UNIVERSIDADE DE LISBOA
FACULDADE DE CIÊNCIAS
DEPARTAMENTO DE BIOLOGIA ANIMAL

ECOLOGICALLY SCALED RESPONSES OF FOREST-DWELLING VERTEBRATES TO HABITAT FRAGMENTATION

Fernando Jorge Portela Martins Ascensão

Doutoramento em Biologia

(Biologia da Conservação)

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«…do we think that we, as landscape-oriented animal ecologists, can beat the weatherman when it comes to predictions? (…) what makes us believe that we should be able to do much better? (…) at some general level of explanation, ecologists may have predictive power regarding the effects of fragmentation, but complexity is likely to make prediction of specifics difficult or impossible. »

in Bissonette and Storch (2002)

NOTA PRÉVIA

Na elaboração desta dissertação são usados resultados de trabalhos já publicados ou submetidos para publicação em revistas científicas internacionais indexadas. Uma vez que estes trabalhos foram realizados em colaboração com outros investigadores, e de acordo com o disposto nos termos do nº 1 do Artigo 45 do Regulamento de estudos Pós-Graduados da Universidade de Lisboa, publicado no Diário da República – 2ª Série nº 65, de 30 de Março de 2012, esclareço que participei integralmente na concepção, obtenção, análise e discussão dos resultados, bem como na redacção dos manuscritos dos capítulos 2 a 5.

Lisboa, 2 de Novembro de 2012

Fernando Ascensão

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ABSTRACT

Road habitat fragmentation has been identified as a major threat for biodiversity conservation. Roads induce a 'barrier effect' by representing a physical obstacle or promoting animal-vehicle collisions, disrupting daily and seasonal movements and consequently leading to the depletion and genetic subdivision of animal populations. However, road verges may also provide resources for some species. This thesis aimed to test for an ecologically scaled response of wildlife to road induced habitat fragmentation. The hypothesis under study is that the biological and ecological traits are determinant in predicting the road effects on species persistence. I expected to detect more pronounced negative road-related effects on species with larger body size, lower reproductive rate and greater mobility. To test this hypothesis I analyzed the responses to the road barrier effect by two model species, the wood mouse (*Apodemus sylvaticus*) and the stone marten (*Martes foina*), accounting for both potential benefits and negative consequences for species persistence. The major goal was to contribute for a sound scientific basis of road impacts on wildlife and generate advice for improving existing management practices that could benefit the maximum number of species, striving towards a better conciliation of road networks with biological conservation. I estimated the abundance of small mammals inhabiting road verges; quantified the gene flow across roadsides for wood mouse; assessed how roads affect the space use and movement of stone martens living in close vicinity of a highway; and modeled the effectiveness of wildlife-vehicle collision mitigation measures directed to medium-sized carnivores. Consistent evidences were obtained that the species' responses to roads follow an ecological scale, where small mammals may benefit with road verge presence, although being more vulnerable to genetic isolation; and medium-sized carnivores are more prone to population depletion. I concluded that this latter group should be the focus of road mitigation plans. Highways should be upgraded with exclusionary fence impermeable to carnivores and larger species, in the most part of their length, combined with a sufficient number of open-span structures. The number of open-span passages to be installed or upgraded should consider not only the connectivity at the local scale but also at the landscape perspective in order to guarantee that the accessible habitat area is sufficient to host viable populations.

Keywords: road ecology, road verges, genetic differentiation, radio-tracking, agent-based models, road mitigation.

RESUMO

A fragmentação dos habitats resultante da presença de estradas e tráfego associado tem sido identificada como uma das principais ameaças à conservação da biodiversidade. As estradas provocam um 'efeito de barreira', uma vez que bloqueiam as deslocações diárias ou sazonais de múltiplas espécies. Este bloqueio faz-se sentir tanto pela colisão dos animais com os veículos, como pelo facto de as estradas e estruturas associadas poderem representar um obstáculo físico à passagem dos animais. Por outro lado, é sabido que as bermas das estradas podem proporcionar recursos a algumas espécies, como abrigo e alimento para invertebrados e pequenos mamíferos.

Esta tese teve como principal objectivo testar se existe uma escala ecológica nas respostas dos organismos à fragmentação dos habitats pelas estradas. A hipótese em estudo considera que as características biológicas e ecológicas das espécies são determinantes para prever os principais efeitos das estradas e o seu impacto na persistência dessas espécies. Era esperado um efeito negativo mais pronunciado das estradas em espécies com maior tamanho, menor taxa reprodutora e maior mobilidade. Para testar esta hipótese, analisei as respostas de duas espécies-modelo ao efeito-barreira resultante de autoestradas: rato-do-campo (*Apodemus sylvaticus*) e a fuinha (*Martes foina*). Nesta tese é assumido que estas duas espécies-modelo representam grupos ecológicos cujas características biológicas (e.g. tamanho, locomoção), ecológicas (e.g. habitat) e (in)tolerância às estradas são relativamente homogéneas. Desta forma, as espécies modelos representam respectivamente roedores e carnívoros de médio porte.

Estas espécies são generalistas, embora geralmente associadas a áreas florestais e em particular ao montado. Sendo generalistas, estas espécies podem fazer um uso

mais amplo da matriz envolvente às manchas de habitat favorável (montado). Assim sendo, considera-se que qualquer resposta destas espécies modelo relativamente à presença de estradas será mais intensificada em espécies com estatuto de conservação mais desfavorável. Por exemplo, a fuinha e gato-bravo (*Felis sylvestris*) partilham muitas características biológicas e ecológicas, mas a segunda espécie tem maiores 'exigências' relativamente à qualidade do habitat.

A tese é composta por quatro estudos, cada um focando um tema diferente mas complementares no seu todo para a compreensão da hipótese em análise. No primeiro estudo as abundâncias de pequenos mamíferos foram estimadas nas bermas das autoestradas A2 e A6 (Alentejo, Portugal) e nas áreas envolventes a estas infra-estruturas. Foi feita também uma comparação da estrutura da vegetação entre os diferentes tipos de habitat amostrados e uma modelação de como a estrutura da vegetação pode influenciar a presença das espécies nas bermas. Foram amostrados 36 sítios, entre os quais onze troços das autoestradas. Um total de 351 indivíduos foi capturado, incluindo 157 ratos-do-campo. Os dados sugerem que as vedações das autoestradas ao evitar o pastoreio permitem que a vegetação cresça e forneça boas condições para a presença de pequenos mamíferos, nomeadamente alimento, abrigo e proteção de predadores.

No segundo estudo examinei a importância relativa do volume de tráfego e da idade da autoestrada no processo de isolamento genético das populações do rato-do-campo. Foram recolhidas amostras de 155 indivíduos genotipadas para nove microssatélites. Foram feitas análises ao nível da estruturação genética, presença de *clusters* recorrendo a técnicas Bayesianas e multivariada, número de migrantes, e parentesco. Os dados foram depois comparados com um modelo de simulação espacialmente explícito para testar qual o nível de permeabilidade da autoestrada aos movimentos do rato do campo mais provável.

