methods and Supplementary Information).

# 6. Dispersal syndromes and the use of life-histories to predict dispersal

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*Résumé en français*

En raison de ses impacts sur l'adaptation locale, le fonctionnement des populations ou la modification des aires de répartition, la dispersion est considérée comme un processus central pour le maintien des populations et l'évolution des espèces. Cependant, mesurer la dispersion est compliqué, ce qui justifie l'utilisation de proxys de la dispersion. Bien qu'attirant, et malgré sa fréquente relation avec la dispersion, la taille corporelle a déjà prouvé ses limites en tant que proxy de la dispersion. Notre hypothèse ici est qu'étant donné l'existence de syndrome de dispersion, des groupes de traits d'histoire de vie pourrait constituer des proxys alternatifs plus appropriés de la dispersion. Nous avons testé cette idée en utilisant les papillons comme modèle d'étude. Nous avons démontré que différents éléments du processus de dispersion (i.e. mobilité individuelle, distance, flux génique) sont corrélés avec différents groupes de traits d'histoire de vie : ces éléments variés de la dispersion forment des syndromes distincts et doivent être considérer comme des caractéristiques spécifiques de la niche de l'espèce. Nous avons alors montré que l'utilisation de ses syndromes permet de prédire précisément la dispersion. L'utilisation des traits d'histoire de vie améliore la qualité des prédictions d'un facteur cinq en comparaison de l'utilisation de la taille des ailes seules. De telles prédictions basées sur les traits d'histoire de vie donnent des résultats crédibles susceptibles d'alimenter les modèles de simulation visant à comprendre les dynamiques évolutives des populations de papillons, et probablement d'autres organismes, en fonction des modifications environnementales afin de favoriser leur conservation.

## Abstract

By affecting local adaptation, population functioning or range shifts, dispersal is a central process for populations persistence and species evolution. However, measuring dispersal is complicated, which justified the use of dispersal proxies. Although appealing, and despite its general relationship with dispersal, body size was however proven unsatisfactory as dispersal proxy. Our hypothesis here is that, given the existence of dispersal syndromes, suite of life- history traits may be alternative, more appropriate proxies for dispersal. We tested this idea by using butterflies as model system. We firstly demonstrate that different elements of the dispersal process (i.e. individual movement rates, distances, and gene flow) are correlated with different suite of life-history traits: these various facets of dispersal are parts of separate syndromes and must be considered as real axes of a species' niche. We then showed that these syndromes allowed accurate predictions of dispersal. The use of life-history traits improved the precision of the inferences made from wing size alone by up to 5 times. Such trait-based predictions thus provided reliable dispersal inferences that can feed simulation models aiming at investigating the dynamics and evolution of butterfly populations, and possibly of other organisms, under the current environmental changes, to help their conservation.

## Introduction

The response of biodiversity to global environmental changes is a subtle blend of three ingredients: tolerate the new conditions or adapt to it, disperse to escape it, or decline locally. Dispersal is key in all these ingredients as the movement of individuals that induces gene flow has a considerable role in evolutionary ecology (Ronce 2007; Clobert et al. 2009; Clobert et al. 2012), for instance on the evolution of local adaptations (Doebeli and Dieckmann 2003), and it is also central to the spatial dynamics of populations and metapopulations (Hanski 1998; 1999a). If we are to accurately predict for instance the distribution shifts or the potential for evolutionary adaptations under climate change, or the spatial functioning of populations in fragmented landscapes, we will need accurate information on dispersal (Berg et al. 2010). But measuring dispersal is challenging, because it is rather unpredictable in space and time (Nathan 2001), and recording movements among local populations is labor intense and might be biased by limits of sampling scales (e.g. Schneider 2003; Franzen and Nilsson 2007).

An appealing solution to overcome this difficulty is to infer unknown dispersal ability for populations or species of interest rather than to measure it directly. An option for doing such inferences is to identify general patterns in the organization of dispersal ability across individuals, populations or species, and then to search for a trait - or a suite of traits - that parallels these patterns, which can then be used as a dispersal proxy. Body size was a first candidate in this quest, as it may relate to dispersal either directly because locomotion is scaling to body size, or because dispersal has causal relationships with other size-dependent traits or processes (Bowman et al. 2002, Clobert et al. 2004). As expected, body size and body shape co-vary with movement rate and dispersal distances in several taxa (moths: Beck and Kitching 2007; birds: Dawideit et al. 2009; plants: Thomson et al. 2010; butterflies: Turlure et al. 2010; Stevens et al. 2012; Sekar 2012). However the power of the predictions that could be obtained from this covariation is low since this relationship is rather noisy, and therefore casts doubt about its use to predict dispersal (Dawideit et al. 2009; Stevens et al. 2012;

Sekar 2012). In line with this, Baguette et al. (2000) showed that difference in dispersal rates of three butterfly species over a common network of habitat patches could not have been predicted from differences in their body sizes. Nevertheless, wing size (a surrogate of body size in butterflies) is still recurrently used as a direct proxy for butterfly dispersal ability (e.g. Fric et al. 2006; Ockinger et al. 2010).

We believe that life-history may offer a better alternative to infer dispersal. Indeed, dispersal is tightly woven into organisms' life-history, encapsulated in syndromes associating different life-history traits both at the within- and at the between-species level (e.g. Li and Margolies 1993; Fjerdingstad et al.; Stevens et al. 2012; Ronce and Clobert 2012). The co-evolution of dispersal and for instance those traits that promote a fast turnover of individuals within populations, which results in a so-called dispersal syndrome, offers the opportunity to predict dispersal from the value taken by other, better informed traits. Here, we will examine if life-histories could be suitable proxies to predict the dispersal ability of butterflies, either alone or in combination with body size.

To uncover the syndrome of life-history associated with dispersal ability and then to measure the quality of the prediction of dispersal that could be obtained by using these syndromes, we used dispersal and life-history data previously published for European butterflies. There are several ways of measuring butterfly dispersal, all uncovering different facets of the process (Stevens et al. 2010b). Here, we described dispersal by four different measurements, pertaining either to the rate and distance of individual movements (measured in the field) or to gene flow among local populations (assessed by population genetics). We considered these four different dispersal measurements sequentially, and modelled their relationships with 18 candidate traits (17 life-history traits and wing size, here taken as an indication of body size) to highlight the syndromes associated with these facets of dispersal.

Then, we retained the combination of traits that gave the best predictive value, and we quantified (by cross-validation) its ability to predict dispersal. In this quantification, we took the inferences obtained from wing size alone as the reference, since wing size was regularly used as a dispersal proxy for butterflies, and we know that its predictive power is low. Finally, we applied the selected predictive model to more than 100 butterfly species for which dispersal was not measured to date, and we explored the general characteristics of dispersal within this group.

## Material and methods

### *Dispersal data*

Butterfly dispersal has been assessed by a variety of methods reviewed in Stevens et al. (2010b) and was available for 50 NW-European species (on 142). The most popular methods include mark-release-recapture (MRR) and inferences from population genetic structure using allozymes. We used the same dispersal data as in Stevens et al. (2010b), here restricted for the sake of statistical power to those measurements available for >15 species. This filtering retained four measurements of dispersal, detailed in Table 1: three were directly related to inter-patch movements assessed in MRR surveys and the last one was the gene flow over space inferred by genetic methods using allozymes.

Dispersal measurements were available for 25 to 30 species each, for a total of 47 species. There were on average 17 species common to each pair of measurements, with 11 species having all four measurements, and 15 having only one.

**Table 1**. The four dispersal measurements available in European butterflies used in this study.

| Dispersal facet | Description of the measurement | Transfo. | N |
|---|---|---|---|

|  |  |  |  |
|---|---|---|---|
| Mean dispersal distance | Mean dispersal distance (km) estimated from a of a negative exponential function of the form $P(D) = e^{-\alpha * D}$ with D=distance (km), fitted to dispersal kernel (density probability of dispersal distances) obtained from mark-release-recapture (MRR) surveys. Mean dispersal distance (x) = $1/\alpha$. | $x'=\log(x)$ | 30 |
| Frequency of long-distance dispersal | Probability of >5km dispersal movements, estimated from a inverse power function of the form $P(D) = a * D^{-b}$ with D=distance (km), fitted to dispersal kernel (density probability of dispersal distances) obtained from mark-release-recapture (MRR) | $x'=\log(x)$ | 28 |
| Dispersal propensity | Propensity to leave a patch, estimated from the proportion of recaptures of marked individuals that occurred in patch of initial capture (residents) in MRR surveys. Dispersal propensity is [1-proportion of residents]. | $x'=-\sqrt{x}$ | 25 |
| Gene flow | Dispersal ability estimated from gene flow across landscapes, as given by the analysis of allozymes spatial redistribution. Corresponds to $[1-F_{ST}]$. $F_{ST}$ quantifies the genetic structuring of populations, and hence is inversely related to gene flow. | $x'=1-\sqrt{x}$ | 26 |

Note. Transfo. is the function ensuring data normality and N is the number of European butterfly species for which the measure is given in Stevens *et al.* (2010b).

### *Life history and morphology*

Butterfly life-histories were described by 17 traits pertaining to demography, specialization and behavior (detailed in Table 2), with species values reported by Bink (1992) and Lafranchis (2000). Ten traits described species demography: the fecundity, the adult lifetime, the voltinism (the number of generations per year), the larval growth rate (averaged over successive generations), the ripe egg load at emergence, the ovigeny index (proportion of eggs already matured at female emergence), the duration of female maturation, the overwintering stage, the flexibility of the life-cycle and the length of the flight period. Four traits described ecological specialization of a species: the thermal tolerance of adults, their habitat range, the dietary niche breadth of larvae, and the strength of a mutual association with ants (myrmecophily). Three behavioral traits were analyzed. For females, we considered the precision in the choice of the laying site (female precision), and the laying strategy that separates single-egg layers from those species that lay batches of ≥ 2 eggs. For males, we retained the strategy of mate location.

Wing size, here summarized by wing length, was used as $18^{th}$ species trait. We used the values reported by Bink (1992), who provided averaged wing size over sexes and generations in cases these were polymorphic. Wing size was on average 11-37.5 mm for NW- European butterfly species. Wing size was log-transformed before analyses, given that allometric relationships are usually power shaped (Peters 1983).

**Table 2**. Life history traits used to predict butterfly's dispersal with generalized linear models. All traits are available for 142 butterfly species, except the laying strategy that is available for 137.

| Trait | Trait description |
|---|---|
| Fecundity | Mean number of eggs laid by females of the species (9 categories). |
| Adult lifetime | Mean duration (days) of the adult stage. Upper limit set at 60 days for species that overwinter as adults: ranges 5-60 days. |
| Voltinism | Annual number of generations, from 0.5 (biannual species) to 3 generation/year. |
| Larval growth rate | Duration (days) of the feeding period for larvae (i.e. without diapause), averaged over successive generations of a year; ranges 16-186 days. |
| Ripe egg load | Number of mature eggs in female's abdomen at emergence (9 levels). |
| Ovigeny index | Proportion of full-grown eggs at emergence (ranges 0-1). |
| Female maturation | Time (days) between female emergence and its first laying: 8 levels, from 1 (1-2 days) to 8 (laying starts after several weeks of diapause). |

| | |
|---|---|
| Overwintering stage | Stage at which the species usually overwinters. 8 categories: from 0 (egg) to 6 (adult), and an additional category for species without overwintering (warm regions). |
| Flexibility of life-cycle | Separates on the one hand species with inflexible life-cycle and on the other hand species with prolonged, shortened, or repeated diapause, with facultative aestivation, or with staggering of emergences, all considered ‘flexible species’. |
| Flight period | Length (in weeks) of flight period (averaged over successive generations where relevant); ranges 3-32 weeks. Results from the interplay between adult lifetime and the synchronization of adult emergences, as shown by a low but significant correlation with lifetime (correlation = 0.34, $P < 0.001$: Stevens et al. 2012). |
| Thermal tolerance | Degree of adult tolerance to temperature extremes and temperature variation (9 levels). |
| Adult habitat range | Number of different ecosystems in which adults of the species are usually found (ranges 1-7). |
| Larval dietary breadth | Number of different host plants caterpillars of the species accept: 4 levels: 1= plants of one species, 2 = plants of one genus, 3 = plants of several genus of the same family, 4 = plants or several families. |
| Myrmecophily | Degree of association with ants, from 0 (no association at all) to 9 (obligate, long association). |
| Female precision | Female precision in egg-laying, 9 levels: from 1 (the female lay where it lands, or even flying) to 9: the female choose the exact position (plant species, plant tissue, height and orientation) before laying each egg or batch of eggs. |
| Laying strategy | Female egg-laying strategy: segregates single-egg layers from those species that lay batches of ≥ 2 eggs. |
| Mate location | Seven levels in the strategy of males mate location, from 1= sit-and-wait strategy to 7 = strong lek forming, through 3 = patrolling and 5 |

=territoriality, and intermediates.

---

*Detection of dispersal syndromes*

Our aim here was to model the various facets of dispersal in butterflies from their life-history traits, while controlling for their wing size. To that purpose, we build models based on the relationships between dispersal measurements and a selection made among the traits presented in Table 2: 10 demographic traits, 4 ecological traits, 3 behavioral traits, and wing size. Wing size was kept in these models as previous studies showed that dispersal is partly dependent on wing size in butterflies (Sekar 2012; Stevens et al. 2012).

In a preliminary step, we analyzed the shape of the relationships between each of the 4 dispersal measurements and each of 16 species traits (all but binomial traits). In particular, we inspected if there was some evidence for non-linear relationships (i.e. U-shape or inverted U- shape relationships) that should be modelled using polynomial terms. We evidenced significant quadratic relationships in 7 cases (of 64), and the quadratic term was marginally significant ($P<0.1$) in 7 other cases (see Table A1): in all those cases, we modelled the effect of the corresponding trait with a polynomial of degree 2; otherwise, only simple-term (i.e. linear) effects were modelled.

We modelled the relationship between dispersal and life-history traits by generalized linear models (GLM). For each dispersal facet, the model selection would start with a full model with the effect of all 18 species traits; in all cases however this model would be saturated. To select a single simpler model, we ran all simpler GLM derived from this full model, with a maximum number of parameters set at 8, in order to avoid saturation. We compared these simpler models through their Akaike Information Criterion corrected for small sample size (AICc: Anderson et al. 1994) using the dredge R-function (Barton 2011). In a second step, in order to identify possible interactions between traits, we built models in which we incorporated the variables retained in the top-ranked models of the first-step of selection (within 2 points of AIC), this time incorporating all first-order interactions. Again, we ran and compared through their AIC all simpler models derived from this model, again with the maximum number of parameters set at 8. The model finally retained was chosen among the models with lowest AIC obtained in this second step of selection (i.e. within 2 points of AIC): we retained only the model with the highest $R^2$ as it captured most of the deviance and hence would be better at predicting the dispersal measurement, which was our goal.

In multi-species comparative studies, it may be important to account for the interdependency of species that arose through common-ancestry. However, some traits (or associations among traits) may be not related with their phylogenetic history (e.g. Gittleman et al. 1996), in which case the application of phylogenetic comparative methods may be unnecessary, and even may induce errors (Martins 2000). To verify that this was the case here too, we performed a preliminary analysis, exactly as described for GLM, but in which dispersal was modelled by phylogenetic generalized least squares method (PGLS, instead of GLM), where the phylogenetic relationships (taken from Cizek et al. 2006) among species is taken into account. In these PGLS, we fitted lambda (the parameter that scales the phylogenetic constrain) by maximum likelihood and verified that its value was negligible (not different from zero). This was the case for all models, so we do not show these PGLS here but instead we only showed GLM, where species are considered independent data points.

### *Quality assessment of the predictions*

After having selected a model for each of the four dispersal measurements, which evidenced the syndrome of life-history traits associated with each of the dispersal facet considered, we assessed their ability to adequately predict the dispersal ability of species. As quality is a matter of comparison, we took the inferences made from wing size only (i.e. a GLM where the only explanatory variable was wing size) as the reference for this comparison.

The quality of the inferences was measured by cross-validation. We used a 75-25% random partitioning of the dataset: 75% of species (i.e. 19-23 species) were the training partition used to parameterize the model (either with the model based on dispersal syndromes, or with the model with wing size only), which was applied to predict dispersal of the remaining species (i.e. 6-7 species in the test partition). Hundred independent random partitions allowed the estimation of standard errors in the predictions.

The performance of the model to predict dispersal was then assessed by the rightness and the imprecision of its predictions. We defined here the rightness as the slope of a regression of (mean) predicted vs. observed dispersal, which ideally should tend to +1. We defined the imprecision of a prediction as the mean absolute difference between (mean) prediction and observed dispersal. For the mean dispersal distance and the probability of long- distance dispersal, this difference was divided by the corresponding observed value, to account for probable scale-dependency in imprecision. We ran 10 independent cross-validations to obtain standard errors of imprecision and rightness for each model.

The gain in precision obtained by predicting dispersal from syndromes of life-history rather than from the only information of wing size was given by the ratio of the imprecision of both methods. The gain in rightness was given by the difference between the slopes of predicted vs. observed regressions obtained with both methods. The statistical significance of these differences was appreciated by GLM, with either imprecision or rightness as the response, and the model type as the independent variable.

Finally, to appreciate the relative importance of each variable for the prediction, we partitioned the $r^2$ of each model among the dependent variables retained, by averaging the increase in $r^2$ due to each variable over all possible orders of the regressors (see Lindeman et al. 1980).

*Dispersal ability of butterflies*

We used the four models selected (one per dispersal measurement) to predict the corresponding dispersal facets for the 142 butterfly species of N-W Europe. Because the predictions might be erroneous if the shape of the dispersal/trait relationship is unknown for a range of trait values, we verified whether the range of values used to parameterize the model (i.e. in species with measured dispersal) corresponded to the range of values in the species for which dispersal would be predicted (all 142 species). In case the effect of a given trait was evidenced on a truncated range of trait values, we restricted our predictions accordingly to the set of species with comparable trait values (see Figs A1, A2 in the online appendix).

## Results

*Dispersal syndromes*

The dispersal ability of butterflies tightly correlated with their life-history traits, a pattern that was independent of wing size for two of the four dispersal measurements (Table 3). Wing size was only correlated with the measure of individual movements obtained in MRR: mean dispersal distance and frequency of long-distance dispersal (Table 3). However, even in these cases, the models where life-history was incorporated explained much better the variation in dispersal ability than did wing size only. In general, wing size was at best of medium importance in the models with life-history traits (Table 3).

**Table 3.** Linear models used to predict the dispersal of butterflies. Four dispersal measurements were modeled from their relationships with a variety of traits (body size, demography, behaviors and ecological specialization were proposed as independent variables). See text for the procedure of model selection. The lower part of the table shows models with wing size as the only regressor, taken for comparison

in this study.

| Response | GLM selected when 17 life-history traits and wing size were proposed | Contribution to $R^2$ | Estimate | F | df | P | Adj.$R^2$ |
|---|---|---|---|---|---|---|---|
| Mean dispersal distance | *Intercept* | | -1.987 | 14.73 | 8-21 | <0.0001 | 0.791 |
| | *Larval growth rate* | 0.231 | -0.005*** | | | | |
| | *Adult habitat range 1* | 0.089 | 0.599* | | | | |
| | *Adult habitat range 2* | 0.067 | 0.174 (ns) | | | | |
| | *Ovigeny index 1* | 0.103 | -1.403** | | | | |
| | *Ovigeny index 2* | 0.040 | 0.412(ns) | | | | |
| | *Log (wing size)* | 0.176 | 0.508** | | | | |
| | *Larval growth rate:ovigeny index 1* | 0.097 | 0.017** | | | | |
| | *Larval growth rate:ovigeny index 2* | 0.046 | 0.003(ns) | | | | |
| Frequency of long-distance dispersal | *intercept* | | -3.214 | 21.45 | 8-19 | <0.0001 | 0.858 |
| | *Length of flight period 1* | 0.058 | -1.273** | | | | |
| | *Length of flight period 2* | 0.335 | -1.906*** | | | | |
| | *Log ( wing size)* | 0.148 | 0.846** | | | | |
| | *Voltinism: adult habitat range* | 0.142 | 0.291* | | | | |
| | *Voltinism* | 0.126 | -0.779(ns) | | | | |
| | *Adult habitat range* | 0.046 | -0.465(ns) | | | | |
| | *Ovigeny index* | 0.024 | 0.328* | | | | |
| | *Larval dietary breadth* | 0.021 | -0.151(ns) | | | | |
| Dispersal propensity | *Intercept* | | -0.586 | 16.79 | 9-15 | <0.001 | 0.856 |
| | *Thermal tolerance, 1* | 0.037 | -0.029 | | | | |
| | *Thermal tolerance, 2* | 0.290 | -0.446*** | | | | |

|  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|
|  | *Overwintering stage* | 0.201 | -0.122*** |  |  |  |  |
|  | *Myrmecophily* | 0.164 | -0.031*** |  |  |  |  |
|  | *Ripe egg load* | 0.096 | 0.036*** |  |  |  |  |
|  | *Female precision* | 0.039 | -0.006*** |  |  |  |  |
|  | *Ovigeny* | 0.029 | 0.286* |  |  |  |  |
|  | *Ovigeny: ripe egg load* | 0.035 | -0.047** |  |  |  |  |
|  | *Ovigeny: female precision* | 0.018 | -0.040* |  |  |  |  |
| Gene flow | *Intercept* |  | 0.515 | 10.09 | 6-19 | <0.0001 | 0.775 |
|  | *Fecundity* | 0.245 | -0.004** |  |  |  |  |
|  | *Female maturation* | 0.173 | 0.037*** |  |  |  |  |
|  | *Voltinism* | 0.122 | 0.120(ns) |  |  |  |  |
|  | *Ripe egg load* | 0.080 | -0.022(ns) |  |  |  |  |
|  | *Fecundity: ripe egg load* | 0.079 | 0.014*** |  |  |  |  |
|  | *Voltinism: ripe egg load* | 0.076 | -0.023** |  |  |  |  |
|  | GLM with wing size only |  | Estimate | F | Df | P | R² |
| Mean dispersal distance | *Intercept* |  | -2.815 | 10.1 | 1-28 | 0.004 | 0.239 |
|  | *Log (wing size)* | - | 0.674 ** |  |  |  |  |
| Frequency of long-distance dispersal | *Intercept* |  | -6.805 | 9.86 | 1-26 | 0.005 | 0.247 |
|  | *Wing size* | - | 1.571** |  |  |  |  |
| Dispersal propensity | *Intercept* |  | -1.708 | 9.87 | 1-23 | 0.005 | 0.270 |
|  | *Wing size* | - | 0.305 ** |  |  |  |  |
| Gene flow | *Intercept* |  | 0.753 | 0.14 | 1-24 | 0.720 | -0.036 |
|  | *Wing size* | - | 0.001 (ns) |  |  |  |  |

***: $P<0.001$; **: $0.001>P>0.01$; *: $0.01>P>0.05$; ns: $P>0.1$. Contribution to R² after the method of Lindeman *et al*. (1980)

A distinct syndrome of life-history traits was associated with each of the four dispersal measurements. Each model built here used up to six different species traits pertaining to demography and ecological specialization: models are detailed in Table 3 and the trait effects are illustrated in Figs A3-A6 of the online appendix. Wing size intervened only in models to predict mean dispersal distance and the frequency of long-distance dispersal, together with adult habitat range and ovigeny (for both measures), and with larval growth rate (for mean dispersal distance) or with voltinism, length of flight period and larval dietary breadth (for long-distance dispersal). Almost completely different suites of traits were retained to explain the variance in the two other dispersal measurements. Dispersal propensity was related to thermal tolerance, overwintering stage, myrmecophily, ripe egg load, female precision and ovigeny. Four traits were needed to explain the variation in gene flow among species: the voltinism and three female traits (the fecundity, the ripe egg load and the female maturation).

### *Quality of predictive models*

Dispersal predicted from the four selected models correctly fitted to the observed measurements (Fig. 1). The predictive ability of these models was much higher than that with wing size only (Table 4). Dispersal in ecological time and gene flow were both well predicted from life-history traits (Table 4, Fig. 1): the slopes of observed versus predicted dispersal ranged between 0.69-0.86, to be compared with the much lower slopes obtained with wing size only (range -0.05 – 0.25), and the predictions obtained were up to 5 times more precise than those obtained with wing size only (Table 4).

**Table 4.** Quality assessment of generalized linear models used to predict dispersal in butterflies. Model description is given in Table 3. Reference level: rightness and precision obtained with a GLM using only wing size.

| | Rightness | | | Imprecision | | |
|---|---|---|---|---|---|---|
| Dispersal measurement | GLM with life-history traits | Reference level | Gain in rightness | GLM with life-history traits | Reference level | Gain in precision |
| Mean dispersal distance | 0.805 ± 0.002 | 0.223 ± 0.003 | + 0.582*** | 0.386 ± 0.001 | 0.629 ± 0.004 | x 1.63*** |
| Frequency long-distance dispersal | 0.831 ± 0.011 | 0.225 ± 0.002 | + 0.606*** | 1.043 ± 0.015 | 5.211 ± 0.003 | x 5.00*** |
| Dispersal propensity | 0.863 ± 0.006 | 0.246 ± 0.004 | + 0.618** | 0.099 ± 0.001 | 0.173 ± 0.0003 | x 1.74*** |
| Gene flow | 0.693 ± 0.006 | -0.056 ± 0.002 | + 0.749*** | 0.0194 ± 0.0001 | 0.031 ± 0.002 | x 1.59*** |

Rightness: slope of a regression of predicted versus observed dispersal. Imprecision: average absolute difference between observed and predicted values (for mean dispersal distance and the frequency of long-distance dispersal, given relatively to observed value to account for scale dependency). Mean ± SE over 10 independent bootstraps. Gain in rightness = rightness trait model – reference. Gain in precision = imprecision imprecision/imprecision trait model. ***: P<0.001 that rightness or imprecision is similar to the reference level.

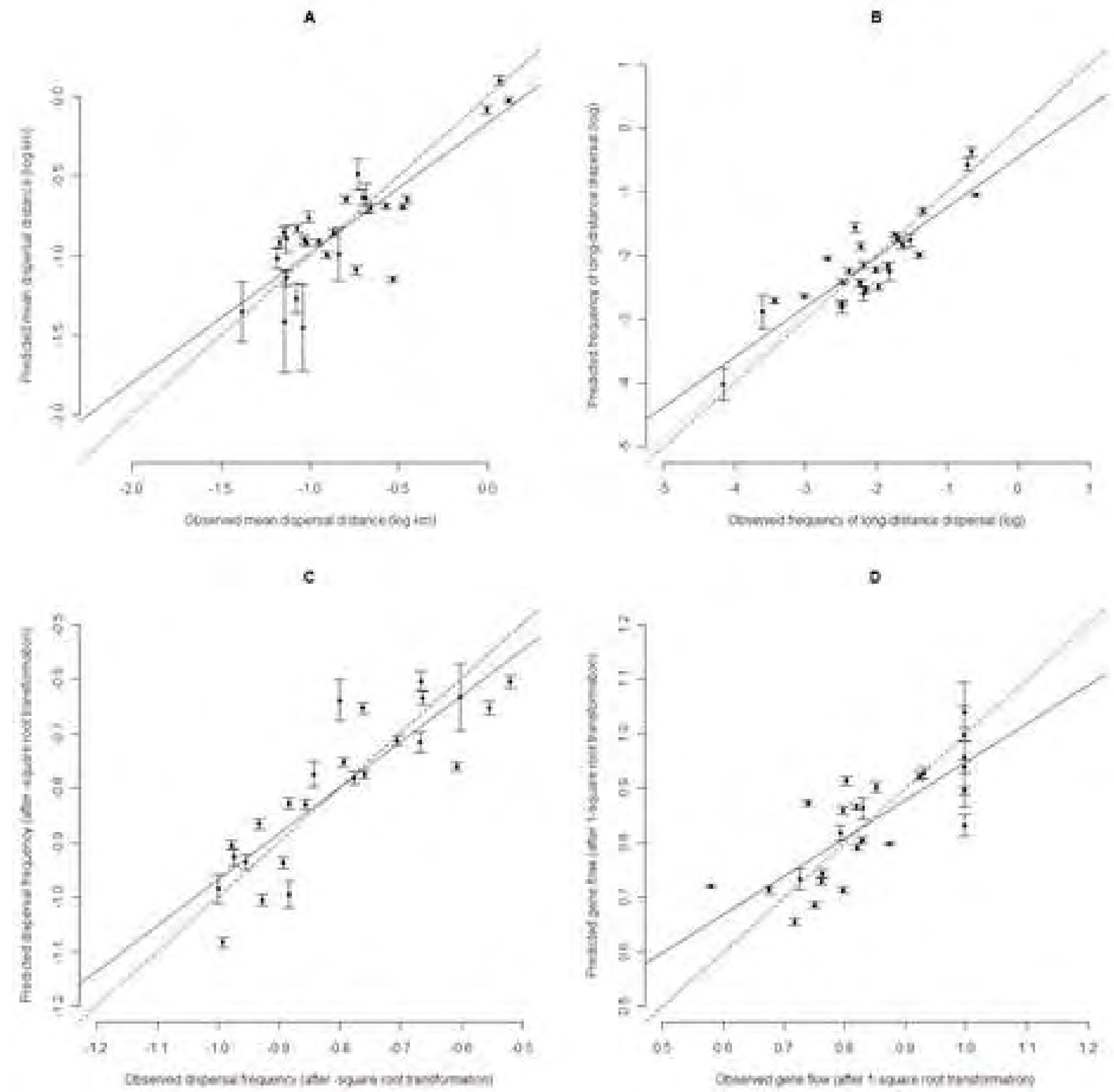

**Figure 1.** Cross-validations of predictive models for butterfly dispersal: predictions obtained from information on multiple life-history traits, together with wing size (Panels A, B) or not (panels C, D) (see Table 3). A: predicted mean dispersal distance; B: predicted frequency of long-distance dispersal; C: predicted dispersal propensity; D: predicted intensity of gene flow, and their respective 95% CI (obtained with 100 random partitions), all plotted against the values of these traits observed for 25-30 butterfly species. Black lines show the linear regressions; for comparison dotted lines show the slope 1:1, and grey line show the regression forced into 0:0.

We used the four models retained to infer dispersal ability for all NW-European butterflies. The comparison of the observed dispersal measurements to those values inferred from these models showed that the distribution of dispersal ability in predictions and in observations generally converged (Fig. 2; Figs A7-A10 in the online appendix).

Our inferences highlight that most butterfly species in Europe move only a few hundred meters on average during dispersal. The mean dispersal distance averaged 158 m in predictions (observed = 155 m), and 90 % of the species were predicted to have mean dispersal distance ≤ 408 m (observed: < 388m). A few species were nevertheless observed (and predicted) to move more than 1 km on average. Very long-distance dispersal however was infrequent, and most species were observed (and predicted to disperser farther than 5 km only rarely: this probability was on average less than 0.01. Here again, a few species appeared to have very high dispersal power, with the probability of such long movements reaching 0.25 in some species (observed; higher predictions reached a probability of 0.37 for long-distance dispersal). By contrast, the dispersal propensity of butterflies was generally high: on average about one third (34 %) of the individuals usually leave their natal patch, or the patch of their first capture, and this (observed) proportion reaches 73% in some species (predicted: 78%). A few species however appeared much more philopatric: dispersal propensity was less than 5% for the 10% less dispersive species. This high frequency of usually short movements resulted in quite high levels of gene flow among populations, and the genetic structuring was generally weak: the higher $F_{ST}$ observed was 0.177, but 90% of the species had observed $F_{ST} \leq 0.078$ (in predictions 90% of species had $F_{ST} \leq 0.082$ and the maximum predicted was 0.117).

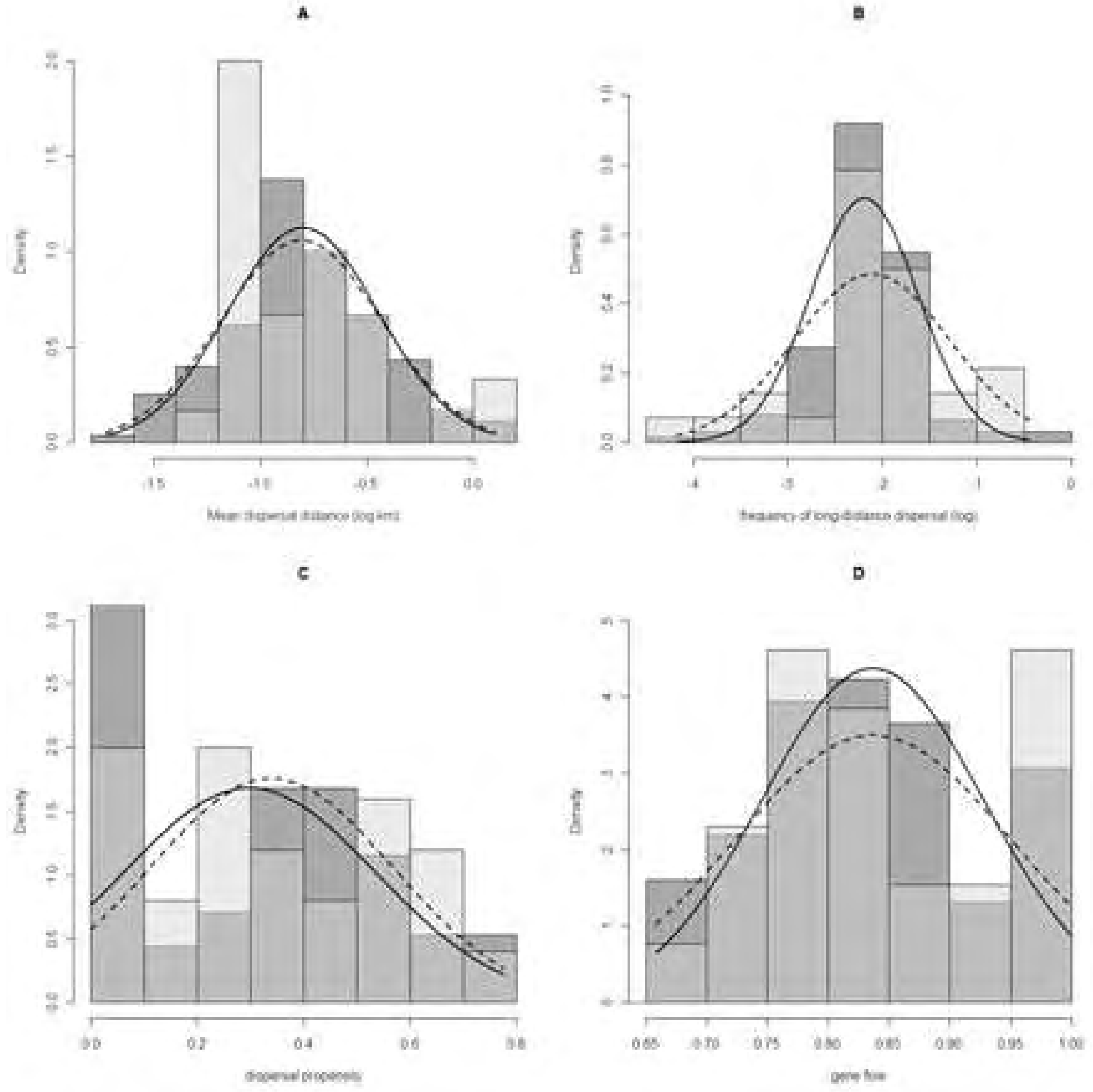

**Figure 2.** Predicted (dark grey, solid curve) and observed (transparent light grey, dotted curve) density probability and corresponding fitted normal distributions of dispersal ability in butterflies. A: mean dispersal distance; B: probability of long-distance dispersal; C: dispersal propensity; D: gene flow. Observations were direct measurement obtained from mark-recapture surveys (A-C) or indirect estimates obtained via population genetics (D). Predictions were obtained from linear models using wing size and three life-history traits (A-B) or only information on four life-history traits (C-D). Predictions were truncated >0 for B, and 0-1 for C and D. Predictions are available for N=124-138 species.

## Discussion

### *The constituting parts of dispersal are embedded in distinct syndromes*

Clearly, as it was predicted from theoretical models (see a review in Ronce and Clobert 2012), dispersal is not evolving independently of other traits, which give rise to predictable syndromes, and consequently a large part of the dispersal variability can be explained by the variation in other phenotypic traits. However, an interesting result of our study was that different facets of the dispersal process correlated with completely different suites of traits. Although theory is unclear on this point (Starrfelt and Kokko 2012; Kisdi et al. 2012), some empirical results already suggested that different combinations of life-history traits can be implied at the different steps of the dispersal process (Massot et al. 2002; see also examples in Bonte et al. 2012). The relative roles of phylogenetic inertia, natural selection, sexual selection, or phenotypic plasticity in explaining these relationships among traits should certainly deserve further investigation.

The way dispersal is measured in fact uncovers different parts of the dispersal process like individual movement rates versus gene flow, possibly corresponding to the different definitions of dispersal existing in the literature (Stevens et al. 2010b). The fact that very different suites of traits were retained to predict these various facets of dispersal can be an indication that they are under partially uncoupled selective pressures, which we already suspected from our previous studies (Stevens et al. 2010a, 2010b). Such differences possibly will result in some opposition between the short-term demographic consequences of dispersal and its long-term genetic effects.

This segregation of the dispersal facets in different syndromes of life-history traits would also have deep consequences for the functional diversity of communities facing environmental changes. Indeed, any selection on a given facet of dispersal will have distinct indirect consequences on life-histories, and the dispersal costs at each of the dispersal steps may be paid independently from the costs incurred at other steps (Bonte et al. 2012). Habitat fragmentation for instance was shown to filter species according to their dispersal ability (e.g. Driscoll and Weir 2005; Van Houtan et al. 2007) and to affect the distance moved (Bonte et al. 2010) or the dispersal propensity (Schtickzelle et al. 2006). Our results show that such filtering, or selection, on the mean dispersal distance would entail the functional diversity within butterfly communities, by having indirect effects for instance on the diversity of specialization (adult habitat range) or of larval growth rate in these communities, but may have no effect on the intensity of gene flow.

In light of this result, dispersal should now be seen as an additional vector in life- history, consisting in several uncoupled (or loosely coupled) dispersal traits (dispersal distance, dispersal frequency, gene flow), which increases the array of potential life-history tactics within communities. Accordingly, we must consider each of these dispersal traits as so many axes of a species' niche.

### *Applications in biodiversity conservation*

The advantage of considering several phenotypic traits, and noticeably life-history traits, to infer dispersal ability is considerable. Life-history indeed appeared to be a very convenient proxy to infer unknown dispersal ability at the species level. For all four dispersal measurements considered here, the inclusion of life-history traits in linear models greatly improved the predictions we would have made from wing size only (Table 4). For two measurements (gene flow and dispersal propensity), wing size was even not retained by model selection. For the two other traits, the relative importance of wing size in structuring the variation in dispersal was low (Table 3). These results highlight the fact that this allometry does not work properly for predicting butterfly dispersal, but the evolution of dispersal syndromes provided valuable alternative to make this inference, useful for planning actions targeted at preserving biodiversity.

Measuring movement rates and distances usually requires long and extensive mark- recapture studies or direct tracking, which is always costly and even may reveal impossible, particularly for rare or endangered species. The traits-based approach developed here proved very useful for inferring mean dispersal distance, dispersal propensity, and even the frequency of long-distance dispersal. Mean dispersal distance is most often needed to feed simulation models, and to help decision making in conservation (Moilanen et al. 2005). For instance, it can be used to infer the spatial grain at which suitable habitats should be distributed in a given landscape to allow a smooth metapopulation functioning (Hanski 1999b; Baguette and Van Dyck 2007; Baguette et al. unpublished). Long distance dispersal can also be very crucial for metapopulation persistence, by hampering genetic drift and its negative effects (Lande 1988), or by allowing (re)colonization of distant habitat patches. These maximal movements however are most often ignored in conservation decisions, because they are often not documented. The traits-based model that we developed here to infer this dispersal measurement is therefore interesting because it requires traits that are quite easy to collect from large-scale monitoring, amateurs' reports, or lab rearing, and hence that can easily be informed for most species.

The inference of gene flow by population genetics is also costly as it requires intensive sampling, coupled with laborious and expensive lab work. Therefore, it can reveal infeasible in some case, especially when conducting multi-species comparisons or feeding multi-species models is the research goal. The traits-based method derived here from the syndromes associating this facet of dispersal to other phenotypic traits offers a reliable alternative to population genetics. Consequently, the relative ability of species to maintain gene flow across space could be inferred for a lot of species, to be integrated, e.g., in designing conservation plans.

It should be interesting to explore to what extent the trait-based approach allows this inference in taxa other than butterflies. Whitmee and Orme (in press) showed that the trait- based approach reliably predicts natal dispersal distances for mammals: both maximal and median distances were satisfactorily predicted with a variety of traits sets. Life-histories of plants predicted reliably the dispersal mode of seeds (ie: ballistic, wind-assisted, transport by animals, etc: Thomson et al. 2010). The extent to which life-histories allow predicting seed dispersal distances, seed dispersal frequency or plant gene flow was however not assessed, probably because their determinants will mainly depend on the dispersal mode the seeds use. In amphibians, we showed that even a poorly informed dataset, with a large amount of missing values, yields accurate predictions of dispersal distances (Trochet et al. unpublished). Trait-based methods thus seem promising to infer unknown dispersal traits.

*How can we improve the inferences on dispersal?*

Our trait-based approach does offer a quick and cheap access to the average dispersal ability of species for which no dispersal data are currently available. This is particularly pertinent in the case of threatened species that may be geographically restricted and for which conservation actions are asked but cannot be implemented while ignoring dispersal. Although even imprecise approximations may strongly improve the power of modelling tools used to predict the fate of populations under changing environmental conditions (Dawson et al. 2011), any solution to refine those predictions is however welcome. We propose here below three ways for such improvement: (1) to go beyond the species level, (2) to explore other species traits, and (3) to make use of population patterns that result from dispersal.

Virtually no life-history trait is entirely fixed at the species level, and most are more or less labile, responding quickly to changed environmental conditions, or according to individual conditions (Roff 2002; Clobert et al. 2004). Even discrete traits like voltinism show some plasticity: observations of additional generations in exceptionally hot years are common in butterflies (Bink 1992; Fischer and Fiedler 2002). Dispersal also has substantial variation within species (Schtickzelle et al. 2006; Stevens et al. 2010a). A solution to take this variation into account and make inferences at the infra-species level could be to identify how dispersal varies according to environmental conditions (e.g. climate, habitat quality, fragmentation, etc.) and to population characteristics (like density, inbreeding or kin density) to refine the predictions made at the species level. However, there is currently too little information available to make such generalizations. For those cases that require very precise estimates of dispersal, for instance where dispersal is suspected to evolve locally like at expanding fronts (Burton et al. 2010), we thus recommend that dispersal should be directly measured or inferred from genetic data collected in situ.

Some traits not considered in this study could be used to refine the inferences of dispersal. Palatability of adults for instance is certainly such a trait. Previous studies showed that unpalatable species and their mimics have different flight patterns than palatable species (Chai and Srygley 1990), probably because both groups are under contrasted pressures from flying predators. Unfortunately, palatability was not measured for European butterflies, which prevented its integration here.

Finally, those elements that are affected by dispersal might also be used to refine the inferences of dispersal, like the geographic range size, or the speed of range expansion. Both relations are however probably obscured by other processes like vicariance and speciation, habitat suitability, host plant distributions, niche breadth of species along abiotic clines, or evolutionary processes at range margins. This is the reason why we did not consider those traits, although their relationships with dispersal distances were evidenced in birds and in mammals (Sutherland et al. 2000; Bowman et al. 2002; Dawideit et al. 2009).

## Conclusion

The importance of dispersal for the functioning and the evolution of populations cannot be ignored, especially now in times of deep environmental changes. Indeed, this key process determines the response of populations and species to many environmental changes, for instance by limiting local adaptation, or by allowing species to change their distribution (Parmesan 2006; Chen et al. 2011).

We showed here that the constitutive elements of dispersal (movement rate, movement distances and gene flow) are parts of different syndromes of life-history, as they related with completely different suite of traits. This implies that each facet of dispersal should be considered a species life-history trait, and an axis of the species' niche. But this also means that the changed selective pressures on one or more facets of dispersal would have distinct side-consequences for functional diversity within communities.

An interesting application of these syndromes is the inference of dispersal: the trait- based approach that relies on these syndromes is convenient to infer dispersal ability when data on dispersal are missing. Generally, the lack of reliable dispersal data is considered as the most important shortcoming in the use of those simulation models that aim at investigating the extinction risks for populations, at predicting the impact of environmental changes or at assessing the relative effects of alternative mitigation scenarios (e.g. Heikkinen et al. 2006). The trait-based approach we introduce here fills this gap by providing sound inferences of the dispersal abilities for species for which it is unknown: life-history information indeed is available for nearly 3 times more butterfly species than is dispersal information. More importantly, this approach allows the explicit consideration of each facet of the dispersal process, as well as its association with other phenotypic traits within syndromes of life- history. Taking these into account is particularly important if we wish to design efficient conservation plans for preserving the whole array of biodiversity (including for instance genetic diversity or functional diversity), facing the combined actions of landscape fragmentation and climate change.

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Supplementary material for "Dispersal syndromes and the use of life-histories to predict dispersal" by Stevens VM, Trochet A, Blanchet S, Moulherat S, Clobert J & Baguette M

The following 12 pages are for an online-only appendix

## 1. Identification of quadratic relationships between dispersal and species traits

A preliminary analysis of the relationships between dispersal measurements and butterfly traits allowed to identify putatively non-linear relationships between traits. When the effect of the quadratic term of a trait was even marginally significant in a GLM (i.e. with $P < 0.10$), the trait effect was modeled with a polynomial effect of degree 2 in the model construction for predictive models. Table A1 summarizes this preliminary analysis.

Table A1. Summary of 64 generalized linear models (GLM) with each of four dispersal measurements as the response and each of 16 species traits and their quadratic effect proposed as explanatory variables.

| | | Mean dispersal distance | | | Frequency of long-distance dispersal | | | Dispersal propensity | | | Gene flow | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Independent variable | Model parameters | Est. | SE | P>\|t\| | Est. | SE | P>\|t\| | Est. | SE | P>\|t\| | Est. | SE | P>\|t\| |
| Fecundity | Intercept | -0.78 | 0.26 | <0.01 | -2.48 | 0.63 | <0.01 | -0.72 | 0.15 | <0.01 | 0.67 | 0.08 | <0.01 |
| | Fecundity | -0.12 | 0.12 | 0.31 | -0.03 | 0.29 | 0.91 | -0.08 | 0.08 | 0.38 | 0.04 | 0.04 | 0.31 |
| | Fecundity² | **0.02** | **0.01** | **0.06** | 0.03 | 0.03 | 0.39 | 0.01 | 0.01 | 0.26 | 0.00 | 0.00 | 0.93 |
| Adult lifetime | Intercept | -2.05 | 0.58 | <0.01 | -4.11 | 1.39 | <0.01 | -1.05 | 0.29 | <0.01 | 0.73 | 0.13 | <0.01 |
| | Adult lifetime | 0.13 | 0.06 | <0.05 | 0.24 | 0.15 | 0.12 | 0.02 | 0.03 | 0.44 | 0.00 | 0.01 | 0.68 |
| | Adult lifetime² | **-0.00** | **0.00** | **0.05** | **-0.01** | **0.00** | **0.09** | -0.00 | 0.00 | 0.50 | 0.00 | 0.00 | 0.89 |
| Voltinism | Intercept | -0.22 | 0.58 | 0.71 | -1.63 | 1.49 | 0.29 | -0.12 | 0.45 | 0.80 | 0.75 | 0.29 | <0.05 |
| | Voltinism | -0.54 | 0.38 | 0.17 | -0.62 | 0.99 | 0.54 | -0.43 | 0.32 | 0.19 | 0.02 | 0.20 | 0.93 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Voltinism² | **0.10** | **0.05** | **0.06** | 0.14 | 0.14 | 0.32 | 0.05 | 0.05 | 0.28 | 0.00 | 0.03 | 0.89 |
| Larval growth rate | Intercept | -0.12 | 0.01 | 0.38 | -0.61 | 0.50 | 0.23 | -0.63 | 0.21 | <0.01 | 0.95 | 0.10 | <0.01 |
| | Larval growth rate | -0.01 | 0.01 | <0.05 | -0.03 | 0.01 | <0.05 | -0.00 | 0.01 | 0.43 | 0.00 | 0.00 | 0.32 |
| | Larval growth rate² | -0.00 | 0.00 | 0.12 | **0.00** | **0.00** | **0.09** | 0.00 | 0.00 | 0.43 | 0.00 | 0.00 | 0.40 |
| Ripe egg load | Intercept | -0.95 | 0.33 | <0.01 | -2.84 | 0.71 | <0.01 | -0.79 | 0.11 | <0.01 | 0.84 | 0.09 | <0.01 |
| | Ripe egg load | 0.09 | 0.14 | 0.54 | 0.37 | 0.33 | 0.27 | -0.02 | 0.06 | 0.67 | -0.03 | 0.04 | 0.50 |
| | Ripe egg load² | -0.01 | 0.01 | 0.46 | -0.04 | 0.03 | 0.28 | 0.00 | 0.01 | 0.51 | 0.01 | 0.00 | 0.18 |
| Ovigeny index | Intercept | -0.60 | 0.10 | <0.01 | -1.94 | 0.25 | <0.01 | -0.78 | 0.05 | <0.01 | 0.90 | 0.03 | <0.01 |
| | Ovigeny index | -1.92 | 0.67 | <0.01 | -2.60 | 1.78 | 0.16 | -0.07 | 0.42 | 0.86 | -0.47 | 0.27 | 0.10 |
| | Ovigeny index² | **1.67** | **0.64** | **<0.05** | 2.26 | 1.71 | 0.15 | 0.03 | 0.39 | 0.94 | 0.34 | 0.27 | 0.21 |
| Female maturation | Intercept | -0.65 | 0.36 | 0.08 | -1.72 | 0.75 | <0.05 | -0.55 | 0.14 | <0.01 | 0.63 | 0.10 | <0.01 |
| | Female maturation | -0.09 | 0.25 | 0.72 | -0.08 | 0.52 | 0.88 | -0.20 | 0.09 | <0.05 | 0.10 | 0.07 | 0.13 |
| | Female maturation² | 0.01 | 0.04 | 0.79 | -0.02 | 0.08 | 0.81 | **0.03** | **0.01** | **<0.05** | -0.01 | 0.01 | 0.32 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Overwintering stage | Intercept | -0.66 | 0.23 | <0.01 | -2.32 | 0.56 | <0.01 | -0.71 | 0.19 | <0.01 | 0.92 | 0.09 | <0.01 |
| | Overwintering stage | -0.19 | 0.12 | 0.14 | -0.06 | 0.30 | 0.84 | -0.05 | 0.21 | 0.82 | -0.08 | 0.06 | 0.22 |
| | Overwintering stage$^2$ | **0.04** | **0.01** | **<0.05** | 0.04 | 0.04 | 0.33 | 0.00 | 0.05 | 0.95 | 0.02 | 0.01 | 0.13 |
| Flight period | Intercept | -2.17 | 0.46 | <0.01 | -5.66 | 0.85 | <0.01 | -0.98 | 0.23 | <0.01 | 0.69 | 0.07 | <0.01 |
| | Flight period | 0.35 | 0.11 | <0.01 | 0.97 | 0.21 | <0.01 | 0.04 | 0.06 | 0.49 | 0.02 | 0.01 | 0.08 |
| | Flight period$^2$ | **-0.02** | **0.01** | **<0.01** | **-0.06** | **0.01** | **<0.01** | -0.00 | 0.00 | 0.56 | -0.00 | 0.00 | 0.23 |
| Thermal tolerance | Intercept | -1.34 | 0.37 | <0.01 | -2.52 | 0.84 | <0.01 | -1.07 | 0.12 | <0.01 | 0.59 | 0.16 | <0.01 |
| | Thermal tolerance | 0.21 | 0.15 | 0.18 | 0.21 | 0.35 | 0.55 | 0.15 | 0.05 | <0.01 | 0.07 | 0.06 | 0.26 |
| | Thermal tolerance$^2$ | -0.02 | 0.01 | 0.24 | -0.02 | 0.03 | 0.52 | **-0.01** | **0.00** | **<0.01** | -0.00 | 0.01 | 0.47 |
| Adult habitat range | Intercept | -0.49 | 0.34 | 0.16 | -1.46 | 0.81 | 0.08 | -0.64 | 0.16 | <0.01 | 0.67 | 0.07 | <0.01 |
| | Adult habitat range | -0.49 | 0.32 | 0.13 | -0.93 | 0.75 | 0.23 | -0.17 | 0.15 | 0.27 | 0.08 | 0.04 | 0.10 |
| | Adult habitat range$^2$ | **0.12** | **0.06** | **0.06** | 0.23 | 0.15 | 0.15 | 0.04 | 0.03 | 0.25 | -0.00 | 0.01 | 0.50 |
| Larval dietary breadth | Intercept | -1.23 | 0.60 | <0.05 | -1.83 | 1.68 | 0.28 | -1.27 | 0.22 | <0.01 | 0.90 | 0.15 | <0.01 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Larval dietary breadth | 0.36 | 0.46 | 0.44 | -0.03 | 1.22 | 0.98 | 0.39 | 0.18 | <0.05 | -0.05 | 0.12 | 0.71 |
| | Larval dietary breadth² | -0.07 | 0.08 | 0.42 | -0.02 | 0.21 | 0.94 | **-0.07** | **0.03** | **<0.05** | 0.01 | 0.02 | 0.73 |
| Myrmecophily | Intercept | -0.79 | 0.08 | <0.01 | -2.12 | 0.18 | <0.01 | -0.78 | 0.03 | <0.01 | 0.83 | 0.02 | <0.01 |
| | Myrmecophily | -0.04 | 0.10 | 0.68 | 0.09 | 0.27 | 0.74 | 0.09 | 0.09 | 0.34 | -0.06 | 0.05 | 0.25 |
| | Myrmecophily² | 0.00 | 0.01 | 0.79 | -0.01 | 0.03 | 0.70 | -0.02 | 0.01 | 0.21 | 0.01 | 0.01 | 0.18 |
| Laying precision | Intercept | -1.00 | 0.32 | <0.01 | -2.28 | 0.73 | <0.01 | -1.05 | 0.20 | <0.01 | 0.76 | 0.13 | <0.01 |
| | Laying precision | 0.04 | 0.14 | 0.79 | -0.06 | 0.34 | 0.86 | 0.19 | 0.12 | 0.12 | 0.02 | 0.06 | 0.69 |
| | Laying precision² | 0.00 | 0.01 | 0.96 | 0.02 | 0.04 | 0.62 | **-0.03** | **0.02** | **0.10** | -0.00 | 0.01 | 0.83 |
| Mate location | Intercept | -0.82 | 0.40 | <0.05 | -2.03 | 0.88 | <0.05 | -0.94 | 0.20 | <0.01 | 0.79 | 0.16 | <0.01 |
| | Mate location | 0.15 | 0.28 | 0.59 | 0.28 | 0.62 | 0.66 | 0.12 | 0.13 | 0.39 | 0.02 | 0.11 | 0.85 |
| | Mate location² | -0.04 | 0.04 | 0.36 | -0.09 | 0.10 | 0.39 | -0.02 | 0.02 | 0.35 | -0.00 | 0.02 | 0.92 |
| Wing size | Intercept | -1.37 | 5.25 | 0.79 | -13.5 | 14.06 | 0.35 | -1.69 | 2.94 | 0.57 | -0.38 | 1.88 | 0.84 |
| | Log(wing size) | -0.29 | 3.51 | 0.93 | 5.99 | 9.29 | 0.52 | 0.29 | 0.92 | 0.88 | 0.80 | 1.28 | 0.54 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Log(wing size)$^2$ | 0.16 | 0.59 | 0.78 | -0.72 | 0.52 | 0.64 | 0.00 | 0.31 | 0.99 | -0.13 | 0.22 | 0.55 |

Bold cells show which effects should be modeled with polynomial effects in models aiming at predicting dispersal in butterflies (see main text).

## 2. Limits to predictions

Predictions from GLM might be problematic in case of skewed range of trait values in the informed dataset (i.e. the species with dispersal measurement available) relatively to the dataset to which the model will be applied. To identify such flaw, we compared the range of values for each trait to the range observed in the whole dataset (Figure A1). This comparison showed that the four informed datasets were skewed for a variety of traits. When traits with skewed range were retained by model selection, we restricted our predictions to butterflies with trait values within the range observed in the dataset from which the predictive model was built (i.e. in species with measured dispersal), as depicted in Fig. A2.

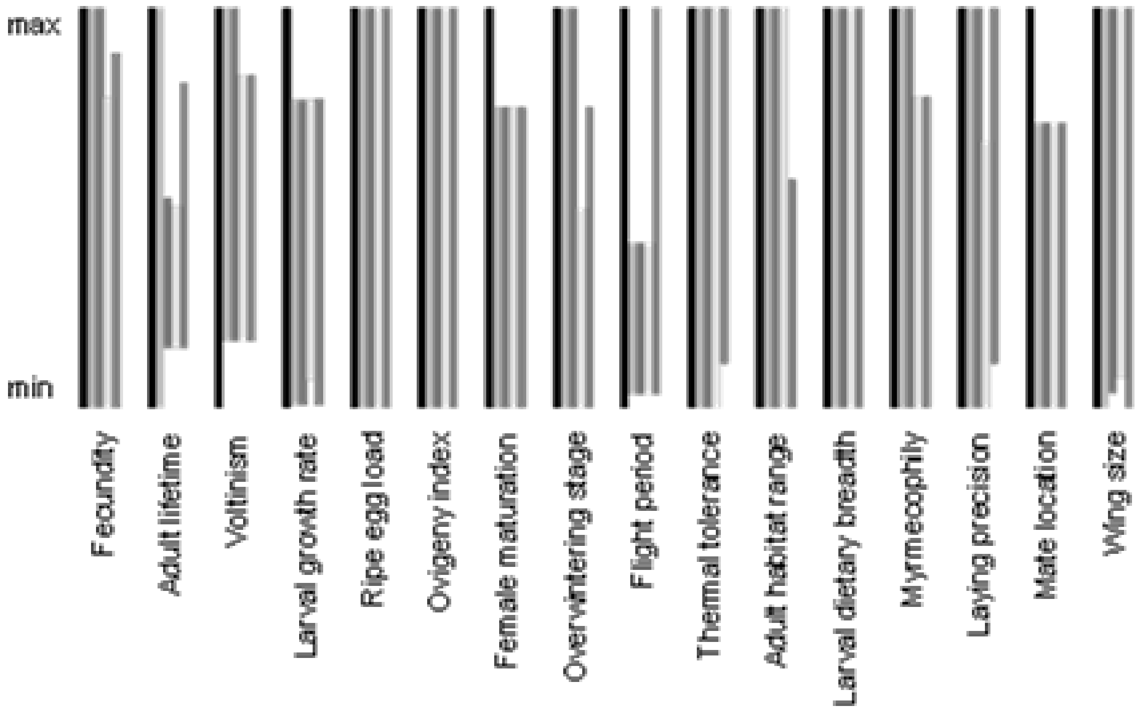


Figure A1. Range of trait values in subset of data comprising butterfly species with measured dispersal compared to range observed in 142 butterfly species of N-W Europe. Black: range with 142 species (scaled for reference); green: range in species with measured mean dispersal distances; dark blue: range in species with estimated probability of long-distance dispersal; light blue: range in species with measured dispersal propensity; orange: range in species with estimated gene flow.

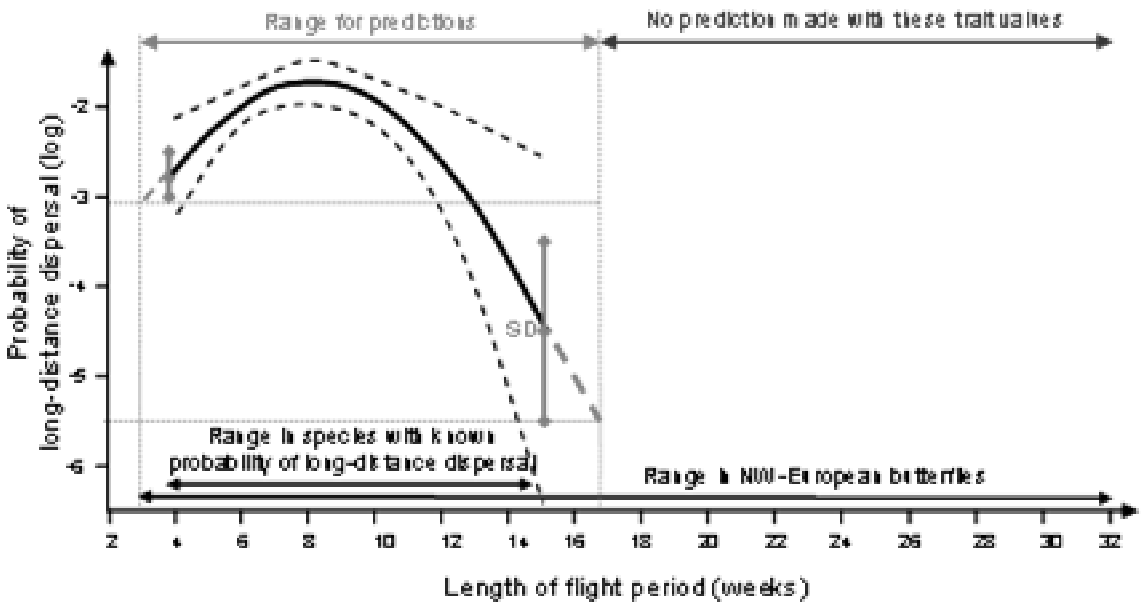


Figure A2. Illustration of a polynomial effect (dashed black curves = 95% CI) of the flight period in a predictive model for the probability of long distance dispersal in butterflies. Butterflies with known probability of long-distance dispersal have short to medium flight periods (4-15 weeks) while this dispersal measurement should be predicted for species with short to very long flight periods (3-32 weeks). Green lines indicate how we enlarged the range of values used for the predictions to 3-17 weeks, based on the standard deviation of the effect.

## 3. Trait models

We selected one single model to predict each dispersal measurement. Four species traits and some interactions among them were used in those predictive GLM. Figs A3-A6 show the effect of each trait retained in these models.

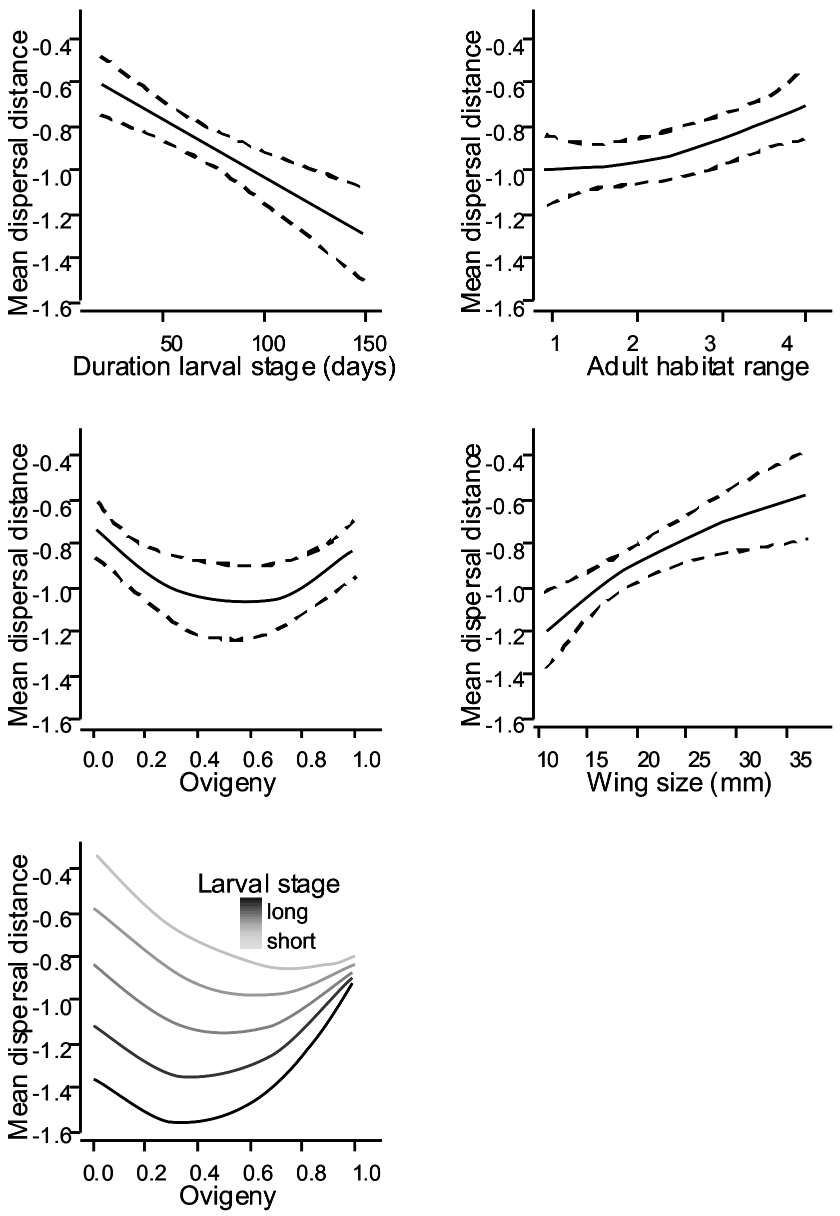


Figure A3. Illustration of the significant effects of traits and interactions between traits retained to predict the mean dispersal distance in butterflies. Mean dispersal distance is shown on a log km scale. The model is detailed in Table 3 of main text. Effects are shown with 95%CI (dashed curves), except for interaction.

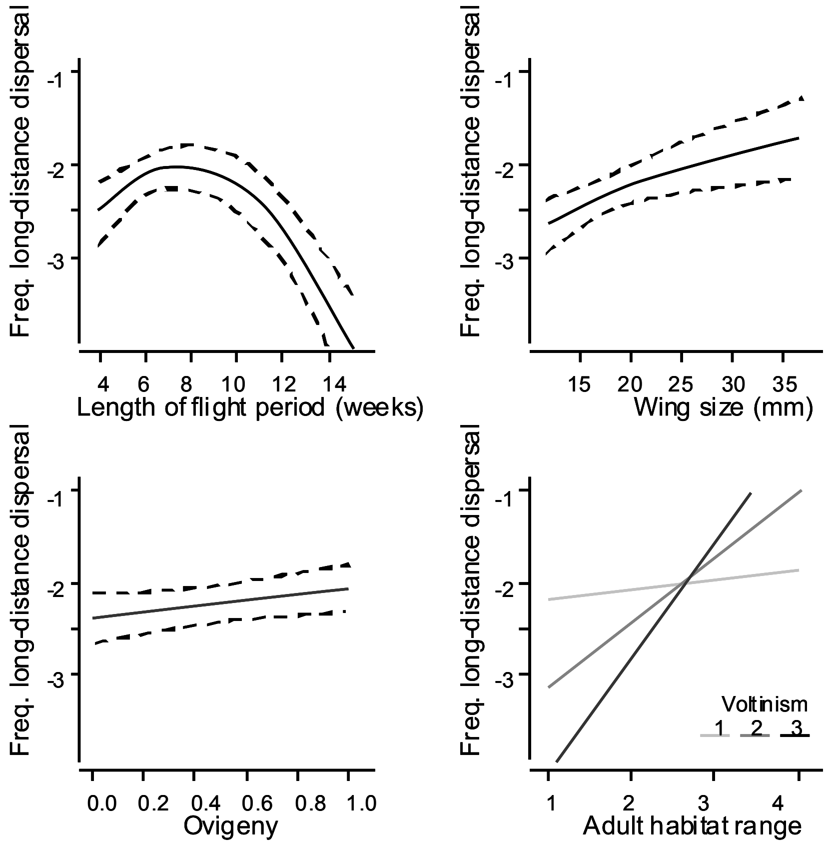


Figure A4. Illustration of the significant effects of traits and interactions between traits retained to predict the frequency of long-distance dispersal in butterflies. Frequency of long-distance dispersal is shown on a log scale. The model is detailed in Table 3 of main text. Effects are shown with 95%CI (dashed curves), except for interaction.

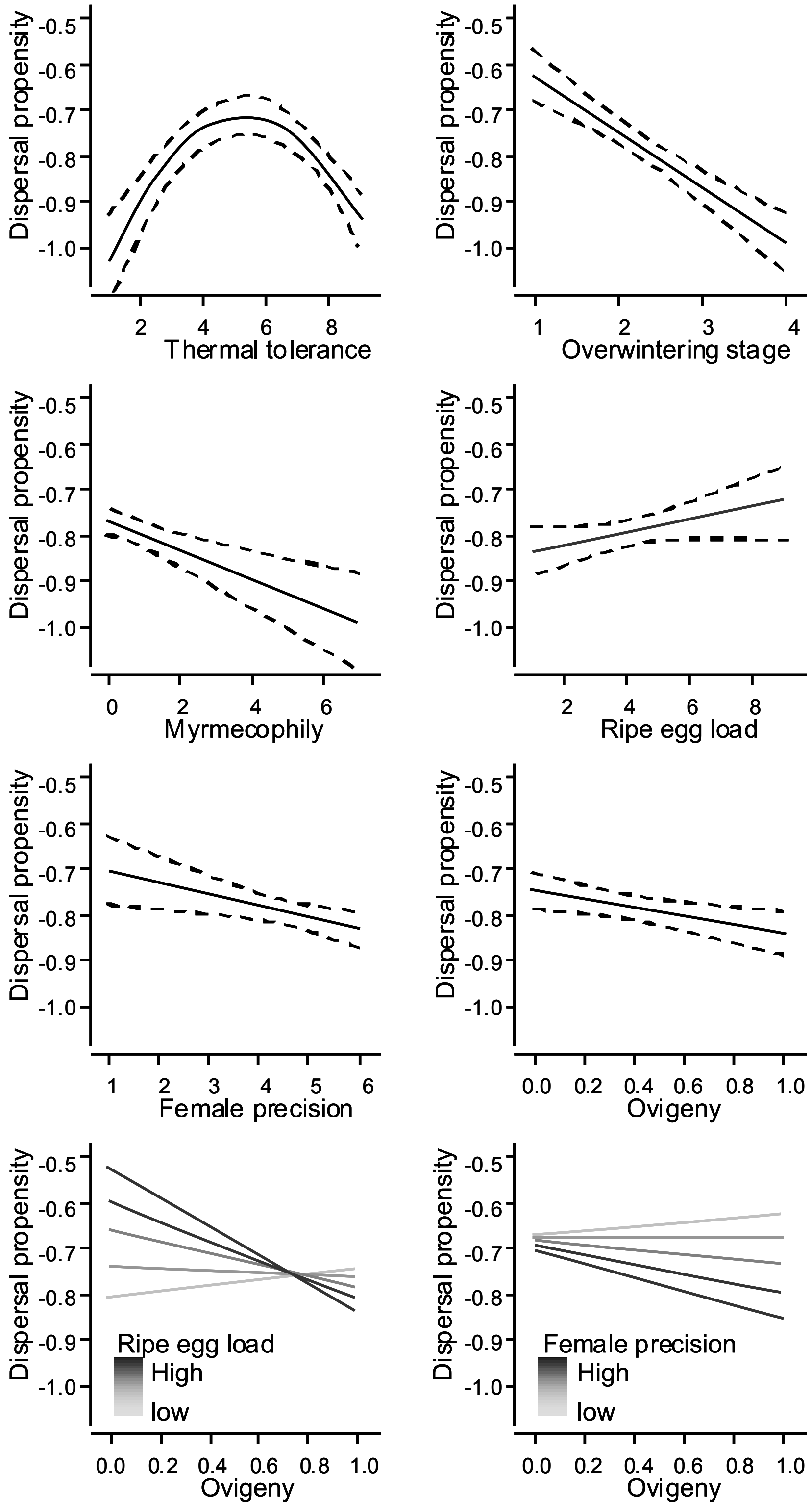


Figure A5. Illustration of the significant effects of traits and interactions between traits retained to predict the dispersal propensity in butterflies. Dispersal propensity is 1-√proportion of residents. The model is detailed in

Table 3 of main text. Effects are shown with 95%CI (dashed curves), except for interactions where CI is not shown.

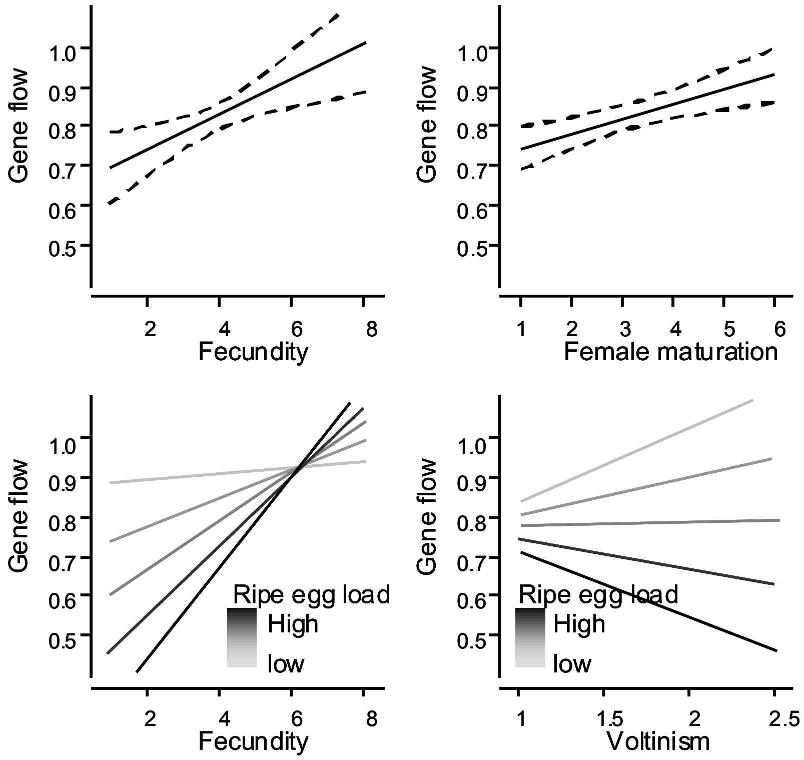


Figure A6. Illustration of the significant effects of traits and interactions between traits retained to predict the intensity of gene flow in butterflies. Gene flow is $-\sqrt{F_{ST}}$. The model is detailed in Table 3 of main text. Effects are shown with 95%CI (dashed curves), except for interactions.

4. Inferences for butterfly dispersal from life-history trait (models shown in Table 3)

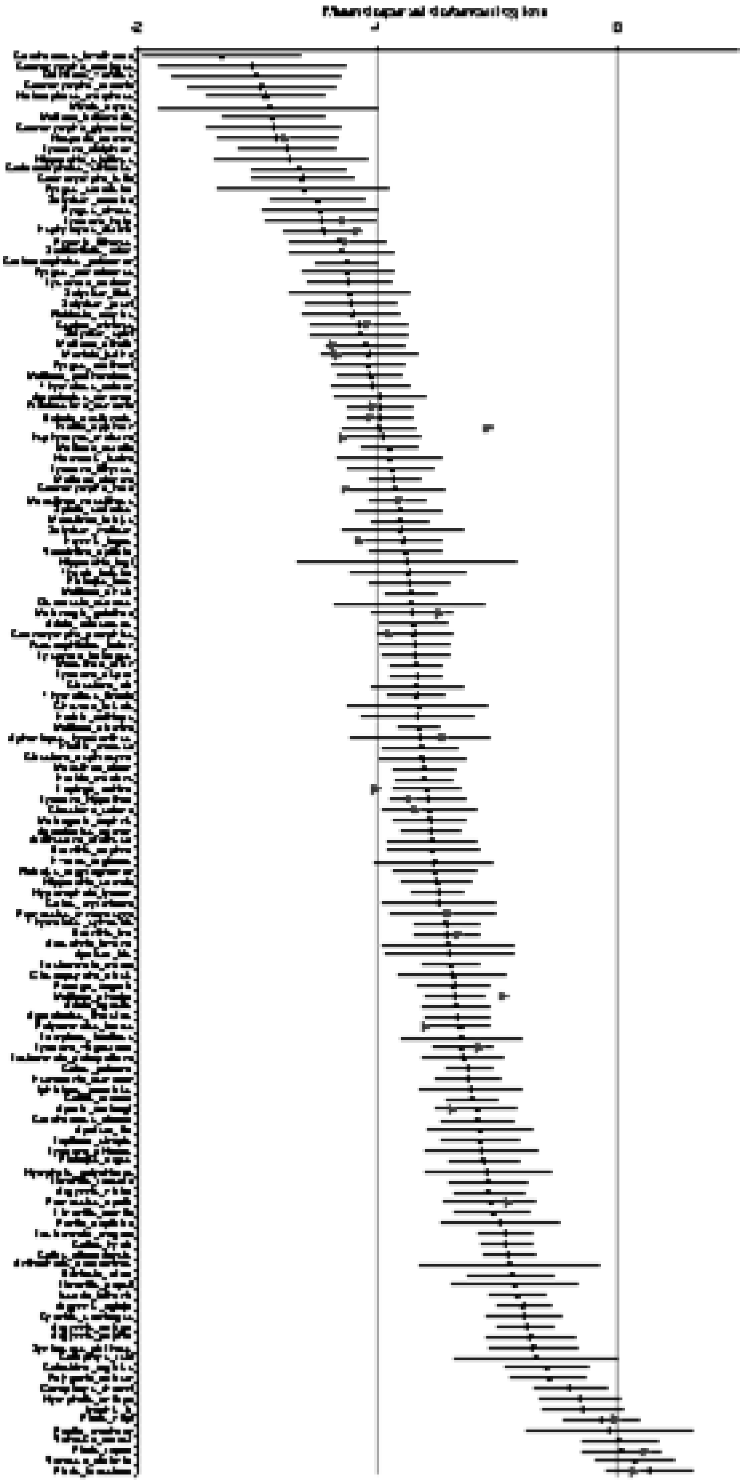


Figure A7. Mean dispersal distance predicted from adult habitat range, larval growth rate, ovigeny and wing size for 138 of the 142 butterfly species of N-W Europe, and 95%CI of the predictions. Red symbols show the observed value for 30 of those species.

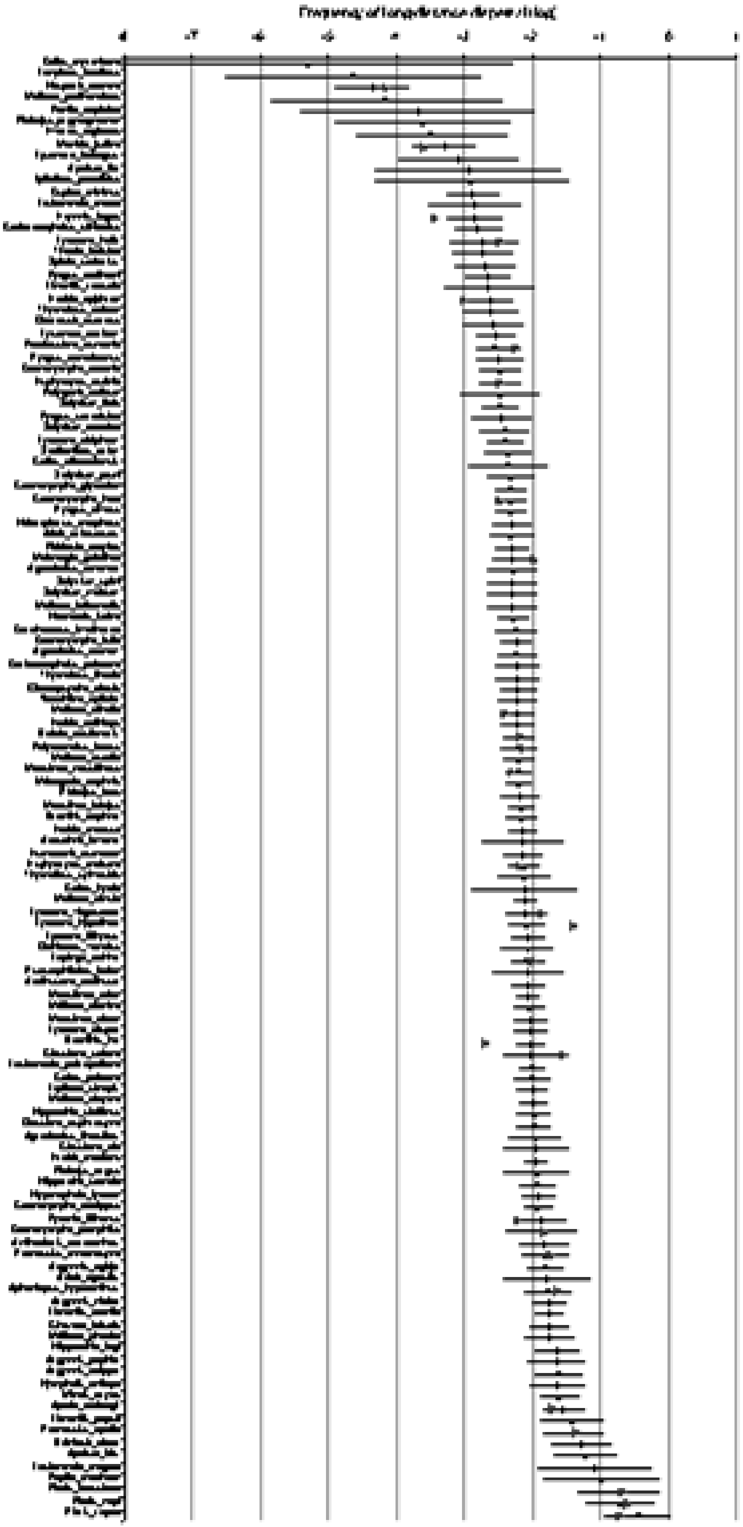


Figure A8. Probability of long-distance dispersal predicted from length of flight period, voltinism, adult habitat range and wing size for 124 of the 142 butterfly species of N-W Europe, and 95%CI of the predictions. Red symbols show the observed value for 28 of those species.

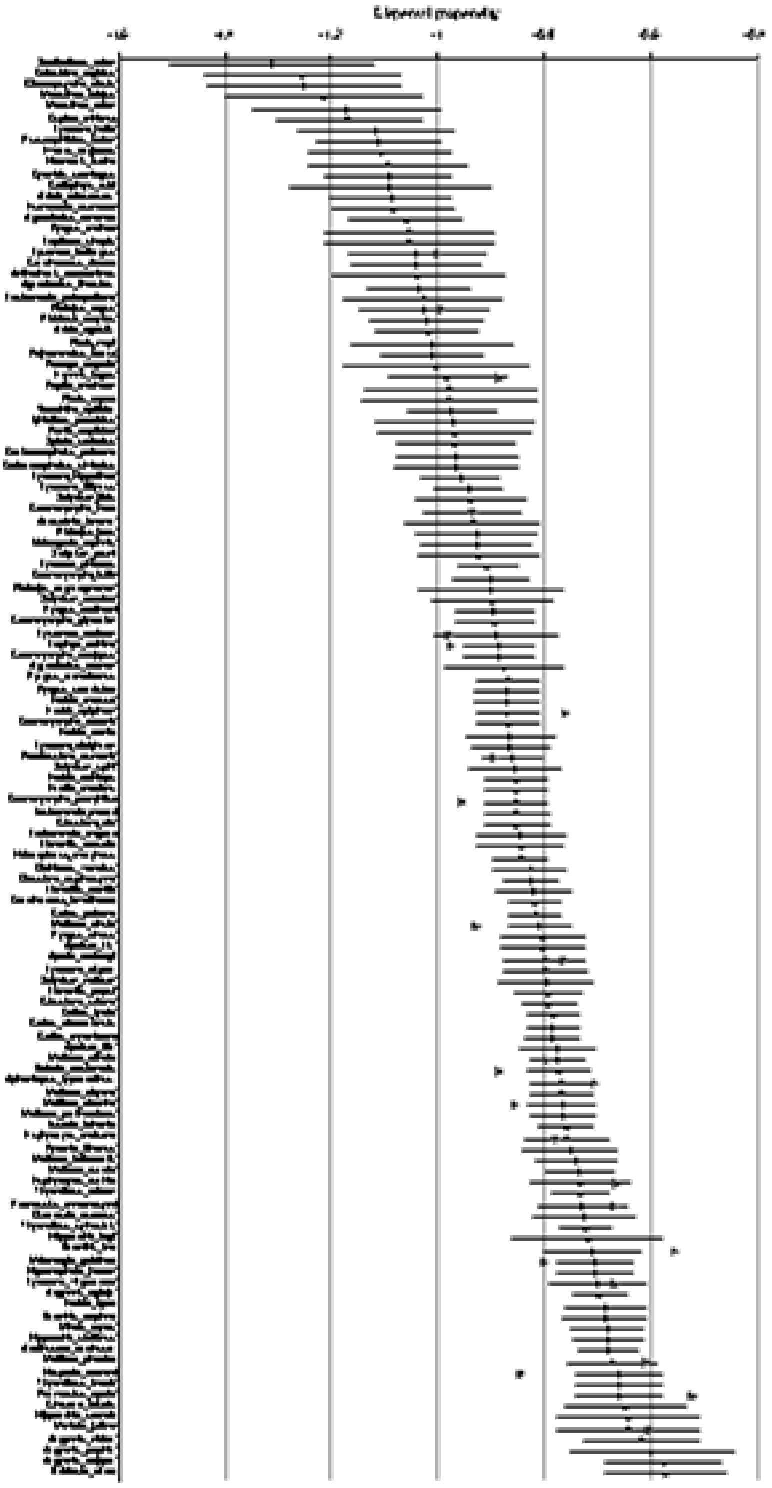


Figure A9. Dispersal propensity predicted from overwintering stage, strength of myrmecophily, adult lifetime and ripe egg load for 127 of the 142 butterfly species of N-W Europe, and 95%CI of the predictions. Red symbols show the observed value for 25 of those species.

Figure A10. Gene flow predicted from female maturation, voltinism, fecundity and ripe egg load for 137 of the 142 butterfly species of N-W Europe, and 95%CI of the predictions. Red symbols show the observed value for 26 of those species.

# 7. Inferring vagility from morphology and life-history traits in European amphibians

Audrey Trochet, **Sylvain Moulherat,** Olivier Calvez, Dirk S. Schmeller, Jean Clobert and Virginie Stevens

Last submission in PlosOne

*Résumé en français*

Dans le contexte actuel de changement climatique et de fragmentation des habitats, il est nécessaire de prendre explicitement en compte la vagilité des espèces afin de définir des stratégies de conservation efficaces. Ceci est particulièrement vrai concernant les amphibiens qui sont fortement menacés partout dans le monde. Ils s'appuient sur des déplacements terrestres pour assurer la dispersion qui maintien le flux de gènes, les recolonisations et les modifications d'aire de répartition, mais aussi pour assurer le bon déroulement de leur cycle de vie comprenant des migrations régulières entre les habitats terrestres et aquatiques. Cependant, généralement, la vagilité est difficile à mesurer et ne l'a été que pour 41% des espèces d'amphibiens européennes. Ici, nous avons voulu tester comment et dans quelles limites nous pouvions utiliser (1) les relations phylogénétiques entre les espèces (2) les relations entre traits à l'échelle spécifique pour inférer la vagilité chez les amphibiens. Nous avons pu observer que la vagilité est liée à la forme du corps, et dans une moindre mesure à la taille et l'âge à la maturité. D'autres relations existent mais n'ont pas été retenues par nos analyses multifactorielles. Nous avons utilisé la relation linéaire entre la vagilité des espèces et des groupes de traits morphologiques et démographiques pour construire une modèle prédictif de la vagilité. Nous avons évalué la qualité des prédictions obtenues par deux méthodes différentes (substitution phylogénique et modèle linéaire) à l'aide de validation croisées basée sur les données connues de 37 espèces d'amphibiens. Bien que les deux méthodes permettent de réaliser des estimations de la vagilité de qualité raisonnable, les prédictions réalisées à l'aide des traits d'histoire de vie s'avèrent de meilleure qualité. Nous avons retenu un unique modèle prédictif robuste ayant des applications directes pour la conservation. Nous avons validé ses performances en utilisant un jeu de données indépendant constitué de 10 espèces nord-américaines. Grâce à notre modèle, nous avons estimé la vagilité de 87 espèces européennes. Nous avons montré que les espèces européennes menacées ont des capacités de déplacement significativement inférieures aux espèces de moindre intérêt conservatoire, et que cette vagilité estimée est sans rapport avec les tendances populationnelles mondiales.

**Abstract**

In the current context of climate change and landscape fragmentation, efficient conservation strategies require the explicit consideration of vagility. This is particularly true for amphibians, highly threatened worldwide. They rely on overland displacements to ensure dispersal that sustains gene flow, recolonization and distribution changes, but also to complete their life-cycle, with regular migrations between aquatic and terrestrial habitats. Generally, however, vagility is hard to measure and was reported for only 41% of the amphibian species in Europe. Here, we aimed to test whether and to what extent we can make use of (1) phylogenetic relatedness among species and (2) trait relationships at the species level to infer vagility in amphibians. We observed that vagility is related to body shape, and to a lesser extent to body size and age at maturity. Relationships with other traits also exist, but are not retained in multi-factorial models. We used the correlations between observed vagility and several morphological and demographic traits to build a linear model to predict vagility. In addition, we used phylogenetic proximity to select substitute species for vagility. We assessed the quality of the inferences made by both methods (phylogenetic substitution and linear models) through cross-validations using the data for 37 amphibian species with known vagility. Although both methods allowed reasonably good approximations of the observed vagility, the precision obtained with trait-based models was much higher. We retained a single robust and efficient predictive model based on only two traits that is of direct applicability to conservation purposes. We validated its performance on an independent set of 10 North-American species. With this model, we inferred the

vagility of the 87 amphibian species of Europe. We showed that threatened European species had significantly lower displacement abilities than species of least conservation concern, and that presumed vagility was unrelated to global population trends.

## Introduction

Vagility (or overland displacements) is an important and globally relevant issue relating to the conservation of amphibians. Amphibian population persistence is known to be tied both to local movement distances (migration), and to longer-distance movements resulting in connectivity among breeding populations (dispersal). Indeed, vagility is a crucial element for the functioning of their populations (Marsh & Trenham 2001; Semlitsch 2000; Stevens & Baguette 2008) especially in inducing genetic mixing (Bohonak 1999; Keller & Waller 2002) and reducing the risk of population extinction or by allowing individuals to track the spatial shift of their climate niche. Because overland's movements play a major role in landscape ecology (Pickett & Cadenasso 1995) and landscape genetics (Storfer *et al.* 2010), vagility might become of major importance in conservation biology (Macdonald & Johnson 2001). These displacements also ensure the mandatory seasonal migration between terrestrial and aquatic habitats. Here, we refer to the ability to make these overland displacements as species' *vagility*. In practice, measuring vagility is often challenging, particularly for rare species, and consequently this information is lacking for most endangered species for which particular conservation efforts should be implemented. However, even an approximation of vagility might significantly increase the predictive power of the models used to explore the viability of populations facing environmental changes (Thuiller *et al.* 2004).