Os níveis de diferenciação genética e parentesco revelaram algum isolamento entre os indivíduos dos dois lados da estrada, sugerindo que as autoestradas diminuem significativamente o movimento dos animais entre os lados da estrada relativamente ao cenário onde a autoestrada não está presente.

No terceiro estudo foi feita uma avaliação da influência da autoestrada A6 no movimento e uso do espaço pela fuinha. Dados de telemetria relativos a sete indivíduos foram analisados num quadro individual. Mais uma vez os resultados foram comparados com um modelo de simulação espacialmente explícito para testar se os movimentos e o uso do espaço foram de alguma forma constrangidos pela presença da auto-estrada. Todas as fuinhas exploraram as áreas adjacentes à autoestrada, sem que nenhuma tenha mostrado uma preferência por zonas da berma com uma estrutura de vegetação mais desenvolvida. Quatro fuinhas cruzaram regularmente a autoestrada, sendo que as suas áreas vitais eram atravessadas pela mesma. Os dados sugerem que o local onde as fuinhas cruzam a autoestrada é influenciado pelo tipo de actividade em curso, a distância à passagem mais próxima e se a fuinha é residente (tem percepção da presença de veículos e locais de cruzamento como as passagens hidráulicas).

No quarto estudo é feita uma análise recorrendo a um modelo de simulação espacialmente explícito para testar qual a melhor forma de mitigação dos atropelamentos de carnívoros de médio porte. Foram criados 125 cenários com diferentes combinações de percentagem de auto-estrada vedada (vedação impermeável à passagem de animais), número de passagens e probabilidade de atropelamento. A eficácia de cada cenário foi avaliada tendo em conta o tamanho da população e nível de diferenciação genético entre as populações dos dois lados da estrada, e comparando estes dados com o cenário onde a estrada não estava presente. Os resultados indicam que o principal factor que determina a diferenciação genética e o tamanho da população é a proporção de autoestrada que é vedada.

No seu conjunto, os quatro estudos fornecem evidências que a resposta das espécies à presença das estradas obedece a uma escala ecológica, onde os pequenos mamíferos beneficiam com a presença das bermas, embora sendo mais vulneráveis aos efeitos de isolamento (genético); e onde os carnívoros de médio porte são mais vulneráveis ao impacto por atropelamento e portanto à diminuiçaõ do tamanho das populações. De acordo com os resultados obtidos, os carnívoros de médio porte devem ser considerados como grupo prioritário em planos de mitigação do impacto das estradas em mamíferos.

Diversas medidas de gestão são sugeridas. Nas autoestradas (e noutras estradas com elevado tráfego) devem ser instaladas redes impermeáveis à passagem de carnívoros de médio porte. As bermas devem ser geridas de forma a proporcionar um habitat favorável à presença de pequenos mamíferos, em particular nas zonas onde a rede impermeável é instalada. Em complemento, devem ser implementadas áreas de exclusão de gado em zonas de sobrepastoreio, para que as populações de micromamíferos possam aí também persistir e proliferar. Estas áreas devem ser ligadas às bermas por elementos lineares naturais ou semi-naturais, como ribeiras e sebes, proporcionando assim uma vasta rede de corredores ecológicos para os pequenos mamíferos. Sendo presas preferênciais para muitos carnívoros, estas medidas de gestão poderão assim beneficiar um grande número de predadores e todo o ecossistema.

Em conjunto com a instalação da rede impermeável, devem ser implementadas ou melhoradas passagens que permitam o cruzamento das estradas por todas as espécies terrestres. Estas passagens devem consistir em viadutos sobre linhas de água, com vegetação abundante para diminuir o uso pelos humanos e aumentar a sua utilização pela fauna silvestre. O número de passagens a instalar deve ter em conta a dinâmica populacional, em particular a área necessária para uma população persistir.

Palavras-chave: road ecology, road verges, genetic differentiation, radio-tracking, agent-based models, road mitigation.

1. General Introduction

1.1. PROBLEM OVERVIEW

The degradation of habitats due to anthropogenic activities has resulted in a generalized loss and fragmentation of pristine areas (Mittermeier *et al.* 1998). Along with climate change, habitat loss and fragmentation have been identified as the primary contemporary threats to biodiversity conservation (Opdam & Wascher 2004; Fischer & Lindenmayer 2007; Araujo *et al.* 2011; Hodgson *et al.* 2012).

The debate focusing on the relative importance in driving population processes, between the available habitat area (or its loss) versus habitat fragmentation, generally suggests that the effects of fragmentation on species occurrence and extinction risk are weaker than the effects of habitat loss (Fahrig 1997). Nevertheless, habitat fragmentation is a major key driver in the dynamics of populations, as it affects the movement or flow of organisms and gametes, and therefore the reachability of resources across landscapes (Taylor *et al.* 1993). In fact, essential evolutionary and ecological processes depend on animal movement, including the ability of species to change or expand their ranges (Morales & Ellner 2002; Kokko & Lopez-Sepulcre 2006). For example, in the case of a metapopulation, separate subpopulations occupying suitable habitat patches undergo repeated extinction and recolonization events; long-term persistence of the metapopulation then arises from a balance between local extinction and (re)colonization through interpatch dispersal movements (Hanski 1998). Therefore, the study of habitat fragmentation effects remains a main topic for conservation biology and is also the core topic of this thesis.

The efforts by the world community for better understanding the fragmentation implications resulted in an immense scientific literature as well the enrolement of the citizens and politicals in the public discussion. Nonetheless, the rate of biodiversity loss does not appear to be slowing and much is due to the habitat fragmentation (Butchart *et al.* 2010). This may result from the resistance in implementing the required conservation measures, for example due to conflict of interests (Chan *et al.* 2006; Webb & Raffaelli 2008; McShane *et al.* 2011) or to an insufficient knowledge on the variable responses of species to habitat fragmentation (Harrison & Bruna 1999) that compromises its efficacy. This thesis intends to contribute to this latter case, aiming to advance the knowledge on how road-related fragmentation impact different ecological models; and how to effectively mitigate the impacts, by including in its conservation management actions the diversity of species responses.

1.2. DEFINING 'HABITAT FRAGMENTATION'

The awareness in the 'western world' of the negative effects of habitat fragmentation dates back to the 1960's, when the 'environmental awakening' began and people got consciousness that human activity could seriously damage the global environment (Haila 2002; Laurance 2008). This communal awakening was naturally linked with a better understanding of the effects of habitat fragmentation. It is widely agreed that the origin of habitat fragmentation concept in ecological research was initiated by MacArthur & Wilson (1963; 1967), with their theory of island biogeography. In their view, the destruction of native habitats results into progressively smaller and more isolated patches, immersed within an unsuitable human-modified matrix, similar to islands surrounded by the ocean.

Recent syntheses confirm that the characteristics of habitat patches (e.g. size, shape and isolation degree) play a primary role in determining how species are distributed in patchy landscapes (e.g. Ewers *et al.* 2007; Prugh 2009). However, this theory has been subjected to several criticisms given its misconception over the matrix-related characteristics and its implication on animal dispersal (see Laurance 2008) or suitability for species persistence (Pereira & Daily 2006). As a result of such, it has been improved in order to incorporate the role of dispersal and of the matrix suitability for species presence and movement (Pereira & Daily 2006; Watling *et al.* 2011), leading to new theories such as the metapopulation (Hanski 1994), source-sink (Pulliam 1988), percolation (Boswell *et al.* 1998) or graph (Urban & Keitt 2001) theories. A concise history of the research on 'habitat fragmentation' can be found in Harrison & Bruna (1999) and Haila (2002).

Meanwhile, the literature on habitat fragmentation and its effects in wildlife became numerous and diversified (for reviews see Saunders *et al.* 1991; Harrison & Bruna 1999; Debinski & Holt 2000; Haila 2002; Fahrig 2003; Ewers & Didham 2007; Fischer & Lindenmayer 2007). Nevertheless, given the amount of research literature, the term 'fragmentation' has also been ambiguously applied. As noted by Fischer et al. (2007), the progress in this field has been hampered by overly restrictive conceptual paradigms (Haila 2002) and the imprecise or inconsistent use of important terminology (Fahrig 2003). Today, the debate focus on whether explicit recognition of 'habitat fragmentation' as an overarching conceptual domain will stimulate or hinder further progress toward understanding and mitigating the effects of landscape change (Didham *et al.* 2012).

As pointed by Fahrig (2003), most researchers still view habitat fragmentation as a process involving the reduction in habitat amount, an increase in the number of habitat patches, a decrease in habitat patch size, and the increase in habitat patch isolation both due to the loss of habitat and the breaking apart of habitat patches (e.g. Andrén 1994). However, all these processes give rise, in turn, to different ecological responses and should therefore be considered separate (Forman 1995; Haila 2002; Fahrig 2003). As resumed by Forman (1995):

> *«Land is transformed by several spatial processes overlapping in order, including perforation, fragmentation and attrition, which increase habitat loss and isolation, but otherwise cause very different effects on spatial pattern and ecological process. (…) Perforation is the process of making holes in an object such as a habitat or land type (e.g., dispersed houses or fires in a forest). Dissection is the carving up or subdividing of an area using equal-width lines (e.g., by roads or power-lines). Fragmentation is the breaking of an object into pieces (that are often widely and unevenly separated). Shrinkage is the decrease in size of objects, and attrition is their disappearance. »*

The fact that most fragmentation research did not differentiate between these processes led to several problems. Not surprisingly, contradictory results were achieved if a researcher only studied some of these ecological processes as they have different effects in wildlife (Fahrig 2003). An obvious misperception is illustrated when 'habitat fragmentation' is used when addressing the size of remnants after the partial removal of adequate habitat. In this case, the term 'habitat loss' is more adequate (Haila 2002; Fahrig 2003). Ironically, this misperception possibly began in the early stages of habitat fragmentation research, when in their famous monograph MacArthur & Wilson (1967) illustrated the concept of habitat fragmentation with Curtis (1956) work on changing forests by grasslands, a clear case of 'habitat loss' (Haila 2002).

In this thesis I use the term 'fragmentation' to refer specifically to the breaking apart of habitat by roads, probably the most visible human-related source of habitat fragmentation (Forman *et al.* 2003). I considered that roads lead to a discontinuity on the spatial distribution of resources and conditions present in an area at a given scale, which in turn may affect the occupancy, reproduction and survival of species (Franklin *et al.* 2002).

1.3. ROADS AS A MAIN DRIVER OF HABITAT FRAGMENTATION

Roads and their associated traffic are known to promote a panoply of negative impacts on wildlife for nearly a century (see Stoner 1925). The urge of understanding and mitigating the road impacts on wildlife lead to a new research field named "Road Ecology" (Forman *et al.* 2003). The investigation has greatly evolved with the emergence of research centers dedicated to road ecology (e.g. Western Transportation Institute created at 1994 at Montana State University), a textbook (Forman *et al.* 2003) and several reviews on road ecological effects (e.g. Forman & Alexander 1998; Spellerberg 1998; Seiler 2001; Coffin 2007; Fahrig & Rytwinski 2009; Taylor & Goldingay 2010), including on genetic consequences due to road fragmentation (Balkenhol & Waits 2009; Holderegger & Di Giulio 2010).

This, together with regular scientific meetings focusing on wildlife and linear infrastructures (e.g. ICOET, IENE) has lead, in the recent years, to an unprecedented state of knowledge on road ecology. In Portugal, the research on road habitat fragmentation effects and mitigation is an emerging concern that has however already given fruitful steps (see review by Grilo 2012).

Among the most important negative impacts, roads are known to fragment the landscapes and habitats by inducing a usually denominated 'barrier effect' (Forman & Alexander 1998). This barrier effect can result from roads disrupting daily and seasonal movements, either due to roads functioning as a physical obstacle (Rondinini & Doncaster 2002; Marsh *et al.* 2005), or by hampering animal displacements due to road avoidance behavior (Dahle *et al.* 2008; McGregor *et al.* 2008) or social relations (Riley *et al.* 2006). Additionally, roads can act as barriers by promoting animal-vehicle collisions, thus eliminating would-be crossers (e.g. Carr & Fahrig 2001; Gibbs & Shriver 2005; Grilo *et al.* 2009).

The road barrier effect, with consequences for a wide range of species across all fauna, impedes the access to resources (e.g. mates, territories, food) and thus affects individual fitness, overall habitat quality and, at a long-term, population persistence. For example, at local scale road permeability is essential for maintaining the natural ranging behavior of animals when their foraging, resting or breeding sites are located in opposite roadsides. It is also crucial for allowing dispersal, thus guaranteeing for the exchange of genetic material among otherwise isolated populations.

At broad spatial and temporal scales, road permeability is also necessary to maintain the continuity of ecological processes and to allow natural range shifts in response to long-term environmental transitions, including ecological adaptation to global climate change (Heller & Zavaleta 2009). Ultimately, the road barrier effect may lead to a genetic subdivision of animal populations (see reviews by Holderegger & Di Giulio 2010), a process known to accelerate the loss of genetic variability due to random drift (Hartl & Clark 1997; Balkenhol & Waits 2009).

In turn, the resulting increased level of inbreeding may decrease the fecundity and increase the probability of local extinction (Saccheri *et al.* 1998; Westemeier *et al.* 1998; Reed *et al.* 2007). Overall, roads may lead to a population decline in the proximity of the infrastructure (Benítez-López *et al.* 2010) and a connectivity breakdown. This will lower the gene flow and consequently increase the probability for individuals to be genetically differentiated across road sides (Balkenhol & Waits 2009).

In spite of the above described negative impacts, a multitude of road ecology studies also report strong evidences that roads may provide beneficial effects to species (see reviews from Spellerberg 1998; Fahrig & Rytwinski 2009; Benítez-López *et al.* 2010). In fact, the interaction animal-road is complex with positive and negative effects coexisting even for the same species (Fahrig & Rytwinski 2009). One paradigmatic example is the effect of road verges. The potential conservation value of road verges has been recognized for decades (e.g. Way 1977). Similar to hedgerows and field margins, road verges are known to be important refuge habitats for many invertebrates and small-sized vertebrates, including ants (Tshiguvho *et al.* 1999), butterflies (Saarinen *et al.* 2005), bees (Hopwood 2008), beetles (Noordijk *et al.* 2009a; Noordijk *et al.* 2009b), birds (Meunier *et al.* 1999) or small mammals (Oxley *et al.* 1974; Bellamy *et al.* 2000). Road verges may also function as corridors linking habitat patches for many wildlife species (Bennett 1990; Vermeulen & Opdam 1995; Doncaster *et al.* 2001; Huijser & Clevenger 2006).