The costs associated with displacements, and particularly with dispersal (Bonte *et al.* 2012) are likely to constrain the allocation of resources among all components of an individual's life which may cause correlations between vagility and several other traits. Dispersal is also a tactic to cope with habitat instability, and as such it may evolve together with other traits that allow species persistence in instable habitats, like a high demographic turnover (Dingle 1996). Several other processes (reviewed in Stevens *et al.* 2012), like for instance the architecture of the genome, may cause apparent correlations between vagility and a variety of traits. If such correlations exist – be they caused by ecological, evolutionary or genetic processes – it should be possible to infer the vagility of a species from the values taken by other traits in this species and the relationship between those traits and vagility across species. Further, species traits and their relationship with vagility may also be constrained by common-ancestry, and phylogenetic proximity may summarize trait resemblance (Losos 2008). On this basis, we might infer unknown vagility by choosing the phylogenetically closest species with known vagility as a substitute for a species of conservation interest for which the data is unavailable.

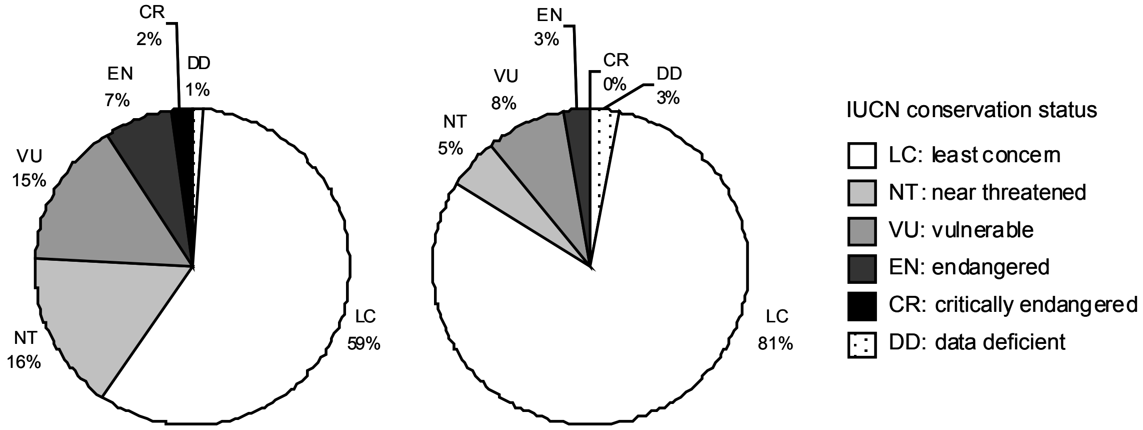
Our aim was to investigate whether (1) the observed relationships among traits at the species level or (2) the phylogenetic proximity among species could be used to infer amphibian vagility. We used published information on European amphibians and investigated the general relationships between vagility and other traits in this group. We built and tested linear models based on relationships between traits and vagility, and used the balance between their robustness and their predictive efficiency to select a single model. We then used it to infer the yet unknown displacement capacity of European amphibians. We also tested the efficiency of a substitution based on phylogenetic proximity. In both approaches, we assessed whether and to which extent the information retained in several alternatives allowed predicting the vagility of amphibians.

## Methods

### *Database description*

Our study is based on a database summarizing 44 traits for 85 European amphibian species from 242 publications collected by searching the Web of Science ®, the Amphibiaweb (2011) and several books (**Appendix S1**). We added two non-European species that recently established populations in Europe in order to increase the number of species with known displacement ability and improve the statistical power of our analyses. Their threat status, from 1 (least concern) to 5 (critically endangered), and their population trends (-1: decreasing, 0: stable or +1: increasing) were extracted from information found on the IUCN website (IUCN 2011). In total, we were able to compile data for 51 anurans and 36 urodels present in Europe (**Appendix S1**). Of these, 75 species are endemic to Europe and 24% have IUCN threat scores ≥ 3 (Vulnerable) (**Fig. 1**; Temple & Cox 2009). In addition, we also collected life history and vagility data on 10 North-American species (5 urodels, 5 anurans) to make an independent validation of our predictive tool (see below).

**Figure 1**. Proportion of species within IUCN categories: left, for 87 species present in Europe; right, for the 37 species in this dataset with migration or dispersal data available (= informed dataset).



Amphibians typically occupy terrestrial and aquatic habitats and perform regular migrations between both. The *vagility* considered here is the maximal displacement recorded for a species, either during dispersal between populations or during migration between habitat parts. We used the maximum distance over the modal distance as long-distance dispersal movements have considerably higher impact on species spread, species persistence and metapopulation functioning (Trakhtenbrot *et al.* 2005) and because vagility data on a landscape scale were unavailable for many species. Therefore, the data availability on amphibian vagility did not allow determining the dispersal kernel of species, which however might be of great interest, particularly if we are to modeling metapopulation functioning at the landscape scale. All vagility values used here resulted from mark-release-recapture or tracking studies. The database informs vagility for 37 European species (24 anurans, 13 urodels), most not threatened (**Fig. 1**), plus 10 North-American species (classified least concern [N=8] or near threatened [N=2]).

To test and then make use of correlations among traits, we considered the traits that have already been shown to vary with dispersal and migration in mammals, birds, insects or plants or that theory predicts they may vary with dispersal (see a review in Stevens *et al.* 2012). For statistical reasons, we imposed a threshold to the number of species (min. 45 of 87) for which the trait must be informed (**Appendix S1**). This procedure retained 7 morphological (**Table 1**) and 2 demographic traits: fecundity and age at maturity. Fecundity is the mean number of eggs laid by a female and by reproductive event. For each trait, individual values were averaged over populations, and then population values were averaged so that only the mean value was kept for each species.

**Table 1**. Loadings of a PCA performed on seven morphological traits for 87 amphibian species.

| Morphological trait | Trait description | N publi. | Contribution to axis (%) | | Correlation with axis | |
|---|---|---|---|---|---|---|
| | | | Axis 1 | Axis 2 | Axis 1 | Axis 2 |
| Mass | Body mass (g) of adults, without distinction between males and females | 1 - 4 | 8.876 | 17.886 | 0.604 | 0.578 |
| SVL_M | Snout-to-vent length (mm) of adult males | 1 - 6 | 23.240 | 0.045 | 0.978 | 0.029 |
| SVL_F | Snout-to-vent length (mm) of adult females | 1 - 7 | 22.339 | 0.368 | 0.959 | 0.083 |
| SVL_ad | Snout-to-vent length (mm) of adults, without distinction between males and females | 1 - 7 | 22.969 | 0.176 | 0.972 | 0.057 |
| Length | Total body length (mm) of adults, without distinction between males and females | 1 - 12 | 13.025 | 21.876 | 0.732 | -0.639 |

|  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|
| HLL | Hind limb length (mm) of adults, without distinction between males and females | 1 - 4 | 1.528 | 46.946 | 0.251 | 0.936 |
| SVL_Meta | Snout-to vent length (mm) at metamorphosis | 1 - 4 | 8.022 | 12.703 | 0.575 | -0.487 |

N publi.: number of publications (per species) from which the trait was averaged. Sexual size dimorphism is often large in amphibians; hence 3 measures of adult snout-to-vent length (SVL) were retained. SVL_ad is the average adult size (not different from sex-specific adult size when there is no information on sexual size dimorphism), whereas SVL_M and SVL_F are the values for males and females, respectively. SVL_Meta is the size at metamorphosis, as no sexual dimorphism was reported at this age.

### *Building and testing the inference tool*

Prior to our analyses, we reconstructed missing traits values (all traits except vagility), as the database was incomplete, with up to 44% of species with missing values (NA) for a given trait (**Appendix S1, S2**). To lower the collinearity among explanatory variables, we summarized morphology by a principal component analysis and used the scores of species along its main axes as synthetic indicators of their morphology. All trait values were standardized before analyses, and vagility was log-transformed to ensure normality. The analyses presented were all performed within the R environment (R Development Core Team 2011).

Our first aim was to investigate how life-history and morphology correlate with the displacement ability at the species level. We investigated this by linear models (LM) and phylogenetic generalized least squares (PGLS). Unlike LM, PGLS incorporate the phylogenetic relationships among species. The comparison of their relative fit (appreciated by AICc: Anderson *et al.* 1994) thus informed the relative importance of phylogeny for traits relationships. The saturated model for both LM and PGLS took the form shown in equation 1. AICc was used to select the model(s) that best fitted the data among all simpler models derived from the saturated model. Similarly, best models (within 2 points of AICc) were then averaged (see **Appendix S2**). We investigated trait relationships separately for anurans and urodeles in a similar fashion.

Eq.1: Vagility ~ morphology (PCA axes) + fecundity + maturity + sampling scale (the length of study sites) + sampling effort (the number of individuals caught) + first-order interactions.

Our second aim was to propose a simple method to infer unknown dispersal. We tested two methods: linear modeling (as described before) and substitutions based on phylogenetic proximity (see below). For each, we assessed the quality of predictions by cross-validation, using a random partitioning of the European dataset, with a ratio of 3:1 for training:test partitions. Confidence intervals of predictions were obtained by repeating 100 times the partition. We regressed the predicted vs. observed vagility and used the slope of this regression to assess the rightness of the prediction, and used the adjusted $R^2$ (when the regression was forced into zero) and the mean absolute residual of the predictions (relatively to this forced regression) to indicate its precision.

When building the predictive linear models, we applied an additional precaution. As we must be confident in the data we use to infer vagility, we should always prefer measured values of species traits over values inferred in a reconstruction step. We thus tested the performance of models constructed on a dataset iteratively purged from its NA richest trait (see **Appendix S2**). At each step, we selected the best of all models derived from the saturated model (as described before), and assessed their quality by cross-validation. Of course, this argument of precaution would not apply when the dataset is complete: in this case, biological arguments should be preferred to select among candidate traits.

The principle for phylogenetic substitutions was to replace ignored vagility values by the value measured in the most closely related species in the training partition (or averaged over several closely related species). Phylogenetic distances were appreciated either by the number of nodes or by the sum of branch lengths separating species in the Amphibian tree (see **Appendix S3**). The sensitivity of predictions to the phylogenetic distance between a species and its substitute was tested with generalized linear models.

### *Patterns of amphibian vagility*

Of the various alternatives tested at the previous step, the method that returned the better predictions was chosen to infer vagility for 87 European amphibians. In this step, we also used the method with the best vagility predictions to infer the vagility of 10 North-American species for which it was repeatedly measured, in order to test the applicability of our predictive model beyond the particular group on which it was built. For this general prediction the sampling scale and the sampling effort were held fixed to the value of their respective upper 95% confidence intervals in order to provide a general picture of displacement ability comparable across species. We then tested for a relationship between vagility and threat status and population trends of European species with generalized linear models.

## Results

### *Correlations among traits*

Only the two first axes of the PCA on morphology had an eigenvalue > 1. These two axes summarized respectively 58 and 27% of the variance in seven morphological traits (**Table 1**). The first axis principally pertained to adult size, with large species having positive scores. The second axis informed body shape: species with high scores on the second axis have relatively long hind limbs, are relatively heavier, show a small total length (i.e. no tail or a short tail), and are relatively small at metamorphosis.

When all traits were included, the best models for vagility were three linear models whose AICc were largely lower than that of concurrent PGLS ($\Delta$AICc = -69.15; **Table 2**), indicating that phylogenetic relationships are not strong predictors of trait relationships in amphibians. All three LM predicted a positive effect of the sampling scale and a positive relationship between vagility and body shape (the second axis of PCA). One predicted that vagility is negatively impacted by body size (the first axis of PCA), and another one predicted a negative effect of the age at maturity. No first order interaction was retained (**Table 2**). Only the negative effect of body shape and the positive effect of the sampling scale were significantly different from zero in the averaged model (**Table 2**).

In anurans, two LMs were retained that outperform concurrent PGLS ($\Delta$AICc = -12.27). Both predicted a positive effect of the spatial scale of sampling, and one predicted a positive association with fecundity. The slope of both effects however had too large confidence intervals in the averaged model to differentiate them from zero (**Table2**).

In urodels, three LMs were retained that outperformed concurrent PGLS ($\Delta$AICc = -7.94). One was the null model (intercept only), one predicted a positive effect of the sampling scale, and another a negative effect of the age at maturity. In the averaged model, these effects had again very large confidence intervals, containing zero (**Table2**).

*Performances of predictions*

After purging step by step the dataset from its NA-richest variable, the average number of reconstructed values gradually passed from 10% (with up to 25% for the less well informed variable) to 0 (**Table 3**). At each of those 9 steps, we selected the candidate LM that best fitted the data. This selection retained 33 models (**Appendix S4**) for which the quality assessment was run.

All 33 models generated a regression of predicted versus observed vagility with very high slopes (range = 0.76 – 0.93; mean = 0.85), and similar for all (Kruskal-Wallis test for a step effect on the slope of predicted/observed regression: $\chi^2_8 = 12.52$, $p = 0.129$). All models however were not equally precise, as shown by the adjusted $R^2$ of predicted and observed regressions forced to zero ($R^2$ = 0.962-0.968; Kruskal-Wallis test for a step effect: $\chi^2_8 = 18.69$, $p = 0.017$). Although the difference only occurred at the third decimal, the precision was significantly lower from step 2 to 5, compared to models at other steps. Among the latter group (steps 1 and 6-9), the precision of the predictions did not change among steps (Kruskal-Wallis $\chi^2_4 = 4.13$, $p = 0.388$).

**Table 2**. Linear models (LM) for amphibian displacement ability: summary of model selection, and model averaging (LM*).

| | | Morphology | | Demography | | Covariates | | Model performance | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Model | Int. | Axis 1 | Axis 2 | Maturity | Fecundity | Sampling scale | Sampling Effort | AICc | ΔAICc | W |
| *All species* | | | | | | | | | | |
| LM_1 | 6.069 | - | 0.748 | - | - | 0.00015 | - | 126.404 | 0.000 | 0.158 |
| LM_2 | 6.086 | - | 0.680 | -0.299 | - | 0.00015 | - | 126.775 | 0.372 | 0.131 |
| LM_3 | 6.096 | -0.205 | 0.782 | - | - | 0.00014 | - | 127.981 | 1.577 | 0.072 |
| LM* | 6.080 (5.45-6.71) | -0.205 (-0.622-0.212) | 0.73 (0.288-1.17) | -0.299 (-0.717-0.118) | - | 0.000147 (0.00007-0.00022) | - | - | - | - |
| *Anurans* | | | | | | | | | | |
| LM_a1 | 6.779 | - | - | - | - | 0.00013 | - | 81.279 | 0.000 | 0.239 |
| LM_a2 | 6.809 | - | - | - | 0.249 | 0.00013 | - | 83.009 | 1.730 | 0.101 |
| LM_a* | 6.79 (5.98-7.60) | - | - | - | 0.249 (-0.255-0753) | 0.00013 (0.000049-0.00021) | - | - | - | - |
| *Urodels* | | | | | | | | | | |
| LM_u1 | 5.540 | - | - | - | - | - | - | 49.320 | 0.000 | 0.252 |
| LM_u2 | 4.775 | - | - | - | - | 0.00021 | - | 49.829 | 0.509 | 0.195 |
| LM_u3 | 5.540 | - | - | -0.447 | - | - | - | 51.204 | 1.884 | 0.098 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| LM_u* | 5.270 (4.06-6.47) | - | - | -0.447 (-1.27-0.377) | - | 0.00021 (-0.00007-0.00050) | - | - | - | - |

Independent variables were standardized so that their estimates can be compared directly. Dashes indicate variables not retained in the model; interactions not retained (only proposed in " all species" models). Int: intercept; Axis 1 and Axis 2: scores along the first and second axis of a PCA on morphology (see text); Sampling scale: longest length of the study site; Sampling effort: total number of individuals for which displacement ability is reported; AICc: corrected Akaike Information Criterion; W: relative weight of the model. LM*: averaged model (in parenthesis, the 95% confidence interval for the estimate of the effect size for each variable in the average model).

**Table 3**. Performance of linear models and phylogenetic substitutions at predicting amphibian vagility.

Linear models

| Step | ΔAIC* | Mean rightness | Mean precision | Robustness |
|---|---|---|---|---|
| 1 | 11.33 | 0.903 | 0.9682 | 0.90 |
| 2 | 12.03 | 0.869 | 0.9658 | 0.92 |
| 3 | 11.64 | 0.827 | 0.9644 | 0.93 |
| 4 | 9.31 | 0.834 | 0.9646 | 0.94 |
| 5 | 7.04 | 0.846 | 0.9649 | 0.95 |
| 6 | 0.00 | 0.880 | 0.9682 | 0.95 |
| 7 | -0.50 | 0.894 | 0.9686 | 0.96 |
| 8 | 0.00 | 0.882 | 0.9617 | 0.97 |
| 9 | 5.30 | 0.902 | 0.9633 | 1 |

Phylogenetic substitutions

| Phylogenetic distance | Rightness | Precision | Sensitivity |
|---|---|---|---|
| Sum of branch lengths | 0.952 | 0.5250 | Rho = 0.272,<br>*P* < 0.001 |
| Number of nodes | 0.955 | 0.5118 | Rho = 0.096,<br>*P* = 0.572 |

Step: step in an iterative process of removing the NA-richest variable (see text, **Appendix S2**). AIC*: the corrected Akaike Information Criterion, here penalized by 2 points for each additional variable needed to build the model (i.e. before a summary by a PCA, see text, **Appendix S2**). Precision: percentage of explained variance in a regression of predicted or substituted vagility vs. observed vagility, forced into zero. Rightness: slope (unforced) of a regression of predicted or substituted vagility vs. observed vagility. Robustness: 1-the proportion of reconstructed data (see **Appendix S2**). Sensitivity: Spearman correlation between absolute differences of observed and substituted vagility and phylogenetic distances. The method is considered sensitive to the phylogenetic distance between a species and its substitute in case this correlation is significantly non-null. Vagility is the log-transformed maximal displacement measured for a species. Phylogenetic distances calculated from the whole composite tree of living amphibians.

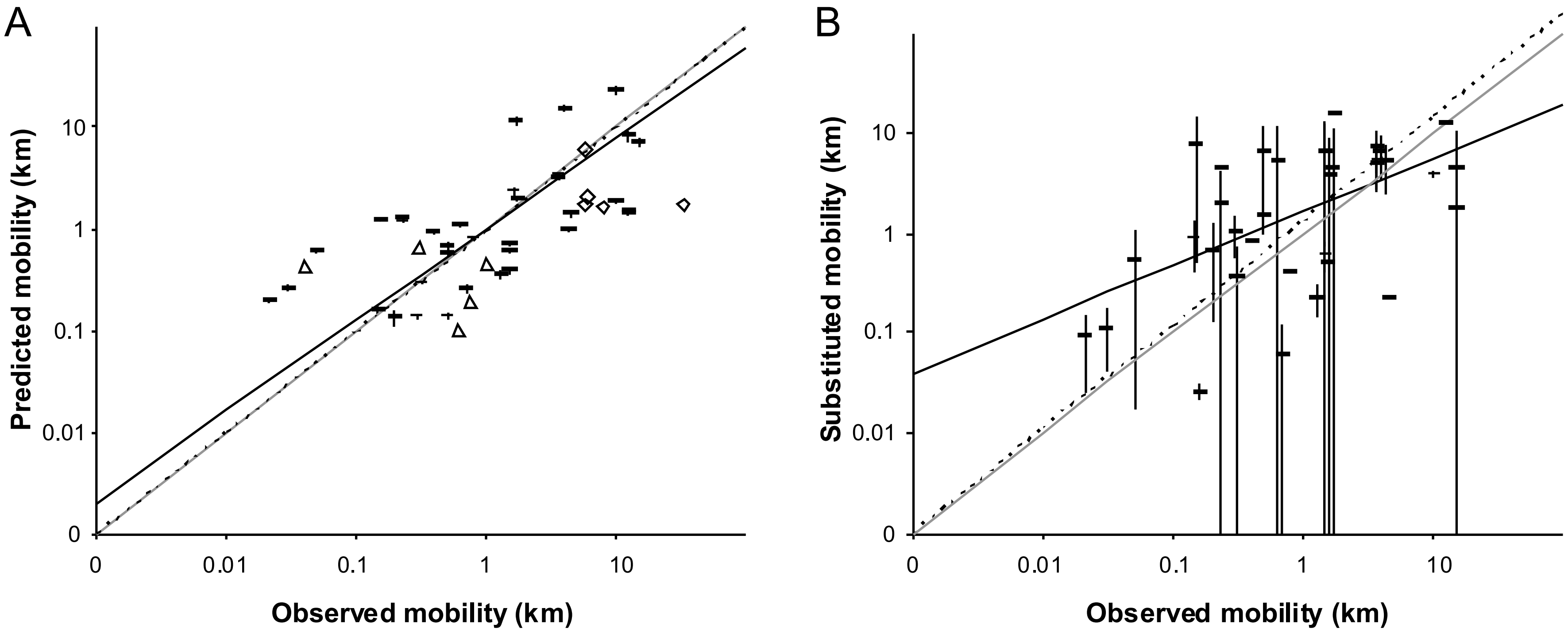
Both phylogenetic substitutions performed quite well, as shown by the regression of substituted versus observed displacement abilities, with high slopes. The precision obtained with substitutes chosen on the number of nodes, rather than on branch lengths, was slightly better, and this method was also less sensitive to the phylogenetic distance between a species and its substitute (**Table 3**).

### *Vagility of European amphibians*

Phylogenetic substitutions were far less precise and less correct than linear models in inferring vagility (**Table 3**, **Fig. 2**), although the inferences made by the best alternative of both methods converged (Pearson correlation for predicted vagility *vs.* substituted vagility = 0.70, $p < 0.001$).

As our main goal was to find a simple and efficient method to predict amphibian vagility, and as all linear models had very high rightness and precision, we selected the model with the lowest AICc for further predictions (here penalized to account for supplementary variable needed to build the PCA: see **Appendix S2**). This linear model (Model 8.1 in **Appendix S4**) modeled the negative effect of total body length and the positive effect of fecundity and of sampling scale on vagility, and has high robustness and precision (detail in **Table 4**), and showed a high performance at predicting vagility of North-American species (cross-validation and application to North-American species illustrated in **Fig. 2A**). Spatial scale appears in all 33 models tested for prediction quality. However, the size of study sites may have been chosen with a good prior on vagility of the study species and hence it may add significant predictive power to the models. On the other hand, its effect may simply be artifactual, as was shown in other studies (e.g. Schneider 2003). Removing the spatial scale from the predictive model (i.e. using model 8.2 in **Appendix S4**) yields predictions with lower rightness and precision, but still with a sufficient performance. Notice that the predictions for North-American species (shown **Fig. 2A**) ignored the sampling scale, which was held fixed at the upper limit of its 95% CI in European studies. Most amphibians have a modest predicted vagility, but for a substantial proportion the predicted vagility was exceeding 5 km (**Fig. 3, 4**). The predicted vagility of European species was not correlated with the species' threat status ($F_{1\text{-}84} = 2.46$, $p = 0.12$) nor with populations trends ($F_{1\text{-}84} = 2.25$, $p = 0.14$). However, observed vagility correlated negatively with the IUCN threat status of species ($F_{1\text{-}34} = 9.68$, $p = 0.004$), but not with population trends ($F_{1\text{-}34} = 0.66$, $p = 0.421$).

**Figure 2**. Cross-validations for predictions of amphibian vagility, obtained (A) with a linear model with fecundity, total length and sampling scale as independent variables or (B) from substitutions based on phylogenetic proximity assessed by the number of nodes separating species. In A, white symbols illustrate the application of the linear model to 10 North-American species that were not considered for building the model. In that case, sampling scale was held fixed at a value corresponding to the upper limit of its 95% CI in the European dataset.



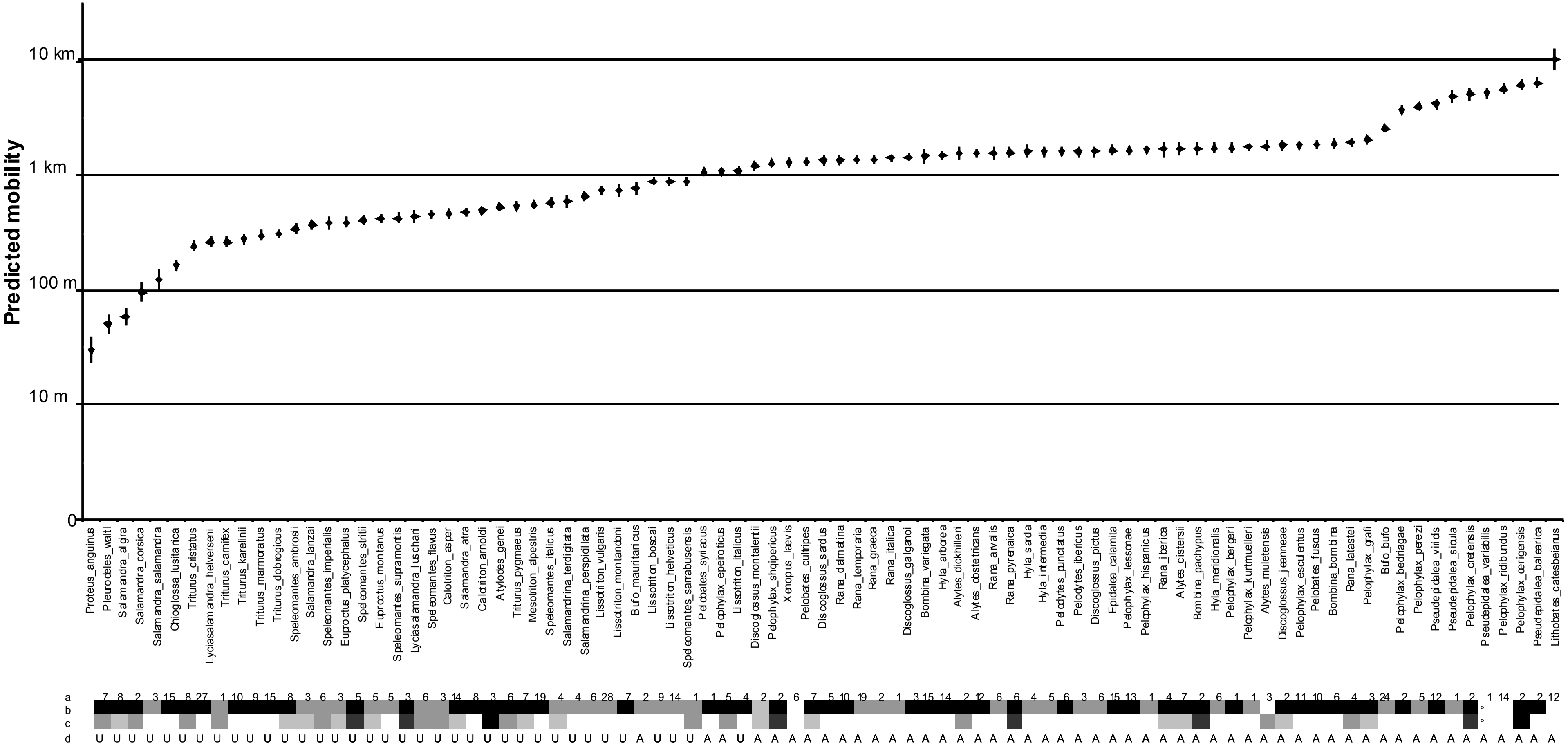
Regressions of predicted (A) or substituted (B) mobility against observed values for 37 European amphibian species (black symbols). Vertical lines: 95% CI. Solid line is the unforced regression; dashed line is the regression forced into zero; grey line is the 1:1 slope, given for comparison. White symbols in A illustrate the application of the predictive linear model to North-American urodels (triangles) and anurans (diamonds) that do not intervene in model building.

**Table 4**. Linear model used for predictions of amphibians' displacement ability. Response is ln-transformed vagility, in m.

| Variable | Estimate | DF | F | *P* | AdjR$^2$ |
|---|---|---|---|---|---|
| Int. | 7.0104 *** | 3-33 | 14.2 | $4.1 \times 10^{-6}$ | 0.5238 |
| Length | -0.5877 ** | | | | |
| Fecundity | 0.5141 * | | | | |
| Sampling scale | 0.8194 *** | | | | |
| | Estimates for variables not standardized | | | | |
| Int. | 6.925 *** | | | | |
| Length | -0.01364 ** | | | | |
| Fecundity | $8.269 \times 10^{-5}$ * | | | | |
| Sampling scale | $1.454 \times 10^{-4}$ *** | | | | |

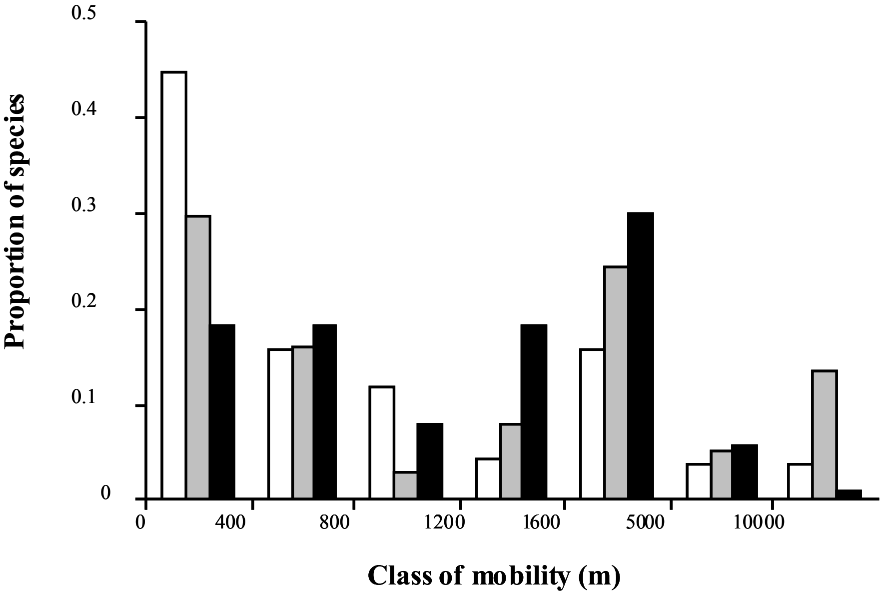
***: P<0.001; **: 0.001<P<0.01; *:0.01<P < 0.05. In the lower part of the table, the estimates that can be used directly from raw data on total body length (Length, in mm) and the number of eggs or offspring per female and per breeding event (Fecundity). The sampling scale is in m, and can valuably be set at 7300 m.

**Figure 3**. Vagility of 87 amphibian species, predicted with a linear model of the form "ln(vagility)= a + b(total length) + c(fecundity) + d(sample scale)", with parameters a, b, c, d as shown in Table 4, and scale fixed to 18 km. Vertical lines: 95% CI for the prediction.



a: number of publications used for each species. b: population trends: black = declining populations, grey = stable populations, white = increasing populations; °: missing data; c: IUCN conservation status (color code like in fig. 1: from least concern in white to critically endangered in black); d: Taxa: U: Urodels, A: Anurans.

**Figure 4**. Proportion of amphibian species with different vagility, as evidenced from field surveys (in Smith & Green 2005: white bars, N=159; this study: grey bars, N=37) or as predicted in this study from a linear model (black bars, N=87).



## Discussion

Vagility of species is an outstanding criterion to assure metapopulation functioning and therefore is a key parameter for conservation decisions. Indeed, the vagility ability of a species might indicate and determine the spatial scale of both population's spatial structure (Bowler & Benton 2005; Thomas & Kunin 1999; Wiens 1989) and population's dynamics (for example in plants see Freckleton & Watkinson 2002). Here, we showed that this trait was rarely measured for European amphibians: we were able to find maximal displacements for less than half the species. Currently, more precise estimates like dispersal kernels are available for only a handful of species. Our analysis, however, strongly suggest that the vagility of amphibians is sufficiently strongly related to their morphological and demographic traits to allow predicting its value from these traits. Moreover, this pattern is relatively independent of the phylogenetic context, as shown by the outperformance of LM relatively to PGLS, so that conservation actors can use the simpler methodology of linear modeling to infer unknown vagility and assist decision making, reserve site selection and reserve site improvements.

Generally, amphibian vagility correlated with body shape, a relationship that, if causal, indicates that loosing its tail at metamorphosis or having relatively long legs can be advantageous to overland displacements. Phillips *et al.* (2006) evidenced the causality of the vagility/leg length relationship in cane toads at expanding fronts, where both traits evolved jointly. The shape/vagility relationship might hence be causal, a question that however requires further investigation.

The negative association between vagility and size at metamorphosis (that was correlated with the second axis in our PCA) is counter-intuitive given the general positive relationship between body size and vagility (see examples in other taxa in Bradbury *et al.* 2008; Paradis *et al.* 1998; Sekar 2012; Stevens *et al.* 2012; Sutherland *et al.* 2000). However, together with the relationship between vagility and fecundity or age at maturity, it might indicate that strong vagility evolved as a means to cope with unstable habitats. Such relationships, classical of the ‘fugitive species’ syndrome (Tilman 1994), were observed among butterflies, where dispersal ability was positively related to several traits pertaining to individual turnover in populations (Stevens *et al.* 2012). Although we noticed a general association between amphibian vagility and several traits allowing their life in ephemeral habitats, we have too little data to directly test the relationship with habitat stability.

The relationship between vagility and fecundity is important for conservation because both traits support species invasiveness. Their positive association across species indicates that a syndrome of invasiveness (with high power of colonization and range expansion) may evolve at expanding fronts. This cocktail of high fecundity and high vagility occurs in *Lithobates catesbeianus* and probably participated to its invasive success in Europe.

In line with previous reports (e.g. Rittenhouse & Semlitsch 2007; Smith & Green, 2005), we showed that anurans have generally higher vagility than urodels (both in observations and in predictions). Smith & Green (2005) showed that most amphibians do not move very long distances, but some anurans were able to move more than 10km (examples of >30 km moves in North-American species are found in Freeland & Martin 1985; Funk & Dunlap 1999). Some European anurans were also capable to perform very long distances (up to 15km in *Pelophylax lessonae*: Tunner 1992). Apart this dichotomy between orders, it is difficult to generalize the pattern of amphibian vagility along phylogenies. Further, the paucity of data did not permit us to model how traits combine at order level, and we obtained very large confidence intervals for slopes in averaged models.

Threatened species have lower observed vagility than species of least conservation concern. Threatened species with low vagility abilities thus probably will be more affected by a given level of habitat fragmentation, through the rupture of functional connectivity among their local populations and the consecutive unbalance in extinction/colonizations in their metapopulations (Cushman 2006). Even if some of these threatened species are spatially-restricted, and dispersal may be a less critical feature of their life history, the current climate change demands an appropriate response of those species. This can either be the local adaptation of species, or the shift of their distribution (Thomas *et al.* 2004). Threatened species appear to be less well disposed to track their shifting climate niche, because they have modest vagility. Identifying and considering explicitly their displacement ability is the needed step toward efficient conservation actions.

The question of whether a high vagility predisposes species to vulnerability has been widely debated. Generally, it is accepted that a high vagility buffers species against the risk of extinction due to fragmentation of habitats by allowing regular dispersal among habitats and to reach new suitable habitats in heterogeneous and dynamic landscapes (e.g. Henle *et al.* 2004; Ockinger *et al.* 2010). This also may explain why vagility is correlated with threat status and not with population trends. The association between vagility and conservation status was lost when all 87 species and their predicted vagility were considered maybe due to the missing data on vagility abilities in threatened species (**Fig. 1**). However, the predicted vagility gradually decreased along the three lower threat categories (least concern, near threatened and vulnerable; **Appendix S5**), but became highly variable among endangered and critically endangered species. Hence, our interpretation should be considered with caution, as additional field data would be needed to verify our notion: vagility was rarely reported for endangered European Amphibians (**Fig. 1**).

### *Performances and limits of the inferences*

Our study reaches the same conclusion as others that used different approaches (Blaustein *et al.* 1994; Semlitsch & Bodie 2003; Smith & Green 2005): most amphibians have modest displacement abilities, with consistently more than 25% of the species reported to move less than 400m, and the majority moving less than 5 km. A substantial proportion of species however may move more than 5 km. Marsh & Trenham (2001) and Smith & Green (2005) already reported that long-distance dispersal is not uncommon in amphibians. The pattern of predicted vagility confirms this distribution, with a lot of low-vagility species, but also a substantial proportion of high-vagility species. The frequency of species with either a very low or very large vagility, as determined by our predictive model, were lower than those reported from field surveys (see **Fig. 4**). This suggests that our model might perform less well for extreme values of vagility and that the frequency of bad and good dispersers was underestimated, although we cannot discard the possibility that field studies were skewed to these species.

Generally, the two methods developed allow inferring vagility of amphibians quite well, but the inferences obtained with the information on traits dependency are far more precise and correct than those obtained from the phylogenetic information. Although both give a reasonably good approximation of vagility, the confidence intervals of the predictions are much larger with phylogenetic substitutions than with the linear model, and LMs should be preferred if one wishes to infer unknown displacement ability. For example, for three species belonging to the genus *Rana*, phylogenetic substitutions yields extremely large confidence intervals for vagility, which precludes the use of closely related species as a surrogate for vagility in this highly diversified, but phylogenetically poorly resolved genus.

In contrast, our linear models appear to deliver vagility approximations with a good precision, as shown by the higher slope of the regression of predicted versus observed regression and the smaller confidence intervals. Further, the selected model only requires two easily collectable parameters, the total body length and the fecundity suggesting that our method could be of general interest for conservation practitioners. However, we need to stress that we took advantage of observed correlations among traits, without consideration of their causality or strength. We insist that these correlations may be indirect, and were retained on the criteria that they are well informed for most species and sufficient to predict accurately their vagility. Other traits might however be more strongly or more directly related to vagility and were discarded because they were less well informed. We believe that other traits might perform as well to predict vagility of amphibians, given the strong correlations observed between dispersal and a large variety of traits in other taxa (e.g. Bradbury et al. 2008; Dawideit *et al.* 2009; Stevens *et al.* 2012), indicating that dispersal is probably a full part of life-history strategies, which makes its relation with other traits predictable (Stevens *et al.* 2012). Apart the model that we finally used, concurrent models based on a wider variety of traits also performed well at predicting vagility. We believe that, provided that other traits will become available for more species, other combinations of a few traits could probably predict dispersal as well, although this can not be tested formally here.

The validation with amphibians of other continents was necessary for a general application of our model, and it confirms that the model may be used for species outside Europe. This validation also confirms that the model can predict vagility without the putatively informative data of sampling scale (see above), as the prediction for North-American species shown in **Fig 2A** did not make use of this information. Nevertheless, we would like to point out that the construction of our model did not include cave-dwelling species. It might hence not apply to such species because vagility may have evolved in response to characteristics of the environment, and hence it might have followed diverging evolutionary routes in cave-dwellers and in other amphibians, possibly resulting in diverging patterns of correlations among traits between both groups.

Our study showed the importance of investigating dispersal/life-history relationships that may help predicting probable values of vagility for species where it is unknown. However, we ignored here the variation of trait values within species due to a lack of data. That variation may be large, particularly for dispersal (see Stevens *et al.* 2010). Within-species variation in vagility, as well as in other traits, may result from variation in the selection on these traits along environmental gradients (like latitude: Alho *et al.* 2011), at invasion fronts (see Phillips *et al.* 2006) or between sexes (sexual polymorphism is ubiquitous in morphology and also in movement abilities: e.g. Austin *et al.* 2003; Berven & Grudzien 1990; Palo *et al.* 2004). We recommend considering the possibility of local adaptation in movement ability and other traits, especially when we are to infer vagility for species experiencing recent change in environmental pressures, like after invasion into new areas. In that case, vagility inferences should be cautious and should use locally-measured traits to feed the model.

## Conclusion

Despite the limits of our predictive model, we believe that it can yield good approximations of species vagility. Information about displacement abilities (both dispersal and migration) is crucial in conservation in amphibian populations and several authors recently asked information and/or tools for modelling amphibian spatial dynamics (Marsh & Trenham 2001; Semlitsch 1998). For example, Brown *et al.* (1990) used this measure to delineate terrestrial ‘‘buffer zones’’, based on spatial requirements of species (i.e. the distance usually moved from wetlands), and included this parameter in their recommendations for wildlife protection in Florida wetlands. They pointed out that the lack of data for most amphibian species forced them to use rough estimates for most species considered. The procedure we identified and tested here would provide the conservation actors with valuable and more precise predictions of vagility from only two traits, widely available and easy to collect, which will allow them to more adequately incorporating space-use considerations in amphibian conservation planning.

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Appendix S1. Summary of phylogenic, morphometric and life-history traits variables used for 87 amphibians species [Taxa: A for Anurans and U for Urodels, adult body mass (Mass), male snout-to-vent length (SVL_M), female snout-to-vent length (SVL_F), adult size (SVL_ad), total length (Length), hind limb length (HLL), sexual maturity (Maturity), size at metamorphosis (SVL_Meta), number of eggs per clutch (Fecundity) and maximum distance of movement found in meters (Mobility)]. Values expected for the life-histories have been averaged between studies (i.e. between populations). Species in bold have available mobility data. * for introduced species.

| *Species* | *Number of publications* | *Taxa* | *IUCN status* | *Mass* | *SVL_M* | *SVL_F* | *SVL_ad* | *Length* | *HLL* | *Maturity* | *SVL_Meta* | *Fecundity* | *Mobility* | *References* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Alytes cisternasii* | 7 | A | NT |  | 36.75 | 38.38 | 37.56 | 37.56 | 45.28 | 2.00 | 25.00 | 60 |  | 1, 2, 3, 4, 5, 6, 7 |
| *Alytes dickhilleni* | 2 | A | VU |  | 44.70 |  | 47.17 | 47.17 |  | 2.00 | 25.00 | 40 |  | 4, 8 |
| *Alytes muletensis* | 3 | A | VU |  | 34.70 | 35.65 | 35.18 | 35.18 |  | 3.00 |  | 20 |  | 4, 7, 9 |
| ***Alytes obstetricans*** | 12 | A | LC | 11.70 | 45.05 | 49.57 | 47.31 | 47.31 | 58.61 | 2.50 | 26.50 | 70 | 500.00 | 2, 3, 5, 6, 10, 11, 12, 13, 14, 15, 16, 17 |
| ***Bombina bombina*** | 6 | A | LC | 3.07 | 38.89 | 40.00 | 39.44 | 39.44 | 52.00 | 1.00 | 15.00 | 300 | 230.00 | 4, 6, 18, 19, 20, 21 |
| ***Bombina pachypus*** | 2 | A | EN |  | 45.75 | 45.25 | 45.50 | 45.50 | 50.75 |  |  | 300 | 230.00 | 6, 19 |
| ***Bombina variegata*** | 16 | A | LC | 6.32 | 45.08 | 44.34 | 44.71 | 44.71 |  | 1.50 | 13.90 | 170 | 4500.00 | 4, 11, 13, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33 |
| ***Bufo bufo*** | 24 | A | LC | 76.54 | 66.11 | 88.46 | 77.28 | 77.28 | 112.65 | 3.00 | 11.75 | 10000 | 4000.00 | 4, 5, 6, 11, 13, 14, 15, 19, 26, 30, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 48, 49, 50 |
| *Bufo mauritanicus* | 2 | A | LC |  | 132.00 | 150.00 | 141.00 | 141.00 |  |  | 12.00 | 10000 |  | 12, 48 |
| *Discoglossus galganoi* | 3 | A | LC | 18.50 | 54.85 | 49.20 | 52.03 | 52.03 |  | 4.00 | 10.00 | 1500 |  | 4, 7, 49 |
| *Discoglossus jeanneae* | 2 | A | NT |  | 39.50 | 40.70 | 40.10 | 40.10 |  |  |  | 1500 |  | 4, 50, 51 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Discoglossus montalentii* | 2 | A | NT | | | | 60.00 | 60.00 | | | | 1000 | | 11, 52 |
| ***Discoglossus pictus*** | 6 | A | LC | 15.85 | 56.49 | 47.86 | 52.18 | 52.18 | 85.00 | 4.00 | 10.00 | 1500 | 1530.00 | 4, 6, 11, 53, 54, 55 |
| *Discoglossus sardus* | 5 | A | LC | | 54.60 | 55.50 | 55.05 | 55.05 | | 3.00 | 15.25 | 1000 | | 4, 11, 52, 56, 57 |
| ***Epidalea calamita*** | 14 | A | LC | 50.22 | 59.03 | 67.13 | 63.08 | 63.08 | 68.17 | 3.00 | 9.66 | 4000 | 4411.00 | 4, 11, 13, 15, 25, 26, 56, 58, 59, 60, 61, 62, 63, 66 |
| ***Hyla arborea*** | 14 | A | LC | 6.04 | 41.19 | 44.03 | 42.61 | 42.61 | 60.84 | 1.00 | 18.00 | 1400 | 12570.00 | 4, 5, 11, 13, 15, 58, 66, 67, 68, 69, 70, 71, 72, 73 |
| ***Hyla intermedia*** | 5 | A | LC | 6.28 | 38.03 | 43.93 | 40.98 | 40.98 | | 1.00 | 20.00 | 1400 | 12600.00 | 4, 57, 58, 67, 74 |
| *Hyla meridionalis* | 6 | A | LC | 3.11 | 38.59 | 39.45 | 39.02 | 39.02 | 65.02 | 1.00 | 14.80 | 1000 | | 7, 11, 12, 13, 58, 75 |
| ***Hyla sarda*** | 4 | A | LC | | | | 35.60 | 35.60 | | 1.00 | 20.00 | 1000 | 12600.00 | 11, 26, 57, 67 |
| ***Lithobates catesbeianus**** | 12 | A | LC | 368.47 | 134.74 | 135.53 | 135.14 | 135.14 | 188.92 | 1.50 | 95.00 | 25000 | 1600.20 | 4, 5, 11, 13, 15, 26, 76, 77, 78, 79, 80, 81 |
| *Pelobates cultripes* | 7 | A | LC | 21.60 | 65.90 | 70.18 | 68.04 | 68.04 | 88.65 | 3.00 | 30.45 | 2500 | | 4, 6, 11, 13, 56, 82, 83 |
| ***Pelobates fuscus*** | 10 | A | LC | 21.30 | 48.91 | 57.13 | 53.02 | 53.02 | 78.00 | 1.50 | 40.00 | 2500 | 500.00 | 4, 5, 6, 11, 19, 26, 84, 85, 86, 87 |
| *Pelobates syriacus* | 1 | A | CR | | | | 90.00 | 90.00 | | 2.00 | 25.00 | 4000 | | 4 |
| *Pelodytes ibericus* | 3 | A | EN | 3.98 | 35.17 | 40.72 | 37.95 | 37.95 | 55.85 | 1.00 | 20.00 | 350 | | 4, 88, 89 |
| *Pelodytes punctatus* | 6 | A | NT | | 36.67 | 43.31 | 45.88 | 45.88 | 63.67 | 1.00 | 20.00 | 1500 | | 4, 11, 13, 15, 58, 89 |
| *Pelophylax bedriagae* | 2 | A | VU | | 58.80 | 61.70 | 60.25 | 60.25 | | 2.00 | | 10000 | | 4, 90 |
| *Pelophylax bergeri* | 1 | A | LC | | 50.00 | 57.50 | 53.75 | 53.75 | | | 25.00 | 3000 | | 4 |
| *Pelophylax cerigensis* | 2 | A | LC | | | | 62.25 | 62.25 | | | | 15000 | | 4, 91 |
| *Pelophylax cretensis* | 2 | A | NT | | | | 72.30 | 72.30 | | | | 15000 | | 4, 92 |
| *Pelophylax epeiroticus* | 5 | A | LC | 48.80 | 72.42 | 83.98 | 78.20 | 78.20 | | 1.00 | | 2575 | | 4, 93, 94, 95, 96 |
| ***Pelophylax esculentus*** | 11 | A | LC | 36.90 | 79.71 | 97.88 | 88.79 | 88.79 | 142.75 | 3.00 | 27.33 | 10000 | 15000.00 | 4, 6, 14, 15, 18, 97, 98, 99, 100, 101, 102 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Pelophylax grafi* | 3 | A | LC | | | | 92.50 | 92.50 | | | | 10000 | | 4, 26, 97 |
| *Pelophylax hispanicus* | 2 | A | LC | | | | 100.00 | 100.00 | | | | 10000 | | 4, 51 |
| *Pelophylax kurtmuelleri* | 2 | A | LC | | | | 100.00 | 100.00 | | 2.00 | | 10000 | | 4, 51 |
| ***Pelophylax lessonae*** | 13 | A | LC | 30.80 | 54.45 | 60.25 | 57.35 | 57.35 | 90.79 | 3.00 | 25.00 | 4000 | 15000.00 | 4, 6, 14, 15, 26, 97, 98, 99, 100, 101, 103, 104, 105 |
| *Pelophylax perezi* | 5 | A | LC | 21.56 | 50.33 | 61.60 | 55.96 | 55.96 | | 2.00 | 27.30 | 10000 | | 4, 5, 26, 97, 106 |
| *Pelophylax ridibundus* | 14 | A | EN | 35.53 | 72.24 | 87.88 | 80.06 | 80.06 | 142.75 | 2.00 | 23.13 | 16000 | 1760.00 | 4, 14, 15, 58, 97, 99, 100, 102, 107, 108, 109, 110, 111, 112 |
| *Pelophylax shqipericus* | 2 | A | LC | | 70.60 | 73.80 | 72.20 | 72.20 | | | | 3000 | | 4, 113 |
| ***Pseudepidalea balearica*** | 2 | A | LC | 26.64 | 67.00 | 64.66 | 65.83 | 65.83 | | 4.00 | 16.00 | 15000 | 3621.00 | 51, 114 |
| ***Pseudepidalea sicula*** | 1 | A | LC | | | | | | | | | 15000 | 3621.00 | 51 |
| ***Pseudepidalea variabilis*** | 1 | A | DD | | | | | | | | | 15000 | 3621.00 | 51 |
| ***Pseudepidalea viridis*** | 13 | A | LC | 33.18 | 69.95 | 78.77 | 74.36 | 74.36 | 92.75 | 3.00 | 18.50 | 15000 | 10000.00 | 4, 11, 12, 26, 30, 51, 58, 90, 115, 116, 117, 118, 119 |
| ***Rana arvalis*** | 6 | A | LC | | 63.25 | 60.25 | 61.75 | 61.75 | 101.13 | 3.00 | 16.25 | 3000 | 640.00 | 4, 11, 15, 19, 58, 120 |
| ***Rana dalmatina*** | 10 | A | LC | 20.99 | 49.17 | 64.57 | 56.87 | 58.35 | 113.25 | 3.00 | 19.00 | 1800 | 1700.00 | 4, 11, 13, 15, 30, 46, 58, 121, 122, 123 |
| ***Rana graeca*** | 2 | A | LC | | 51.75 | 58.00 | 54.88 | 54.88 | 98.75 | 3.00 | | 800 | | 4, 58 |
| *Rana iberica* | 4 | A | NT | 6.03 | 37.71 | 45.09 | 41.40 | 41.40 | 84.00 | | 13.00 | 450 | | 4, 5, 58, 123 |
| *Rana italica* | 1 | A | LC | | | | 60.00 | 60.00 | | | | 2000 | | 4, 51 |
| ***Rana latastei*** | 4 | A | VU | | 42.42 | 45.44 | 43.93 | 43.93 | 100.84 | 3.00 | 15.00 | 400 | 150.00 | 4, 58, 124, 125 |
| *Rana pyrenaica* | 6 | A | EN | | 38.50 | 43.00 | 40.75 | 40.75 | | | 12.00 | 150 | | 4, 11, 13, 26, 126, 127 |
| ***Rana temporaria*** | 19 | A | LC | 42.24 | 69.45 | 70.83 | 70.14 | 70.14 | 122.25 | 3.00 | 13.75 | 4000 | 10000.00 | 4, 5, 11, 13, 14, 15, 19, 26, 44, 58, 120, 121, 126, 128, 129, 130, 131, 132, 133 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Xenopus laevis*** | 6 | A | LC | 33.41 | 62.49 | 73.30 | 67.90 | 67.90 | | 2.00 | 15.00 | 2500 | 1500.00 | 5, 11, 26, 134, 135, 136 |
| *Atylodes genei* | 6 | U | VU | 3.92 | 53.29 | 49.00 | 51.15 | 98.24 | 16.33 | | 20.00 | 10 | | 4, 138, 139, 140, 141, 142 |
| *Calotriton arnoldi* | 3 | U | CR | | 58.95 | 58.45 | 58.70 | 103.95 | 18.27 | | | 20 | | 139, 142, 143 |
| ***Calotriton asper*** | 14 | U | NT | 6.62 | 64.97 | 61.67 | 63.32 | 116.70 | 16.50 | 3.00 | 53.38 | 30 | 50.00 | 4, 11, 13, 139, 140, 141, 144, 145, 146, 147, 148, 149, 150, 151 |
| ***Chioglossa lusitanica*** | 8 | U | VU | 2.00 | 45.09 | 45.94 | 45.51 | 159.00 | 14.04 | 4.00 | 70.00 | 20 | 700.00 | 4, 139, 140, 141, 152, 156, 157, 158 |
| *Euproctus montanus* | 5 | U | LC | 2.50 | 56.00 | 58.00 | 57.00 | 116.50 | 20.50 | 3.00 | 50.85 | 60 | | 4, 7, 139, 141, 157 |
| *Euproctus platycephalus* | 5 | U | EN | 4.00 | 59.85 | 43.55 | 51.70 | 120.00 | 16.50 | 2.50 | 60.00 | 220 | | 4, 139, 141, 157, 158 |
| *Lissotriton boscai* | 9 | U | LC | 1.77 | 32.18 | 38.12 | 35.15 | 72.49 | 13.50 | 3.00 | 32.95 | 250 | | 4, 139, 140, 141, 159, 161, 162, 163, 164 |
| ***Lissotriton helveticus*** | 14 | U | LC | 1.30 | 34.50 | 39.64 | 37.07 | 77.24 | 14.00 | 2.00 | 35.00 | 460 | 400.00 | 4, 11, 13, 15, 26, 59, 139, 140, 141, 150, 163, 164, 165, 166 |
| *Lissotriton italicus* | 4 | U | LC | 0.98 | | | | 67.28 | | | 25.00 | 400 | | 4, 139, 159, 167 |
| *Lissotriton montandoni* | 7 | U | LC | 2.15 | 38.79 | 45.04 | 41.91 | 83.85 | 14.17 | 3.00 | 30.00 | 250 | | 4, 139, 140, 141, 159, 168, 169 |
| ***Lissotriton vulgaris*** | 28 | U | LC | 2.68 | 39.98 | 41.38 | 40.68 | 84.23 | 14.50 | 2.88 | 39.67 | 300 | 800.00 | 4, 11, 14, 15, 18, 19, 26, 44, 59, 139, 140, 141, 150, 164, 169, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184 |
| *Lyciasalamandra helverseni* | 2 | U | VU | | | | | 140.00 | | 3.00 | | 2 | | 139, 182 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Lyciasalamandra luschani* | 6 | U | VU | | 62.83 | 61.34 | 62.08 | 118.65 | 22.19 | 3.00 | | 2 | | 4, 140, 184, 185, 186, 187 |
| ***Mesotriton alpestris*** | 19 | U | LC | 2.96 | 45.59 | 53.84 | 49.72 | 95.06 | 17.00 | 3.00 | 47.86 | 260 | 1500.00 | 4, 11, 14, 15, 19, 26, 59, 140, 141, 142, 151, 164, 165, 176, 188, 189, 190, 191, 192 |
| ***Pleurodeles waltl*** | 8 | U | NT | 25.20 | 87.40 | 82.25 | 84.83 | 218.30 | 33.50 | 1.50 | 49.38 | 1400 | 200.00 | 4, 7, 12, 140, 141, 142, 177, 193 |
| *Proteus anguinus* | 7 | U | VU | 13.20 | | | 169.90 | 257.00 | 17.60 | 7.00 | 38.00 | 70 | | 4, 140, 141, 142, 148, 149, 194 |
| *Salamandra algira* | 2 | U | VU | | | | | 226.00 | | | | 18 | | 12, 140 |
| ***Salamandra atra*** | 8 | U | LC | | 66.95 | 71.23 | 69.09 | 117.00 | 23.50 | 3.00 | 47.50 | 11 | 30.00 | 4, 11, 140, 141, 142, 151, 195, 196 |
| *Salamandra corsica* | 3 | U | LC | | | | | 200.00 | | 6.00 | 55.50 | 23 | | 26, 140, 197 |
| ***Salamandra lanzai*** | 6 | U | VU | 12.20 | 82.55 | 83.68 | 83.11 | 138.00 | | 5.00 | 55.00 | 6 | 21.00 | 11, 26, 140, 198, 199, 200 |
| ***Salamandra salamandra*** | 15 | U | LC | 35.23 | 129.75 | 155.25 | 142.50 | 175.25 | 41.00 | 3.00 | 63.25 | 54 | 503.00 | 11, 13, 14, 15, 19, 140, 141, 142, 151, 200, 201, 202, 203, 204, 205 |
| ***Salamandrina perspicillata*** | 6 | U | LC | | 31.00 | 40.33 | 35.66 | 92.95 | 11.50 | 4.00 | 27.50 | 60 | 315.00 | 140, 142, 206, 207, 208, 209 |
| *Salamandrina terdigitata* | 4 | U | LC | | | | 33.31 | 94.00 | 10.68 | | 30.00 | 60 | | 4, 140, 141, 208 |
| *Speleomantes ambrosii* | 3 | U | NT | 2.26 | 53.31 | 55.93 | 54.62 | 125.00 | 17.01 | 4.00 | 20.00 | 10 | | 4, 139, 140 |
| *Speleomantes flavus* | 3 | U | VU | | 60.00 | 63.92 | 61.96 | 112.50 | 21.25 | | 20.00 | 10 | | 4, 139, 140 |
| *Speleomantes imperialis* | 3 | U | NT | 3.92 | 60.85 | 62.16 | 61.51 | 121.61 | 21.07 | | 20.00 | 10 | | 4, 139, 140 |
| *Speleomantes italicus* | 4 | U | NT | 2.88 | 52.20 | 56.23 | 54.22 | 94.61 | 17.32 | | 20.00 | 10 | | 4, 139, 140, 141 |
| *Speleomantes sarrabusensis* | 1 | U | VU | | | | | | | | 20.00 | 10 | | 4 |
| *Speleomantes strinatii* | 5 | U | NT | | 58.48 | 63.59 | 61.03 | 119.50 | | 3.00 | 25.00 | 11 | | 4, 11, 26, 140, 211 |
| *Speleomantes supramontis* | 3 | U | EN | 5.08 | 60.47 | 63.60 | 62.04 | 116.59 | 20.99 | 2.50 | 20.00 | 10 | | 4, 139, 140 |
| ***Triturus carnifex*** | 10 | U | LC | 8.84 | 70.19 | 72.28 | 71.24 | 144.42 | | 2.90 | | 400 | 299.00 | 4, 18, 36, 140, |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | 160, 176, 197, 212, 213, 214 |
| ***Triturus cristatus*** | 26 | U | LC | 7.34 | 64.33 | 69.28 | 66.81 | 140.05 | 26.50 | 2.67 | 61.25 | 400 | 1290.00 | 4, 11, 15, 18, 19, 26, 44, 56, 59, 140, 141, 142, 151, 160, 165, 173, 174, 175, 176, 177, 181, 215, 216, 217, 218, 219 |
| *Triturus dobrogicus* | 8 | U | NT | 4.70 | 62.99 | 66.69 | 64.84 | 132.08 | 20.01 | 3.20 | 35.00 | 250 | | 4, 18, 85, 140, 160, 176, 177, 220 |
| *Triturus karelinii* | 9 | U | LC | | 68.84 | 71.12 | 69.98 | 136.89 | 25.94 | 4.00 | 40.00 | 250 | | 4, 140, 142, 160, 176, 177, 221, 222, 223 |
| ***Triturus marmoratus*** | 15 | U | LC | 8.91 | 65.74 | 72.43 | 69.08 | 138.30 | 28.00 | 4.17 | 43.13 | 400 | 146.00 | 4, 11, 13, 18, 26, 56, 140, 142, 160, 164, 215, 216, 217, 224, 225 |
| *Triturus pygmaeus* | 7 | U | NT | 2.94 | 49.49 | 43.90 | 46.70 | 101.07 | 18.55 | 2.00 | 46.50 | 150 | | 4, 140, 141, 160, 224, 226, 227 |
| ***Number of missing values*** | | | | **38** | **18** | **19** | **7** | **3** | **35** | **24** | **22** | **1** | **50** | |

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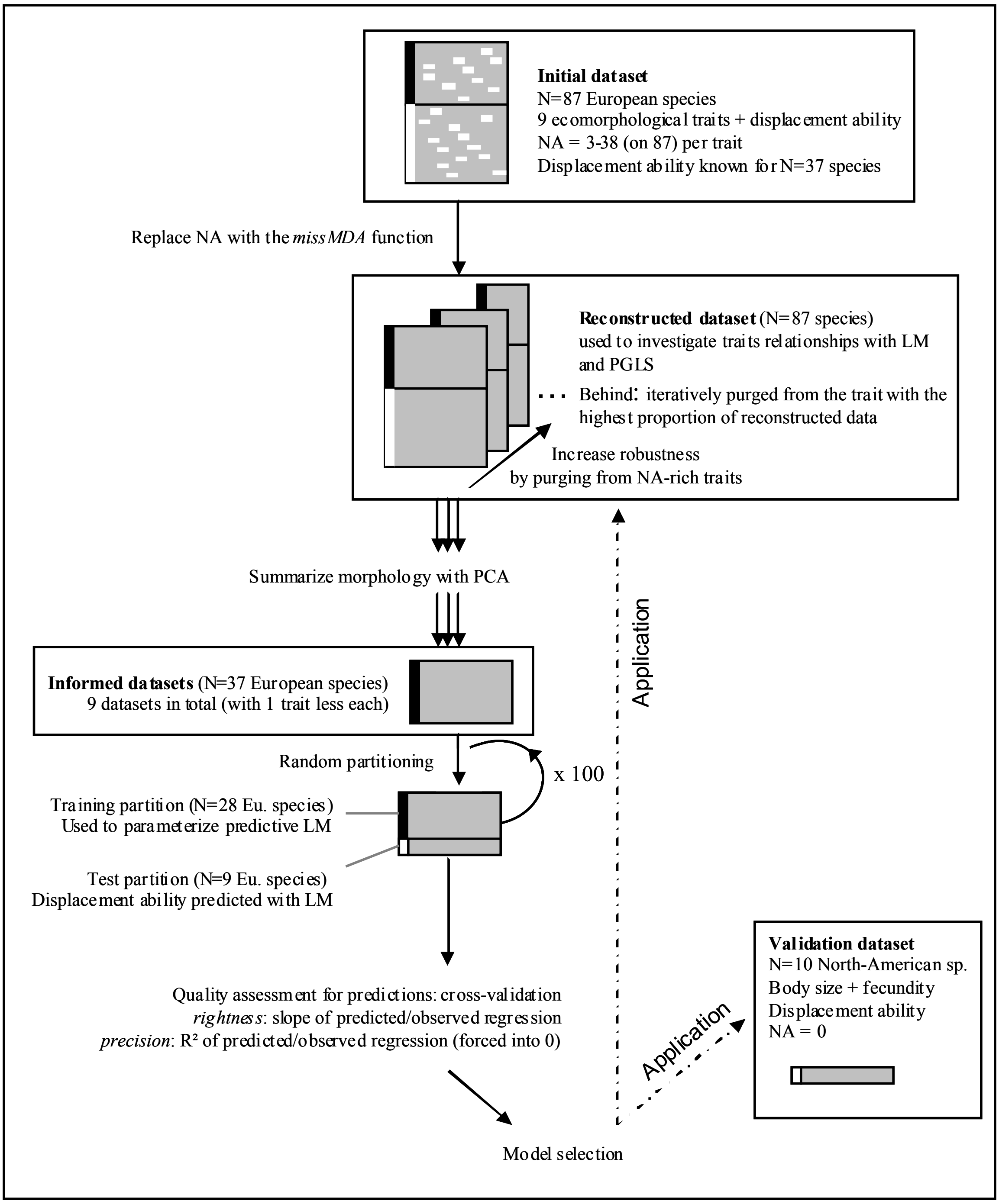
Appendix S2. Analytical procedure used to build and select predictive models for amphibian mobility.

The figure here below illustrates the analytical procedure we used in this study to investigate (1) which traits correlated with mobility and (2) the extent to which these correlations allowed predicting mobility across species.



Black and white bars left of the illustration of a dataset show if displacement ability is known (black), or unknown or ignored (white). Grey parts illustrate morphological and demographic traits, with white inserts showing the presence of missing data (NA). LM: linear model; PGLS: phylogenetic generalized least squares; NA: missing values; PCA: principal component analysis.

A first complication of our analyses was the frequent occurrence of missing values (hereafter NA) ranging from 3% to 44% per variable (see Appendix S1). In a preliminary step, we thus imputed the missing data using the regularized iterative principal component analysis (PCA) algorithm as implemented in the missMDA R-package (Josse *et al.* 2009) to generate a reconstructed dataset (see figure). This function first replaces missing values by the mean of each variable and performs PCA on the completed dataset, then, it imputes the missing values with the reconstruction formulae and iterates until convergence.

Similarly to life-history and morphology data, information on mobility was only available for 37 species (42.5%), considerably reducing the statistical power of models of the form "mobility ≈ a combination of traits + interactions among them". To circumvent this second limitation, we ran a PCA with the 7 morphological traits and then used species coordinates on the two first PCA axes (which had an eigenvalue > 1) as explanatory variables of mobility. All 87 species in the reconstructed dataset were used in this PCA. Then, we investigated how species' mobility relates to demography and morphology using analysis of variance. The fecundity, the age at maturity, the two first axis of the PCA on morphology, and their interactions were used as explanatory variables. As we suspected the sampling scale (see Schneider 2003; Franzén & Nilsson 2007; Stevens *et al.* 2010) and the sampling effort to impact the maximal displacement recorded, we included the distance of study sites and the number of individuals caught or tracked as covariates in our model. The complete (saturated) model was hence of the form:

$$\ln(\text{mobility}) \sim (\text{morphology axis 1} + \text{morphology axis 2} + \text{maturity} + \text{fecundity})^{\wedge}2$$

$$+ \text{sampling scale} + \text{sampling effort} + \text{error}$$

This model was used to investigate how life-history and morphology correlate with the displacement ability at the species level. Each variable entered into this model was standardized so that we could compare directly the scale of their effects on mobility. The relative goodness-of-fit of all simpler models derived from the saturated model was assessed by their AICc (corrected Akaike Information Criterion: Anderson *et al.* 1994). Models with ΔAICc < 2 from the model with the lowest AICc were retained and averaged as implemented in the MuMIn R-package (Barton 2011). To investigate trait relationships separately for anurans and urodels, we proceeded similarly as for the full dataset, but excluded first order interactions due to data limitation at that taxonomic scale.

Comparative studies at the species level must consider phylogenetic inter-dependency among species. Stevens *et al.* (2012) however showed that trait correlations might be relatively independent from phylogeny. We thus consistently compared linear models (LM), where species were considered as independent data points, to PGLS (phylogenetic generalized least squares: Rohlf 2001) of the same form, where phylogenetic correlations were accounted for. The relative importance of the phylogeny in structuring the data was appreciated by the comparison between the PGLS' AICc and the LM's AICc. In PGLS, the correlation structure due to phylogenetic non-independence was calculated under the hypothesis of a Brownian motion. The phylogenetic reference was the composite tree of living amphibians, extracted from the Tree of Life (Cannatella 2008), to which branch lengths were computed with Grafen's (1989) method.

### *Predictions from relationships among traits*

We then used LM to predict mobility from the values of other ecomorphological traits. As we mentioned previously, our database initially contained many NA, which were replaced through the missMDA R-function. However, for statistical robustness of predictions, a model based on NA-poor variables should be preferred over a model using NA-rich variables. To increase the robustness we thus iteratively removed the NA-richest variable from the reconstructed dataset (i.e. before summarizing the morphology with PCA: see figure). Then, with this new dataset, we proceeded as follows: summarizing the morphology with a PCA (if more than 2 morphological traits remained), running the LM, selecting the best model(s) with AICc, and cross-validating their predictions.

At each step of this iterative process we used a cross-validation to assess the efficiency of each model to predict amphibian's mobility. In this process, the available mobility data were randomly split in two: 75% of species were the training partition (28-29 species) from which the model was parameterized and 25% were the test partition (9-10 species) for which mobility was ignored and predicted with the model (Anderson et al. 2009 ; Thuiller 2003). This partition was repeated 100 times to get confidence intervals for the predictions. The predictive quality of each model was then defined as its ability to a) minimize the adjusted $R^2$ of the regression of predicted against observed mobility, with the intercept constrained to zero (model's *precision*) and b) to provide a slope close to 1 when regressing predicted mobility against observed mobility (model's *rightness*) (this time the intercept was not constrained).

### *Inferences from phylogenetic substitutions*

To evaluate the efficiency of phylogenetically-based substitution to infer unknown mobility we performed again cross-validations with a 75-25% random partitioning (repeated 100 times). This time, the ignored mobility of each species in the test partition was simply replaced by that of the phylogenetically closest species present in the training partition. Two alternative rules were considered in this substitution, as the phylogenetic distance was appreciated by either (i) the number of nodes, or (ii) the sum of branch lengths separating the two species in the phylogenetic tree of living amphibians (with 1342 taxa considered). When several species in the training partition had similarly low phylogenetic distance with a species and can be considered similarly good candidates for the substitution, their mobility were averaged. The *rightness* of the predictions made by these phylogenetic substitutions was assessed by examining the slope of the regression of substituted *vs*. observed mobility, and its *precision* by the $R^2$ of a regression with intercept forced to 0. Moreover, we evaluated if the predictions obtained were *sensitive* to the phylogenetic proximity between a species and its substitute(s) by testing for correlation between the deviation of the prediction (absolute difference between observed and substituted mobility) and the phylogenetic distance between both species.

### *Inferring mobility for European amphibians, and validating with independent data*

The single best method for inference (either a substitution or a linear model) was applied to the whole reconstructed dataset with 87 European species. To compare among alternative phylogenetic substitutions, we considered their rightness, their precision and their sensitivity. To select among the alternative LM, we considered the balance between their robustness (i.e. the proportion of data that were initially missing and were reconstructed), their fit to the data (AICc), and the quality of their predictions (rightness and precision). The AIC is like a measure of the price/quality ratio of a model, as it corresponds to its log-likelihood penalized by 2 points for each parameter entered in the model. In the particular case of our study, the real "cost" of a LM not only depends on the number of variables retained, but also on the number of traits needed to perform all the analysis, i.e. how many traits were summarized by the PCA on morphology when at least one PCA axis was retained in LM. We thus adjusted the AICc values to take this into account, with a penalty of 2 per "supplementary" variable needed to build the model.

After having selected the best alternative to predict the displacement ability in amphibians, it was applied (1) to 87 European species present in the reconstructed dataset, and (2) to a sample of 10 North-American amphibians (5 anurans + 5 urodels = validation dataset: see figure), all with mobility repeatedly measured but ignored here. This application to independent data ensured the validity of our model. This general prediction was based on a linear model, where the covariate (sampling scale) was held fixed for all species to the value of its upper 95% confidence interval in the European dataset, because the covariate value was of course unavailable for those species for which displacements were never measured, and also in order to provide a general picture of displacement ability, comparable across all species. This also allowed us assessing the robustness of the model to the ignorance of the spatial scale, by comparing measured mobility for North-American species to their predicted mobility, with a prediction that ignored the spatial scale that was chosen by "experts" to measure their mobility.

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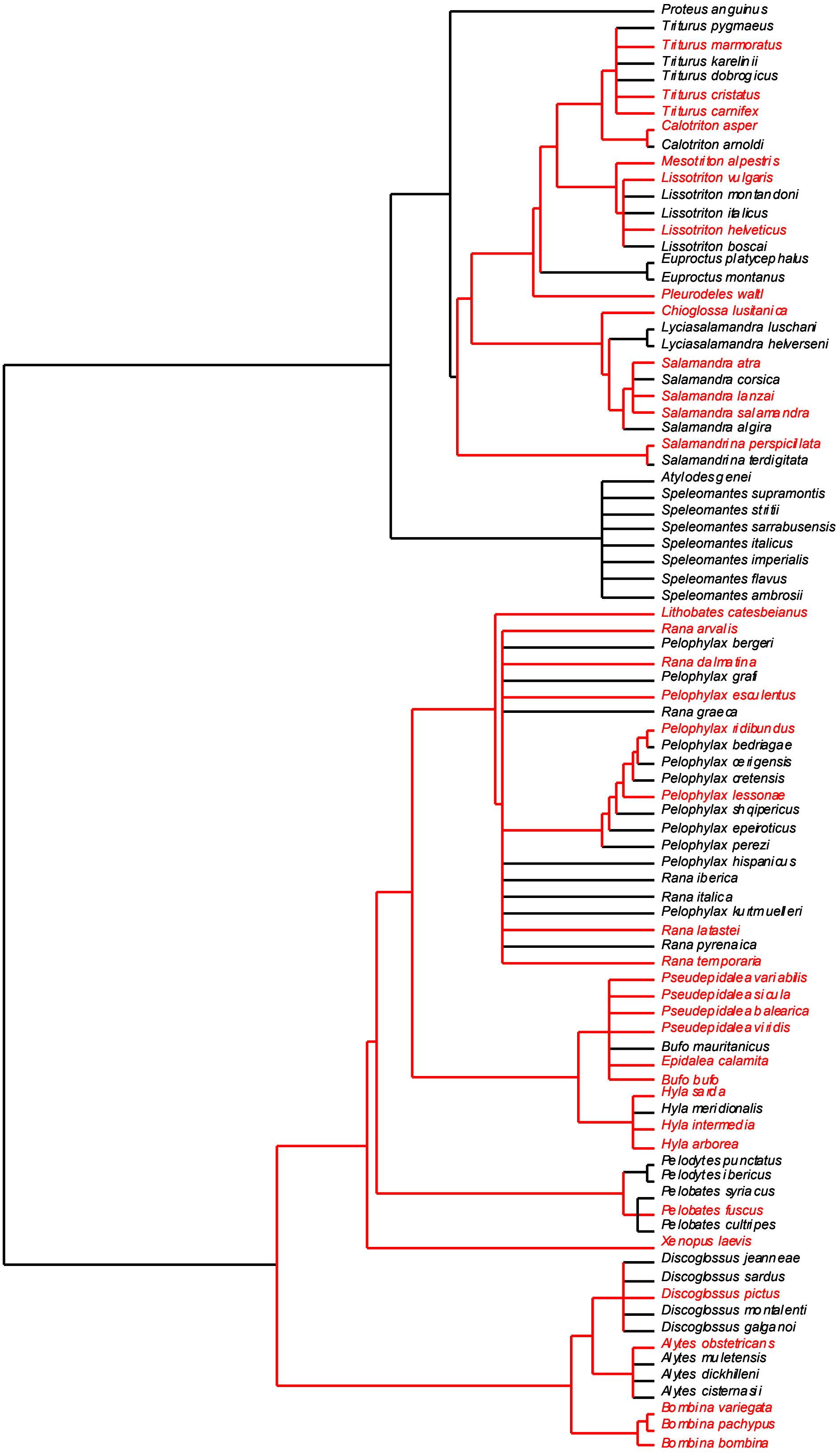
Appendix S3. Phylogenetic tree of the 87 amphibian species used in analyses. In red: species for which mobility was reported in literature. In black: species with unknown mobility.



**Appendix S4**. Summary of the construction of a predictive model for amphibian vagility, based on linear models (LM) with information from 1 to 9 species traits. At each step, one trait was removed from the database to increase the robustness of the dataset. At each step, morphological traits, demographic traits and their first order interactions, as well as the spatial scale of the study (S. scale) and the sampling effort (S. effort: the number of individuals for which mobility was measured) were proposed as independent variables with fixed effects on species mobility (maximal displacement measured). For steps 1-5, the demography was summarized by a PCA from which the two first axes were proposed as the independent variables for morphology in the LM. For steps 6-9, demographic traits were proposed directly. Squares illustrate variables proposed in the model at each step, and circles illustrate the data considered indirectly at each step (and summarized by two axes of a PCA: Axis 1 and Axis 2). Variables retained in the model are illustrated with black squares; variables not retained in the model are illustrated with white squares. At each step, all models with □AICc <2 from the model with the lowest AICc (Akaike Information Criterion, corrected for small sample size) were retained, and their performance was assessed through cross-validation (see text). The rightness (rightn.) is the slope of a regression of predicted vs. observed displacement ability. The precision obtained with the prediction is given by the adjusted R², i.e. the explained variance of a regression of predicted vs. observed mobility, forced into zero. *AICc is model's AICc, penalized by 2 points per supplementary variable used, depending on the difference between the number of variables summarized in the PCA and the number of demographic axes retained in the model. Numbers below trait names are the number of species (of 37) for which the trait value was obtained via a reconstruction with iterative PCA algorithm (see text). At each step, the trait that had the highest number of species with reconstructed value was discarded (see text and **Fig. 2**). Model 8.1 was considered the most robust and efficient for predicting displacement ability in amphibians.

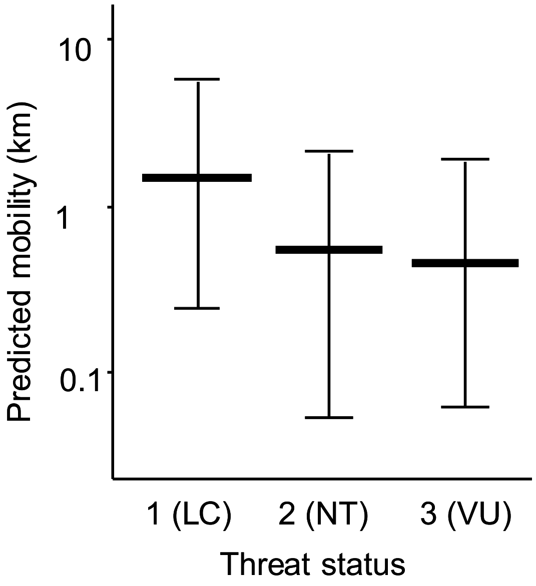
| | | Morphology | | | | | | | | | Demography | | Covariates | | Model performance | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Step | LM | Mass | SVL_M | SVL_F | SVL_ad | Length | HLL | SVL_Meta | Axis 1 | Axis 2 | Fec. | Matur. | S. scale | S. effort | AICc | *AICc | Rightness (slope) | Precision (Adj.R²) |
| | | *8* | *3* | *3* | *2* | *2* | *9* | *4* | | | *0* | *3* | | | | | | |
| 1 | 1.1 | ● | ● | ● | ● | ● | ● | ● | □ | ■ | □ | □ | ■ | □ | 127.1 | 139.1 | 0.9255 | 0.9682 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1.2 | ● | ● | ● | ● | ● | ● | ● | □ | ■ | □ | ■ | ■ | □ | 127.0 | 139.0 | 0.9040 | 0.9683 |
| | 1.3 | ● | ● | ● | ● | ● | ● | ● | ■ | ■ | □ | □ | ■ | □ | 127.2 | 137.2 | 0.8794 | 0.9681 |
| 2 | 2.1 | ● | ● | ● | ● | ● | | ● | □ | ■ | □ | □ | ■ | □ | 129.5 | 139.5 | 0.8804 | 0.9661 |
| | 2.2 | ● | ● | ● | ● | ● | | ● | □ | ■ | □ | ■ | ■ | □ | 130.2 | 140.2 | 0.8580 | 0.9654 |
| | 2.3 | ● | ● | ● | ● | ● | | ● | ■ | ■ | □ | □ | ■ | □ | 129.7 | 137.7 | 0.8688 | 0.9659 |
| 3 | 3.1 | | ● | ● | ● | ● | | ● | ■ | □ | ■ | □ | ■ | □ | 131.5 | 139.5 | 0.8107 | 0.9642 |
| | 3.2 | | ● | ● | ● | ● | | ● | ■ | □ | ■ | ■ | ■ | □ | 129.1 | 137.1 | 0.8321 | 0.9664 |
| | 3.3 | | ● | ● | ● | ● | | ● | □ | ■ | □ | ■ | ■ | □ | 133.8 | 141.8 | 0.8038 | 0.9619 |
| | 3.4 | | ● | ● | ● | ● | | ● | □ | ■ | ■ | ■ | ■ | □ | 130.0 | 138.0 | 0.8471 | 0.9656 |
| | 3.5 | | ● | ● | ● | ● | | ● | ■ | ■ | ■ | □ | ■ | □ | 132.1 | 138.1 | 0.7671 | 0.9636 |
| | 3.6 | | ● | ● | ● | ● | | ● | ■ | ■ | ■ | ■ | ■ | □ | 131.3 | 137.3 | 0.7906 | 0.9643 |
| | 3.7 | | ● | ● | ● | ● | | ● | □ | ■ | ■ | □ | ■ | □ | 129.7 | 137.7 | 0.8984 | 0.9658 |
| | 3.8 | | ● | ● | ● | ● | | ● | □ | ■ | □ | □ | ■ | □ | 132.4 | 140.4 | 0.8662 | 0.9633 |
| 4 | 4.1 | | ● | ● | ● | ● | | | □ | ■ | □ | □ | ■ | □ | 130.5 | 136.5 | 0.8612 | 0.9651 |
| | 4.2 | | ● | ● | ● | ● | | | □ | ■ | □ | ■ | ■ | □ | 129.3 | 135.3 | 0.8872 | 0.9662 |
| | 4.3 | | ● | ● | ● | ● | | | ■ | ■ | ■ | □ | ■ | □ | 130.0 | 134.0 | 0.8058 | 0.9656 |
| | 4.4 | | ● | ● | ● | ● | | | ■ | □ | ■ | □ | ■ | □ | 134.2 | 140.2 | 0.7620 | 0.9615 |
| | 4.5 | | ● | ● | ● | ● | | | ■ | ■ | □ | □ | ■ | □ | 130.3 | 134.3 | 0.8588 | 0.9653 |
| | 4.6 | | ● | ● | ● | ● | | | ■ | □ | ■ | ■ | ■ | □ | 132.6 | 138.6 | 0.7963 | 0.9631 |
| | 4.7 | | ● | ● | ● | ● | | | □ | ■ | ■ | □ | ■ | □ | 130.0 | 136.0 | 0.8679 | 0.9656 |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 5.1 | ● | ● | ● | □ | ■ | □ | □ | ■ | □ | 130.4 | 134.4 | 0.8709 | 0.9652 |
|  | 5.2 | ● | ● | ● | □ | ■ | □ | ■ | ■ | □ | 129.9 | 133.9 | 0.8839 | 0.9657 |
|  | 5.3 | ● | ● | ● | ■ | □ | ■ | □ | ■ | □ | 132.6 | 136.6 | 0.7956 | 0.9631 |
|  | 5.4 | ● | ● | ● | ■ | ■ | ■ | □ | ■ | □ | 130.8 | 132.8 | 0.7872 | 0.9649 |
|  | 5.5 | ● | ● | ● | ■ | □ | ■ | ■ | ■ | □ | 131.5 | 135.5 | 0.7928 | 0.9641 |
|  | 5.6 | ● | ● | ● | ■ | ■ | □ | □ | ■ | □ | 129.9 | 131.9 | 0.8857 | 0.9657 |
|  | 5.7 | ● | ● | ● | □ | ■ | ■ | □ | ■ | □ | 129.9 | 133.9 | 0.9066 | 0.9657 |
| 6 | 6.1 |  | □ | ■ |  |  | ■ | □ | ■ | □ | 126.8 | 126.8 | 0.8885 | 0.9684 |
|  | 6.2 |  | □ | ■ |  |  | ■ | ■ | ■ | □ | 127.4 | 127.4 | 0.8718 | 0.9679 |
| 7 | 7.1 |  | □ | ■ |  |  | ■ |  | ■ | □ | 126.6 | 126.6 | 0.8940 | 0.9686 |
| 8 | 8.1 |  |  | ■ |  |  | ■ |  | ■ | □ | 127.1 | 127.1 | 0.8819 | 0.9682 |
|  | *8.2* |  |  | ■ |  |  | ■ |  | □ | □ | *139.8* | *139.8* | *0.7641* | *0.9552* |
| 9 | 9.1 |  |  |  |  |  | ■ |  | ■ | □ | 132.4 | 132.4 | 0.9020 | 0.9633 |

Mass: species adult mean body mass; SVL: average snout-to-vent length (_M: for adult males; _F: for adult females; _ad: for adults; _meta: at metamorphosis); length: total length of adults (including tail); HLL: length of hind-limb for adults; fec.: fecundity = average number of eggs (or larvae) per female per breeding event; matur.: age at maturity); Numbers in italic are the number of species (of 37) for which the trait value was obtained via a reconstruction with iterative PCA algorithm (see text and **Appendix S2**).

**Appendix S5**. Mean predicted mobility (and 95%CI) of amphibians of the three lowest threat statuses: LC: least concern; NT=near threatened; VU=vulnerable.



# 8. Trait database of reptile life histories

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In preparation (last draft 29/04/2014)

*Résumé en français*

Les bases de données portant sur les traits d'histoire de vie des espèces sont essentielles pour un large panel de disciplines telles que la biologie évolutive, l'écologie et la biologie de la conservation. Alors que des données d'histoire de vie sont disponibles en ligne pour de nombreuses espèces notamment de plantes, les données concernant les traits d'histoire de vie des reptiles eurpéen sont largement éparpillées à travers l'europe, dans différentes langues et ne sont généralement disponibles qu'à travers des média imprimés. Pour cette raison, nous avons généré une base de données complète des traits d'histoire de vie couvrant 149 espèces de reptiles européens. Les données ont été compilées en réalisant une recherche bibliographique, des interviews d'experts et des présentations de conférences. Nous n'avons cependant inclus dans la base de données que les donées publiées. La base de données couvre toute l'Europe et les pays africains et asiatiques adjacents. Les traits inclus dans la base de donnée sont répartis en cinq champs principaux: activité et énergétique, phénologie, dispersion, age à la maturité sexuelle and morphométrie. Pour assurer la standardisation des données, nous avons clairement défini les traits utiliser avant la compilation. Toutes les entrées ont été vérifiées par au moins une personne supplémentaire. Le jeu de données fourni une source unique d'informations utilisables pour réaliser des meta-analyses, de la modélisation en écologie ou des études de biologie de la conservation.

## Abstract

Life-history data are essential for a wide range of evolutionary, ecological, and conservation biological issues. While life history data for many species, especially plants, are available online, life history traits of European reptiles are available only widely scattered in different languages and primarily in printed media. For this reason, we generated a comprehensive trait database covering all 149 European reptile species. Data were compiled through literature surveys, expert interviews, and conference presentations but we included exclusively published data. The database covers whole Europe and the adjacent Asian and African countries. Traits were categorised under five main headings: activity and energy, phenology, dispersal, age at sexual maturity, and individual measurements. To secure standardization of data, we defined trait data specifically before we started compiling data. All entries were checked by at least one further person. The dataset provides a unique source for meta-analyses and modelling in ecology and conservation biology.

## Key words

Activity, dispersal, Europe, life history traits, lizards, phenology, Reptilia, snakes, tortoises

## Data resources

## Data published through Dryad

## Project details

**Project title:** Securing the conservation of biodiversity across administrative levels and spatial, temporal, and ecological scales (SCALES) (Henle et al. 2010)

**Subproject:** Trait database of reptile life histories

**Personnel:** Klaus Henle (Project Coordinator, Taxonomic Expert, and Data Compilation), Annegret Grimm (Data Compilation and Data Manager), Ana María Prieto Ramírez (Data Compilation), Sylvain Moulherat (Data Compilation), Julie Reynaud (Data compilation).

**Funding:** FP7 integrated project SCALES; EU Grant no. 226852

## Taxonomic coverage

**General taxonomic coverage description:** The coverage of this database spans the class of Reptilia in the Kingdom Animalia. The database collates species traits of all 149 European species belonging to 43 (SEH Species Name, http://www.seh-herpetology.org/Distribution_Atlas/Reptiles_in_Europe (Gasc et al. 2004)) respectively 59 (current species name) genera.

Taxonomy started from the names used by Societas Europaea Herpetologica (SEH) in their atlas (http://www.seh-herpetology.org/Distribution_Atlas/Reptiles_in_Europe) (Gasc et al. 2004). Since the atlas was published, many names changed both at the generic and sub-generic level, primarily by splitting previous taxa. Whereas some new taxa are based on thorough evidence using more than one character set, comprehensive data sets that were evaluated in detail statistically, and biogeographic evidence, others are poorly defined. While many new or resurrected taxa were accepted by other authors without any discussion, controversies exist about other taxa. Unfortunately, this is only partially related to the extent of evidence provided for the initial taxonomic decision. Because of this instability we had to make own decisions on which new taxonomic arrangements to follow and which not. We accepted recent changes (named current taxonomy in the following) only, if they were backed by large samples on more than one character set that were analysed with appropriate statistical methods; if only one character was used, we accepted taxonomic changes only if combined with strong biogeographical evidence.

**Taxonomic ranks:**

**Kingdom:** Animalia.

**Phylum:** Chordata.

**Class:** Reptilia.

**Order:** Testudines, Squamata (Amphisbaenia, Sauria, Serpentes).

**Family:** Agamidae, Amphisbaenidae, Anguidae, Blanidae, Boidae, Chamaeleonidae, Cheloniidae, Colubridae, Dermochelyidae, Emydidae, Gekkonidae, Geomyidae, Lacertidae, Lamprophiidae, Natricidae, Phyllodactylidae, Scincidae, Sphaerodactylidae, Testudinidae, Typhlopidae, Viperidae.

**Common Name:** Reptiles, Snakes, Lizards, Tortoises.

## Spatial coverage

**General spatial coverage:** Case studies and handbook data cover 49 European countries. Additional studies were included from adjacent countries in Asia (Armenia, Azerbaijan, Georgia, Iran, Israel, Kazakhstan, Kyrgyzstan, Lebanon, Russia, Syria, Tajikistan, Turkey, Turkmenistan, and Uzbekistan) and Africa (Algeria, Morocco). Moreover, case studies from USA about the invasive European gecko *Hemidactylus turcicus* were included.

## Temporal coverage

1966 – 2013.

## Methods

**Method step description:** Our literature survey was conducted as focal species surveys of all European reptiles. A first basis was found in German (Böhme et al. 1981ff), French (Arnold and Ovenden 2010), and Spanish (Escarré and Verricard 1981, Salvador and Marco 2009) handbooks data. We searched for additional case studies and reviews in ISI web of knowledge, Google Scholar, and Google published in English, German, French, and Spanish. Additionally, we searched in experts' libraries and institutions to include grey literature (i.e.

literature that has not been formally published). We tried to trace any potentially relevant sources cited in the publications found through these searches. Moreover, we presented our project on herpetological conferences to expand our sources of literatures from experts. However, we entered data into the database exclusively from published literature. The publications that provided entry data for the database are listed in Table 1.

**Study extent description:** All European reptile species are covered in the database without specific temporal patterns of sampling.

**Sampling description:** Before reading the literature, we decided the structure (described below) and the main topics to be covered by the database. Main topics selected were activity and energy traits, phenological traits, dispersal traits, age at sexual maturity, and individual measurements. Literature was scanned following specific key words linked to these topics. A detailed description of the metadata is given below.

**Quality control description:**

All data entries were checked by at least one person other than the one who entered the data. This check included a plausibility check of the original data. In the few cases that doubts remained were excluded from the database.

Further data will be checked by us before they will be entered to the database.

**Table 1:** References of results and data for the compilation of information on reptile life history traits presented in this article and the database.

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**Dataset descriptions**

Our database comprises two sections: basic information and actual trait data. In the basic section, general information about taxonomy, references, and countries is stored. The trait data section is divided into the following ecological parts: activity, phenology, sexual maturity, dispersal, and individual measurements.