Given the recent expansion of the road network in the Iberian Peninsula, road ecology studies are becoming more necessary. Since the integration of Portugal and Spain in the European Union (EU), in 1986, both countries experienced a notably increase in this network, particularly that of highways (Fig. 1.1): from 196 km to 2,737 km in Portugal and from 2,154 km to 14,262 km in Spain (data for 2010; source Eurostat 2010, URL: http://nui.epp.eurostat.ec.europa.eu).

The responses to roads and traffic is expected to be highly variable among different species (Fahrig & Rytwinski 2009; Benítez-López *et al.* 2010). While it is impossible to assess the impacts and best mitigation measures for all species, it is nevertheless vital to understand to which ecological profiles the mitigation of the road negative effects is more critical. Also, it is essential to understand how we can take advantage of any positive consequence from the road presence, and for which ecological profile, in order to improve the conservation management plans.

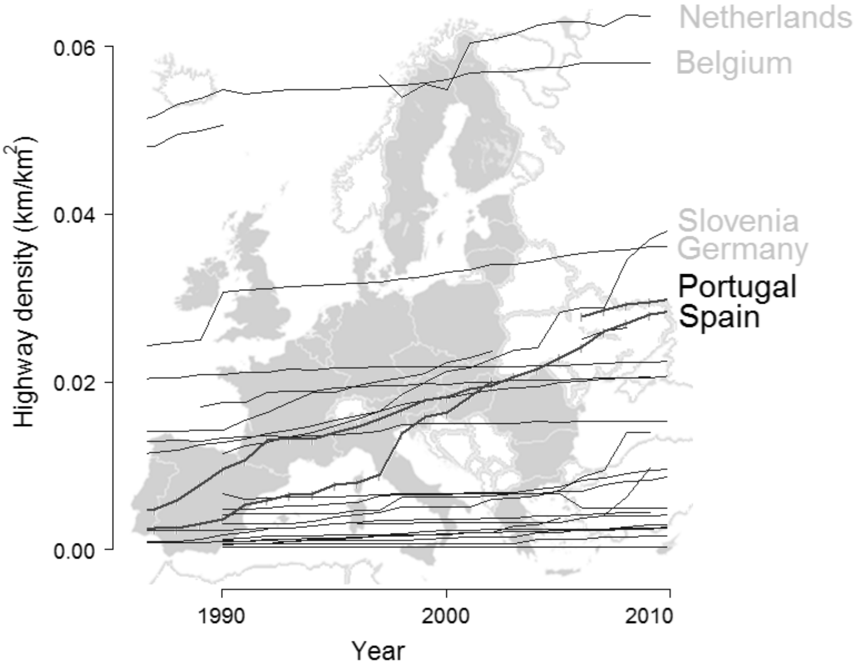

Fig. 1.1 – Highway density (Km/Km^2) for the 27 EU countries since 1986 (year that Portugal and Spain joined EU). Data for both Iberian countries is highlighted (source Eurostat 2010, http://nui.epp.eurostat.ec.europa.eu). Countries with higher highway density are indicated.

1.4. AN ECOLOGICALLY SCALED APPROACH

In order to explain interspecific differences in response to habitat fragmentation, Vos et al. (2001) proposed the use of *ecologically scaled landscape indices*. These indices presume that whether a given pattern of habitat is connected greatly depends on the species' ability to move through different land uses. Therefore, habitat fragmentation is expected to be a function of the species' *morphological/life history traits* and *behavioral responses* (Nupp & Swihart 1998, 2000; Virgós 2001; Vos *et al.* 2001; Goodwin & Fahrig 2002; Virgós *et al.* 2002; Gehring & Swihart 2003; Turner 2005). The same rationale can be applied when studying the ability of species to cope with the road presence and associated traffic. In fact, these two criteria – *morphological/life history traits* and *behavioral responses* – have been used to characterize the species most negatively affected by roads (Rytwinski & Fahrig 2012).

The first set of criteria (*morphological/life history traits*) suggests that, in general, larger species should be more negatively affected by roads than smaller species. This is so because larger species are more mobile than smaller ones, generally occur at lower densities, and have lower reproductive rates and longer generation times (Rytwinski & Fahrig 2012). As larger species are often predators of smaller species, it is also possible that negative effects of roads on populations of large animals could lead to reduced predation on small animals in areas of high road density, and thus indirectly reduce the impact of roads on smaller animals (Rytwinski & Fahrig 2012).

The second set of criteria (*behavioral responses*), suggests that species behavioral responses to roads and traffic are the main drivers that moderate the population-level effects of roads. Two important works, by Jaeger *et al.* (2005) and Fahrig & Rytwinski (2009), considered four main behavioral responses of animals towards roads:

i) *Avoidance of the road pavement* (*or roads functioning as a physical barrier*), which reduces the animal mortality on roads but also reduces the accessibility to habitats and other resources;

ii) *Avoidance of traffic disturbance* (noise, lights, chemical emissions), similar to the previous response except that it is expected to vary with the level of disturbance. For example, the higher the amount of traffic on the road the more habitat is effectively lost to the species;

iii) *Vehicle avoidance*, where animals are able to move out of the path of an oncoming vehicle;

iv) *Attraction to the road*, which is expected to increase the frequency with which animals enter the road and consequently their mortality risk.

The first two responsive behaviors make species less susceptible to traffic mortality but inversely may cause population subdivision into smaller and partially isolated population units that are more vulnerable to extinction. This is the case for species that clearly avoid the road pavement (e.g. McGregor *et al.* 2008) because the road will remain a barrier to movement even when there is no traffic. This was referred as "fence effect" by Jaeger & Fahrig (2004). Species sensitive to traffic disturbance will suffer an additional loss of habitat (at a distance beyond the road itself) since the habitat near the road becomes unusable or of lower quality (Eigenbrod *et al.* 2008).

On the other hand, species that are able to avoid oncoming vehicles should have low road mortality and should be able to cross the road when traffic volumes are not too high. Consequently, roads should be less detrimental for these species than for those in the first two avoidance categories. Some species however, may be attracted to a road in response to the presence of a given resource such as food (road-killed animals, e.g. scavenging raptors), nesting sites (e.g. turtles) or heat to thermoregulate (e.g. snakes) (see Rytwinski & Fahrig 2012 for examples and respective references). Species that are attracted to roads or that move onto roads irrespective of traffic are more susceptible to road mortality (Forman *et al.* 2003) unless they are also able to avoid oncoming vehicles (vehicle avoidance).