**Object name:** Trait database of reptile life histories

**Character encoding:** UTF-16

**Format name:** Microsoft Access Database

**Format version:** Microsoft® Access® 2010 (14.0.7104.5000) SP2 MSO (14.0.7116.5000) as part of Microsoft Office Professional Plus 2010

**Distribution:** http://scales.ckff.si/scaletool/db_reptile.php

**Publication date:** 2014-01-21

**Language:** English

**Licenses of use:** This database is entitled "Trait database of reptile life histories", a user-friendly interface has been made available within our project webpage (www.scales-project.net), and the database has been uploaded to DRYAD. Data have been made available under the Creative Commons CC-Zero Waiver: http://creativecommons.org/publicdomain/zero/1.0/

**Metadata descriptions**

Our relational database is divided into a basic section and the actual trait section. The basic section comprises three tables: *Species*, which list all 149 European reptile species according to their SEH name and their current species name; *References*, listing all handbooks, reviews, and case studies used in the database (165 in total); *Countries*, listing 49 European, 14 Asian, and 2 African countries and the USA where reptile trait data stem from including their ISO 2, ISO 3, and ISO No codes.

In the trait data section, five main tables were created following the five main topics (activity and energy, phenology, dispersal, sexual maturity, individual measurements). All tables are provided with species ID, county ID, country specifications (if published), altitude (if published), latitude (if published), longitude (if published), and reference ID such that each data point can be tracked correctly. The definitions and contents of the five tables are described in the following. Word in italic symbolise column headings.

*Activity, Energy, Habitat*: Data about *daily activity* describe activity peaks during the day including activity switches within the year. These activity patterns were defined as ten different categories: (1) *cn:* crepuscular/nocturnal; (2) *dn:* active whole day, no circadian rhythm (diurno-nocturnal); (3) *tn:* nocturnal, but thermoregulation during day possible; (4) *hu:* humidity dependent, no circadian rhythm; (5) *1:* one activity peak during the day throughout the year (unimodal); (6) *2:* two activity peaks during the day throughout the year (bimodal); (7) *as1a:* activity switch: summer: one peak during dusk or night (crepuscular or nocturnal), spring/autumn one peak during the day (diurnal) [it is possible that species show bimodal activity between the switch diurnal to nocturnal]; (8) *as1b:* activity switch: one peak during the day throughout the year, but switched to morning during summer; (9) *as2a:* activity switch: in summer two peaks during the day, spring/autumn: one peak during the day but diurnal throughout the year; (10) *as2b:* activity switch: summer: one peak during dusk or night (crepuscular/nocturnal), spring/autumn: two peaks during the day (morning/afternoon).

Additionally, data about *energy accumulation* denote when species accumulate energy for reproduction (spring, summer, or autumn). Moreover, their *habitat* and their *longevity* were listed.

Phenology: Phenological traits refer to four *phases*: first and second breeding season, aestivation, and hibernation. Each phase is specified by a specific *start* and *end month*. This range represents the maximum duration if several start or end months were given for the specific country (region) in a specific reference. This results in maximal breeding time (earliest start month and latest end month for a specific country reported in a specific reference) and minimal hibernation time (latest start month and earliest end month reported). Note that for aestivation no rounding problems occurred.

*Dispersal*: *Dispersal* covers, true dispersal, home range movement and seasonal migration because most references do not sufficiently differentiate between them. However, if data were sufficiently explicit, we used only dispersal data. Dispersal data above 250 m were rounded to 250 m. The reference time span is not necessarily one year but depends on the study described in the original paper. The data in the database are the highest values for a specific country found in specific literature. If a range of maximum dispersal was given in the reference, we put the lower value as *maximum dispersal low* and the higher value as *maximum dispersal high*. If no range was given, data were allocated to the later one. Furthermore, *passive dispersal* and ways of *locomotion* reported in literature are given.

*Sexual Maturity*: Age at sexual maturity was defined as *minimum, median*, or *mean age* for *males* respectively *females*. The minimum age refers to the lowest age at sexual maturity given in a specific reference for a specific country. The median age is usually the age in which most individuals reach sexual maturity. Mean age is given only if explicit values were provided by the relevant publication.

*Individual Measurements*: In the last section, individual measurements as *minimum*, *maximum*, and *mean* values (depending on the data given in literature) were recorded. If published, a *sample size* was listed. *Traits* were specified as mass, length, width, number, or size. These traits always refer to denoted parts of the population (*Who*), such as females, males, hatchlings, juveniles, eggs, or clutches. This structuring allows maximal possibilities of combinations using minimal memory space.

Since our database was built as a relational database, IDs between the basic and the traits tables were linked to each other for fast searches and queries.

**Metadata language:** English

**Date of metadata creation:** 2013-12-10

**Hierarchy level:** Database

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# Related SCALES reports

In this section I will only join the English abstract of the deliverable I contributed. And the short cases study I provided to the SCALETOOL Work Package.

# 1. Review manuscript on dispersal traits and distances, including the underlying trait database (Gotzenberger et al. 2011)

W.P.2.1.3, 114pp

Gotzenberger, L., Zobel, M., Baguette, M., Blanchet, S., Calvez, O., Clobert, J., Lange, R., **Moulherat, S.**, Pe'er, G., Schmeller, D.S., Stevens, V.M., Tamm, R., Trochet, A.

The dispersal of organisms and their propagules is a fundamental process that influences all levels of ecological organization, from the individual to the ecosphere. Dispersal can generally be defined as the movement of individuals away from their place of birth. It can be captured by dispersal curves, i.e. the probability of an individual to disperse to a certain distance. Obviously, the shape of a dispersal curve is highly dependent on the kind of organism we are looking at. Which feature of the dispersal curve is important for an ecological process depends to a large degree on the spatial scale under consideration. For instance, for large scale migration rates it is much more important to know the maximum dispersal distance an individual can travel, whereas processes at a more local level can better be addressed by mean dispersal distances or knowledge about the entire dispersal curve. In a conservation biology framework, information on dispersal is crucial for assessing the connectivity of local populations in a landscape, as well as for predicting arrival of invasive species and species range shifts under global change scenarios. The investigations carried out in SCALES regarding dispersal distances were twofold. Firstly, for three different groups of organisms, predictive models were developed and their capability of predicting dispersal distances was evaluated.

Secondly, a cross taxa study focused on the comparability of dispersal distances and dispersal curves estimated with different methods for different animal taxa. Predictive models for dispersal distance within taxa. We analyzed the relationship between species traits related to dispersal, and the dispersal distance of the species, based on data from previously published literature and data bases, as well as data collected by the involved SCALES partners. Not all of the different focal taxonomic groups of WP 2.1 (plants, birds, amphibians, butterflies, bees) had sufficient data available and the types of data were heterogeneous among groups. We therefore provided the overall review as a collection of separate studies for different taxonomic groups that have been produced by different partners within WP 2.1. The presented results and discussions reflect the current state of the analyses and future work within the SCALES project will add further insights.

A general result across the single studies is that it seems possible and feasible to achieve at least rough dispersal distance estimates from species traits. The predictive capability that could be achieved with the traits analyzed is clearly dependent on the availability of data within a certain taxonomic group. We expect that the future collation of data and improvement of the statistical models to relate dispersal distances to traits will further improve the precision with which dispersal distances can be estimated. As data for more taxonomic groups become available, the general approach can of course also be applied to other groups.

In conclusion, the estimation of dispersal distances from species traits can be seen as an important step forward in improving models that rely on dispersal distances as input to predict connectivity and migration of species. Dispersal across taxa and methods Though dispersal distance as expressed in simple mean or maximum dispersal distance is an important feature of dispersal curves, data on the entire dispersal curves are often helpful. In particular, such data can be used as input in individual based models of population connectivity. Thus, this part of the deliverable had a more general approach than the predictive models, collating data on distances but also on whole dispersal curves from a variety of animal taxa (amphibians, beetles, birds, butterflies, damselflies, grasshoppers, mammals, snails). These data were analyzed with respect to the methods that were used to obtain the dispersal data. Different mathematical functions for depicting the species dispersal curve were described. These functions were then evaluated regarding their capability to describe a given dispersal curve given the patch-related focus of studies (intra-patch, inter-patch, or combination of both), methodology applied (Capture Mark Recapture vs. telemetry), movement mode of the animals (walking vs. flying), and duration of the study. Results showed that patch related focus and duration of the studies did affect the shape of the dispersal curve. Also, contrary to discussions in the literature, a Poisson function was found to be the best description of most dispersal curves. Additionally, mean and maximum dispersal distances were analysed. Although a significant positive correlation between mean and maximum dispersal exists, such a relationship is not valid within all of the studied taxa. Measures of dispersal distance were also affected by sex and developmental stages of the target individuals. In consequence, efforts to model connectivity have now a more solid basis for including dispersal distances. However, care is still needed with respect to what is the best function for particular taxa for solving the particular modelling task.

## 2. Development of dispersal response categories under different ecological scenarios (Baguette et al. 2012a) W.P.2.3.1, 127pp

Baguette, M., Blanchet, S., Clobert, J., Legrand, D., **Moulherat, S.**, Stevens, V.M., Turlure, C.

The conservation of many species in European landscapes depends on the existence of large populations guarantying the persistence of sufficient numbers of individuals. Theory indeed predicts that the extinction probability of a population is conversely related to its size. As the re-allocation of large amount of space to nature is rarely an option in most European landscapes, an alternative strategy would be to promote the maintenance of groups of local populations connected by the exchanges of local populations. Such metapopulations would avoid the extinction of species at the landscape level. The metapopulation theory thus provides a solid framework for the conservation of species in heavily fragmented landscapes. The linkage strategy, corollary of the metapopulation theory, aims at facilitating the dispersal of individuals among local populations, either by the creation of corridors or stepping stones that bind local habitat patches into functional ecological networks.

Dispersal is thus central to the strategies of biodiversity conservation. However, there is a critical gap between the recent findings in dispersal ecology and evolution, and the implementation of these strategies of biodiversity conservation. Most of them are based on the implicit and untested assumption that linkages among similar ecosystems would promote the metapopulation functioning of the species living in these ecosystems. However, dispersal is a highly complex, multi-causal process, which is extremely variable both between and within species. This idiosyncratic nature of the dispersal process precludes the use of estimates of landscape structural connectivity aiming at linking like with like. Rather, the solution would be to design functional networks for umbrella species that are representative of other species living in the same ecosystem. The ecological network in a given landscape will then be designed by stacking up such linkages designed for several species living in different ecosystems.

The linkage strategy requires accurate data of the dispersal of individuals of a given species in a given landscape. Its precise assessment using genetic data and the methods of landscape genetics is an essential prerequisite of the spatial planning of functional ecological networks. However, we show here that dispersal is tangled in life history traits in one taxonomic group. This relationship paves the way for predictions from dispersal estimates that could be used for uninformed species, if less precise dispersal estimates are sufficient. Finally, we show that the dispersal responses of individuals confronted to contrasted ecological structures depends more on their displacement mode than on dispersal rates. We show also that changes in landscape grain generate non linear and unpredictable changes in dispersal. This scaling issue reveals that the choice of study grain should be dictated by species perceptions of landscape structures rather than by the remotely sensed data available.

## 3. **Repport on scaling properties of declining and expanding populations in relation to underlying pressure and on area requirements for Viables populations** (Clobert et al. 2012) W.P.2.3.2, 213pp.

Clobert, J., Baguette, M., Brotons, L., Bullock, J.M., De Caceres, M., Fall, A., Fortin, M.J., Franz, K., Heikkinen, R.K., Heliola, J., Henle, K., Hooftman, D.A.P., Kopsova, L., Kuussaari, M., Mazaris, A., Matsinos, Y., **Moulherat, S.**, Pe'er, G., Perea, R., Poyri, J., Prudhomme, C., Saarinen, K., Storch, D., Tansey, C., Tsianou, M., White, S.M.

In this deliverable we aimed at examining the scaling properties of decliniong and expanding populations in relation to underlying pressures. In the second part, we also aimed at studying the effect of area requirements, and possibly its interaction with underlying pressure and scaling properties, onto population viability. The method to achieve these goals has been either to review the literature available or to conduct some innovative studies on the pressure, the scaling properties, and/or the minimum area requirements. We were limited in our investigation, first because it was a huge topic, and also because the data available for such studies are limited or difficult to acquire. One of the first conclusions of the report is therefore the urgent need for acquiring good data on as many species, or taxonomic groups as possible. This is a key since most of the advices that can be made are relaying on model simulations with little or no validation.

**The first study is a review of what we know about the effect of climate change on the organism's distribution.** We examined in particular the role of dispersal since this life history trait is an important component of the ability a species has to meet the climate warming challenge. Indeed, climate conditions can induce flexible or plastic changes in dispersal behavior, which may speed up or slow down range expansion. As many others, our review shows clearly that some climate chage effects are already occurring. Some of the observed demographic effects are positive (enhanced growth and fecundity, earlier maturation and higher reproductive success, enhanced survival) but others are negative (lower survival, increased water and heat stress, reduced dispersal). Even though, many species are sensitive to thermal conditions, their demographic reponses to climate change will depend on other climate factors, such as humidity, on landscape structure and on biotic interactions with other species. So far, there has been tendency to counfound the realized niche of a species with its climate niche, which is in contracdiction with theory and data from biogeography and community ecology.

The two following studies will examine more closely whether declining populations exhibit sparser distributions than those with expanding distributions using Finnish butterflies and the effect of dispersal power changes due to climate warming using UK plants.

Previous theoretical and empirical studies have suggested that species distributional patterns are similar across multiple scales and species distributional trend may be reduced from spatial patterns: expanding species show more clumped distributional pattern than declining species. We tested these predictions in our **second study using two independent comprehensive empirical datasets on Finnish butterflies** covering local (0.1 – 1 km) and regional (10 – 100 km) scales ans by comparing distributional patterns etween two time periods, 2000-2002 and 2009-2011. We observed that a majority of butterfly species showed declining areas of occupancy (AOO) and fractal dimension (Dij) between the two study periods at the regional scales but not at the local scales. The change of AOO10 was negative in most distributional classes and main breeding habitat except species recently colonized southern coast of Finland and species inhabiting field margins, respectively. In contrast to predictions, AOO10 and D10-100 showed negative impacts on the change of AOO10 at regional scales, and observation that may be explained by the high proportion of declining species in our data. Butterfly species with the highest values of fractal dimension at regional scales (D10-100) calculated for the period 2000-2002 showed positive long-term distributional trends and greatest recent northern range limit shifts (between the periods 1992-1996 and 2000-2004). Interestingly, species with a southern distribution pattern but contrasting distributional trends (declining species vs. recent colonizers) were indistinguishable from their regional scale fractal dimension (D10-100), which may be a reflection of the rare and stochastic colonization events over the Gulf of Finland to the south coast of Finland. Finally, we were not able to find a relationship between the change in AOOs and fractal dimensions (Dij) and the recent habitat availability change derived from individual butterfly species from Corine Land Cover classes.

Big changes in area occupancy and connectivity clearly should be considered as an as important driver as climate warming. The ability of a declining or expanding species to resist pressures clearly will depend on direct and indirect interactions among drivers, as already seen in the review on reptiles.

If climate change impacts on habitat suitability and demography are often studied, the direct effects on a dispersal vector is rarely considered. This is especially important for **wind dispersed species, such as plants and was investigated in the third study.** To address this issue, we analysed climate model projections of future wind speeds and modeled their possible impacts on dispersal and spread of dispersed plants. Projections for 17 Global Climate Models and three emission scenarios suggested great uncertainty about wind speeds in southern England by the period 2070-99. Projections ranged from -90% to +100% changes in the mean wind speed, although, the average projection was for large falls in both summer and winter wind speeds. Using a novel method for converting projected changes in mean wind speed to new seasonal wind speed distributions, we parameterized a mechanistic model of seed dispersal by wind using baseline and changes in mean wind speed from -80% to +80%. This mechanistic seed dispersal model was combined with demographic data in analytical model of plant spread. This was carried out for three Brtitish native and three non-native species, whch represented a range of life-forms. Dispersal kernels and population spread rates were affected disproportionately by changes in wind speed, demonstrating nonlinear propagation of uncertainty in wind speed projection through to modeled plant spread rates. Sensitivity analyses showed differences among the plant species in which demographic transitions were most important in determining spread rates. By contrast, sensitivity of spread rates to dispersal parameters showed great consistency among species, with seed release height being more important than seed terminal velocity. Plant populations will need to shift their geographic ranges to keep pace with climate change-driven habitat loss. This study shows that climate change may affect that ability by decreasing the dispersal distances of wind-dispersed plants and thus their potential spread rates. However, the modeling approach presented here illustrates that uncertainty in climate models leads to an even greater uncertainty about how dispersal and spread will change in future climates. Caution should therefore be execised in making predictions as to how fast plant species may spread in response to climate change. Such incertitude caused by the lack of appropriate data and the existence of indirect effect of climate change on specific art of species life cycle are clearly diminishing our ability to make prediction and examine properly the scaling effects of a combination of pressures. This conclusion also emerged from the other studies.

The prediction of species spatial responses to global change requires therefore a good understanding of the ecological mechanisms mediating such responses. This point will be illustrated in a **fourth study using data of species re-colonization after fire**, a mechanism which is akin to the colonization of the cold margin of a species distribution facing climate warming. As already stated, dispersal is a key mechanism in determining species responses to environmental change especially in dynamic landscapes affected by interactions between land use changes and fires. Using novel methodologies to link monitoring data to hybrid species distribution models explicitly accounting for species dispersal capacity, we assessed the capacity of early succession bird species in Mediterranean habitats to respond to fire impact at the landscape scale. The results indicate that strong dispersal constraints will limit the colonization of new fire-related habitats and mediate the interactions between species dynamics and andscape changes. In this case, species persistence in the long term will be related to the degree of spatial autocorrelation of the fire disturbance and to which degree this matches the scale at which species are able to disperse. Since this response is not only dependent on species dispersal capacity but also on landscape dynamics, our results have strong implications for the scaling of changing species distributions in relation to particular global change drivers.

The analyses of species changes at large scales are likely to not taking into account key interactions between ecological processes such as dispersal and landscape dynamics, leading to poor capacity in predicting species capacity to persist in a given region. This is especially relevant in regions in which contrasting fine-grained impacts of disturbance are likely. It is suggested that this may be an increasingly common case due to the number of potentially interacting factors affecting species distribution dynamics in real landscapes. If our results are extrapolated to different systems, large scale exercises of species distribution changes may be strongly biased in areas where the impact of climate change is affected by other, more fined grained, disturbances such as fire.

As a conclusion of the above four studies, we might stated that: 1) we have not enough data and more data should be acquired at the appropriate scale (at the species dispersal scale) in order to fully incorporate the neede details to make accurate species distribution projections; this is true for almost all taxonomic groups; 2) we need to have more model systems wher dispersal process is known in deail; 3) processes are clearly location and scale dependent; 4) Processes at work at cold and warm margin(expanding and retracting) populations are most likely not being the same; we therefore need more model systems that are studied on the entire distribution area, militating for an European effort in this respect; 5) interactions among pressure should be more deeply studied, first because they caused nonlinearities in the species response to pressures, but also direct and indirect interactions among drivers are common; 6) almost nothing is known about the effect of species interaction and pressures; we need to support research in this area; 7) clearly dispersal, and behavioural or physiological plasticity are important factors in determining a species response to pressure; not much is known on this important life history traits for many species, and developing more model system to study it, and finding surrogate for this trait are of prime importance if we really want to adequately predict species distributional changes as well as devising appropriate conservation strategy (connectivity, assised colonization, modification of the landscape and habitat structure).

One idea in this framework is to use the possibility that dispersal will be linked to other life history trait. The latter can then be used as a surrogate for assessing dispersal capacity of a species whenever this life history trait is not available or difficult to measure. Home range size or other area size related trait might be such a possibility.

Indeed, Policy-makers and landscape planners often need simple measures or rules of thumb that can direct effective measures for nature conservation despite gaps in knowledge or uncertainty. Two examples for policy-relevant measures are the Minimum Viable Population size (MVP), and the concept of Minimum Area Requirement (MAR). Minimum Area Requirement of species is an index that defines the amount of space (suitable habitat) that is required for the long term persistence of populations. It is a potentially useful concept since it explicitly addresses area per se, and therefore it can be used for reserve site selection, design, and assessment. Nonetheless, application of the MAR concept is quite limited, and values are available for only a small number of species compared to the MVP.

**In the last chapter of this report we reviewed in part one the literature in terms of main sources of information related to the MAR, main methods to evaluate it, and factors that affect the estimates.** We consider three approaches to identify the MAR of species: a) Population Viability Analysis (PVA) studies that explored a range of areas; b) PVAs that contained the information on MVP alongside population density or information on the study area, thus enabling one to calculate the MAR, or alternatively provided the MAR value as is yet without exploring area per se; and c) empirical studies that calculated the area requirement of species based on observed patterns of occupancy across a range of habitat areas. We evaluated the performance of each in terms of the correlation between MAR and body size, and explored the effect of a range of variables including species' traits and environmental conditions in predicting MAR.

Most of the studies focused on birds and mammals, with only some insects and reptiles – and not a single amphibian or other taxa. These results indicate that the MAR concept is currently used in a relatively narrow context and only for a handful of taxonomic groups.

Despite major differences in the source of data and taxonomic groups, and despite the fact that MAR values provided by studies varied greatly even for a given study, we found relatively strong correlations between body mass and MAR, and simple measures such as trophic level (feeding guild). Our results indicate that some approaches yield higher correlation with body mass compared to others. Firstly, empirical observations on occupancy showed no significant patterns regarding body mass, but instead were better explained by life history traits and trophic levels, a result found in studies on butterflies and amphibians. These results likely reflect the fact that various factors affect occupancy, and therefore occupancy patterns may not be appropriate for estimating the area requirement of populations. Translating MVP into MAR through density yielded medium correlations with body mass, but other analyses indicated not support of theory, as only significant factor was found to be the feeding guild of species. This, in our opinion, reflects some oversimplification of the relationship between population size and area through density. We find a particularly risky simplification in the form of assuming a fixed MVP and deriving the MAR only from home range size. In this case, high correlations with body mass (and other factors) are obtained, but these reflect an attribute of home-ranges rather than the MAR. Finally, PVAs that explored a range of areas yielded comparably high correlation between MAR and body mass, and the feeding guild as a second factor that explains the area requirement of species. In our opinion, these are promising results since they are emergent from simulation model that try to capture important attribute of species, using empirical data but concomitantly incorporating uncertainty through sensitivity analysis or inspection of alternative scenarios. Furthermore, the design of PVAs includes consideration of the question whether the relevant ecological level is the population or the emetapopulations. Therefore, we see PVAs as accounting for several important sources of uncertainty that may diminish the reliability of other estimations.

There are many caveats that must be considered if using the MAR concept for applied conservation. A conservative approach must be taken where one should employ the maximum estimate available. Considering the limitations and simplifications involved with the MAR concept, authors of ecological studies seem to be reluctant in providing MAR values. The consequence, however, leads to discrepancy between policy needs and the outputs of scientific investigations. Considering that human pressure and conservation area planning often involves spatial questions, we see the MAR as a potential important metric that deals with landscapes rather than population attributes. It can be used to provide important information to decision-makers, and an important measure for estimating conservation areas for their functionality across species.

To facilitate the process of making the MAR concept more operable in nature conservation, we offer several guidelines:

a. Use the MAR values that are outcomes of models and not superimposed on them
b. Consider whether the relevant MAR is on population or metapopulation level
c. Consider how edge effects and management of the matrix affect the effective area available for species; and
d. Apply caution intranslating individual-level information into MAR

Finally, we delineate current applications of the concept, some challenges and prospects, and potential directions that may facilitate the future usability of the MAR concept. These include a) a need to identify better means to link individual-level and population level parameters, b) extension of the MAR concept to metapopulations, c) a need to assess the minimum area requirements versus minimum habitat available, d) a need to develop a dynamic MAR concept in light of dynamic conservation challenges, e) a need to revise the effects of dispersal and connectivity on MAR, and finally, f) need to develop robust means for validation of estimates.

**The second part of chapter 4 in our review in offered a different angle to the use of area as an alternative to measure dispersal and prefer to infer from historical and present day maps to infer future distribution of species.** Indeed, Intensification of human activities has caused drastic losses in semi-natural habitats, resulting as well in declining connectivity between remaining fragments. Successful future restoration should therefore increase both habitat area and connectivity. The first steps in a framework for doing so are addressed here, which involve the mapping of past habitat change. We present a method which is unique in : the large area covered (2500 km²), the high resolution of the data (25 x 25 m), the long period assessed (70 years), and the system translation of land use maps into Broad Habitat Type using soil survey. We digitized land use maps from the 1930s for the county of Dorset in sourthern England. The resulting map was compared to the UK Land Cover Map of 2000. Four our example area, land use shifted dramatically to more ontensive agriculture: 97% of all semi-natural grasslands were converted into agriculturally-imrpoved grassland or arable land as were large proportions of heathlands and rough grasslands (-57%). The other important driver of change was afforestation (+25%). The larger habitats areas became fragmented, with average fragment size of different habitats falling by 31-94%. Furthermore, the connectivity between fragments dropped drastically, by up to 98%. Analyses such as those presented here not only quantify the scale and pattern of habitat loss, but are important to inform land-use planning to restore biodiversity by both increasing the available habitat and facilitating dispersal among habitat fragments.

As we have seen, we have explored many ways of studying the interplay between scales, pressures, and population dynamics without coming with a clear-cut strategy or theory that encapsulate all issues raised in this repport. Although they are clearly some methods which might be used in case of urgent needs in term of conservation (such as surrogate measure of dispersal, using historical maps, using meliorated niche of physiological models, etc), none are a panacea and have still strong limitations. Here we want to advocate for an alternative strategy to handle such complex issues to predict really whet will be the future of species distribution under pressures and which scales are pertinent for which species. This is an engineering approach. Alike when constructing a bridge, the engineer uses general principles (theory) which are adapted to the place where the bridge has to be built (the species, community, landscape, etc). Many such tools have been devised in the course of SCALES, going from genetic, to behavioural to dynamical to spatial (scales) and to social, which can be understood as general principles or the tool box solving conservation issues. The engineer in environment will then use these tools to apply them to a more specific case or question. It clearly remains to construct this tool box with tools which are made compatible among themselves and to train future engineers to use them to solve environmental questions.

Conservation issues are idiosyncratic by virtue and only an engineering approach, in close contact with researchers in the relevant scientific fields, will help implementing more efficient conservation plans which are valid from the local to the European scales.

# 4. **Report on scalling trade-offs in conservation goals and cross-scale conservation principles** (Gunton et al. 2013) W.P.2.6.2, 37pp

Gunton, R., Marsh, C., **Moulherat, S.**, Rosindell, J., Kunin, W.

Nature conservation is a task that imposes both ethical and technical challenges. Priorities must be assigned to a range of entities such as species, populations, communities, biodiversity and ecosystem functions. While there can be no simple solution to the question of what to conserve and how, existing policy recommendations tend to be based on assessments that are essentially subjective, or that ignore important subjective foundations on which calculations are based. This report describes an attempt to synthesize empirical patterns across a range of conservation criteria focused on biodiversity and ecosystem function. Drawing on both quantitative simulation modelling and expert assessment, it assesses a range of habitat configurations such as might occur in European landscapes at two spatial scales, according to criteria of (i) within-species genetic diversity, (ii) population viability; (iii) species richness and (iv) agricultural ecosystem service, taking pollination as a case-study. These criteria are considered for four contrasting groups of species: small mammals, butterflies, songbirds and plants (both trees and grassland herbs). The results show that the number and spacing of habitat patches can be more important than the overall area of habitat in a landscape, and neither complete fragmentation nor complete amalgamation of habitat is optimal. The relative values of patterns depend on the spatial scale at which they occur, on the conservation criterion being assessed and on the kind of species concerned. Nevertheless, some promising cross-criteria and cross-species patterns emerge at each spatial scale, suggesting that certain types of fragmented patterns, with a wide range of patch sizes, are particularly beneficial. Our results are supported by agreement between expert assessments and simulation models in most cases.

## 5. Assessment of uncertainty of simple connectivity estimates

(Baguette et al. 2012d) W.P.3.2.2, 62pp

Baguette, M., Clobert, J., Matsinos, Y., **Moulherat, S.**, Pe'er, G., Schmeller, D.S., Schober, L., Trochet, A., Turlure, C.

Landscape connectivity results from the interaction of individuals with the features of the landscape in which they move. The review of several methodologies aiming at predicting landscape connectivity showed that simple connectivity estimates based on landscape geometrical properties (size of suitable habitats or inter-habitat distances) are much too uncertain to be used in conservation. Alternative methods that either predict movements by applying movement rules to dispersing individuals or track movements by assessing gene flow between populations proved to be more reliable.

Two simple, structural connectivity estimates are compared to the observed movements in a quasi-experimental situation. Movements between local populations of two specialist butterflies living in discrete habitat patches within the landscape are neither predicted by the Euclidean distances between populations, nor by this distance weighed by the cost associated with the crossing of unsuitable matrixes. It is concluded that these two simple estimates are too uncertain to reliably predict landscape connectivity for butterflies.

## 6. **Population viability across scales** (Baguette et al. 2012c) W.P.3.2.3, 88pp

Baguette, M., Clobert, J., Curtis, J.M.R., Franz, K., Henle, K., Johst, K., Malinowska, A.H., Matsinos, Y., **Moulherat, S.**, Naujokaitis-Lewis, I., Pe'er, G., Radchuk, V., Turlure, C., Wintle, B.A..

Population viability analyses (PVAs) contribute to conservation theory, policy and management. Most PVA studies focus on the dynamics of a single species in a given landscape in relation to particular threats and/or management options, utilizing a case-specific set of performance criteria. Recent attempts to synthesize and generalize the results of multiple PVA studies have failed to overcome the methodological idiosyncrasies and find meaningful generality in meta-analysis. These studies highlight the problem of case-specificity in PVA methods and reporting and identify the need for some degree of standardization.

The aims of this study are therefore to: (i) examine whether PVA implementation and reporting have changed or improved over time, (ii) assess whether published guidelines for PVA design and implementation have been used or offer solutions, and (iii) offer a omprehensive protocol for design, application and communication of PVAs.

# 7. Scale Tools

*Functional connectivity of the natterjack toad (*Bufo calamita*) for regulatory study of an industrial area in southwestern France. (Moulherat 2013a)*

In south-western France (44°11’36.32”N, 0°31’14.23”E), MetaConnect was used in the context of regulatory study for implantation of economic area (ZAC) and terrestrial transport infrastructure (high-speed railway). We used MetaConnect to determine the potential impact of such infrastructures on the functional connectivity in *Bufo calamita.* We also evaluate the cumulative impact of the infrastructures on the functional connectivity (Art. L122-1 modified by Loi n° 2010-788 du 12 juillet 2010 – art. 230 of the French environmental legislation).

## I. MetaConnect setting up

The philosophy of this analysis was not to determine the absolute functional connectivity for *B. calamita* but rather to estimate the landscape potentialities in regard to *B. calamita* functional connectivity.

Habitat patches were determined *a priori* based on expert assessment. Landscape rugosity coefficient were derived from those experimentally determined by Stevens et al.(Stevens et al. 2006a). *B. calamita* was assumed to disperse following the stochastic-movement-simulator (SMS) assumptions (Palmer et al. 2011). The rational for this choose is that, even though, Stevens et al.(Stevens et al. 2006a) found that least-cost-path assumption is consistent with the population genetic structure of *B. calamita* in their study site, Palmer & Stevens recently improved the fit between simulated dispersal of *B. calamita* and the observed population genetic structure using the SMS model (2012, unpublished datas). Finally, MetaConnect was setup with LHTs values found in the literature.

## II. Functional connectivity for *B. calamita*

Expert survey of the site performed for the regulatory study (cabinet ECTARE) confirmed the presence of *B. calamita* (adults and eggs) on the most important habitat patches *a priori* assumed from habitat suitability (figure 1). Simulation results showed that the building of the ZAC will probably not change strongly the persistence of *B. calamita* within the study site. Even though, the habitat patch which would be destroyed by the building of the ZAC (figure 1) plays an important role in the local functioning of the meta-population as a relay for the dispersal of *B. calamita* along the North-South axis (figure 2), this destruction will have little influence on *B. calamita* persistence because this role would be ensured by a patch nearby (figure 1, 2). In contrast, the addition of the high-speed-railway will disrupt the flow of individual along the North-South axis which would increase patches extinction probabilities and total extinction probabilities (figure 1) and would greatly reduce *B. calamita*'s movements within the study site (figure 2).

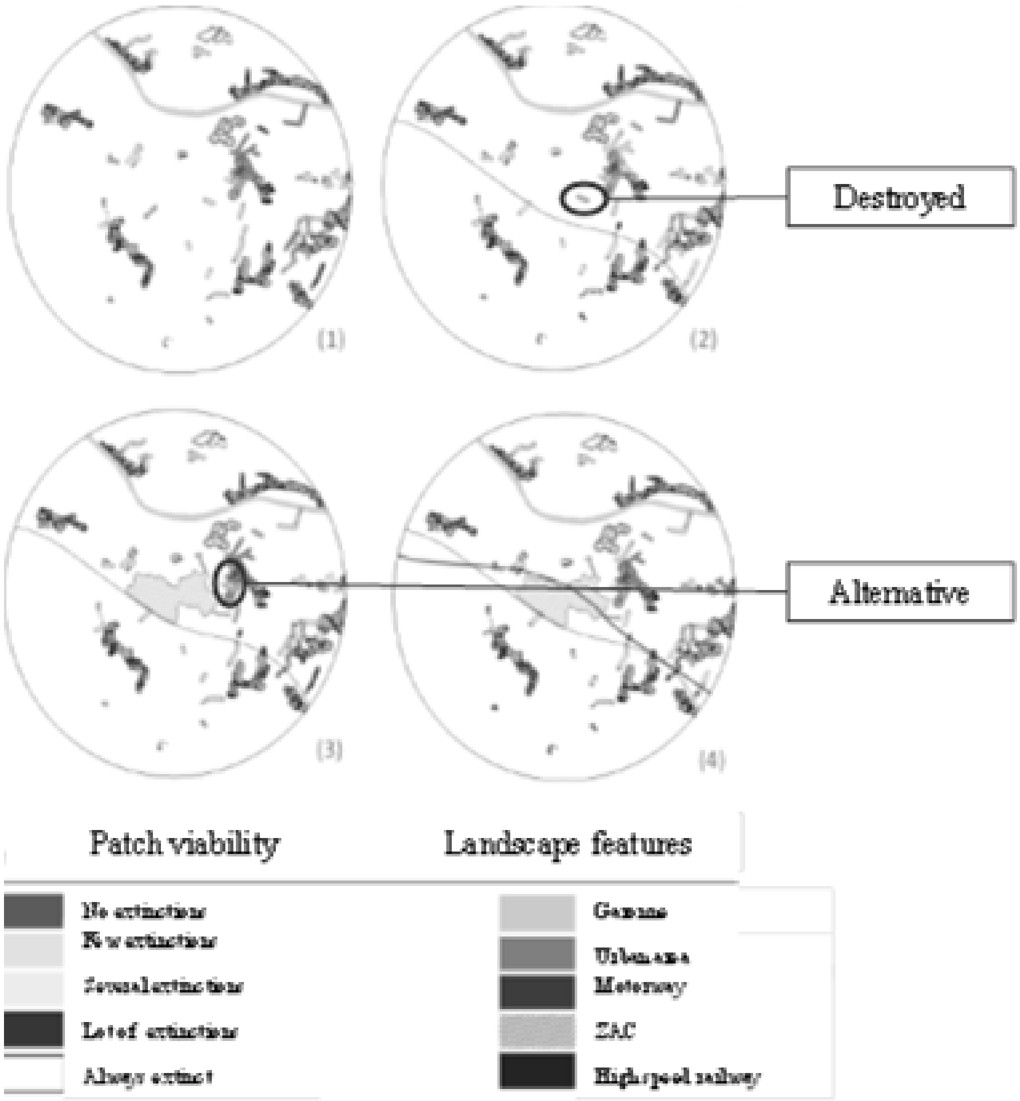

Figure 1: Patch viability of *Bufo calamita* estimated with MetaConnect 5 km around the ZAC project. The patch viability for *B. calamita* depends on the presence of artificial structures (motorway (2), ZAC (3), high speed railway (4)) but do not reduce significantly from scenario (1) (without any artificial structure) to scenario (3). The high speed railway building would seriously threaten the maintenance of *B. calamita* within the study site.

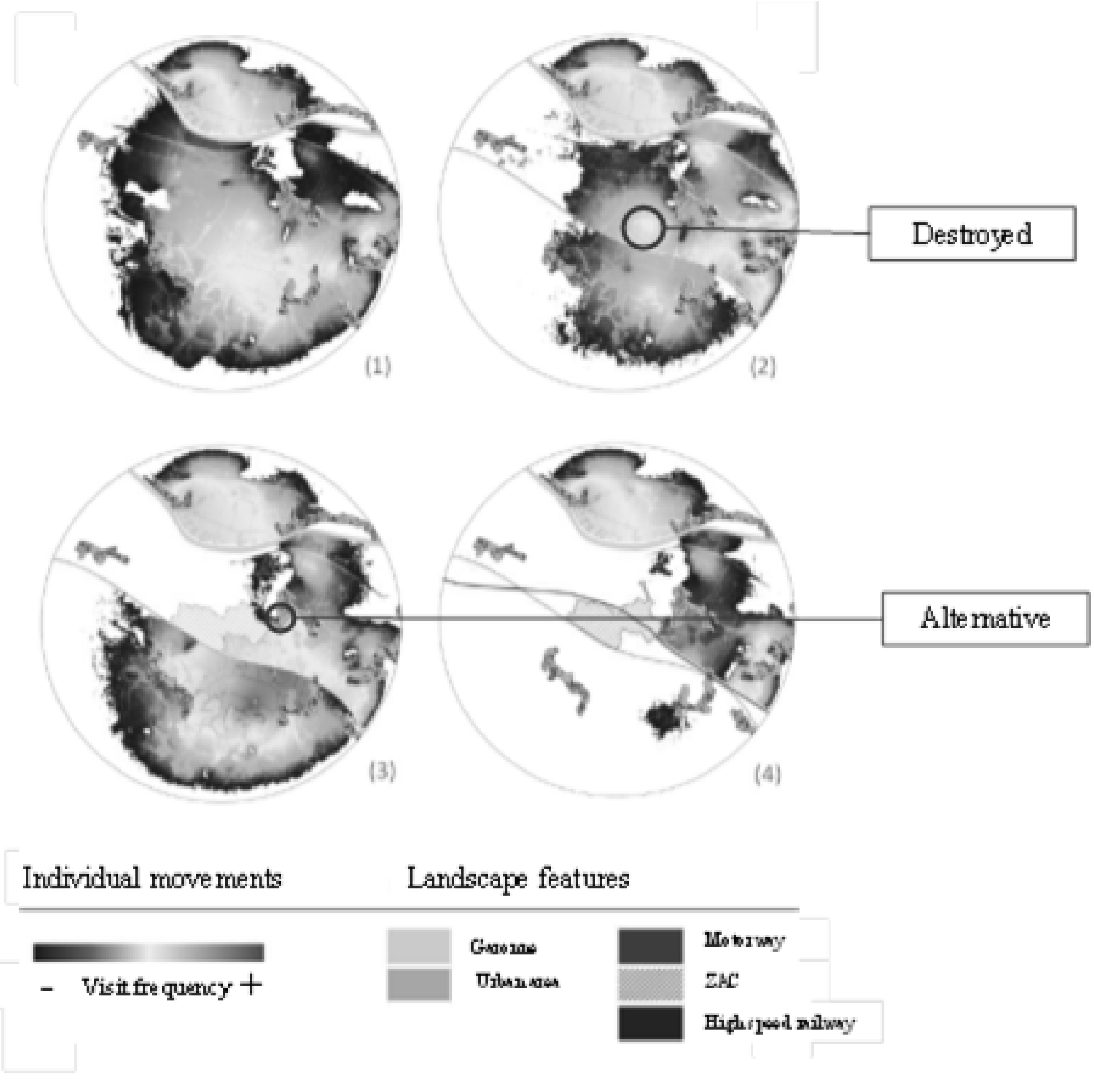

Figure 2: Movements of *B. calamita* estimated with MetaConnect under the SMS model (scenario legend as for figure 1). The ZAC building would concentrate the flow of individual between the ZAC and the urban area at its east rendering the "alternative" patch crucial for

individual flow in the north-south axis. The high speed railway building would strongly affect the individual flow on the north-south axis.

## III. Conclusion

The use of MetaConnect detected that the ZAC will destroy an important patch for the functional connectivity of the natterjack toad in the study site. However, the functional redundancy of this patch with an eastern patch (figure 1, 2) suggests that this other patch would functionally replace the destroyed patch.

In addition, the detection of this second patch as a key patch will allow informing the city nearby as required by the French legislation. This patch would then benefit from the compensatory measure induced by the ZAC build to ensure its conservation and functionality.

## IV. References

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2 Palmer, S. C. F., Coulon, A. & Travis, J. M. J. Introducing a 'stochastic movement simulator' for estimating habitat connectivity. *Methods in Ecology and Evolution* (2011).

*Population viability of the keeled skimmer (*Orthetrum coerulescens*) within the bog network of the Montselgues plateau in France. (Moulherat 2013b)*

Fifty years ago, the Montselgues plateau in France was covered by heathland and bogs. Then forestry replaced the breeding sheep economic activity leading to the reduction of the heathland and bog area and their fragmentation by forestry pine (figure 1). In 2005, the site benefits a LIFE project (http://www.life-montselgues.eu/) to restore the bog network of the Montselgues plateau. In 2006, at the starting of the project, the site's conservation managers (Conservatoire des Espaces Naturels de Rhône-Alpes (CEN RA) and the Parc Naturel des Monts d'Ardèche (PNR MA)) used dragonflies populating as an indicator of the bog network habitat quality(Jullian and Coïc 2002). In 2010, by the end of the LIFE project, they contacted the station d'écologie expérimentale du CNRS à Moulis (SEEM CNRS) to assist them in evaluating whether the population of *O. coerulescens* was viable within the site. The SEEM proposed them to test MetaConnect (Moulherat et al. submitted-a) in an applied conservation context.

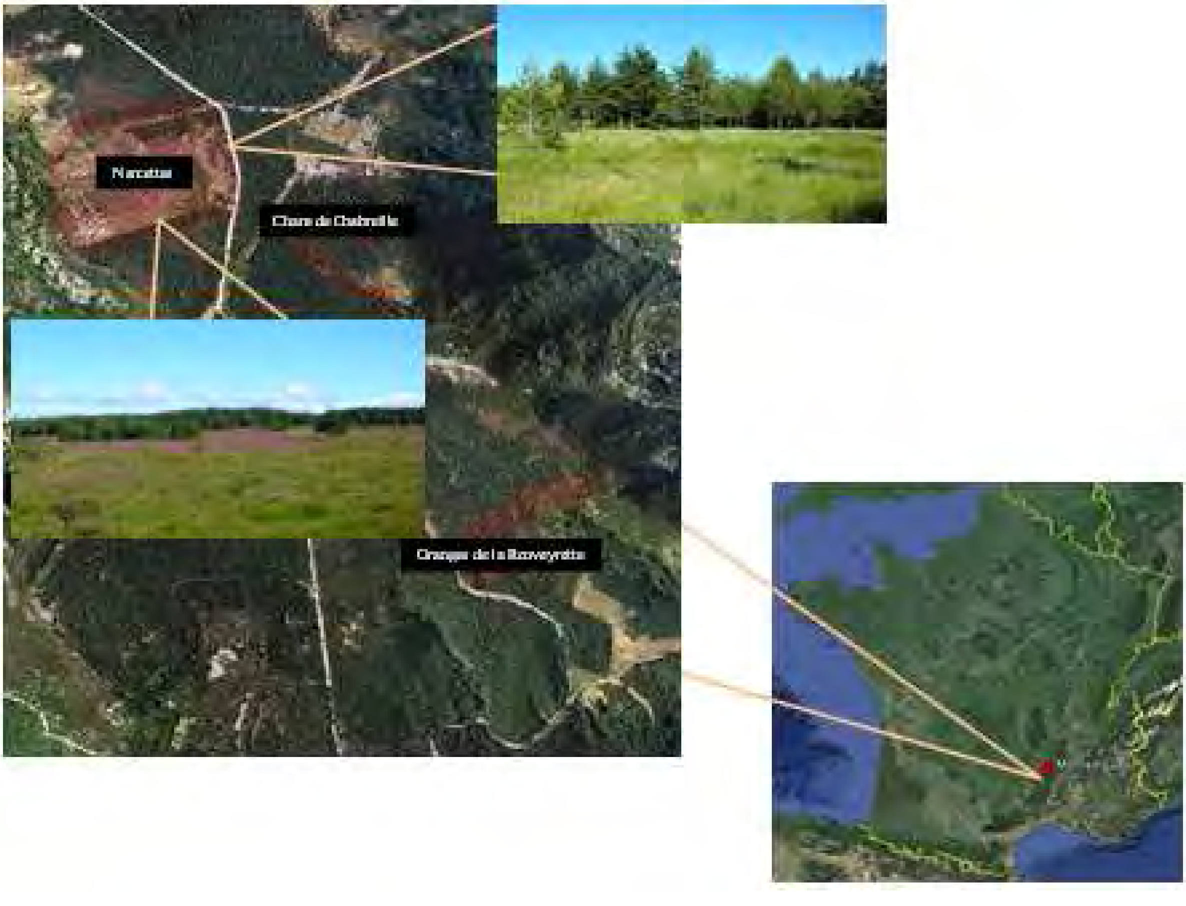

Figure 1: The bog network (red shade) of the Montselgues plateau in France (France, Ardèche, 44°30’13.63”N, 4°00’32.29”E, alt. 1032 m) is covered by heathland and bog fragmented by pine forestry.

## I. Acquiring the demographic characteristics of the local keeled skimmer population

To estimate the demographic parameters necessary to setup MetaConnect to perform a population viability analysis (PVA), we conducted a mark-release-recapture (MRR) survey(Lebreton and Pradel 2002) of *O. coerulescens*. The MRR study provided us for survival (Φ), population size ($N$) and individuals flows between bogs of the network (Ψ) within the 9 capture sessions (i.e. weeks) of the field work (table 1).

Table 1: Survival, population size and dispersal probabilities estimated using the MARK software(White and Burnham 1999) with model POPAN(Arnason et al. 1979) to estimate $N$ and multistate models(Doligez et al. 2002) to estimate Φ and Ψ for each bog of the Montselgues plateau (Narcettes: N, Cham de Chabreille: C and Granges des Rouveyrettes: R). Results are presented as mean ± SE.

| **Site** | **Φ** | ***N*** | **Ψ** | | |
|---|---|---|---|---|---|
| | | | **N** | **C** | **R** |
| **N** | $0.62 \pm 0.05$ | $434 \pm 16$ | NA | $0.02 \pm 0.01$ | $0.004 \pm 0.003$ |
| **C** | $0.62 \pm 0.05$ | $350 \pm 23$ | $0.02 \pm 0.01$ | NA | $0.007 \pm 0.004$ |
| **R** | $0.62 \pm 0.05$ | $519 \pm 19$ | $0.004 \pm 0.003$ | $0.006 \pm 0.003$ | NA |

## II. Running MetaConnect

We setup and run MetaConnect with the demographic parameters estimated above to perform the PVA analysis of keeled skimmer within the bog network of the Montselgues plateau. We repeated 100 times a 200 time steps (200 years) simulation of the metapopulation dynamic. The runs were performed on the total network (3 populations) and for each patch independently to estimate the patch dependency in regard to the species maintenance and gene flow. To perform the PVA, we checked for extinction probability and genetic structure of the population(Baguette et al. 2012b; Pe'er et al. 2013).

At the scale of the complete network, the extinction probability over the 200 time steps is null. In addition, the flow of individual prevents from a genetic structure within the bog network (figure 2).

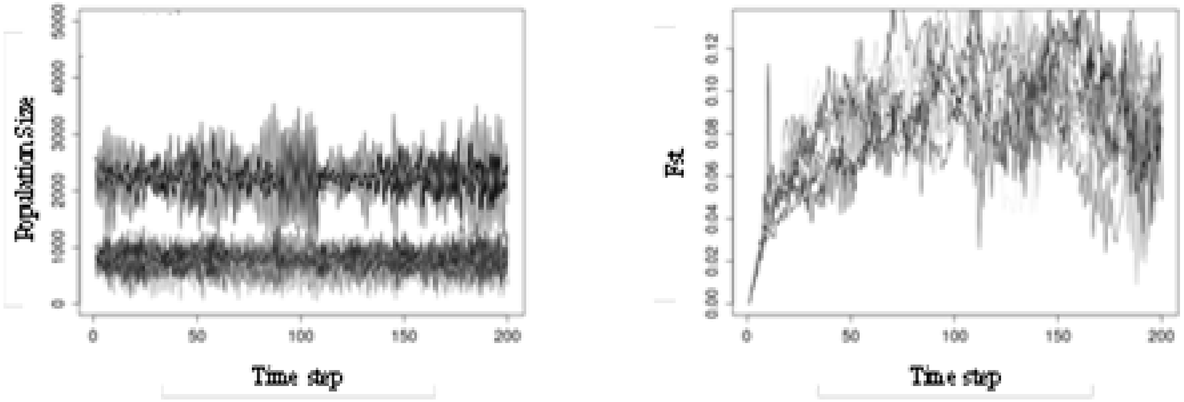

Figure 2: Examples (10 runs show) of individual population dynamic (left panel) and genetic structure (Fst, right panel) of *Orthetrum coerulescens* within the bog network of the Montselgues plateau in France simulated with MetaConnect. At the global scale (black line) and for each bog of the network separately (Narcettes: red, Chabreille: green and Rouveyrettes: blue), the keeled skimmer population is viable and stable over the 200 time steps simulations. In addition, the low asymptotic value of Fst (0.1), shows that the actual landscape would probably have the ability to structure the population genetic.

However, if the bog network is viable, simulations considering each bog independently show that if the population size is stable over the 200 time steps, the heterozygosity ($H_O$) level is crashing down in Chabreille (figure 3) when Narcettes and Rouveyrettes are not threatened by inbreeding by their own. This results suggests that the smallest bog by its area and population size, should be protected from isolation.

## III. Conclusion

MetaConnect permitted us to assist efficiently conservation managers by:

1) Answer the question whether the population of *Orthetrum coerulescens* is viable within the bog network of the Montselgues plateau.

2) Identify the site (the Cham de Chabreille) that first requires a special attention to prevent a part of the meta-population from inbreeding.

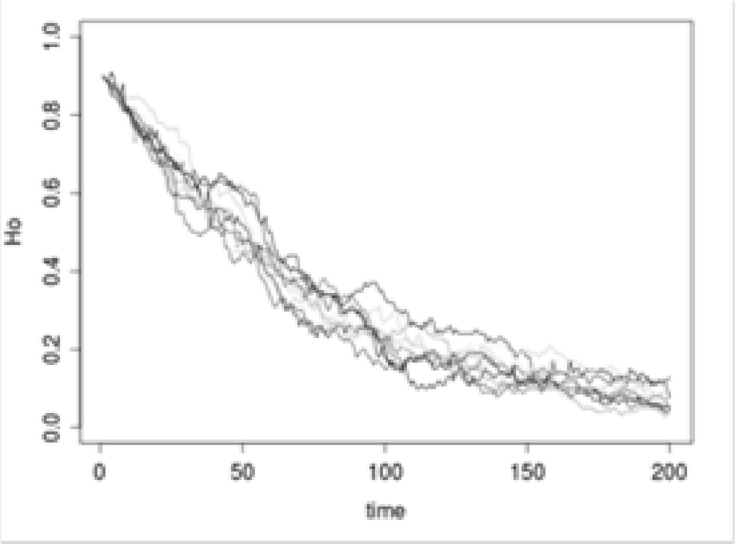

Figure 3: Simulated (10 runs show) heterozygosity in the Cham de Chabreille over 200 time steps for *Orthetrum coerulescens*. The inbreeding depression in Chabreille tends to homogenize the population genotypes.

## IV. References

1 Moulherat, S., Chabbert, R., Pascault, B., Dupieux, N. & Clobert, J. Population viability of the keeled skimmer (O. coerulescens) within the bog network of the Montselgues plateau in France. (In prep).

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# Related TerrOïko report

In this section I join an illustrative example of TerrOïko's activity and how I use the skills I developed during my PhD to assist conservation managers.

TerrOïko

Rapport Final-2013

Analyse du suivi des déplacements
d'Azurés des Mouillères sur le réseau de
tourbières du plateau de Montselgues

# SOMMAIRE

# 1 Contexte de l'étude et propositions faites par TerrOïko

Cette partie reprend les propositions techniques ainsi que les protocoles de terrain proposés par TerrOïko dans le cadre de l'appui méthodologique et l'analyse des données concernant l'effet des ruptures de continuités écologiques sur l'Azuré des mouillères au sein du réseau de tourbières du plateau de Montselgues.

## *1 / Proposition technique (06/05/2013)*

## Introduction et objet du document :

Ce document constitue la proposition technique et financière élaborée par TerrOïko, pilote de l'opération, en réponse à la consultation du Parc Naturel Régional des Monts d'Ardèche pour un appui méthodologique et analyse des données concernant l'effet des ruptures de continuités écologiques sur les populations d'azuré des mouillères sur le réseau de tourbières du plateau de Montselgues.

Notre proposition se veut synthétique et pédagogique sans pour autant négliger la rigueur qu'elle nécessite. Nous restons néanmoins disponibles pour répondre aux questions techniques, spécialement si malgré nos explications, il reste des interrogations concernant les outils et les méthodes que nous utilisons qui sont communément utilisés par les chercheurs mais malheureusement rarement accessible aux gestionnaires.

## Notre compréhension du contexte et de vos objectifs :

### *1 / Des enjeux écologiques :*

Le réseau de tourbières du plateau de Montselgues a subi une fragmentation des milieux ouverts (landes et tourbières) récente très importante (figure 1). Cette fragmentation par les massifs boisés pourrait être fortement préjudiciable à la qualité des milieux d'intérêts (zones humides et tourbières) en raison de l'assèchement des milieux par les résineux autour et au sein des tourbières et zones humides d'une part mais aussi en causant des ruptures de continuités écologiques entre les tourbières potentiellement préjudiciables à la survie des espèces vivants dans ces milieux (odonates, lépidoptères, lycopodes, drosera,...) présents dans le réseau de tourbières du plateau de Montselgues. Le CEN Rhône-Alpes et le PNR des Monts d'Ardèche ont réalisé des travaux de restauration des tourbières (restauration du fonctionnement hydraulique, ouverture de milieux,...), des travaux de restauration des connectivités (corridors écologiques) et des suivis de populations d'odonates et azuré des mouillères grâce à un financement LIFE (2005-2010).

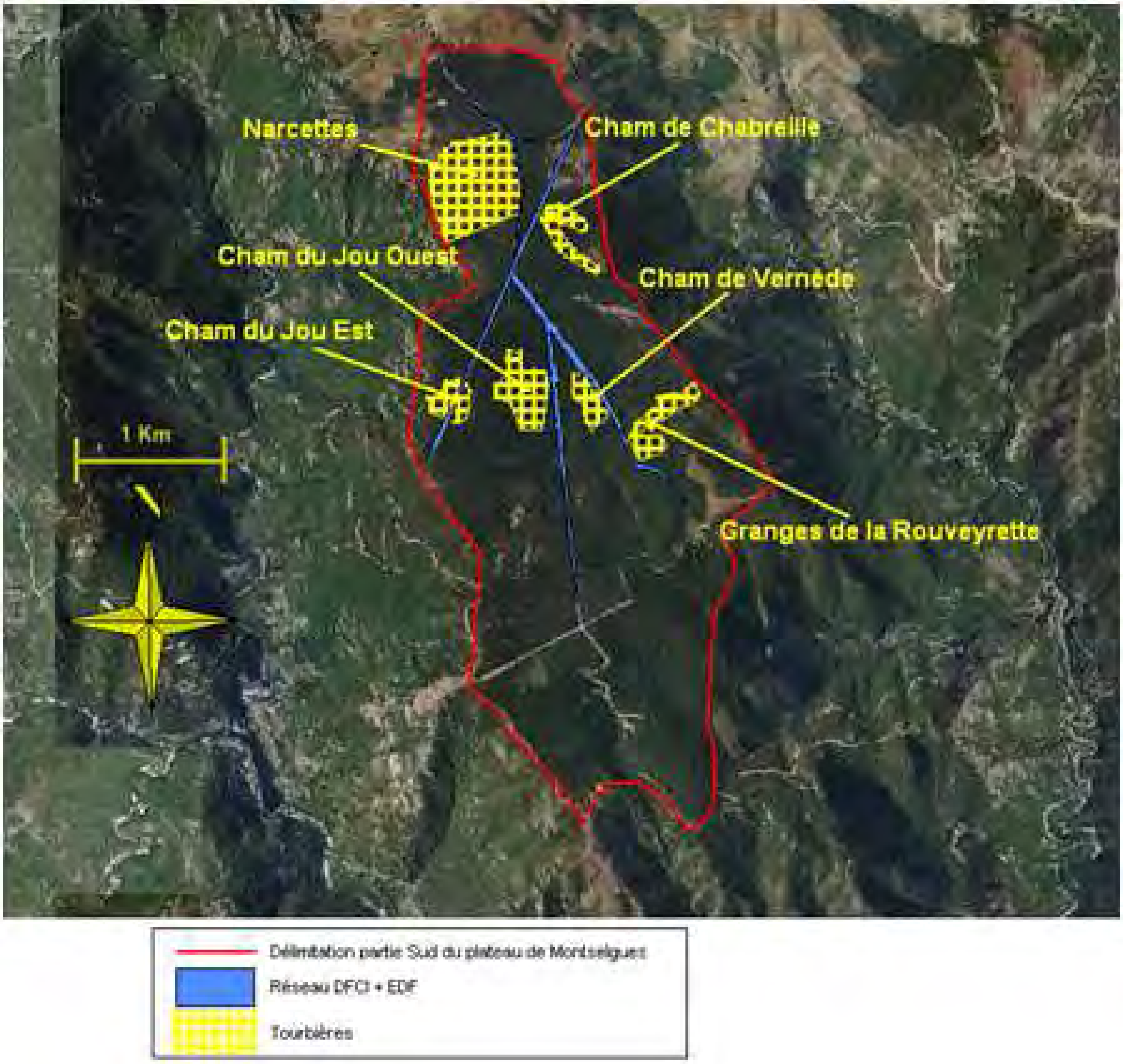


Figure 1 : photo aérienne annotée du site de l'étude.

Les précédentes études sur la fonctionnalité des corridors pour les populations de libellules réalisées en collaboration avec la station d'écologie expérimentale du CNRS à Moulis ont d'ores et déjà pu mettre en évidence que :

- les populations d'odonates ne sont pas particulièrement menacées au sein du réseau de tourbières.

- les corridors écologiques réalisés ont un effet sur les périodes de déplacement des libellules entre les tourbières (possibilité de déplacement lors des jours ventés).
- les corridors écologiques réalisés n'augmentent pas significativement le flux d'individus entre tourbières.

Bien que cette précédente basée sur les populations d'odonates ait montré que ce groupe d'espèces n'a que très peu de risque d'extinction au sein du réseau et que les flux d'individus permettent une brassage génétique acceptable, il en est probablement autrement pour des espèces a plus faibles capacités de dispersion tels que les lépidoptères.

Pour étudier le groupe des lépidoptères, il a été décidé d'utiliser l'azuré des mouillères comme espèce parapluie. Se focaliser sur une espèce parapluie a le double avantage d'améliorer les connaissances sur une espèce d'intérêt patrimonial tout en transposant, tout ou en partie, les conclusions spécifiques de l'étude à d'autres espèces, notamment de lépidoptères, inféodées au même milieu.

L'azuré des mouillères est une espèce patrimoniale bénéficiant d'un plan national d'action (PNA). Dans le cadre des plans de gestion du réseau de tourbières du plateau de Montselgues, l'azuré des mouillères bénéficie d'une attention particulière (suivi annuel réalisé par Yves Rozier, suivi des stations de gentianes pneumonanthes, décompte des œufs sur les gentianes, suivi des fourmis (2 espèces du genre *Mirmyca*) nécessaires pour l'accomplissement du cycle de vie de l'azuré des mouillères).

Afin de préserver au mieux, la population d'azuré des mouillères sur le plateau de Montselgues, il est nécessaire de :

- comprendre sa dynamique démographique
- l'impact de la structure paysagère sur les échanges d'individus entre les tourbières du réseau

Pour ce faire le parc désire connaître précisément les caractéristiques démographiques et les capacités de dispersion de l'azuré des mouillères au sein du réseau de tourbières du plateau de Montselgues. Cette connaissance permettra de réaliser une gestion optimale du site pour cette espèce d'une part et d'autre part est essentielle pour proposer des aménagements adaptés pour la construction des corridors biologiques prévus dans les prochaines années.

TerrOïko grâce à son équipe composée de docteurs en écologie et son partenariat avec la station d'écologie expérimentale du CNRS de Moulis spécialisée dans ce type de problématique est en mesure de proposer un traitement original, efficace, scientifiquement validé et transposable de cette problématiques.

## 2 / Des enjeux d'optimisation des travaux à réaliser :

Les travaux d'aménagement de corridors sont importants et coûteux. Une bonne connaissance des espèces susceptibles d'utiliser ces corridors permet de comprendre au mieux comment elles peuvent les utiliser. Cette connaissance approfondie apporte l'assurance de réaliser des travaux et aménagements réellement fonctionnels pour les espèces cibles. Il est donc nécessaire de réaliser une étude de qualité sur ces interactions afin d'optimiser fonctionnalité des aménagements et leurs coûts financiers. TerrOïko est en mesure de proposer une telle optimisation grâce à l'utilisation de son outil MetaConnect qui lui permettra de comparer la qualité et la fonctionnalité des différents scenarii d'aménagements proposés par le PNR des Monts d'Ardèche et CEN Rhône-Alpes.

# Proposition technique :

## 1 / Précision technique :

TerrOïko propose une méthodologie innovante, issue des derniers travaux du CNRS de Moulis, ainsi que l'exclusivité d'un outil d'analyse de viabilité des méta-populations, développé par ce même laboratoire, basé sur la dynamique des populations et la connectivité fonctionnelle du territoire pour définir les enjeux liés à la viabilité des populations animales et végétales.

La méthode développée par TerrOïko présente l'intérêt :

- d'**estimer la viabilité des populations** au sein du réseau de tourbières
- de **quantifier les flux de populations** entre les différentes structures paysagères.
- de **tester en prospective l'effet d'aménagements potentiels** de restauration de corridors.

Pour le compte de TerrOïko, cette étude sera réalisée par Sylvain Moulherat (doctorant TerrOïko/CNRS, voir CV joint) spécialisé dans les études de connectivité et concepteur d'outil d'aide à la décision pour les aménagements destinés à restaurer les connectivités écologiques. Il a d'autre part déjà collaboré avec le PNR des Monts d'Ardèche pour le compte du CNRS en 2011 dans le cadre de l'étude de la fonctionnalité des corridors biologiques du plateau de Montselgues pour les populations de libellules. Il avait alors participé à l'encadrement du stagiaire du parc (Romain Chabbert), à l'élaboration des protocoles de terrain et avait réalisé l'analyse des données récoltés.

## 2 / Détermination des interactions entre la structure paysagère et le cycle de vie de l'azuré des mouillères.

**Le projet se déroulera en 4 étapes** pour déboucher sur la détermination des coefficients de friction de l'azuré des mouillères dans les différents habitats du plateau de Montselgues, l'analyse complète de la viabilité de la méta-population (probabilités d'extinction locale et globale) d'azuré des mouillères, les flux d'azurés des mouillères au sein du réseau de tourbières et la détermination du scenario de restauration des connectivités entre tourbières du réseau le mieux adapté s'il s'avère que des connectivités entre tourbières sont altérées :

1. ***Détermination des coefficients de frictions/rugosité des différents habitats pour Sphengaris alcon***
2. ***Suivi par capture-marquage-recapture de la population d'azuré des mouillères du plateau de Montselgues***
3. ***Intégration des informations recueillies lors des étapes 1 et 2 à MetaConnect***

### 4. Test des différents scenarii d’aménagements possibles et détermination du scenario le plus pertinent.

### *Etape 1 : Détermination expérimentale des coefficients de friction/rugosité*

*Objectif : associer à chaque habitat les capacités de déplacement de l'azuré des mouillères*

Les coefficients de friction/rugosité seront déterminés expérimentalement suivant la méthode développée par Camille Turlure (Turlure et al. 2012) et adaptée au contexte du réseau de tourbières du plateau de Montselgues. Dans ce but, le stagiaire devra réaliser sur le terrain un suivi individuel d'azuré des mouillères. Le protocole consiste à longer une lisière d'habitat. Au cours du déplacement le long de ce transect, chaque individu observé est suivi physiquement à une distance de 10 m pendant 20 min. Un repère est posé à chaque changement de trajectoire ou arrêt de l'individu. Pour chaque repère, les coordonnés GPS et l'heure sont notés sur des fiches de terrain. Il sera aussi noté sur la fiche, le comportement du papillon face au changement d'habitat lors d'un déplacement (pénétration dans le nouvel habitat, retour en arrière,…). Le suivi s'arrête lorsque le papillon passe plus de 5 min inactif. Les suivis seront réalisés jusqu'à ce que chaque type d'habitat ait été parcouru par une vingtaine d'individus (10 mâles, 10 femelles) afin d'être en mesure de réaliser des analyses statistiques robustes.

A partir de ces données de trajectométrie, nous serons en mesure de déterminer les vitesses de déplacement de l'azuré des mouillères dans chacun des habitats du réseau de tourbières ainsi que son affinité pour ceux-ci. Ces deux informations seront intégrées pour la détermination des coefficients de friction/rugosité.

Dans un souci de qualité de sa prestation, TerrOïko sera assisté dans cette tâche par C. Turlure.

### *Etape 2 : Capture-Marquage-Recapture (CMR) d'azuré des mouillères, caractéristiques démographiques de la population d'azuré des mouillères sur le plateau de Montselgues.*

*Objectif : Estimer les caractéristiques démographiques, tailles de populations et flux de l'azuré des mouillères au sein du réseau de tourbières du plateau de Montselgues*

Les caractéristiques démographiques seront déterminées grâce à l'analyse complète des données de CMR. Afin d'optimiser le temps d'expérimentation du stagiaire sur le terrain, les campagnes de captures seront réalisées en même temps que les suivis visant à calculer les coefficients de friction/rugosité. Les individus suivi pour cette première expérience seront capturés en fin de suivi, marqué, sexé, et leurs mesures morphométriques réalisées (taille, masse).

Les sessions de CMR seront organisées en 10 sessions de 3 jours ce qui nous permettra d'optimiser au mieux l'adéquation entre l'effort de prospection et le cycle de vie de l'espèce (survie de l'ordre de 3-4 jours). L'effort de capture sera équitablement réparti par demi-journée en fonction de la superficie des différentes tourbières (1 demi-journée par tourbière sauf pour les Narcettes qui devront être prospectées pendant 2 demi-journées par session). Pour chaque session de 3 jours, l'ordre de prospection des tourbières sera choisi aléatoirement.

A la fin du suivi de CMR, les histoires de captures seront analysées à l'aide du logiciel MARK en utilisant les modèles POPAN pour obtenir les tailles de populations d'azurés des mouillères des différentes tourbières, et des modèles multi-états afin d'analyser les flux d'individus existants. D'autre part, ces analyses nous fourniront les caractéristiques démographiques de l'azuré spécifiques au plateau de Montselgues. Ces analyses couplées aux résultats de l'étape 1 devraient nous permettre d'estimer les tailles de populations d'azuré des mouillères des années précédentes (Turlure et al. 2010) et déterminer les tendances populationnelles à moyen terme.

En parallèle au suivi d'azuré, le stagiaire devra relever les coordonnés GPS des stations de gentianes pneumonanthes (*Gentiana pneumonanthe*) et le cas échéant, la présence d'œufs d'azurés des mouillères et éventuellement le nombre d'œufs.

### *Etape 3 : Intégration des résultats des étapes 1 et 2 à l'utilisation de MetaConnect*

*Objectif : Réaliser les études de viabilité de la population d'azuré des mouillères*

Les résultats de l'analyse de la CMR de la population d'azuré des mouillères fournira les informations locales des caractéristiques démographiques de l'espèce (taille de population, survie adultes, flux d'individus,...) dans le réseau de tourbières. Au cas où les populations d'azuré sur le site seraient trop petites pour permettre une analyse robuste de la CMR, les caractéristiques démographiques seront extraites de la littérature scientifique existante sur le sujet. Ces données démographiques seront utilisées dans MetaConnect pour simuler la dynamique de la population d'azuré des mouillères sur le plateau de Montselgues.

Les coefficients de friction/rugosité une fois déterminés seront cartographiés à l'aide de la cartographie précise des habitats fournie par le CEN Rhône-Alpes et utilisés comme données d'entrée de MetaConnect. Ces données nous serviront lors de la simulation de la dispersion de l'azuré des mouillères au sein du réseau de tourbières. De même, les stations de gentianes pneumonanthe seront utilisées comme données d'entrée de MetaConnect.

Les simulations réalisées avec MetaConnect fourniront les probabilités d'extinction de l'azuré des mouillères sur chaque tourbière ainsi que pour l'ensemble du réseau étudié. Ces simulations fourniront aussi le potentiel de structuration génétique du paysage actuel. Ces résultats fourniront donc à la fois l'information quant à la viabilité de la population d'azuré des mouillères au sein du réseau de tourbière du plateau de Montselgues et déterminer les zones nécessitant une amélioration de la qualité du milieu pour optimiser la survie de l'espèce, mais aussi la dynamique des flux d'individus au sein du réseau et par là même permettra d'identifier les besoins et localisations de reconnexion des tourbières.

### *Etape 4 : Utilisation des simulations MetaConnect afin de tester puis optimiser les travaux à mener.*

*Objectif : Choisir le meilleur scenario d'aménagement parmi les scenarii réalisables*

Les cartographies d'habitat seront modifiées en fonction des différents scenarii de travaux envisagés par le CEN Rhône-Alpes concernant l'amélioration de la qualité de l'habitat pour l'azuré des mouillères ou l'amélioration des connectivités. De nouvelles simulations seront alors réalisées, à nouveau les probabilités d'extinctions et la structuration génétique seront de nouveau évaluées permettant ainsi au gestionnaire de choisir le scenario le plus adapté en connaissance de leurs impacts probables sur la dynamique de la population, le flux d'individus et la structuration génétique de la population.

## 3 / Déroulement du projet

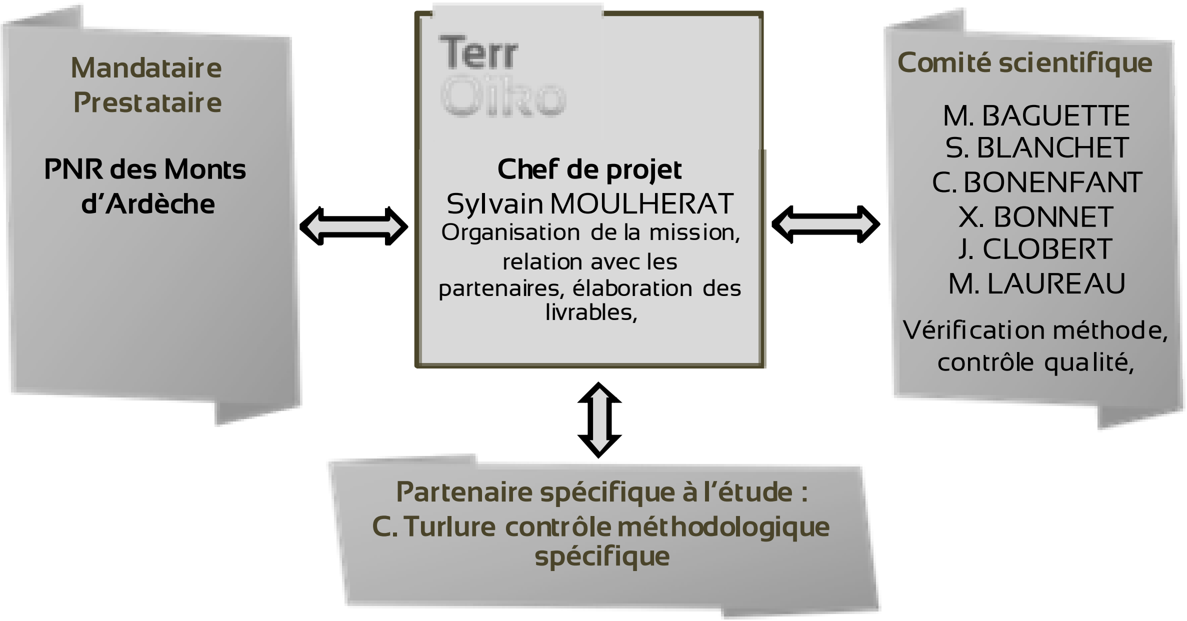
| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Organisation de la mission | | | | | | |
| **Etape 1 :** appui méthodologique | | | | | | |
| ***Détermination expérimentale des coefficients de friction/rugosité pour* Sphengaris alcon** | | | | | | |
| Définition des protocoles | ░ | ░ | | | | |
| Formation du stagiaire aux protocoles | ░ | ░ | | | | |
| Réalisation des expériences | | █ | █ | █ | | |
| **Suivi CMR** | | | | | | |
| Définition du protocole | ░ | ░ | | | | |
| Formation du stagiaire aux méthodes d'analyse de la CMR | ░ | ░ | | | | |
| Réalisation des sessions de captures | | █ | █ | █ | | |
| **Etape 2 :** analyse des données | | | | | | |
| ***Détermination expérimentale des coefficients de friction/rugosité pour* Sphengaris alcon** | | | | | | |
| Calcul des coefficients de friction/rugosité | | | | ░ | ░ | |
| Spatialisation des coefficients de friction/rugosité | | | | █ | █ | |
| ***Suivi CMR*** | | | | | | |
| Estimation des paramètres démographiques | | | | ░ | | |
| Spatialisation des données démographiques et des stations de gentianes pneumonanthes | | | | █ | █ | |
| **Etape 3 :** Etude de viabilité et flux d'individus | | | | | | |
| Intégration des données récoltées en étapes 1 et 2 | | | | ░ | ░ | |
| Etude de viabilité de *Sphengaris alcon* au sein du réseau de tourbière | | | | ░ | ░ | ░ |
| Etude des connectivités pour l*Sphengaris alcon* au sein du réseau de tourbières | | | | ░ | ░ | ░ |
| **Etape 4 :** Test des scenarii d'aménagements | | | | | | |
| Etude prospective des connectivités | | | | | ░ | ░ |

|  | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| **Réunions, rédaction de rapport** |  |  |  |  |  |  |
| Réunion de lancement du projet | ■ |  |  |  |  |  |
| Réunion de restitution des premiers résultats et définition des scenarii d'aménagements à tester |  |  |  | ■ | ■ |  |
| Encadrement du stagiaire pour la rédaction de son rapport | ░ | ░ | ░ | ░ |  |  |
| Rédaction du rapport d'étude |  |  |  |  | ░ | ░ |

Les cases vertes claires correspondent aux activités de TerrOïko, les cases vertes foncées correspondent aux activités du stagiaire PNR des Monts d'Ardèche pour lesquelles TerrOïko supervisera l'aspect technique, méthodologique. Les cases jaunes correspondent aux activités de TerrOïko qui impliqueront l'ensemble des partenaires concernés par l'étude (PNR des Monts d'Ardèche, CEN Rhône-Alpes,...).

## *Dispositions d'organisation :*

### *- Organisation du projet :*



**<u>Composition du Comité Scientifique :</u>**

M. BAGUETTE ->Directeur de recherche MNHN, dispersion des organismes, viabilité des populations
S. BLANCHET -> Chargé de recherche CNRS Moulis, peuplements piscicoles, trame bleue, épidémiologie,
C. BONENFANT -> Chargé de recherche CNRS Lyon, biologie de la conservation, mammologie
X. BONNET -> Directeur de recherche CNRS Chizé, herpétologie
J. CLOBERT -> Directeur de recherche, CNRS Moulis, dispersion des organismes, herpétologie, ornithologie
M. LOREAU -> Directeur de recherche CNRS Moulis, fonctionnalité des écosystèmes

**Partenaire spécifique :**

C. Turlure -> Post-doctorante FNRS Liège, dispersion, papillons, détermination expérimentale de coefficients de friction

## *2 / Protocoles de terrain (27/06/2013, mis à jour le 11/07/2013)*

# Objectifs expérimentaux

## *1 / Détermination des coefficients de friction pour* Phengaris alcon *dans le réseau de tourbière du plateau de Montselgues.*

L'objectif de cette expérience est de mesurer expérimentalement les coefficients de frictions associés aux différents types de couvert végétal présent dans le réseau de tourbières du plateau de Montselgues. Dans la plupart des études réalisées utilisant des coefficients de friction ou coefficients de perméabilité ou encore coefficients de rugosités, ces coefficients sont évalués sur dire d'experts (Turlure et al. 2011). Pour certains groupes d'espèces, des méthodes expérimentales ont été développées afin de mesurer ces coefficients (Stevens and Baguette 2008; Turlure et al. 2011). C'est notamment le cas en ce qui concerne les papillons (Turlure et al. 2011). Concernant ce groupe, la comparaison entre les estimations réalisées par des experts et les mesures réalisées expérimentalement a permis de mettre en évidence une très faible capacité des experts à estimer la valeur (même relative) de ces coefficients (Turlure et al. 2011; Baguette et al. 2012b). Or il se trouve que ces coefficients sont à la base des études de connectivité (i.e. chemin de moindre coût) (Belmont et al. 2010). Il est donc nécessaire, lorsque l'espèce étudiée le permet, de mesurer expérimentalement ces coefficients afin de réaliser des études pertinentes de la connectivité sur papillon. C'est pourquoi dans le cadre de cette étude sur *Phengaris alcon*

(*Maculinea alcon*), nous avons décidé de mettre en place le protocole de mesure des coefficients de friction développé par Camille Turlure (Turlure et al. 2011) consistant à suivre individuellement les papillons (en excluant la méthodologie qu'elle propose basée sur des transects n'étant pas adaptés aux relativement faibles densités de papillons attendues à Montselgues).

Les coefficients de friction ainsi calculés serviront à paramétrer dans un second temps MetaConnect (Baguette et al. 2012c; Gunton et al. 2013) afin de réaliser étude de viabilité, étude des connectivités et évaluation des propositions d'aménagements sur site visant à protéger la population de *Phengaris alcon* du plateau de Montselgues.

## *2 / Estimation des paramètres démographiques pour* Phengaris alcon *dans le réseau de tourbière du plateau de Montselgues.*

L'objectif de cette expérience est de déterminer :

1. La taille de la population de *Phengaris alcon* sur les tourbières étudiées.
2. Leurs caractéristiques démographiques (survie adulte, détectabilité, recrutement).
3. Mesurer les flux d'individus (entre tourbières et individus venant de l'extérieur du système étudié).

Les paramètres démographiques seront estimés grâce à l'analyse des résultats du suivi de la campagne de Capture Marquage Recapture prévue en parallèle de l'expérience visant à mesurer les coefficients de friction pour *Phengaris alcon*. Ces résultats seront analysés à l'aide de Mark et des modèles POPAN (permettant d'estimer les tailles de populations) (Arnason and Schwarz 2002) et Multi-strate (permettant de quantifier les flux d'individus) (Kendall and Nichols 2002). Les flux d'individus venant de l'extérieur du système et le recrutement seront dérivés de l'analyse conjointe des deux types de modèles.

Les estimations des paramètres démographiques serviront quant à eux à réaliser l'étude de la viabilité de la population de *Phengaris alcon* sur le plateau de Montselgues.

A ce niveau de l'expérience, l'estimation des paramètres démographiques sera étroitement liée à la quantité d'individus capturés et marqués. Une attention particulière devra donc être faite quant à l'organisation des sessions de capture qui devront être optimisées dans l'objectif de maximiser le taux de recapture.

Malheureusement, pour des raisons administratives et règlementaires (autorisation de capture pour *Phengaris alcon* non délivrées dans les temps) les individus ne pourront pas être marqués et la CMR ne pourra pas être réalisée. Deux méthodes alternatives ont été étudiées pour parer à cette difficulté :

- Le *Distance sampling* n'est pas une méthode qui permettra d'obtenir des informations fines sur la démographie de *Phengaris alcon* sur le plateau de Montselgues.
- L'occupation de site, ne donnera pas d'information concernant la démographie de l'espèce et les estimations de probabilité d'extinction par site ne seraient pertinentes dans le cadre de notre étude que si elles étaient réalisées sur plusieurs années avec le même protocole.

Il semble donc que les données démographiques que nous utiliserons par la suite seront extraites de la littérature scientifique qui contient de nombreuses informations précises et de bonne qualité sur la démographie de cette espèce.

# Protocoles détaillés

## *1 / Préparation expérimentale : Cartographie du site*

Le CEN RA dispose d'une cartographie de la végétation du site assez détaillée. L'objectif de ce travail de préparation est d'affiner la cartographie du site afin de déterminer les limites des différentes zones (types de milieux) à traiter (voir section 2.2), de localiser les secteurs à gentianes pneumonanthes (*Gentiana pneumonanthes*) et produire les supports cartographiques qui serviront à réaliser l'analyse de viabilité et des flux de *Phengaris alcon* au sein du réseau de tourbières du plateau de Montselgues. L'opérateur devra, avant le début des expériences, réaliser la mise à jour cartographique des données CEN RA et relevé au GPS les stations de gentiane (1 point au centre de la station). Pour chaque station, il devra relever le nombre de pieds de gentianes et la surface de la station (coordonnées des coins de la station puis calcul de surface sous logiciel SIG).

Les zones forestières seront caractérisées par la densité en arbres 2-5 quadrats (suivant la surface de la zone forestière) de 10m X 10m seront réalisés. Dans chacun de ces quadrats, le nombre d'arbres, leur essence et leur circonférence seront relevés.

Par la suite, au cours des expériences, les nouvelles stations de gentianes devront être relevées et l'ensemble des stations devront être prospectées afin de compter le nombre d'œufs moyens par station de gentiane (comptage des œufs sur 1-10 pied(s) de gentiane suivant la taille de la station).

## 2 / Coefficients de frictions

Les coefficients de frictions seront déterminés à partir de 2 mesures distinctes (Stevens et al. 2004; Stevens et al. 2006a; Stevens et al. 2006b; Baguette and Van Dyck 2007; Baguette et al. 2012b):

- Résistance : correspond à la capacité qu'a un individu de traverser le milieu (~performances locomotrices)
- Préférence : correspond à la réaction des individus face à un nouveau milieu (entre dans le nouveau milieu ou rebrousse chemin)

Le protocole mis au point par Camille Turlure (2011) concernant les papillons consiste à suivre individuellement les individus et prendre les coordonnés GPS de chacune des bifurcations de trajectoires.

Protocole détaillé par étapes :

- Prospection à l'interface entre 2 milieux (Tableau 1)
- Repérage d'un *Phengaris alcon* proche de la lisière, note de son comportement (franchissement : o/n) et prise du 1$^{er}$ point GPS (fiche terrain + si possible mémoire GPS).
- Suivi de l'individu à distance raisonnable pour ne pas perturber son comportement ni le perdre de vue (10-15 m). A chaque changement de direction ou arrêt, relevé des coordonnées GPS (possibilité de marqué le point à l'aide d'un repère au cas où difficulté de suivi ou problème d'enregistrement mais plutôt déconseillé pour le suivi complet).
- Arrêt du suivi lorsque le papillon est considéré inactif (7-10 min immobile).

- Le nombre d'individus suivi sera dépendant du nombre de cas et des besoins statistiques de l'analyse (si possible ~20 individus par type d'interface).

La description fine de l'habitat sera simplifiée et les différents types de couvert végétal seront assignés à 4 catégories de milieux (figure 1) comme suggéré par Camille Turlure (com. Pers.). Cette simplification de la description se fera en lien avec les caractéristiques physiques du milieu (hauteur de la strate végétale et présence de ressources (Turlure et al. 2011)). Pour les fins de cette étude une catégorie corridor sera ajoutée correspondant aux pistes, corridors déjà réalisés et la ligne à haute tension. D'autre part, 2 catégories de forêts seront distinguées (dense ou non dense). La distinction sera effectuée grâce aux indices de densité calculés grâce aux quadrats forestiers (section 2.1). La répartition des différents milieux utilisés par le CEN RA au sein des classes définies pour cette étude sera réalisé par le stagiaire avant le 10 juillet et soumis à l'expertise de M. Rozier.

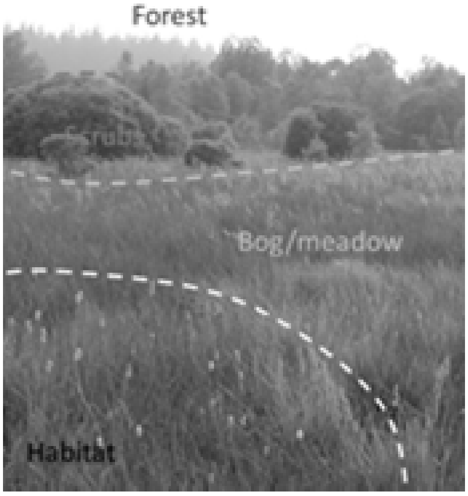


Figure 2 : Classes d'habitats utilisés (d'après Turlure et al. 2011). La classe habitat correspond aux milieux dans lesquels on trouve de la ressource (*Gentiana pneumonanthe*). La classe bog/meadow (tourbière/prairie) correspond aux milieux similaires physiquement à la classe habitat mais sans gentiane. La classe scrubs (buissons) correspond aux zones de buisson (landes à genêts par exemple). La classe forest (forêts) sera composée des différents types de forêts présents sur la zone d'étude.

Tableau 1 : Couverts végétaux présents dans le réseau de tourbières de Montselgues.

- Mare (eau peu minéralisé)

- Prairie paratourbeuse à joncs
- Prairie humide à molinie
- Prairie à molinie boisée par le pin sylvestre
- Bois de bouleaux humides
- Tourbière haute active
- Dépression à rhynchospore blanc
- Lande à callune et genêts
- Lande à genêt à balais
- Lande à genêt purgatif
- Lande à genêt à balais et genêt purgatif
- Lande à fougère aigle
- Lande à callune et genêt boisée en pin sylvestre
- Chemins, pistes, zones rudérales
- Lande à genêt purgatif sous forêt mixte
- Lande à genêt purgatif boisée en pin sylvestre
- Lande à fougère aigle sous forêt de frênes et d'aulnes
- Pelouse à Nard et fétuques
- Hêtraie sèche
- Reboisement de pin sylvestre
- Forêt mixte
- Forêt de frênes et d'aulnes
- Bois de bouleaux humide mélangé à la hêtraie sèche
- Plantation de conifères
- Eboulis

## 3 / Capture Marquage Recapture (CMR)

Les sessions de capture seront réalisées sur des cycles de 3 jours (10 sessions) afin d'optimiser le taux de recapture compte tenu de l'espérance de vie courte de cette espèce mesurée dans la littérature (Dupont 2010).

L'effort de capture sera équitablement réparti par demi-journée en fonction de la superficie des différentes tourbières (1 demi-journée par tourbière sauf pour les Narcettes qui devront être prospectées pendant 2 demi-journées par session). Pour chaque session de 3 jours, l'ordre de prospection des tourbières sera choisi aléatoirement (Tableau 2).

Tableau 2 : planning par ½ journée des sites à échantillonner (N : Narcettes, J : Cham du Jou, R : Grange des Rouveyrettes, V : Cham de Vernède, C : Cham de Chabreille)

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| J | N1 | R | V | N2 | C | J | N1 | R | V |

| 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|
| N2 | C | J | N1 | R | V | N2 | C | J | N1 |

| 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|---|---|---|
| R | V | N2 | C | J | N1 | R | V | N2 | C |

| 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 |
|---|---|---|---|---|---|---|---|---|---|
| J | N1 | R | V | N2 | C | J | N1 | R | V |

| 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 |
|---|---|---|---|---|---|---|---|---|---|
| N2 | C | J | N1 | R | V | N2 | C | J | N1 |

| 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 |
|---|---|---|---|---|---|---|---|---|---|
| R | V | N2 | C | J | N1 | R | V | N2 | C |

Les captures avec marquage seront réalisées en fin d'expérience de suivi individuel. Les recaptures intervenant aussi en fin de suivi individuel, seront réalisées sans capture physique de l'individu si possible (lecture du code à distance avec jumelles si nécessaire).

# Résultats escomptés

## 1 / Coefficients de frictions

Les valeurs mesurées sur le terrain pour préférence et résistance des milieux seront analysées suivant la méthode statistique adéquate (Stevens et al. 2006a; Turlure et al. 2011) et ces deux informations seront intégrées pour attribuer le coefficient de friction à chacun des milieux étudiés.

Ces résultats seront ensuite cartographiés et serviront de base aux analyses réalisées avec MetaConnect.

## 2 / Paramètres démographiques

Suite à l'analyse des résultats faite à l'aide de Mark, les paramètres démographiques (survie, flux, taille de population, recrutement,...) seront utilisés pour configurer les traits d'histoire de vie de *Phengaris alcon* dans MetaConnect ce qui permettra d'obtenir les analyses de viabilité et de connectivité spécifique des populations de *Phengaris alcon* du plateau de Montselgues. Cette étape est primordiale dans la mesure où les papillons en général présentent des adaptations locales très marquées (Turlure, Baguette Com. Pers.).

Dans la mesure où l'étude CMR ne sera pas réalisable, nous utiliserons les données disponibles dans la littérature scientifique de la même façon.

## 3 / Bilan de l'activité de TerrOïko pour le projet.

Comme prévu dans la proposition technique, TerrOïko par l'intermédiaire de Sylvain Moulherat a réalisé les protocoles expérimentaux (fournis le 27/06/2013), participé à l'encadrement du stagiaire (formation théorique (1j) le 22/04/2013, formation terrain pour application et ajustement des protocoles le 03/07/2013). La formation statistique du stagiaire n'a pu être réalisée dans la mesure où le stagiaire n'a pas pris contact avec TerrOïko comme il était convenu (réunion du 22/04/2013) avant la fin de son stage dans ce but. TerrOïko a participé à toutes les réunions de pilotage du projet (S. Moulherat) prévues dans le planning initial plus une réunion de mi-parcours afin de participer aux ajustements nécessaires. Enfin, TerrOïko a analysé (J. Cornuau et S. Moulherat) et extrait les informations prévues à partir des données fournies par le PNR MA *via* David Viratel (stagiaire). A l'heure actuelle les deux dernières parties de la proposition technique (Etude de viabilité et test des scenarii d'aménagements) restent à réaliser car TerrOïko n'a pas reçu les données permettant de réaliser ces analyses.

# 2 Bilan du stage de David Viratel (M2 Paris VII)

Le stage de David Viratel devait permettre l'acquisition des données permettant une analyse fine des interactions entre *Phengaris alcon* et les différents milieux constitutifs de son habitat lors de ses déplacements (coefficients de friction) ainsi que les données permettant l'analyse de la viabilité de la population d'azurés des mouillères ainsi que des flux d'individus au sein du réseau de tourbières du plateau de Montselgues.

## *1 / Données récoltées*

Lors de ce stage, les données suivantes ont été récoltées :

- Mise à jour de la cartographie des habitats (initialement réalisée par le CEN RA).
- Localisation GPS des stations de gentianes et dénombrement du nombre de pied par station.
- 36 trajectoires d'azuré des mouillères récoltées suivant le protocole décrit.
- Classification des habitats par leur structuration verticale (supervision Yves Rozier)

# 2 / Données manquantes

Par rapport au protocole fourni, plusieurs données n'ont pas été récoltées sur le terrain entrainant l'impossibilité pour TerrOïko de traiter certaines des questions soulevées initialement par l'étude. D'autre part, certaines données ont été sous échantillonnées réduisant ultérieurement la puissance des analyses statistiques.