In spite the handful of studies focusing the behavioral responses of species to roads [in a recent review Rytwinski & Fahrig (2012) summarized the behavioral responses of seventeen *taxa* to roads], there is still a lack of data to allow robust inferences and detection of general patterns. In this thesis I considered the first set of criteria above described when addressing my main research question - IS THERE A RELATION BETWEEN SPECIES' *MORPHOLOGY/LIFE HISTORY TRAITS* AND THE EFFECTS OF ROADS?

1.5. FROM INFERENCES TO PATTERNS: AGENT-BASED MODELS AS A TOOL IN CONSERVATION BIOLOGY

As referred by McGarigal & Cushman (2002), inferences on fragmentation effects on wildlife need to be replicated across an adequate sample of experimental units, in order to obtain a reliable and consistent cause-effect pattern. This can be extremely difficult when applied to road ecology studies, given the need of studying areas with identical environmental conditions and similar road characteristics. Moreover, collecting data of species responses to road fragmentation, particularly for wide ranging species, is often extremely costly and time consuming (Holderegger & Di Giulio 2010).

To circumvent these constrains, in three papers from this thesis I made use of agent-based modeling (ABM). ABM is a simulation technique that considers individuals in a given landscape as agents and implements some of their life history variability, individual resource use and behaviors into a coherent model to solve or better understand a given research problem. In contrast with classical theoretical ecology, which typically works top-down, stops at the population level and does not consider the individual variability of the species, ABM is a bottom-up approach that explicitly considers the components of a system (e.g. individuals as agents and landscape units as cells) and attempts to understand how the system's properties emerge from the interactions among these components (DeAngelis & Mooij 2005; Railsback & Grimm 2011).

This emphasis on interactions between agents and their environment allows the use of ABMs for exploring complex phenomena that are ill-suited to classic analytic approaches (Grimm *et al.* 2005; Barton *et al.* 2009; Grimm & Railsback 2012). Hence, ABM can be a valuable tool in connectivity research (Barton *et al.* 2009; Palmer *et al.* 2011). However, although ABMs have been used for some time in ecological studies (DeAngelis & Mooij 2005; Grimm *et al.* 2005; Railsback & Grimm 2011) and their versatility and utility in modeling animal movement is well known (McLane *et al.* 2011), they have been scarcely applied in connectivity research, including in road-related studies (but see Kramer-Schadt *et al.* 2004; Revilla *et al.* 2004; Wiegand *et al.* 2005; Revilla & Wiegand 2008; Pe'er *et al.* 2011).

1.6. RATIONALE OF THE THESIS

As mentioned previously, there are strong evidences that species with larger body size, lower reproductive rate and greater mobility are most negatively affected by roads and/or traffic. These evidences were recently supported in the meta-analyses performed by Benítez-López *et al.* (2010) and Rytwinski & Fahrig (2011; 2012). However, their focus was on road effects on population size/density and, as referred in previous sections, another main effect linked to road fragmentation involves the genetic differentiation amid roadside populations (Balkenhol & Waits 2009; Holderegger & Di Giulio 2010).

In order to assess how the *morphology/life history traits* of species is likely to be related to these two main road negative effects (population depletion and genetic differentiation), and thus give support to my thesis rationale, I performed an exploratory ordination analysis making use of available information for a large set of non-flying terrestrial mammal species. The basis were the recent reviews of road effects on population size (Fahrig & Rytwinski 2009) and genetic differentiation (Balkenhol & Waits 2009; Holderegger & Di Giulio 2010) to which I added information regarding biological and ecological traits obtained from Jones *et al.* (2009) (Tables 1.1 and 1.2).

Table 1.1 - Variables used in PCA. Variable codes are the same as presented in Jones *et al.* (2009). Variable definitions are adapted from Jones *et al.* (2009).

Variable name	Variable code	Variable Definition	Units
Weight	5-1_AdultBodyMass	Body mass	g
Length	13-1_AdultHeadBodyLen	Total length from tip of nose to anus or base of tail of adult	mm
AgeFirstBirth	3-1_AgeatFirstBirth	Age at which females give birth to their first litter	Day
HomeRange	22-1_HomeRange	Size of the area within which everyday activities of individuals or groups (of any type) are typically restricted	Km^2
LitterSize	15-1_LitterSize	Number of offspring born per litter per female	Nº of offspring
LittersPerYear	16-1_LittersPerYear	Number of litters per female per year	N º of litters
Longevity	17-1_MaxLongevity	Maximum adult age	Month
Density	21-1_PopulationDensity	Number of individuals	N /Km^2

When more than one effect was reported by Fahrig and Rytwinski (2009), I reclassified as follows: 'neutral/negative' was set to 'negative' and 'neutral/positive' was set to 'positive' - the assumption was to favor any evidence for a positive or negative effect. To summarize the data, a Principal Component Analysis was then applied using the R package 'ade4' (Dray & Dufour 2007). Results are presented in Fig. 1.2.

Table 1.2 - Data used in PCA using species' *morphology/life history traits* variables. Only mammal species were considered. PopSize: effect on population size, adapted from Fahrig and Rytwinski (2009); GenDiff: effect on genetic differentiation, compiled from Balkenhol and Waits (2009) and Holderegger and Di Giulio (2010). Class: species were grouped into four classes according to their weight. Remain variables refer to species' weight, length, home range, litter size, litters per year, longevity and density, respectively, from Jones et al. (2009). See Table 1.1 for details on these variables. Species are sorted by weight.

Order	Scientific name	PopSize	GenDiff	Class	Weight	Length	AgeBirth	HR	LitterSize	LittYear	Longevity	Density
Rodentia	*Peromyscus leucopus*	positive	--	1	1.8E+01	9.5E+01	1.1E+02	8.0E-04	4.3E+00	4.5E+00	3.8E+01	1.4E+03
Rodentia	*Mus musculus*	positive	--	1	1.9E+01	--	1.4E+02	4.8E-04	5.5E+00	4.5E+00	7.2E+01	4.9E+03
Rodentia	*Chaetodipus formosus*	neutral	--	1	2.0E+01	--	--	--	5.3E+00	--	--	--
Rodentia	*Clethrionomys gapperi*	--	yes	1	2.0E+01	1.0E+02	--	4.3E-04	5.3E+00	2.4E+00	2.0E+01	1.2E+03
Rodentia	*Peromyscus maniculatus*	positive	yes	1	2.0E+01	9.6E+01	8.7E+01	1.8E-03	4.8E+00	2.6E+00	--	6.6E+02
Rodentia	*Clethrionomys glareolus*	--	yes	1	2.1E+01	1.1E+02	8.3E+01	8.6E-04	4.2E+00	3.5E+00	5.9E+01	3.7E+03
Rodentia	*Ochrotomys nuttalli*	positive	--	1	2.3E+01	9.2E+01	--	3.0E-03	2.6E+00	--	3.0E+01	3.0E+02
Rodentia	*Peromyscus boylii*	negative	--	1	2.4E+01	--	--	2.5E-03	3.1E+00	--	1.8E+01	5.3E+02
Rodentia	*Microtus arvalis*	--	no	1	2.7E+01	1.1E+02	4.9E+01	4.8E-04	4.9E+00	6.0E+00	3.6E+01	1.8E+04
Rodentia	*Dipodomys merriami*	neutral	--	1	3.8E+01	--	9.7E+01	1.6E-03	2.5E+00	1.8E+00	2.4E+01	3.7E+02
Rodentia	*Microtus ochrogaster*	positive	--	1	4.3E+01	1.2E+02	--	2.4E-04	3.8E+00	4.1E+00	--	9.1E+03
Dasyurom.	*Antechinus flavipes*	--	no	1	4.5E+01	--	--	--	8.5E+00	1.0E+00	4.2E+01	6.9E+02
Rodentia	*Dipodomys microps*	positive	--	1	5.6E+01	1.1E+02	--	3.0E-02	2.4E+00	1.0E+00	--	1.2E+03
Rodentia	*Microtus californicus*	positive	--	1	5.7E+01	1.4E+02	--	5.8E-05	4.3E+00	5.0E+00	--	1.3E+04
Rodentia	*Tamias ruficaudus*	--	yes	1	6.0E+01	1.2E+02	3.7E+02	--	4.9E+00	1.0E+00	9.6E+01	--
Rodentia	*Tamias striatus*	positive	--	1	--	1.5E+02	--	--	--	--	--	--
Rodentia	*Ammospermophilus leucurus*	neutral	--	1	1.0E+02	1.5E+02	--	4.0E-02	8.2E+00	1.3E+00	7.0E+01	1.5E+01
Rodentia	*Rattus rattus*	neutral	--	1	1.4E+02	1.8E+02	1.4E+02	2.1E-03	5.9E+00	3.8E+00	5.0E+01	2.7E+03
Rodentia	*Neotoma lepida*	neutral	--	1	1.4E+02	1.6E+02	--	1.0E-03	2.7E+00	3.5E+00	--	6.7E+02
Rodentia	*Sciurus lis*	--	yes	1	2.6E+02	--	--	1.1E-01	--	--	--	--
Rodentia	*Cynomys ludovicianus*	positive	--	2	8.0E+02	2.9E+02	--	1.7E-03	4.5E+00	1.0E+00	1.0E+02	1.8E+03

Order	Scientific name	PopSize	GenDiff	Class	Weight	Length	AgeBirth	HR	LitterSize	LittYear	Longevity	Density
Erinaceom.	*Erinaceus europaeus*	negative	--	2	8.0E+02	2.4E+02	3.5E+02	7.0E-02	4.6E+00	1.5E+00	1.7E+02	1.2E+02
Carnivora	*Martes Americana*	neutral	--	2	8.8E+02	3.8E+02	3.7E+02	3.3E+00	2.6E+00	--	2.3E+02	6.7E-01
Lagomorpha	*Lepus europaeus*	negative	--	2	3.8E+03	5.3E+02	--	3.1E-01	2.2E+00	4.4E+00	1.4E+02	--
Carnivora	*Vulpes vulpes*	negative	--	2	4.8E+03	6.3E+02	--	3.1E+00	4.6E+00	--	1.8E+02	1.1E+00
Carnivora	*Lynx rufus*	negative	yes	2	6.4E+03	6.9E+02	5.5E+02	2.8E+01	2.8E+00	--	3.9E+02	1.0E-01
Diprodontia	*Phascolarctos cinereus*	negative	--	2	6.6E+03	--	7.3E+02	1.0E-02	1.0E+00	1.0E+00	2.4E+02	1.6E+02
Carnivora	*Canis mesomelas*	negative	--	2	8.3E+03	7.1E+02	--	1.1E+01	3.6E+00	--	1.7E+02	7.4E-01
Carnivora	*Lynx pardinus*	negative	--	2	1.1E+04	9.7E+02	--	6.4E+00	2.5E+00	--	--	3.2E-01
Carnivora	*Meles meles*	negative	--	2	1.2E+04	7.1E+02	--	1.2E+00	3.0E+00	--	1.9E+02	2.5E+00
Carnivora	*Canis latrans*	--	yes	2	1.2E+04	8.8E+02	3.7E+02	1.7E+01	5.7E+00	--	2.6E+02	2.5E-01
Carnivora	*Lynx lynx*	negative	--	2	1.9E+04	8.6E+02	--	1.2E+02	2.3E+00	1.0E+00	3.2E+02	2.0E-02
Artiodactyla	*Pecarita jacu*	neutral	--	3	2.1E+04	8.8E+02	3.5E+02	1.7E+00	2.0E+00	--	2.9E+02	7.9E+00
Artiodactyla	*Capreolus capreolus*	negative	yes	3	2.2E+04	--	7.3E+02	4.5E-01	1.8E+00	--	2.0E+02	7.7E+00
Artiodactyla	*Tayassu pecari*	neutral	--	3	3.1E+04	1.1E+03	--	9.9E+00	2.0E+00	--	2.5E+02	1.6E+00
Carnivora	*Canis lupus*	negative	--	3	3.5E+04	1.1E+03	5.5E+02	2.2E+01	5.0E+00	2.0E+00	3.5E+02	1.0E-02
Artiodactyla	*Catagonus wagneri*	negative	--	3	3.5E+04	1.0E+03	7.3E+02	7.4E+00	2.5E+00	--	1.1E+02	9.2E+00
Artiodactyla	*Redunca redunca*	negative	--	3	4.3E+04	1.1E+03	--	--	1.0E+00	--	2.2E+02	1.0E+00
Artiodactyla	*Aepyceros melampus*	neutral	--	3	5.2E+04	1.4E+03	7.3E+02	2.2E+00	1.0E+00	--	2.1E+02	1.3E+01
Carnivora	*Puma concolor*	negative	no	3	5.4E+04	1.4E+03	6.2E+02	1.2E+02	2.7E+00	7.5E-01	2.4E+02	2.0E-02
Artiodactyla	*Ovis canadensis*	--	yes	3	7.5E+04	1.6E+03	3.0E+02	1.6E+01	1.0E+00	--	2.9E+02	1.5E+00
Artiodactyla	*Phacochoerus africanus*	negative	--	3	8.3E+04	1.4E+03	--	--	--	--	1.8E+02	--
Artiodactyla	*Odocoileus hemionus*	negative	--	3	8.4E+04	1.5E+03	5.5E+02	2.0E+00	1.6E+00	--	2.6E+02	1.6E+01
Artiodactyla	*Sus scrofa*	negative	--	3	8.5E+04	1.4E+03	3.1E+02	5.4E+00	4.6E+00	1.5E+00	2.5E+02	3.6E+00
Artiodactyla	*Rangifer tarandus*	negative	--	4	1.1E+05	2.2E+03	--	2.4E+03	1.0E+00	--	2.4E+02	2.6E+00
Carnivora	*Ursus americanus*	--	no	4	1.1E+05	1.4E+03	1.8E+03	2.8E+01	2.3E+00	--	3.8E+02	7.3E-01