Les données manquantes sont :

- Les données de capture marquage recapture (CMR) en raison du non renouvellement des autorisations de capture par le PNR MA.
- Présence/absence et dénombrement des pontes d'azurés des mouillères sur les gentianes pneumonanthes.
- Densité d'arbres dans les habitats forestiers
- Informations météorologiques journalières

Les données sous échantillonnées sont :

- Trajectoires d'azurés des mouillères (14 jours réalisés sur 30 initialement prévus dû aux mauvaises conditions météorologiques ayant affecté la phénologie de la majorité des espèces de papillons français ; retard d'émergence d'environ 15j, Baguette com. pers.).

## 3 / *Récapitulatif des données disponibles*

Tableau 2 : Récapitulatif des données disponibles et des noms de dossiers dans lesquels elles se trouvent

| Données | Récoltée | Non récoltée | Sous-échantillonnée | Nom du dossier |
|---|---|---|---|---|
| Habitat | X | | | Trajectoire |
| Gentianes | X | | | Gentiane |
| Densités arbres | | X | | ∅ |
| CMR | | X | | ∅ |
| Trajectoire | | | X | Trajectoire |
| Pontes | | X | | ∅ |
| Météorologie | | X | | ∅ |

Etat des données à récoltées d'après le protocole fournis et état des données reçues par TerrOïko. Les localisations correspondent aux noms de dossier dans lequel se trouvent les données généralement sous format de table attributaire des données cartographiées.

# 3

# Analyse des données récoltées

## 1 / *Analyse descriptive*

### A / Données spatialisée : lien entre position des gentianes et trajectoire des azurés des mouillères

Les répartitions de gentianes (figure 5, p.23) et les changements de trajectoire des papillons lors de leurs déplacements ont été analysées comme étant des processus ponctuels poissoniens (figure 3). Les positions des gentianes et des changements de trajectoires des papillons ont été analysés seuls. Puis ont été mis en relation. Les *Phengaris alcon* n'ont été détectés pendant la période de terrain que sur 3 des 5 tourbières (Narcettes, Rouveyrettes et Vernèdes) (figure 3) et essentiellement dans les habitats défini par Yves Rozier comme étant Habitat (classe 1 : molinaie avec présence de *Gentiana pneumonanthes*), zones de nourrissage (classe 2 :

landes à calunes ou bruyères) et en forêt dense (N=2, classe 4 : sylviculture) (figure 4). Aucune trajectoire n'a été relevé dans les autres milieux (classe 3 : lande à genet à balais, classe 4 : forêt peu dense (hêtraies), classe 6 : corridors, piste, LHT).

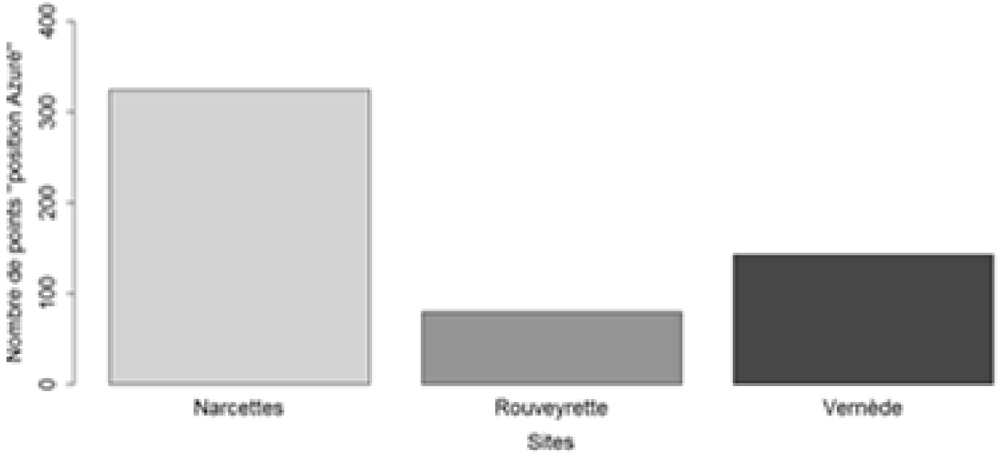


Figure 3 : Nombre de changements de trajectoires enregistrés par site du réseau de tourbières de Montselgues.

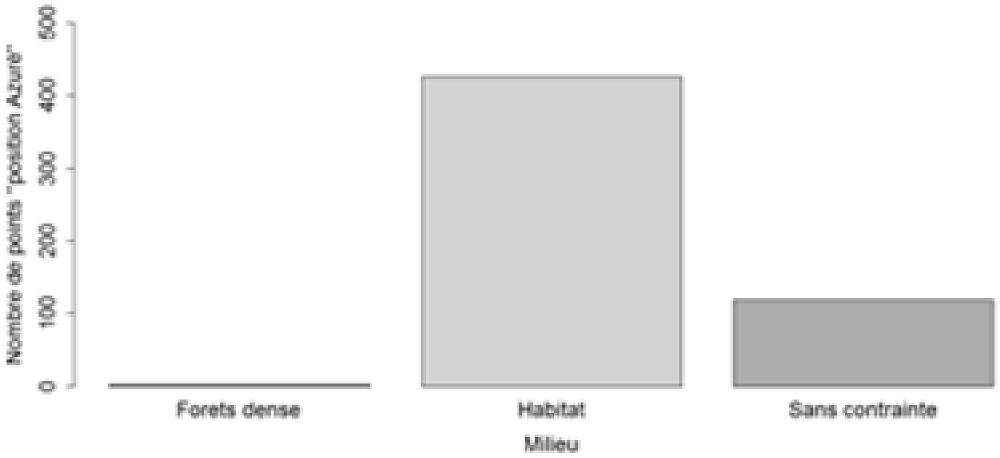


Figure 4 : Nombre de changements de trajectoires enregistrés par classe de milieux (dénomination utilisée dans le rapport de stage de David Viratel). Forêt dense=classe 5, Habitat Milieu=classe 1 et sans contrainte=classe 2 (voir texte pour plus de détails). La dénomination « sans contrainte » ne sera plus employée par la suite pour éviter toute ambiguïté car elle présente une description subjective de ce milieu et les termes utilisés correspondent au sujet de l'étude.

<u>Figure 5 :</u> Répartition de la densité des gentianes pneumonanthes au sein du réseau de tourbières du plateau de Montselgues (extrait du rapport de stage de David Viratel).

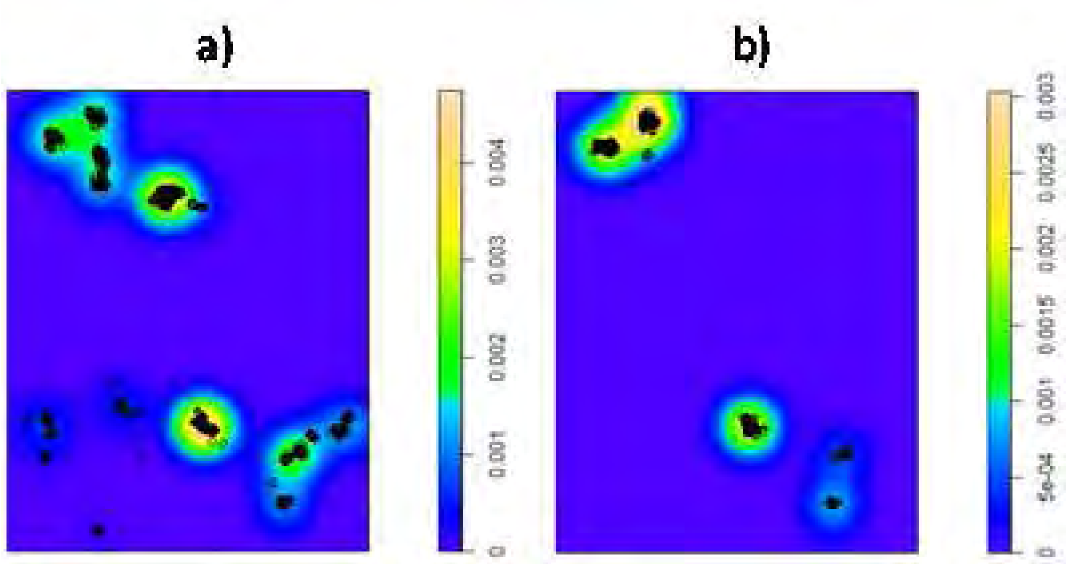


Figure 6 : Zones de présence des gentianes pneumonanthes (a) et des zones patrouillées par l'azurée des mouillères (b).

Comme escompté, les zones de présence des gentianes pneumonanthes et les zones patrouillées des azurés des mouillères (figure 6) présentent des distributions agrégées (Encadré 1, figure 7 et 8) à l'échelle du patch (des tourbières < 300m).

### *Encadré 1 : Fonction F, G, J et K pour l'analyse des données spatialisées*

*Les fonctions F, G et J informe de la* ***structure spatiale des données à petite échelle*** *(dans ce cas jusqu'à 100 m) par rapport à une distribution aléatoire (poissonienne).*

*La fonction K de Ripley, permet d'identifier* ***les structurations spatiales à grande échelle*** *(dans ce cas jusqu'à 400m) par rapport à une distribution aléatoire (poissonienne).*

*Les résultats obtenus à l'aide de ces outils statistiques se lisent de la même façon qu'il s'agisse d'un jeu de données simple (papillons seuls) ou une mise en relation entre deux jeux de données (papillons par rapport à gentianes). Seule l'interprétation change.*

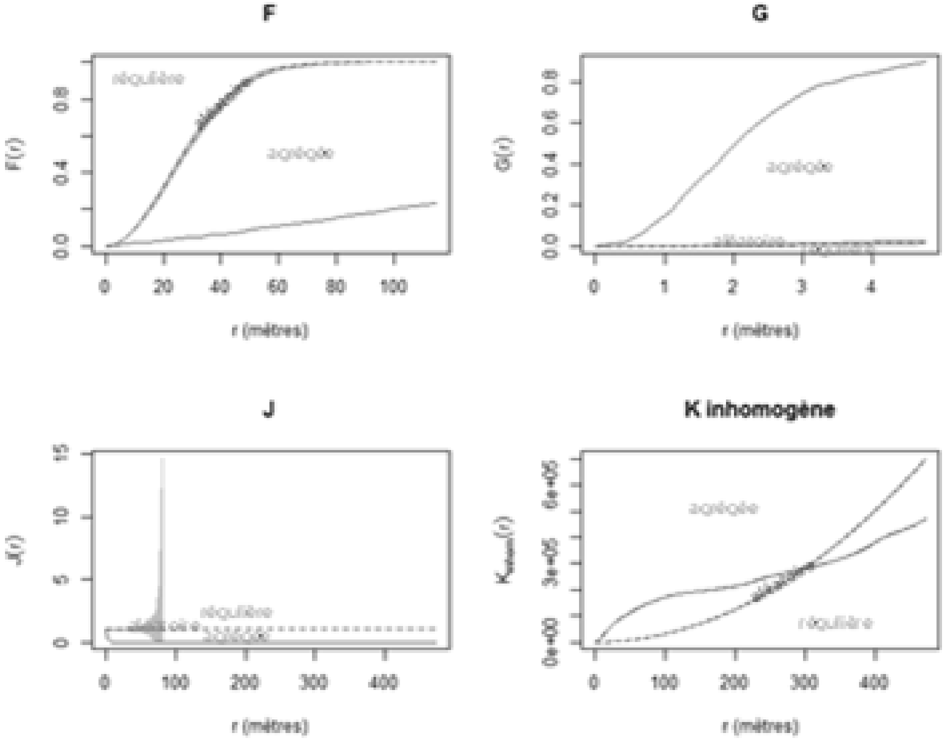


Figure 7 : Etude de la structuration spatiale des gentianes pneumonanthes.

La courbe verte en ligne continue représente la distribution observée. Elle se situe dans la zone du graphique correspondant à une distribution agrégée. La structuration spatiale des gentianes pneumonanthes est clairement agrégée entre 0 et 300m.

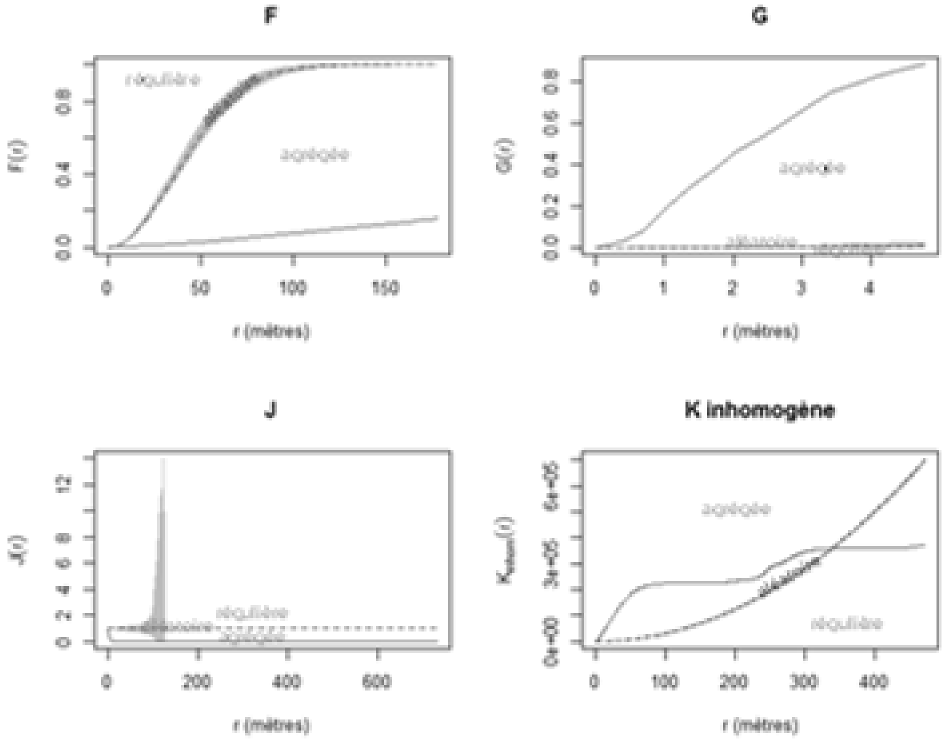


Figure 8 : Etude de la structuration spatiale des trajectoires d'azuré des mouillières.

La courbe verte en ligne continue représente la distribution observée. Elle se situe dans la zone du graphique correspondant à une distribution agrégée. La structuration spatiale des trajectoires d'azurés des mouillères est clairement agrégée entre 0 et 300m.

La mise en relation (figure 9) entre les positions de gentianes pneumonanthes et les trajectoires de l'azuré des mouillères montrent que, comme escompté, l'azuré ne se trouve que dans des secteurs où la gentiane est présente (association) mais que les trajectoires des azurés ne sont pas déterminées par la présence des gentianes (indépendance).

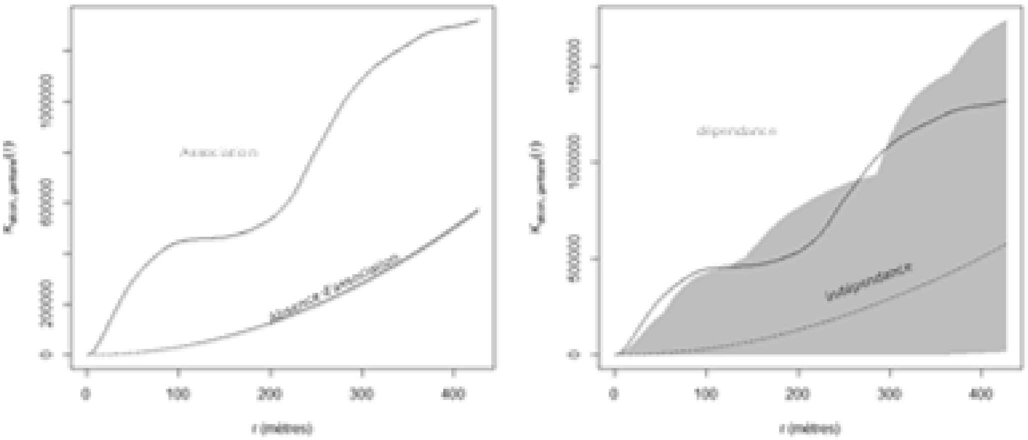


Figure 9 : Relation spatiale entre la gentiane pneumonanthe et l'azurée des mouillères. Cette relation spatiale est représentée par la courbe grise. Les azurés des mouillères sont situés dans des secteurs présentant de la gentiane pneumonanthe (graphique de gauche), cependant les trajectoires des azurés sont indépendantes de la présence de gentiane (graphique de droite).

## B / Analyse des trajectoires : kernels de déplacements journaliers

L'analyse des types des trajectoires (trajectoires quotidiennes) réalisées par les azurés des mouillères informe sur les distances que peuvent parcourir les azurés lors d'un déplacement. L'histogramme de la distance euclidienne réalisée, informe sur la distance couverte lors de trajets alimentaires journaliers (figure 10). La distance totale cumulée informe sur les capacités de vol de l'espèce (figure 11). Enfin la distance entre chaque changement de trajectoire indique comment l'azuré des mouillères se déplace (figure 12). Les informations de distances sont résumées dans le tableau 3 pour chaque *Phengaris alcon* suivi. Le déplacement des azurés semble affecté par la structure verticale du milieu qu'il traverse (tableau 4). Les distances parcourues entre 2 arrêts se modifient en fonction de la nature du milieu (augmentation des distances parcourues entre 2 arrêts entre l'habitat et les zones de nourrissage, test de Wilcoxon, $p = 0.05$).

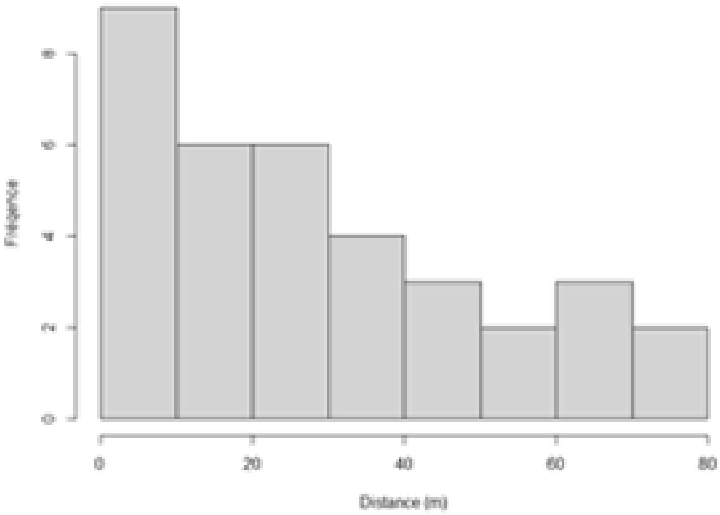


Figure 10 : Distribution des distances euclidiennes (distance entre le premier et le dernier point de l'enregistrement) parcourues pour chacun des trajets journaliers (alimentaires) de 36 *Phengaris alcon* au sein du réseau de tourbières.

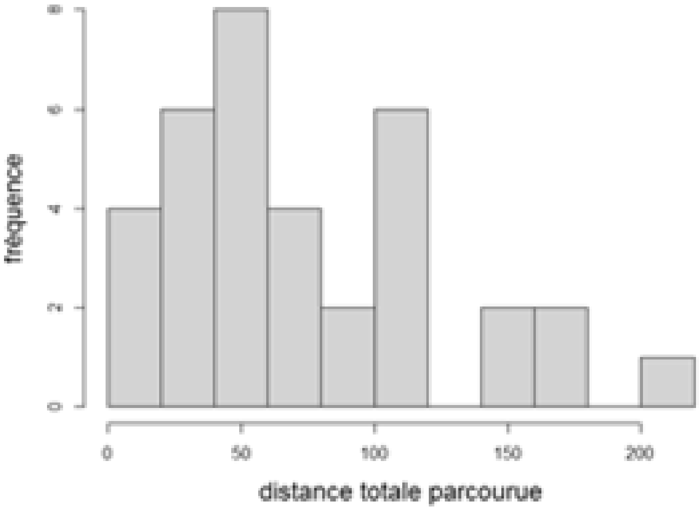


Figure 11 : Distribution de la distance totale journalière obtenue en cumulant l'ensemble des trajets récoltés en un jour pour chacun des 36 *Phengaris alcon* suivis au sein du réseau de tourbières. Les distances sont exprimées en m.

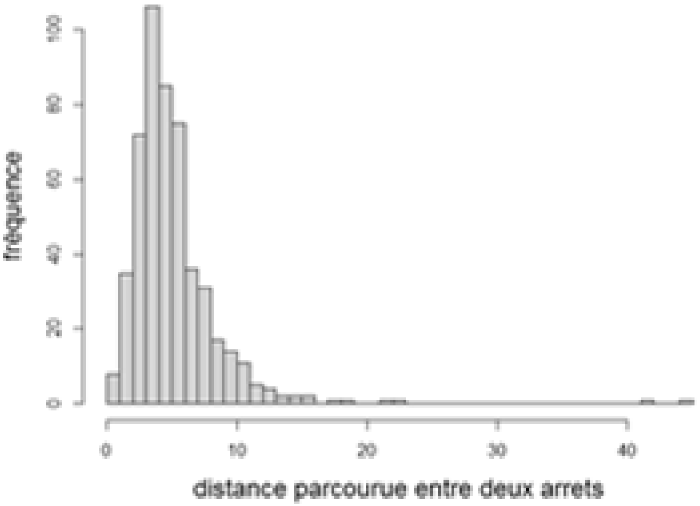


Figure 12 : Distribution des distances parcourues entre 2 arrêts par 36 *Phengaris alcon* lors de trajets alimentaires au sein du réseau de tourbières du plateau de Montselgues.

Tableau 3 : Résumé des distances parcourues au cours des 36 trajectoires de *Phengaris alcon* enregistrées au sein du réseau de tourbières du plateau de Montselgues. Toutes les distances sont exprimées en m.

| identifiant | minimum | médiane | moyenne | maximum | distance totale | Distance euclidienne |
|---|---|---|---|---|---|---|
| Alcon_1 | 2.525 | 4.975 | 4.977 | 7.316 | 39.814715 | 3.875087 |
| Alcon_10 | 2.889 | 7.828 | 7.438 | 17.2 | 96.694179 | 61.3858 |
| Alcon_11 | 1.487 | 5.186 | 4.855 | 9.724 | 72.82334 | 5.841313 |
| Alcon_12 | 0.9779 | 7.558 | 7.6 | 15.97 | 91.201179 | 39.88895 |
| Alcon_13 | 1.842 | 4.158 | 4.364 | 9.244 | 100.378258 | 40.04138 |
| Alcon_14 | 1.674 | 5.811 | 6.888 | 11.56 | 48.214604 | 32.12284 |
| Alcon_15 | 2 | 3.857 | 4.238 | 6.129 | 38.144054 | 23.19919 |
| Alcon_16 | 2.072 | 5.639 | 5.912 | 10.3 | 23.64734 | 18.41227 |
| Alcon_17 | 1.229 | 4.057 | 4.048 | 7.306 | 48.574276 | 15.37358 |
| Alcon_18 | 2.574 | 5.197 | 5.676 | 10.29 | 68.110608 | 6.18573 |
| Alcon_19 | 3.384 | 4.871 | 5.904 | 12.54 | 53.138504 | 0.518668 |
| Alcon_2 | 3.126 | 3.198 | 3.202 | 3.282 | 9.606005 | 8.549717 |
| Alcon_20 | 1.17 | 3.944 | 4.367 | 11.4 | 170.294113 | 79.35758 |
| Alcon_21 | 3.039 | 3.089 | 3.284 | 3.725 | 9.852744 | 6.786893 |
| Alcon_22 | 0.9028 | 3.586 | 4.042 | 11.7 | 218.254946 | 43.56473 |
| Alcon_23 | 0.6519 | 3.779 | 4.489 | 18.64 | 112.222791 | 77.88697 |
| Alcon_24 | 4.552 | 6.724 | 7.632 | 13.26 | 53.4261 | 36.33305 |
| Alcon_25 | 1.162 | 3.817 | 4.075 | 8.86 | 118.187915 | 56.55482 |
| Alcon_26 | 0.7761 | 3.965 | 3.735 | 7.058 | 18.676326 | 14.89556 |
| Alcon_27 | 3.892 | 4.973 | 5.276 | 7.776 | 36.93417 | 31.50816 |
| Alcon_28 | 1.921 | 3.369 | 3.82 | 14.34 | 57.297124 | 6.662378 |
| Alcon_29 | 0.9542 | 5.296 | 5.373 | 12.04 | 102.092862 | 8.890733 |
| Alcon_3 | 0.8421 | 6.749 | 14.1 | 44.29 | 155.105786 | 67.5522 |
| Alcon_30 | 0.7493 | 3.186 | 3.891 | 7.321 | 62.262727 | 16.43263 |
| Alcon_31 | 0.9439 | 3.237 | 3.567 | 6.211 | 42.800377 | 20.85385 |
| Alcon_32 | 1.552 | 3.146 | 4.855 | 14.08 | 53.409728 | 27.86024 |
| Alcon_33 | 2.46 | 5.356 | 5.729 | 9.218 | 63.016902 | 46.20356 |
| Alcon_34 | 1.096 | 5.775 | 6.62 | 22.07 | 112.545496 | 52.77955 |
| Alcon_35 | 2.553 | 5.352 | 5.717 | 10.78 | 114.335566 | 17.56658 |
| Alcon_36 | 4.003 | 6.845 | 6.721 | 8.862 | 33.607131 | 24.26969 |
| Alcon_4 | 2.848 | 4.623 | 4.903 | 6.441 | 34.323209 | 14.33974 |
| Alcon_5 | 1.793 | 4.88 | 5.217 | 10.45 | 161.719851 | 26.03681 |
| Alcon_6 | 1.335 | 4.816 | 5.189 | 13.66 | 150.472928 | 64.85258 |
| Alcon_8 | 3.692 | 4.005 | 4.46 | 5.683 | 13.379776 | 8.964042 |
| Alcon_9 | 1.783 | 3.349 | 5.068 | 11.17 | 40.541423 | 21.14654 |
| **minimum** | 0.6519 | 3.089 | 3.202 | 3.282 | 9.606 | 0.5187 |
| **médiane** | 1.793 | 4.816 | 4.977 | 10.3 | 57.3 | 23.1992 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **moyenne** | 2.012891429 | 4.748457143 | 5.349485714 | 11.4256 | 75 | 29.3341 |
| **maximum** | 4.552 | 7.828 | 14.1 | 44.29 | 218.3 | 79.3576 |

Tableau 4 : Résumé des distances parcourues entre 2 arrêts en fonction du type de milieu dans lequel ont évolués les 36 *Phengaris alcon* du réseau de tourbières du plateau de Montselgues. Toutes les distances sont exprimées en m.

| | minimum | médiane | moyenne | maximum |
|---|---|---|---|---|
| **Foret dense (N=2)** | 3.277 | 3.715 | 3.715 | 4.153 |
| **Habitat (N=426)** | 0.6519 | 4.509 | 5.234 | 44.29 |
| **Zone d'alimentation (N=118)** | 1.096 | 3.973 | 4.816 | 2207 |

# 2 / Coefficients de friction

Il n'existe pas dans la littérature scientifique de définition consensuelle du coefficient de friction. Les termes, perméabilité, transparence, coefficient de rugosité et coefficients de friction sont généralement redéfinis systématiquement et ont généralement des usages identiques ou tout au moins extrêmement proches. Deux aspects bien distincts du comportement des animaux en déplacement dans un milieu peuvent être déterminés grâce au protocole mis en place (Stevens et al. 2004; Stevens et al. 2006a). On appellera par la suite coefficient de perméabilité le coefficient illustrant le comportement d'un individu face au changement de milieu (traverse, longe ou rebondi au niveau de l'interface). Le coefficient de friction quant à lui, correspond dans cette étude aux capacités de déplacement des individus dans les différents types de milieu (performances locomotrices au regard du milieu dans lequel évolue l'individu).

## A / Coefficients de perméabilité

A l'interface entre deux milieux distincts, les azurés des mouillères ont des comportements différents suivant le type d'interface (figure 13). D'autre part, ce comportement semble lié à la structuration verticale du milieu d'arrivée (figure 14, AFC (Analyse Factorielle des Correspondance), $\chi^2$=24.7519, ddl=8, p=0.002).

L'analyse de l'AFC, montre que 98.4% de la variabilité des comportements face aux différents interfaces est porté par l'axe 1 (figure 14). Avec un tel poids explicatif (il explique 98.4% de ce qu'on observe), on peut considérer raisonnablement que cet axe est référent pour calculer les coefficients de perméabilité des milieux pour les azurées des mouillères. Pour ce calcul, on réalise les projetés orthogonaux des points représentatifs des interfaces sur ce premier axe (tableau 5).

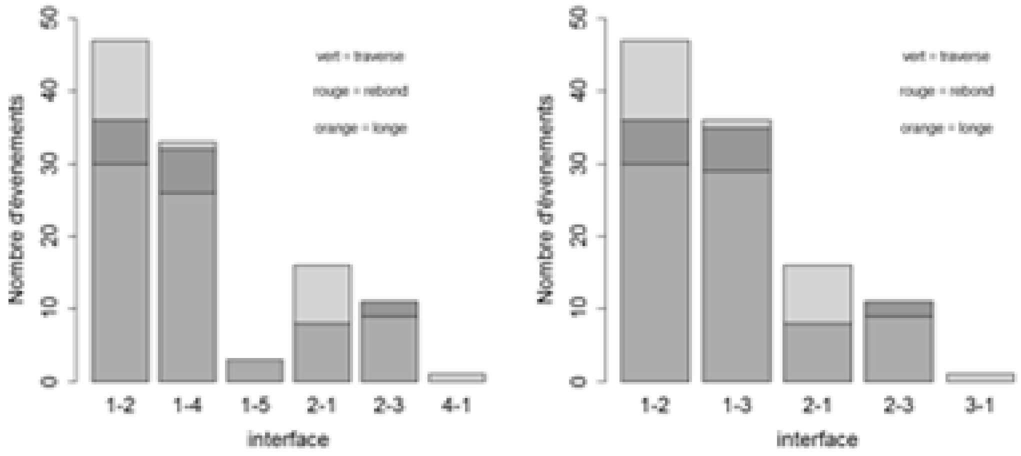


Figure 13 : Distribution des comportements de 36 *Phengaris alcon* dont les trajectoires ont été suivies au sein du réseau de tourbières du plateau de Montselgues. Les habitats 3, 4 et 5 étant très largement sous représentés (graphe de gauche), ils ont été rassemblés au sein d'une même classe 3 pour la suite des analyses (graphe de droite). Si les échanges de l'habitat (classe 1) vers les zones de nourrissage (classe 2), sont assez fréquents, les échanges entre la classe 1 et la classe 3 (couvert végétal élevé) sont beaucoup plus rare. A l'inverse, les azurés ont tendance à revenir dans le milieu 1. La très forte fréquence des comportements de « longe » entre les habitats 1-3 et 2-3 suggère que la structuration verticale du milieu à tendance à diriger les individus.

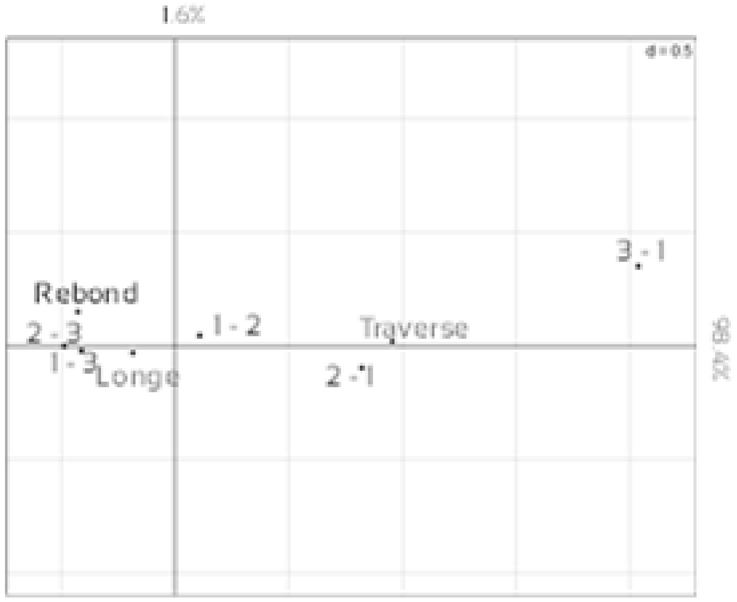


Figure 14 : Représentation sur le premier plan factoriel de l'analyse factorielle de correspondance (AFC) qui associe les comportements des 36 *Phengaris alcon* suivis au sein du réseau de tourbières du plateau de Montselgues aux différentes interfaces de milieux rencontrés (1 : habitat, 2 : zone de

nourrissage, 3 : forêts et landes à genêts à balais, 1-2 représente l'interface individu venant de 1 et arrivant en lisière de 2). Les individus ont tendance à plus rebondir lorsqu'ils arrivent en lisière de milieux avec une structuration verticale importante. Le comportement de longe est plus courant, que le rebond et un peu moins caractéristique des milieux très structurés verticalement. Ce comportement. Les comportements de traverse, sont caractéristiques du retour vers le milieu 1 (2-1, 3-1).

Tableau 6 : Coefficients de perméabilité de l'azuré des mouillères face aux différents interfaces rencontrés lors des enregistrements de trajectoires de 36 *Phengaris alcon* au sein du réseau de tourbières du plateau de Montselgues. Ces coefficients correspondent aux coordonnés projetés sur le premier axe de l'AFC associant comportement des azurés et type d'interface (figure 14).

| | Arrivée en 1 | Arrivée en 2 | Arrivée en 3 |
|---|---|---|---|
| Départ en 1 | | 0.108 | -0.414 |
| Départ en 2 | 0.822 | | -0.489 |
| Départ en 3 | 2.04 | NA | |

## B / Coefficients de friction

Les coefficients de frictions ont, dans cette étude, été mesurés à partir des performances locomotrices des azurés des mouillères dans les différents milieux rencontrés. Pour ce faire, pour chaque portion de trajectoire de chaque azuré suivi, nous avons extrait, la vitesse de déplacement des individus et l'avons mise en relation avec le type de milieu. Nous avons alors réalisé des modèles linéaires généralisés avec effets aléatoires (GLMM) avec le milieu et le site comme effet fixe et l'individu comme effet aléatoire explicatifs de la vitesse enregistrée suivant une distribution poissonnienne, sur lesquels nous avons effectué une sélection descendante (Burnham and Anderson 2004) sur critère d'Akaïke (Akaike 1974). Aucune des variables utilisées ne permet d'expliquer la variance des vitesses enregistrées (figure 15). Cependant, la figure 15, tend à suggérer que si les classes 1 et 2 ne présentent pas de différence significative au regard des performances locomotrices des azurés des mouillères, la classe 3 semble ralentir leur déplacements. Nous avons donc extrait les coefficients de friction de ce modèle linéaire et les avons normalisés en prenant comme référence la classe 1 (habitat). Les coefficients de friction ainsi déterminés sont répertoriés dans le tableau 7.

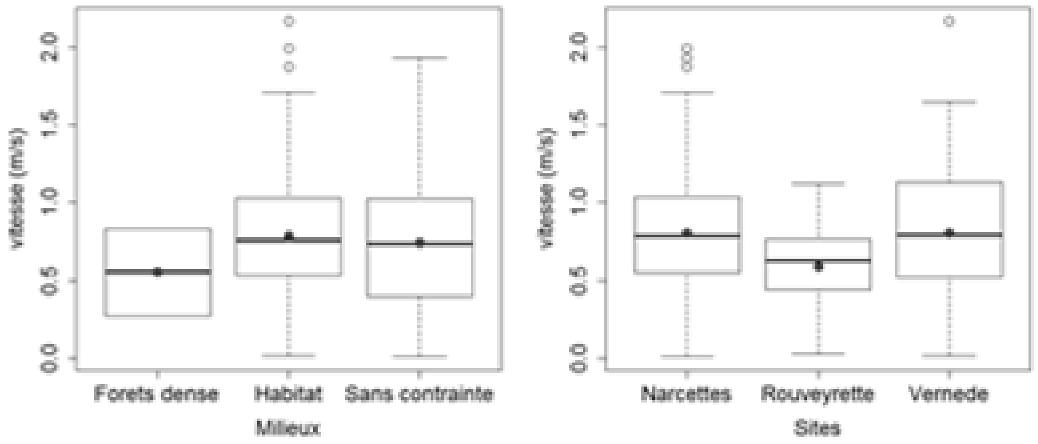


Figure 15 : Vitesse de déplacement de 36 *Phengaris alcon* suivi au sein du réseau de tourbières du plateau de Montselgues.

La vitesse ne présente de différence significative ni en fonction du site (graphe de droite) ni en fonction du type de milieu (graphe de gauche). Cependant une tendance se dessine en fonction du type de milieu qui demanderait à être affinée (N=2 pour forêt dense).

Tableau 7 : Coefficients de frictions normalisés déterminés à partir des trajectoires de 36 *Phengaris alcon* suivis au sein du réseau de tourbières du plateau de Montselgues. L'analyse fine des résultats montre que les azurés sont ralentis dans les zones de nourrissage par rapport à leurs zones d'habitat en raison d'un temps passé à butiner plus long dans ces milieux. Concernant les milieux plus fermés (classe 3), les temps d'arrêts sont plus courts mais la vitesse de déplacement est réduite par rapport à la classe 1.

| Milieu | 1 (Habitat) | 2<br>(Zone de nourrissage) | 3<br>(Forêt et Landes à genêts à balais) |
|---|---|---|---|
| Coefficient de friction normalisé | 0 | -0.01312 | -0.27718 |

## 3 / Paramètres démographiques

Dans la mesure où la CMR n'a pas pu être réalisée, nous avons pris le parti d'estimer la taille de la population de *Phengaris alcon* suivant la méthode décrite par Camille Turlure et al. (2010). Cette méthode consiste à estimer le nombre d'individus présents dans une population à partir des ressources disponibles localement (Encadré 2). Turlure et al. (2010) ont montré que cette méthode permet de très bonnes estimations des tailles de populations chez les papillons, bien plus réalistes que celles réalisées à partir des espaces vitaux des individus.Malheureusement, les données disponibles ne permettent pas d'estimer la taille des populations suivant ces méthodes.

# 4 Conclusions

## *1 / Implication des résultats dans le cadre de la gestion du site*

Les travaux réalisés ont permis de déterminer comme convenu les coefficients de friction associés aux différents milieux présents sur le site d'étude lorsque les données étaient de qualité suffisante. D'autre part, l'analyse comportementale des azurés des mouillères ainsi que celle de leur trajectométrie lors de leurs déplacements journaliers (alimentaires) ont permis de mieux comprendre le lien entre comportement et structure paysagère. D'après nos résultats, il semble donc que l'azuré des mouillères a besoin de patchs contenant de la *Gentiane pneumonanthes* à des distances inférieures à 80m pour qu'un flux intense d'individus lié à l'activité journalière (déplacements pour le nourrissage) puisse advenir. D'autre part, afin de favoriser les déplacements d'azurés des mouillères, il faut limiter la présence d'éléments structurant verticaux au sein des corridors. Par contre, la présence de tels éléments en lisière de corridor (ou de patch) semblerait canaliser le flux d'individus (tendance à longer les lisières). Une structuration linéaire ou en pas japonais des patchs d'habitats ne semble pas impacter les déplacements journaliers des azurés des mouillères mais un habitat alternant zones d'habitat et zones de nourrissage semble être un bon élément de mobilité de l'azuré des mouillères (tendance à se déplacer dans des zones hétérogènes entre habitat et zone de nourrissage).

## Encadré 2 : *Estimation des capacités de charges des différentes tourbières du plateau de Montselgues à l'aide de la méthode développé par Turlure et al. (2010).*

La méthode développée par Turlure et al. (2010) permet d'estimer la taille d'une population de papillons à partir des ressources disponibles et exploitables sur le site d'étude (figure ci dessous).

Dans notre cas précis, nous avons séparé la ressource en 2 parties distinctes :

- La ressource reproductive (*Gentiana pneumonanthes*)
- La ressource alimentaire (surface de milieu de classe 2 exploitable).

Nous avons utilisé les distributions de distances journalières effectuées pour définir les surfaces de zones exploitables pour l'alimentation et conservé deux valeurs susceptibles de constituer la limite d'exploitation (20m et 80m). Les estimations de tailles de populations ont donc été réalisées pour ces deux valeurs.

Figure : Représentation schématique de l'acquisition de l'information « ressource » à partir des données géolocalisées de gentianes et d'habitat.

# *2 / Poursuite du projet*

Dans la mesure où les données démographiques issues de la CMR n'ont pas pu être collectées et où une méthode alternative d'estimation de la taille potentielle des populations a été déployée (moins précise), il semblerait judicieux de réaliser la CMR sur *Phengaris alcon* au sein du réseau de tourbières du plateau de Montselgues. Un tel suivi nous permettrait en plus d'estimer les paramètres démographiques de cette population (Lebreton et al. 1992), de discriminer parmi les trajectoires obtenues cette années celles correspondant à des trajets alimentaires et celles correspondant à des trajets dispersifs et d'affiner grandement l'analyse réalisée cette année (2013). D'autre part, une telle information permettrait d'estimer précisément les distances maximales parcourues entre zones d'habitat, permettant ainsi de mieux de déterminer comment placer les aménagements de type corridors.

Les analyses de viabilité seront réalisées ultérieurement à la demande du PNR MA et du CEN RA s'ils estiment que la méthodologie proposée par TerrOïko le 18/10/2013 à Vogue lors de la présentation des résultats permet une analyse de viabilité suffisamment pertinente (Encadré 3). Cette analyse sera réalisée dans le courant de l'hiver 2013-2014 si elle est jugée nécessaire. Dans le cas où cette analyse de viabilité était réalisée, le test des aménagements potentiels sera réalisé en même temps si les cartes sont fournies dans les temps par le CEN RA.

***Encadré 3 :*** *Méthodologie proposée le 18/10/2013 à Vogue pour étudier la viabilité de la population de* Phengaris alcon *dans le réseau de tourbières du plateau de Montselgues.*

La Méthodologie proposée est basée sur une étude théorique appuyée par les données de la littérature concernant l'espèce dans d'autres sites européens.

Les caractéristiques démographiques de cette espèce sont bien documentées et ne présentent qu'assez peu de variabilité inter-régionale. Le point critique réside donc dans l'estimation de la capacité de charge du site de Montselgues. TerrOïko propose donc de réaliser non pas une analyse de viabilité mais un groupe d'analyse en faisant varier la capacité de charge du site entre 10 indiv/ha (Dupont 2010) et 50 indiv/ha (estimation réalisée par Nowicki et corrigée par Turlure et al 2010). Afin de réaliser une estimation de la probabilité d'extinction en fonction de la capacité de charge. Les résultats préliminaires tendent à montrer que le site de

Montselgues serait dans des gammes de capacités de charges entre 10 et 20 indiv/hectare.

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# SOMMAIRE

# 1

# Contexte de l'étude

## *1 / La proposition technique*

Ce document constitue la suite des résultats du projet proposé par TerrOïko dans le cadre de l'appui méthodologique et l'analyse des données concernant l'effet des ruptures de continuités écologiques sur l'Azuré des mouillères au sein du réseau de tourbières du plateau de Montselgues (Moulherat 2013).

La proposition technique que nous vous avions faite comportait 4 étapes différentes :

5. ***Détermination des coefficients de friction/rugosité des différents habitats pour Phengaris alcon***
6. ***Suivi par capture-marquage-recapture de la population d'azuré des mouillères du plateau de Montselgues***
7. ***Intégration des informations recueillies lors des étapes 1 et 2 à MetaConnect***
8. ***Test des différents scenarii d'aménagement possibles et détermination du scenario le plus pertinent.***

L'analyse des étapes 1 et 2 ont déjà été réalisées (Moulherat 2013). Le présent rapport traite de la partie 3 sur l'intégration des informations recueillies lors de l'étape 1 et 2 à MetaConnect. Un rappel de la proposition est faite ci-dessous.

### *Etape 3 : Intégration des résultats des étapes 1 et 2 à l'utilisation de MetaConnect*

*Objectif : Réaliser les études de viabilité de la population d'azuré des mouillères*

Les résultats de l'analyse de la CMR de la population d'azuré des mouillères fournira les informations locales des caractéristiques démographiques de l'espèce (taille de population, survie adultes, flux d'individus,...) dans le réseau de tourbières. Au cas où les populations d'azuré sur le site seraient trop petites pour permettre une analyse robuste de la CMR, les caractéristiques démographiques seront extraites de la littérature scientifique existante sur le sujet. Ces données démographiques seront utilisées dans MetaConnect pour simuler la dynamique de la population d'azuré des mouillères sur le plateau de Montselgues.

Les coefficients de friction/rugosité une fois déterminés seront cartographiés à l'aide de la cartographie précise des habitats fournie par le CEN Rhône-Alpes et utilisés comme données d'entrée de MetaConnect. Ces données nous serviront lors de la simulation de la dispersion de l'azuré des mouillères au sein du réseau de tourbières. De même, les stations de gentianes pneumonanthe seront utilisées comme données d'entrée de MetaConnect.

Les simulations réalisées avec MetaConnect fourniront les probabilités d'extinction de l'azuré des mouillères sur chaque tourbière ainsi que pour l'ensemble du réseau étudié. Ces simulations fourniront aussi le potentiel de structuration génétique du paysage actuel. Ces résultats fourniront donc à la fois l'information quant à la viabilité de la population d'azuré des mouillères au sein du réseau de tourbière du plateau de Montselgues et déterminer les zones nécessitant une amélioration de la qualité du milieu pour optimiser la survie de l'espèce, mais aussi la dynamique des flux d'individus au sein du réseau et par là même permettra d'identifier les besoins et localisations de reconnexion des tourbières.