Order	Scientific name	PopSize	GenDiff	Class	Weight	Length	AgeBirth	HR	LitterSize	LittYear	Longevity	Density
Carnivora	*Panthera tigris*	negative	--	4	1.6E+05	1.8E+03	1.2E+03	5.9E+01	2.5E+00	1.0E+00	3.2E+02	3.0E-02
Artiodactyla	*Connochaetes taurinus*	negative	--	4	2.0E+05	2.0E+03	7.3E+02	--	1.0E+00	--	2.6E+02	7.8E+00
Carnivora	*Ursus arctos*	negative	no	4	2.0E+05	1.5E+03	1.5E+03	2.8E+02	2.0E+00	--	6.0E+02	2.0E-02
Artiodactyla	*Cervus elaphus*	--	yes	4	2.4E+05	2.1E+03	9.9E+02	5.1E+01	1.0E+00	--	3.2E+02	2.9E+00
Artiodactyla	*Equus quagga*	negative	--	4	4.0E+05	--	--	--	--	--	2.4E+02	--
Artiodactyla	*Alces alces*	negative	--	4	4.8E+05	2.7E+03	1.2E+03	6.4E+01	1.3E+00	1.7E+00	3.2E+02	4.0E-01
Artiodactyla	*Taurotragus oryx*	negative	--	4	5.6E+05	2.7E+03	9.6E+02	3.5E+01	1.0E+00	--	2.8E+02	1.6E+00
Artiodactyla	*Giraffaca melopardalis*	neutral	--	4	9.6E+05	4.2E+03	1.8E+03	8.4E+01	1.0E+00	1.0E+00	4.4E+02	1.2E+00
Proboscidea	*Loxodonta africana*	negative	--	4	3.9E+06	--	3.5E+03	1.2E+03	1.0E+00	--	9.6E+02	6.7E+00

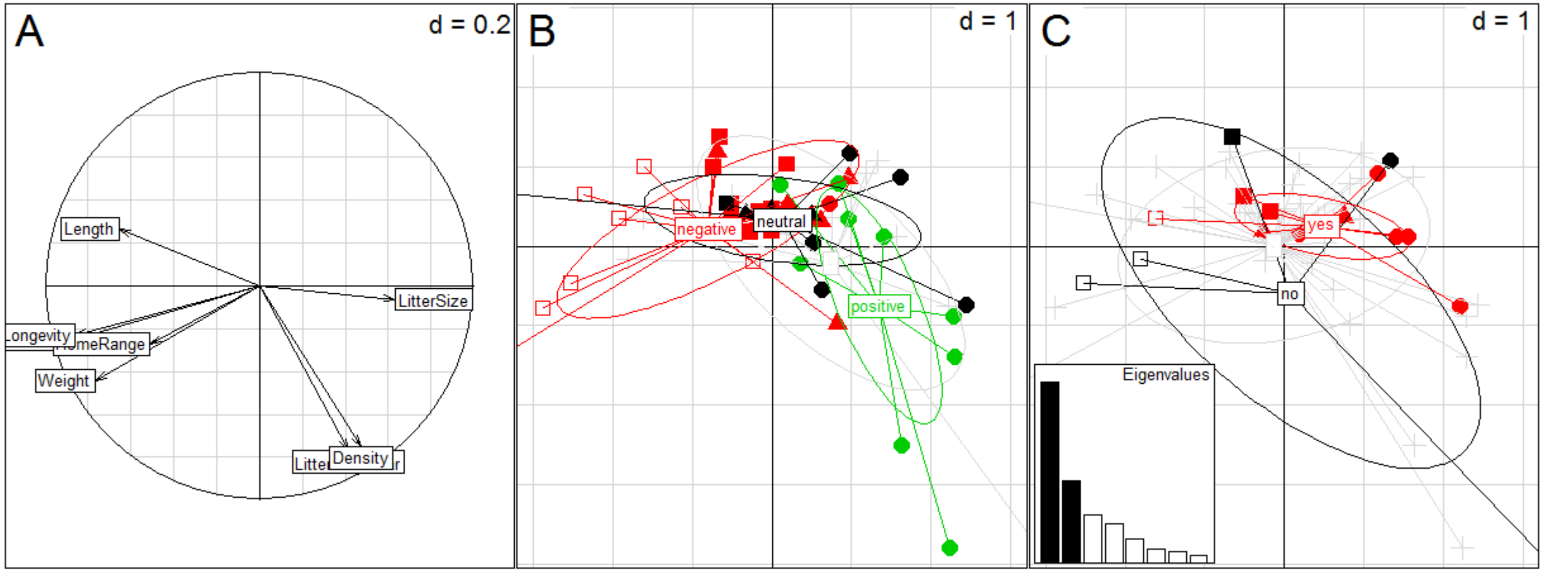

Fig. 1.2 - Results from a PCA including *morphology/life history traits* of mammal species. The two Principal Components account for 44.6% and 20.2% of total variation. A – Correlation circle plot showing the relationships among variables.Each variable is shown as a vector, which signals the combined strength of the relationships between the measured variable and the two Principal Components (vector length), and whether these relationships are positive or negative (vector direction). The angle between two vectors signals the degree of correlation between two measured variables. A right angle indicates that two variables are completely uncorrelated; zero or 180 degrees between two variables indicates complete positive or negative correlation. B and C – Scatter diagrams representing the species ordination, colored according to the road effect on population size ("positive", "neutral" and "negative") in B; and genetic differentiation ("yes" or "no") in C. In B and C, circles, triangles, filled squares and empty squares stand for small, medium, large and very large mammals (class 1 to 4 in Table 2.1), respectively. Species for which no data is available for one of the effects are represented by a cross and in grey color. Barplot of PCA eigenvalues is presented in C.

Results obtained suggest an overall pattern of road effect on population size, where smaller species may benefit from the road presence, while larger species are more negatively affected. This finding is concordant with previous studies by Fahrig & Rytwinski(2009) and Rytwinski & Fahrig (2011, 2012) and suggests a relation between species' ecological traits and their response to roads (Fig. 1.2, panel B). Nevertheless, no pattern emerges when focusing on road genetic differentiation effects (Fig. 1.2, panel C).

1.7. MAIN AIMS AND OUTLINE OF THE THESIS

This thesis aimed to test for an ecologically scaled response of wildlife to road induced habitat fragmentation. The hypothesis under study is that the biological and ecological traits are determinant in predicting the road effects on species persistence. In particular, I expected to detect more pronounced negative road-related effects on species with larger body size, lower reproductive rate and greater mobility.

To test this hypothesis I analyzed the responses of two model species to the road barrier effect, accounting for potential benefits and negative effects for species persistence resulting from the road presence. The two model species were the wood mouse (*Apodemus sylvaticus* Linnaeus, 1758) and the stone marten (*Martes foina* Erxleben, 1777) (see following section). Accordingly, I expected to detect more pronounced negative road-related effects on the stone marten relatively to the wood mouse. The overall scheme of the research conducted is illustrated in Fig. 1.3.

The first experiment aimed to compare the abundance of small mammals inhabiting road verges comparatively to that found in main land uses of southern Portugal (using small mammals as target species, wood mouse included – Chapter 2). In the second study I used genetic-based analyses to assess the gene flow across roadsides and the level of barrier effect for wood mouse (Chapter 3). Regarding stone marten research, by combining radio-tracking and agent-based modeling, I firstly assessed how roads affect the space use and movement of individuals living in close vicinity of the roads (Chapter 4). In the subsequent chapter I modeled the effectiveness of wildlife-vehicle collision mitigation measures directed to medium-sized carnivores (Chapter 5).