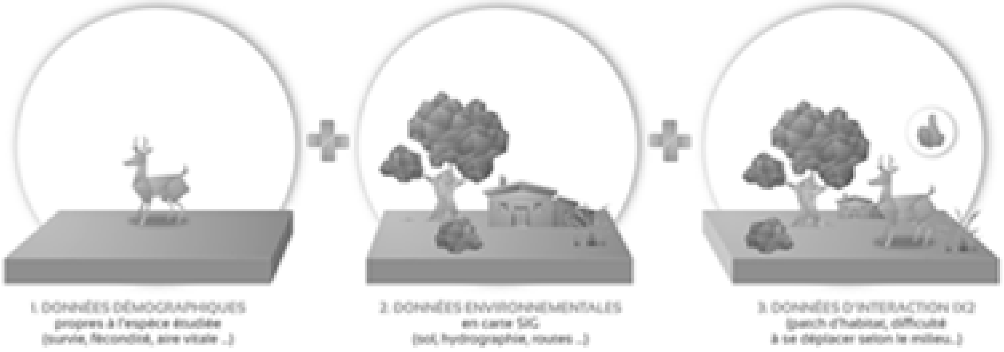
## *2 / Bilan de l'activité de TerrOïko pour le projet.*

Comme prévu dans la proposition technique, TerrOïko (Sylvain Moulherat et Jérémie Cornuau) a réalisé les simulations avec MetaConnect. Les données d'entrée ont été extraites dans la mesure du possible à partir des travaux de terrain du PNR MA *via* David Viratel ou Yves Rozier (Moulherat 2013). Quand les données n'étaient pas disponibles nous avons eu recourt à la littérature scientifique tirée en grande partie des synthèses réalisées dans le cadre du Plan National d'Action *Maculinea* (Dupont 2010, Merlet et Dupont 2012). A l'heure actuelle la dernière partie de la proposition technique (test des scenarii d'aménagements) reste à réaliser en fonction des décisions d'aménagements que vous souhaiterez nous soumettre.

# 2

# Données entrées dans les simulations

Notre outil de simulation de la biodiversité et de la connectivité fonctionnelle (MetaConnect) intègre des données démographiques, des données environnementales et l'interaction de l'écologie avec l'environnement (figure ci-dessous). Ces données d'entrée proviennent en grande partie des travaux du PNR MA (Moulherat 2013).



# 1 / Données sur la démographie

MetaConnect a été structuré en fonction des traits d'histoire de vie de l'espèce. Nous avons paramétré le cycle de vie à partir des informations du plan national d'action *Maculinea* et de nos bases de données (Figure 1 et tableau 1).

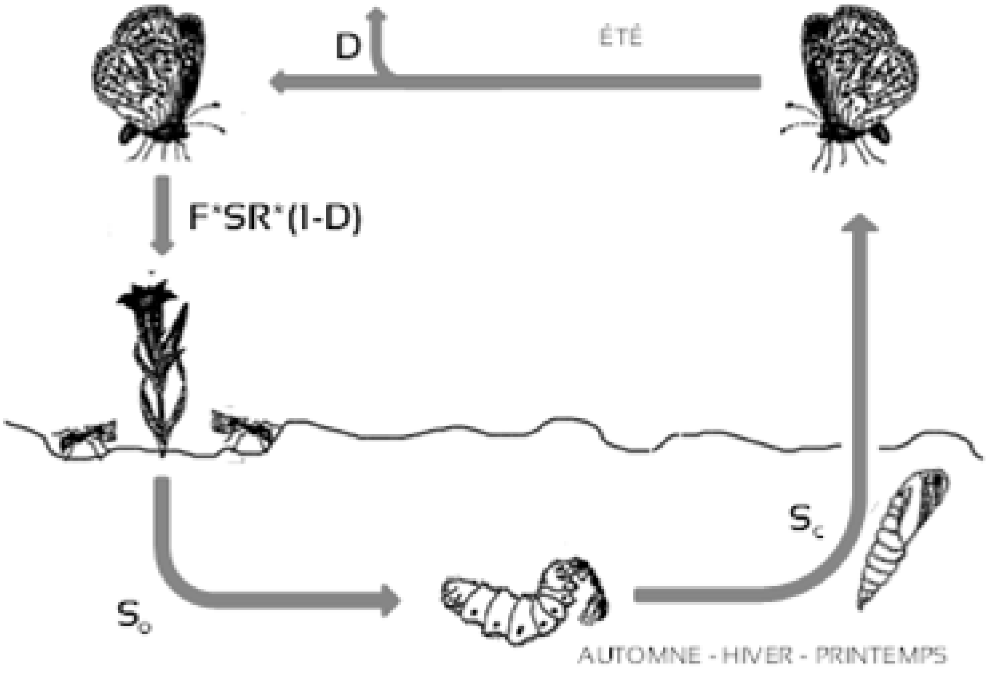


Figure 1. Cycle de vie de *Phengaris alcon* (d'après Rozier 1999 et Lhonoré 1996).

Tableau 1. Paramètre rentrés dans le cycle de vie de *Phengaris alcon*.

| Paramètre du cycle de vie | valeur |
|---|---|

| | |
|---|---|
| survie des œufs ($S_o$) | 0.15[a] |
| survie des chenilles ($S_c$) | 0.15[a] |
| probabilité de dispersion (D) | 0.05[b] |
| sex ratio (SR) | 0.5[b] |
| fécondité (F) | 120[b] |

[a] *d'après Als et al. 2001*

[b] *d'après Dupont 2010*

Remarque : Thomas et al. (1998) ont montré que seulement une partie de la génération (25%) se nymphose à la fin du printemps suivant et que l'autre partie (75%) reste une année supplémentaire dans la fourmilière. La présence de ces deux cohortes de chenilles dans la fourmilière a été prise en compte dans le cycle de vie (non-représentée sur la figure 1).

## 2 / Données sur l'environnement

Nous avons utilisé les cartes de la matrice paysagère sous format SIG (Figure 2). Nous avons utilisé les cinq milieux différents identifiés par le PNR MA (David Viratel) et Yves Rozier lors des travaux de terrain du PNR MA de l'été 2013 (Moulherat 2013, Tableau 2).

Tableau 2. Matrice paysagère

| **Habitat** | **couleur** |
|---|---|
| Classe 1 (dont moliniaies) | vert clair |
| Classe 2 | rose |
| Classe 3 | jaune |
| Classe 4-5 | vert foncé |
| Classe 6 | blanc |

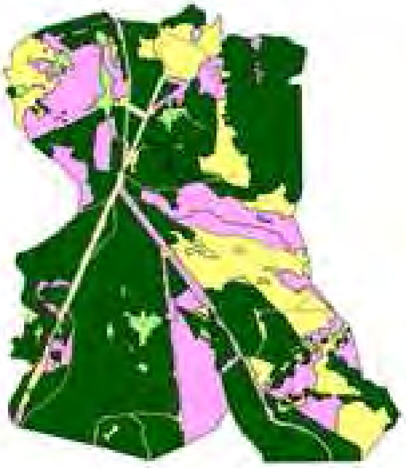


Figure 2. Carte de la matrice paysagère du réseau de tourbières du plateau de Montselgues. La description complète des classe
précéd
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Figure 3. Carte des patchs d'habitat définis à partir des données de terrain recueillies durant l'été 2013 sur le plateau de Montselgues.

Les patchs d'habitat favorable ont été défini à partir des données recueillies sur le terrain (localisation GPS des pieds de gentianes pneumonanthes et distances moyennes parcourues par les azurés) comme étant les zones de 10.4 m autour des pieds de gentianes répertoriés et géo référencés (Moulherat 2013). Nous avons donc distingué 36 patchs d'habitats potentiels (Figure 3).

# *3 / Données sur l'interaction démographie/environnement*

### 1) Qualité des patchs d'habitats.

Nous avons quantifié la qualité des patchs d'habitat en fonction de la densité en pied de gentiane, du nombre d'œufs moyen par gentiane et du pourcentage de gentiane occupé par des œufs. Nous avons calculé la densité de pieds de gentiane pour chacun des patchs d'habitat à partir des données de terrain du PNR MA (Moulherat 2013). Nous avons quantifié la capacité d'accueil des patchs d'habitat à partir des données de Yves Rozier collectées sur le plateau de Montselgues (années 2000 à 2009). L'analyse des données d'Yves Rozier nous a permis de déterminer que le nombre moyen d'œufs par gentiane est de 6 et que le pourcentage de gentiane avec des œufs est en moyenne de 19%. C'est donc sur la base de ces données locales que nous avons configuré MetaConnect.

### 2) Le mode de dispersion.

Les données sur la dispersion de *Phengaris alcon* et chez les *Maculinea* en général ne sont pas encore précisément connues. Nous avons donc décidé de réaliser des simulations basées sur 3 sources d'informations concernant les capacités maximales de dispersion de *P. alcon* (distance maximale de dispersion). Nous avons retenu des scenarii avec une très faible distance de dispersion (30 mètres) une distance moyenne de dispersion (300 mètres) et une grande distance de dispersion (3000 mètres). Les trois distances ont été choisies à partir des données récoltées sur le terrain pendant la campagne de terrain de l'été 2013 (30m), du plan national d'action (300m, Dupont 2010, Merlet & Dupont 2012) et de la littérature scientifique (3000m, Nowicki 2005). Le mode de dispersion choisi est le SMS (stochastic movement simulator) (Palmer et al 2011, Coulon et al. en préparation) qui est le plus adapté pour *Phengaris alcon* (Baguette et Stevens com. Pers.).

### 3) Les coefficients de friction.

Nous avons utilisé les coefficients de friction précédemment mesurés par les travaux de terrain du PNR MA (Moulherat 2013, Tableau 3).

Tableau 3. Coefficient de friction.

| Habitat | coefficients de friction |
|---|---|
| Classe 1 | 0 |
| Classe 2 | -0.01312 |

| | |
|---|---|
| Classe 3 | -0.27718 |
| Classe 4-5 | -0.27718 |
| Classe 6 | -0.01312 |

# 3 Analyse des données simulées

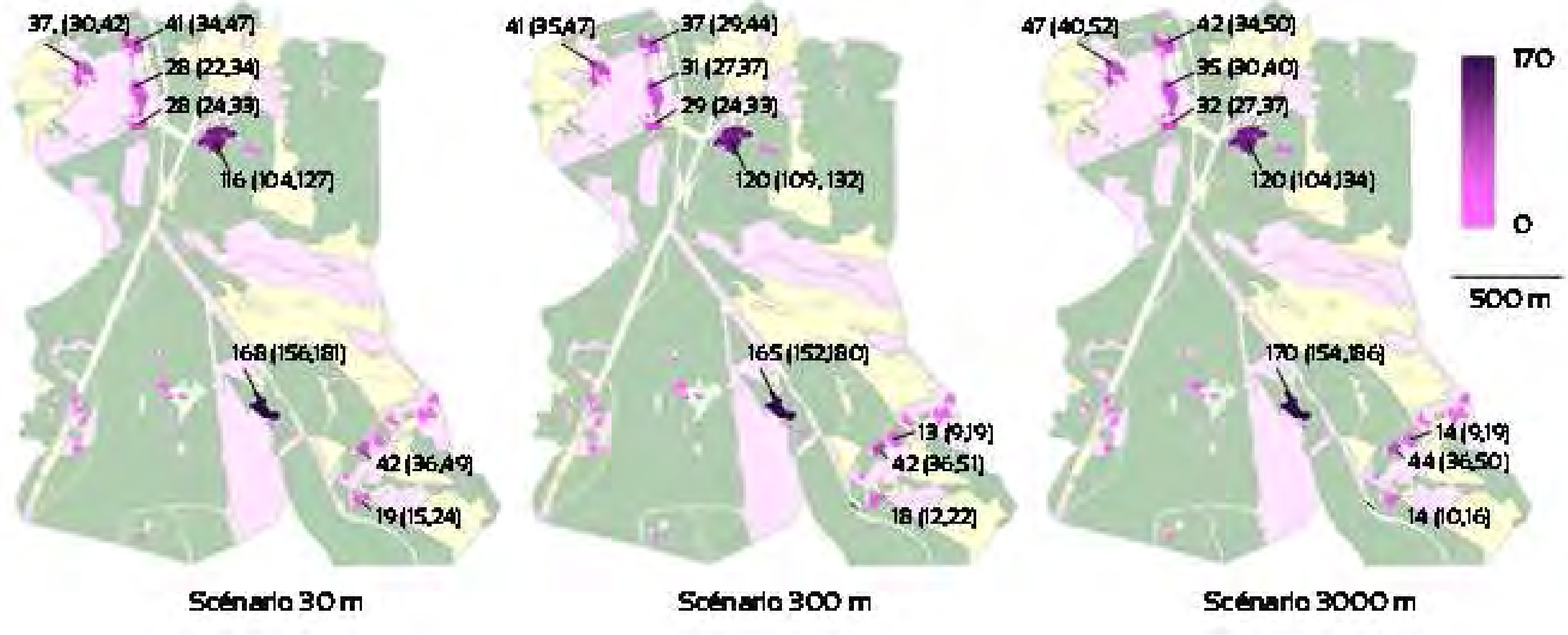
## 1 / Probabilité de survie, viabilité de la population

Les cartes ci-dessous présentent les probabilités de survie des populations de *Phengaris alcon* sur le plateau de Montselgues suivant trois scénarii de capacité de dispersion (30, 300 et 3000 mètres).

Les probabilités d'extinction sont relativement stables entre les différents scénarii. Globalement des populations d'azuré des mouillères ont de grandes chances de se maintenir sur les Narcettes, la cham de Chabreille, la cham de Vernède et sur deux patchs d'habitat des granges de Rouveyrette. A contrario les populations de la cham du Jou ont de fortes chances de disparaitre, excepté sur sa partie Est pour des capacités de dispersion de 3000 mètres.

## 2 / Tailles des populations

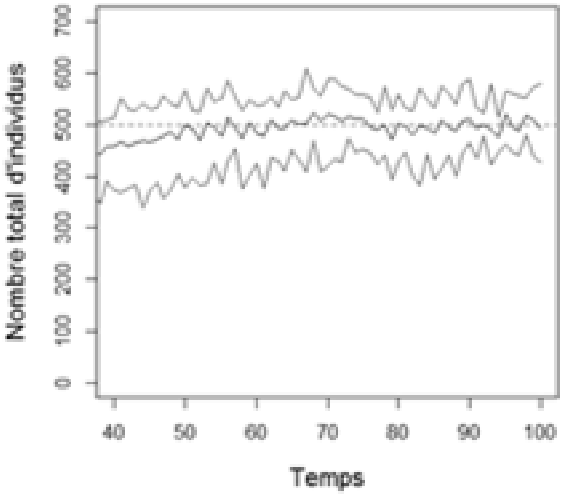
Les cartes ci-dessous présentent les tailles des populations espérées de *Phengaris alcon* sur le plateau de Montselgues suivant trois scénarii de capacité de dispersion (30, 300 et 3000 mètres). Les nombres représentent les tailles de populations moyennes avec entre parenthèse les limites basses et hautes de l'intervalle de confiance à 95%. Seules les populations > 10 individus adultes sont représentées.



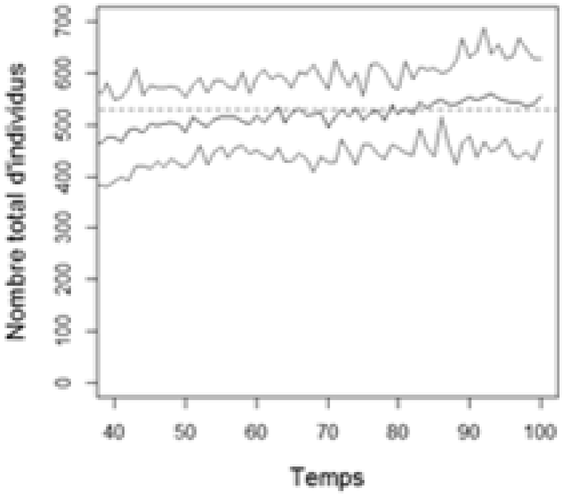
Scénario 30 m Scénario 300 m Scénario 3000 m

Les tailles de population sont relativement stables entre les différents scénarii. Globalement trois grandes populations sont présentes à Chabreille, Vernède et les Narcettes. Une population plus faible est présente à Rouveyrette. Les populations de Jou Est et Ouest sont trop petites et instables pour être représentées (<10 individus).

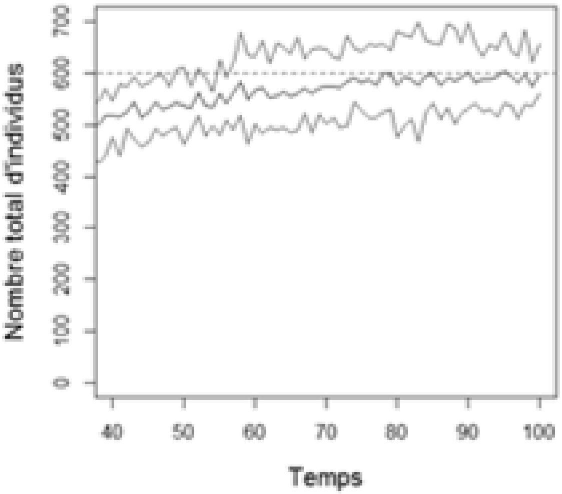
Les graphiques ci-dessous présentent les tailles des populations en fonction des années simulées (0 à 100 ans) de *Phengaris alcon* sur le plateau de Montselgues suivant trois scénarii de capacité de dispersion (30, 300 et 3000 mètres). Les années 0 à 40, correspondent au temps de stabilisation du simulateur et ne sont pas prises en compte dans l'analyse. L'axe des ordonnées représente le nombre moyen d'individus, l'axe des abscisses représente le temps en années. La courbe noire représente la moyenne et les courbes rouges représentent l'intervalle de confiance à 95%.



Scénario 30 m



Scénario 300 m

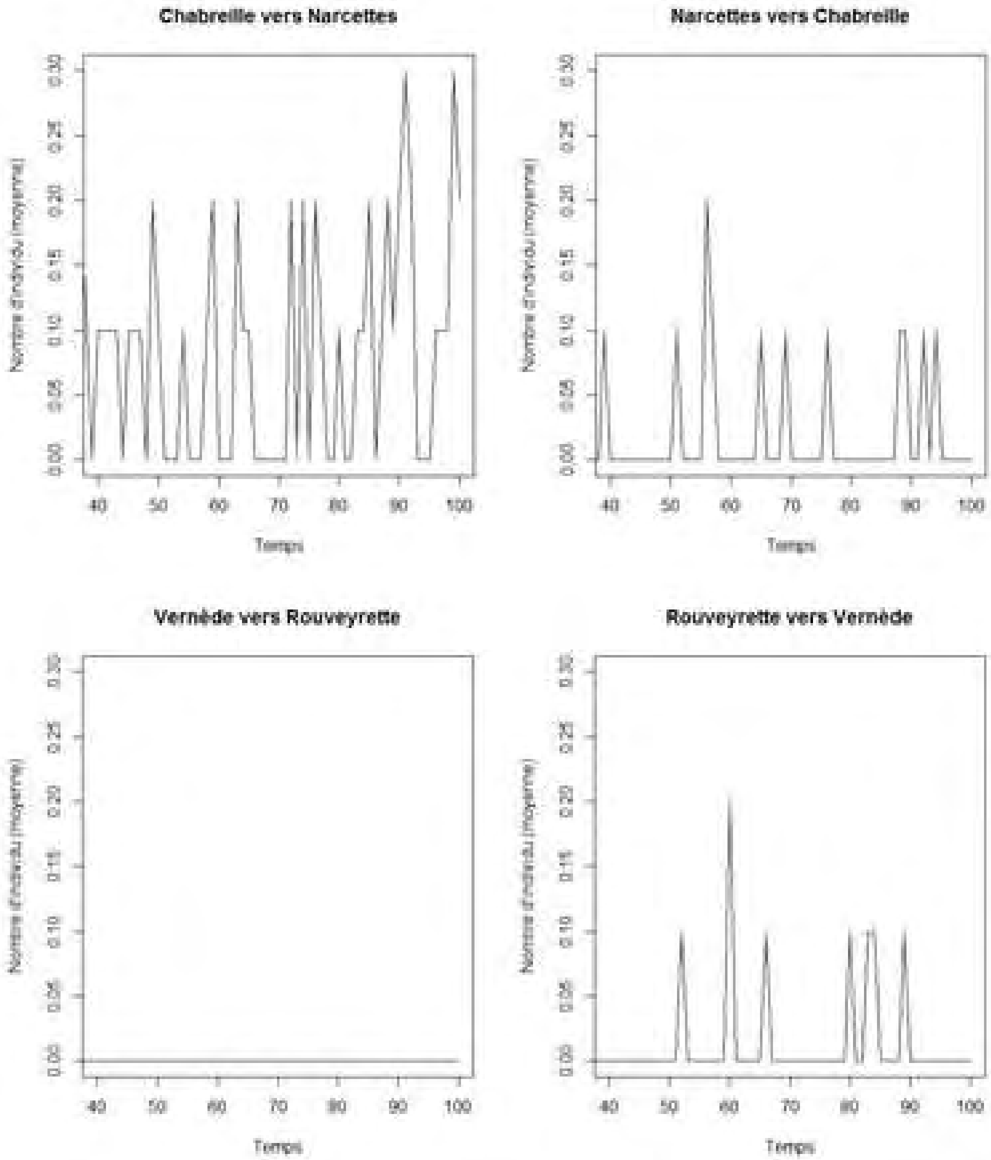


Scénario 3000 m

Les tailles de population globale sont relativement similaires entre les différents scénarii. Les populations globales se stabilisent autour de 500 papillons pour le scénario 30, et augmente progressivement avec l'augmentation des capacités de dispersion (moins d'individus morts en dispersion quand les capacités de dispersion augmentent).

## 3 / Flux de papillons sur le territoire

Les cartes ci-dessous présentent les flux moyen de *Phengaris alcon* suivant les trois *scénarii* de capacité de dispersion (30, 300 et 3000 mètres). Les flux sont déterminés à partir de la fréquentation des papillons simulés ayant réussi leur dispersion (ayant atteint un patch d'habitat différent de celui dont ils sont partis). En d'autres termes, un flux de 50 papillons signifie que sur 100 générations, 50 passages ont été observés dans la cellule (correspondant donc à 0.5 passage par an). L'interprétation des valeurs doit également se faire de manière relative en comparant les cellules entre elles.



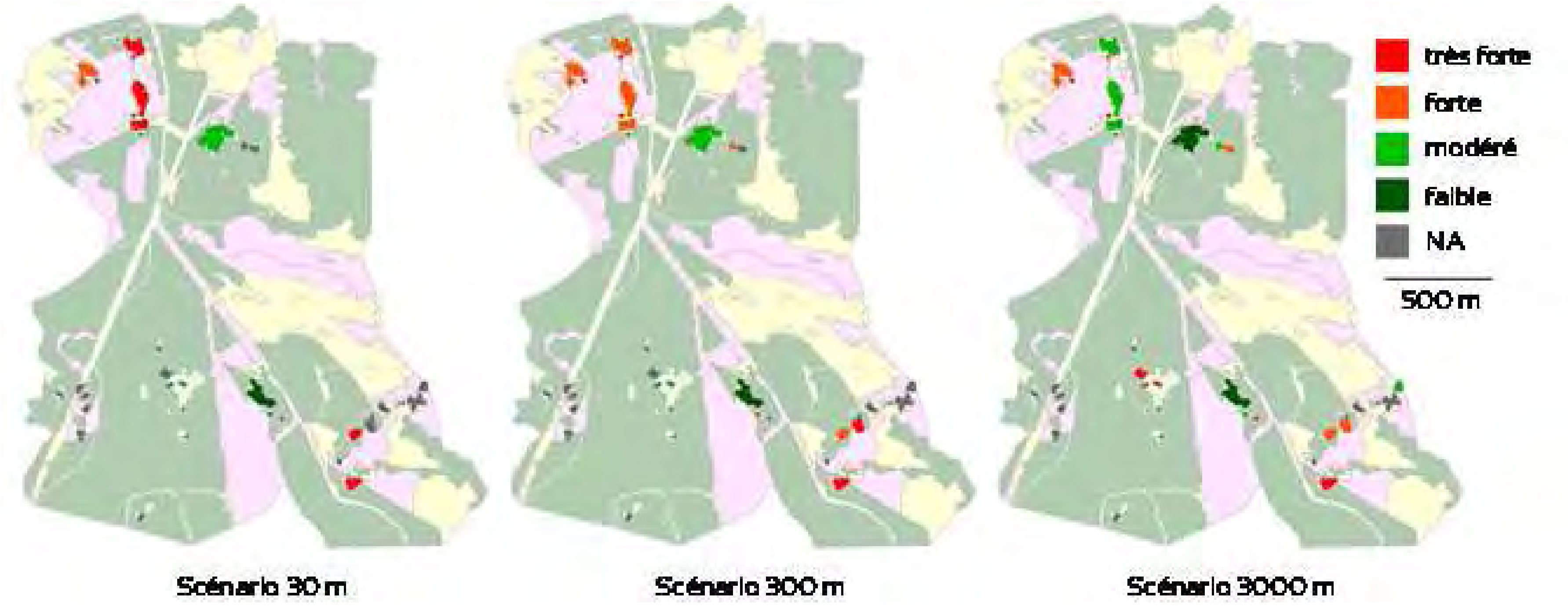
Le gradient de capacité de dispersion (30, 300 et 3000 mètres) que nous avons choisi est très bien représenté au niveau des flux d'individus allant de l'absence de flux entre les patchs d'habitat (scénario 30) à une présence de flux limité entre les patchs et une séparation Nord-Sud nette des populations (scénario 300) jusqu'à une présence de flux entre l'ensemble des patchs d'habitat (scénario 3000).

D'après les mesures effectuées sur le terrain et leur interprétation au regard des connaissances sur l'espèce, la valeur la plus plausible des capacités de dispersion de *Phengaris alcon* à Montselgues se trouveraient autour de 300 mètres. Dans ce scénario les Narcettes/Chabreille et Vernède/Rouveyrette ne sont pas connectés par un flux d'individus. La connexion entre Chabreille et les Narcettes et entre Vernède et Rouveyrette est à surveiller car le flux d'individu étant relativement faible (<0.3 passages par an, Figure ci-contre).

Les zones utilisées par *Phengaris alcon* (rouge foncé, figure page précédente) dans le scénario où les capacités de dispersion sont placées à 3000 mètres montrent que sous cette hypothèse, les azurés utiliseraient la ligne à haute tension et la piste pour canaliser leurs déplacements. (Remarque : le secteur >100 passage sur la partie centrale à l'Est de la carte est issue d'un artefact de simulation lié au fait qu'une piste « mène » au bord de la carte. Ce secteur n'est donc pas à interpréter comme étant un carrefour de dispersion de *Phengaris alcon* mais reste dans la continuité des observations précédentes à savoir que la ligne à haute tension et la piste semblent favoriser le déplacement des azurés).

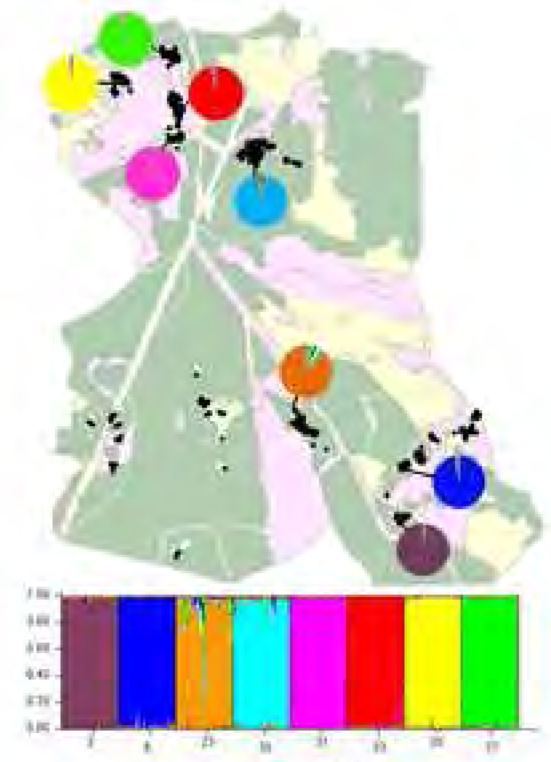
## 4 / Structuration génétique

Les cartes ci-dessous présentent les indices de diversification génétique des populations (Fst) des populations de *Phengaris alcon* sur le plateau de Montselgues suivant trois *scénarii* de capacité de dispersion (30, 300 et 3000 mètres). Les patchs non viables sont en gris (NA). Si 0 < Fst < 0.05 : la différenciation entre les populations est faible (pas de consanguinité) ; si 0.05 < FST < 0.15 : la différenciation entre les populations est modérée (consanguinité modérée) ; si 0.15 < Fst < 0.25 : la différenciation entre les populations est importante ; si Fst > 0.25 : la différenciation entre les populations est très importante (forte consanguinité).

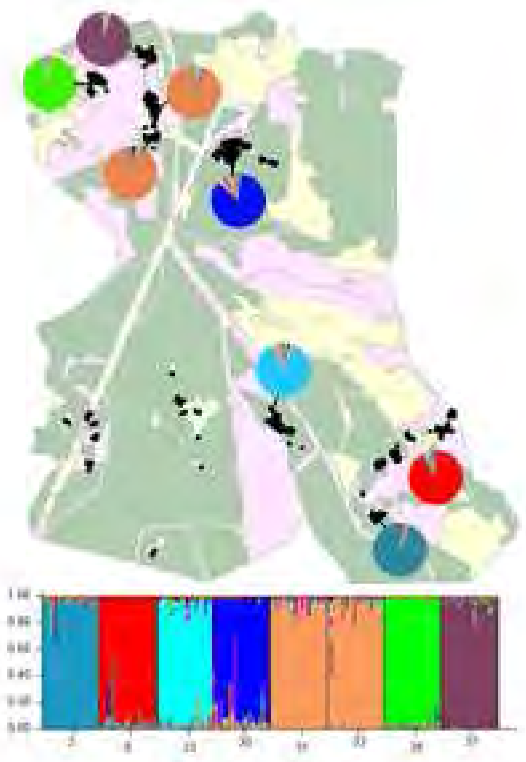


Globalement les indices de diversification des populations (Fst) sont relativement haut, signe d'une différenciation importante entre les différents patchs de *Phengaris alcon*. Nous observons comme attendu que lorsque les capacités de dispersion augmentent (de 30 à 3000 mètre) les Fst diminuent signe que des aménagements permettant de meilleurs déplacements d'individus sur le territoire permettraient de rétablir des échanges génétiques convenables. Les populations des granges de la Rouveyrette montrent une forte différenciation liée à un faible flux de gènes entre les populations. Ce résultat est retrouvé sur les Narcettes bien qu'il soit moins prononcé. Curieusement les Fst de la cham de Vernède et de la cham de Chabreille montrent que ces populations ne sont pas très différenciées. Cependant aux vues des flux d'individus observés précédemment ce résultat est imputable à la grande taille de ces deux populations et non à des échanges génétiques importants.

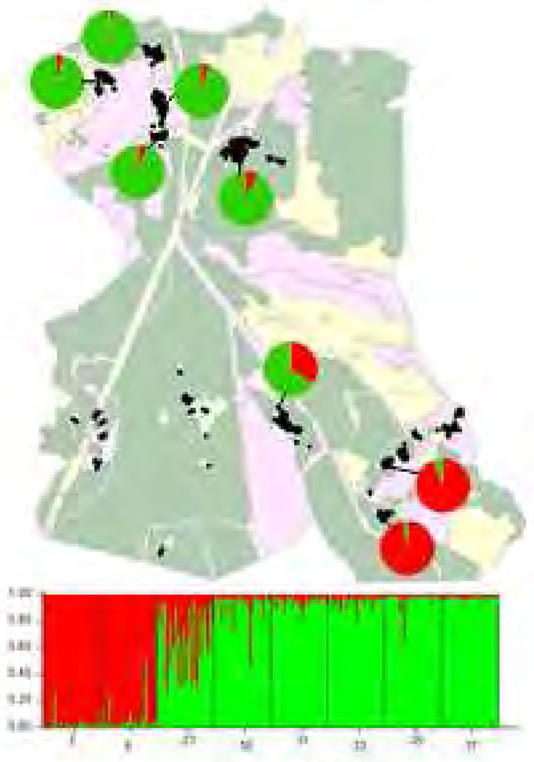
Nous avons testé l'existence d'une structuration génétique des populations à l'aide du logiciel STRUCTURE (Pritchard et al. 2000). Les groupes identifiés par STRUCTURE comme étant potentiellement des populations différenciées sont représentés sur les cartes ci-dessous. Chaque population identifiée par STRUCTURE est représentée par une couleur différente. Les camemberts représentent le degré d'appartenance des individus d'un patch à la population identifiée par STRUCTURE. Lorsque deux patchs ont les mêmes couleurs cela signifie que STRUCTURE les identifie comme une seul population génétique.



Scénario 30 m



Scénario 300 m



Scénario 3000 m

Les résultats de STRUCTURE montrent que sur une fine échelle il est possible d'observer des différenciations génétiques entre les différents patchs d'habitat. Ainsi pour les scénarii à 30 mètres et à 300 mètres même les Narcettes, les granges de la Rouveyrette, la cham de Chabreille et la cham de Vernède peuvent être considérés génétiquement comme des populations différentes. Pour ces mêmes scénarii Les Narcettes et les granges de la Rouveyrette se trouvent structurées en plusieurs populations. A l'inverse pour le scénario à 3000 mètres, seules deux populations au sens génétique sont trouvées. On observe alors une différenciation entre les granges de la Rouveyrette et le reste des patchs. Il est à noter que nous pouvons observer un *continuum* génétique entre les populations où la cham de Vernède permet de faire le lien entre génétique entre les granges de la Rouveyrette et les Narcettes/Chabreille..

# 4 Synthèse et propositions

## 1 / Synthèse

Les résultats issus de nos simulations et réalisés à partir de données provenant de votre territoire nous ont permis d'obtenir des indications pertinentes sur la connectivité structurelle sur le plateau de Montselgues. Globalement les densités de population sont faibles tout comme les flux d'individus conduisant à une différenciation forte entre les patchs d'habitat. Cependant les probabilités de survie des Narcettes de la cham de Chabreille et de la cham de Vernède sont très élevées. Il est à noter que peu de sites disposent de données d'occupation des gentianes aussi précises que celles dont nous avons disposé dans cette étude et qui nous a permis de déterminer un taux moyen d'occupation de 19%. Cette remarque implique que nos simulations sont réalisées avec des hypothèses pessimistes notamment concernant la qualité du milieu et que les résultats sont donc à resituer dans ce contexte.

Les tailles de populations espérées de *Phengaris alcon* sur le plateau de Montselgues sont relativement faibles. La présence d'habitat favorable (ici calculée à partir d'un *buffer* de 10,4 mètres autour des points de gentiane) est de 7.2 hectares pour un territoire de 376.9 hectares. Le nombre total de papillons est d'environ 550 individus (scénario 300) ce qui correspond à une densité d'environ 76 individus/hectare d'habitat favorable et 1.46 individus/hectare en prenant en compte la totalité du territoire. Ces valeurs sont faibles comparées à la littérature. En effet la densité de population du genre *Maculinea* varie entre 10 et 1000 individus/hectare et est estimé à 300 papillons/hectare en milieu favorable chez *Phengaris alcon* (Mouquet et al. 2005, Nowicki et al. 2005).

Bien que les densités de population soient faibles les probabilités de survie des populations sont plutôt bonnes sur le territoire excepté pour la cham du Jou et une partie des granges de la Rouveyrette. Ce résultat est en accord avec d'autres résultats d'études de terrain similaires disponibles dans la littérature où le maintien de très petites populations (10 papillons) est observé (Habel et al. 2007). Cependant les simulations montrent (en accord avec la littérature existante) que dès que la taille des populations d'un patch est inférieure à 10 individus leurs chances de survies diminuent fortement (Habel et al. 2007). Habel et al. 2007 expliquent que ces extinctions de population peuvent être endiguées si d'autres populations sont présentes à proximité et peuvent recoloniser le milieu. C'est exactement ce que nous observons sur la cham du Jou Ouest où plus la dispersion est importante plus la probabilité de survie des populations est importante. Il faut toutefois noter qu'avec des capacités de dispersion les plus plausibles (300 mètres) les populations de la cham du Jou ne sont pas viables.

L'absence de potentielle recolonisation à la cham du Jou Est illustre le fait que les populations sont extrêmement isolées sur le plateau de Montselgues. Les flux d'individus observés entre les différents patchs d'habitat sont quasi inexistants pour un scénario de dispersion moyen (300 mètres). De plus, même avec un scénario où les capacités de dispersion sont idéales les flux d'individus restent faibles (autour de 1 à 3 individus par génération). Globalement, le manque de flux entre les différents patchs d'habitat peut être attribué à :

- la distance trop importante qui les sépare. En effet, l'exploitation des données de terrain de l'été 2013 a montré une faible capacité de déplacement de *P. alcon* au sein du réseau de tourbières du plateau de Montselgues (Moulherat, 2013). Ces résultats sont appuyés par d'autres études qui suggèrent que chez *Phengaris alcon* deux patchs d'habitat peuvent être généralement considérés comme distincts lorsque la distance qui les sépare est de 250 à 500 mètres (Wallisdevries 2004, Habel et al. 2007). Nowicki et al. 2007 ont observé que les échanges entre patchs d'habitat étaient très faibles chez *Phengaris alcon* pour des distances variant entre 300 à 700 mètres. Le plan national d'actions en faveur des *Maculinea* estime qu'au-dessus de 1 kilomètre les flux d'individus ne sont plus possibles.
- des tailles de population par patch d'habitats faibles (peu d'individus par patch donc mécaniquement, peu d'individus dispersants).

# 2 / Bilan et propositions

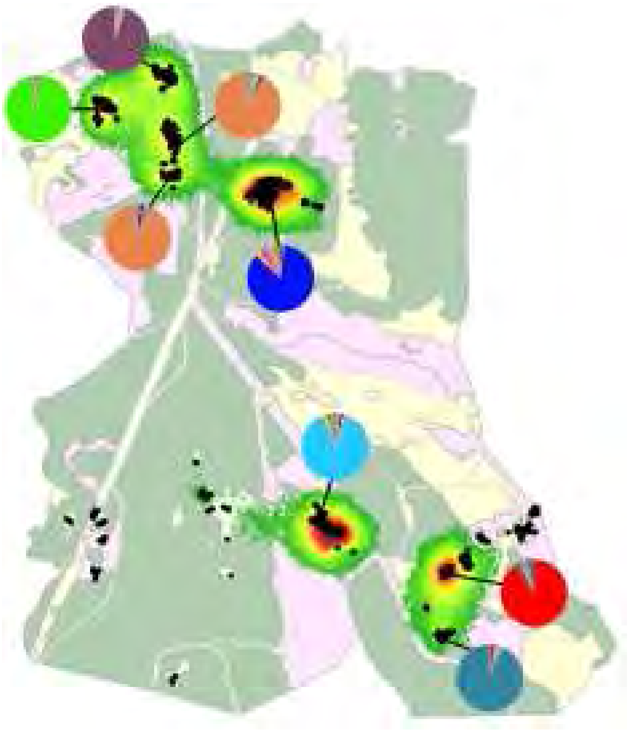


Figure 4 : Bilan des résultats

Les résultats de simulation (figure 4) suggèrent que :

- Au sein des Narcettes, le secteur de l'exclos, celui de la mare nord et celui du secteur sud proche de la parcelle pâturée par les chevaux semblent n'avoir que très peu d'échanges entre eux. Il semble que bien que la couverture végétale soit favorable au déplacement des azurés, l'absence de sites relais (présence de gentiane) et/ou la faible densité de population sur ces différents secteurs soit un frein aux échanges. Une semblable observation explique l'isolement des secteurs les plus au sud des granges de la Rouveyrette (voir cercle rouge sur figure 5 pour des propositions de localisation de secteur où favoriser le développement de la gentiane pneumonanthe).

- Les secteurs nord (Narcettes et cham de Chabreille) et sud (Cham du Jou, cham de Vernède et granges de la Rouveyrette) sont deconnectés malgré la présence de structures paysagères qui sembleraient favorables au déplacement des azurés (piste et ligne haute tension). Il semble difficile de reconnecter ces deux secteurs car il faudrait créer un réseau de patchs d'habitats favorables entre ces deux secteurs.
- La structure actuelle du paysage permet des flux (limités) d'individus entre les Narcettes et la cham de Chabreille favorisés par la présence du corridor (signalé par les lignes rouges de la figure 5). Cependant ces flux sont encore insuffisants pour assurer un brassage génétique suffisant pour éviter la hausse de la consanguinité sur chacun de ces sites. Cette augmentation persistante de la consanguinité pourrait être atténuée par un renforcement des populations de ces sites (augmentation du nombre de gentianes et meilleure circulation des individus aux seins des Narcettes et de la cham de Chabreille)
- La construction de corridor (ici considéré comme ayant un couvert végétal de classe 2 (lande à calune)) semble favoriser les échanges, d'azurés des mouillères entre les Narcettes et la cham de Chabreille. Il est donc probable que la construction de corridors similaires entre la cham de Vernède et les granges de la Rouveyrette aient des effets similaires (signalé par la ligne discontinue rouge sur la figure 5). Par contre, la construction de corridors dans le but de connecter la cham du Jou au reste du réseau risque d'être inefficace car les distances à franchir sont importantes et supérieures aux capacités de déplacement de l'azuré des mouillères. Il faudrait donc associer la construction/aménagement des corridors à la création de patchs relais (signalé par les pointillés rouges). Le cas échéant il serait aussi nécessaire d'améliorer la qualité du milieu sur la cham du Jou afin que les populations d'azuré des mouillères puissent s'y installer de façon pérenne (cercles en ligne discontinue sur la figure 5).

L'ensemble des propositions faites ici (figure 5) pourront faire l'objet d'une étude de comparaison de *scenarii* d'aménagement avec MetaConnect.

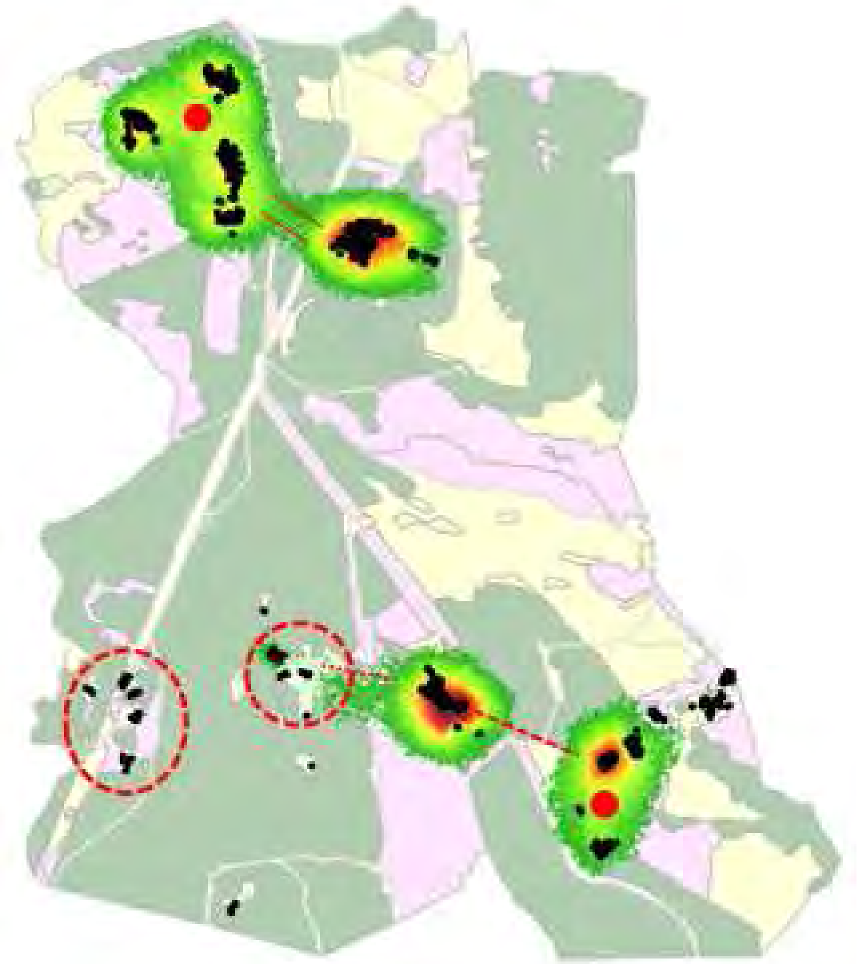


Figure 5 : aménagements et priorisations d'aménagement susceptibles d'améliorer la viabilité et la connectivité entre les tourbières du plateau de Montselgues. Les cercles rouges pleins correspondent à la localisation de zones relais qui permettraient de consolider les populations et les échanges au sein des Narcettes et des granges de la Rouveyrette (zones pour lesquelles il serait nécessaire de favoriser le développement des gentianes). Les cercles en lignes discontinues rouges correspondent aux deux secteurs de la cham du Jou qui nécessiteraient des aménagements visant à améliorer la qualité du milieu pour l'azuré des mouillères (pour pouvoir accueillir des populations viables d'azurés). Du point de vue des restaurations de connectivité, le corridor entre les Narcettes et la cham de Chabreille est signalé par les traits pleins rouges. Il semblerait prioritaire de restaurer la connectivité entre les granges de la Rouveyrette et la cham de Vernède (lignes discontinue rouge) afin de renforcer les populations de ces deux sites. La restauration de la connectivité entre la cham de Vernède et la cham du Jou semble moins prioritaire (ligne pointillée rouge) dans la mesure où les populations la cham du Jou ne sont pas viables et nécessiteraient dans un premier temps une amélioration de la qualité du milieu.

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