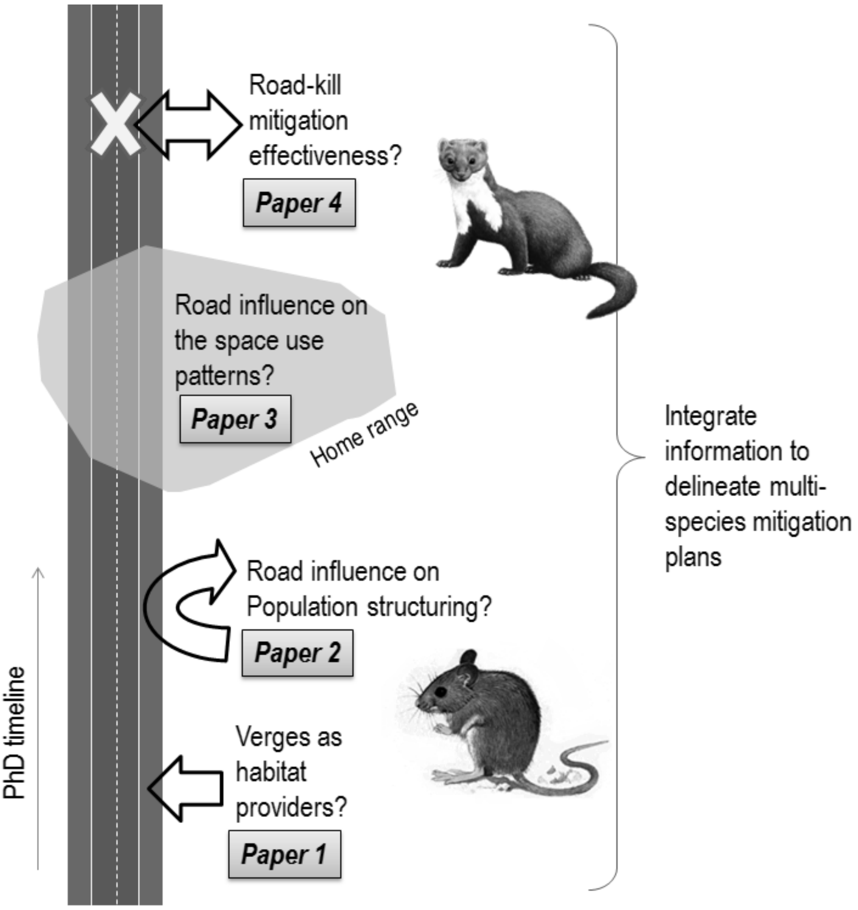

Fig. 1.3 – Overall scheme of the thesis: two mammal species, representing different morphological and life-history traits, were studied in order to assess their responses to road-induced habitat fragmentation. Response assessment included the study of verges as alternative habitat for small mammals, the genetic structuring of populations for wood mouse, the road influence on space use and movement patterns of stone marten and the effectiveness of mitigation measures to diminish the number of road-kills of stone marten. This information is integrated and discussed in Chapter 6.

The goal in studying the processes lying beneath the road barrier effect upon ecologically distinct species was to contribute to a holistic perspective of road-wildlife interaction. Ultimately, I intended to improve the knowledge regarding the effectiveness of road mitigation procedures towards the upgrade of landscape connectivity, in a multi-species angle. The specific objectives and main methodological approaches of each experiment/analytical approach are further described in the respective paper/chapter.

1.7.1. LIST OF PAPERS

This thesis comprises the papers listed below, corresponding to chapters two to five:

Ascensão F, Clevenger AP, Grilo C, Filipe J, Santos-Reis M. 2012. Highway verges as habitat providers for small mammals in agrosilvopastoral environments. Biodiversity and Conservation [DOI: 10.1007/s10531-012-0390-3].

Ascensão F, Mata C, Mullins J, Ruíz-Capillas P, Silva C, Santos-Reis M, Clevenger AP, Malo J & Fernandes C. Highways as barriers to gene flow in small mammals: a genetic and agent-based modeling approach. Manuscript to be submitted to Molecular Ecology.

Ascensão F, Clevenger AP, Grilo C, Tracey J, LaPoint S, Santos-Reis M. Road effects on marten space use and movement: combining radio-tracking with agent-based simulation models. Manuscript submitted to Animal Conservation.

Ascensão F, Clevenger AP, Santos-Reis M, Urbano P, Jackson N. Wildlife-vehicle collision mitigation: partial fencing is an answer? An agent-based model approach. Manuscript submitted to Ecological Modeling.

2. Model species

A major problem in conservation management is how to integrate the habitat requirements of an array of species that greatly differ in their biological/ecological characteristics and response to habitat fragmentation (Vos *et al.* 2001; Gehring & Swihart 2003). Likewise, successful road mitigation plans is expected to cover the maximum number of species' requirements while targeting the main management efforts to the most vulnerable *taxa.* One possible way to manage this issue is to use model species which by their biological and ecological traits, namely habitat requirements, represent clusters of species (ecological profiles).

Accordingly, I collected and analyzed data from mainly two species – wood mouse and stone marten – which I considered as ecological profiles and assumed as having characteristics (e.g. vagility, habitat requirements) or tolerance/avoidance to roads representative from a larger group of species (small mammals and mammalian predators, respectively). The selected species are habitat generalists (Delibes 1983; Diaz *et al.* 1999; Rosalino *et al.* 2011), although closely associated to forested habitats, including *montado* areas (Santos-Reis *et al.* 2004; Rosalino *et al.* 2011).

Throughout this thesis, forest refers mainly to *montado* areas. *Montado* is a traditional Mediterranean agrosilvopastoral system (Blondel *et al.* 2010), mostly concentrated in the Alentejo province of Portugal and the Extremadura province of Spain, covering over 6 million ha in the Iberian Peninsula (Gaspar *et al.* 2007; Blondel *et al.* 2010). These areas are characterized by a mosaic of scattered trees in varying densities mainly dominated by cork oak (*Quercus suber*) and holm oak (*Q. ilex*), crops, grassland, fallow lands, shrubland and extensive animal grazing and cultivation (Pinto-Correia & Mascarenhas 1999).

Given that generalists species can presumably make more complete use of non-matrix habitats when moving through a fragmented landscape (Gehring & Swihart 2003; Pereira & Daily 2006), I assumed that any significant effect found for these model species are most probably exacerbated in specialist species, which significantly augment the importance of applying mitigation measures toward decreasing the road impact. Furthermore, being generalists and common species, I expected to avoid sample size bias. Moreover, although I planned the trapping procedures to minimize injuries on captured individuals, those procedures were not risk-free. Therefore, choosing rare or low-density species would be unethical in my point of view. Also, I intentionally selected species from different trophic levels, a small mammal and a carnivore, to further address the predator-prey relation when discussing the effectiveness of mitigation measures.

Therefore, I considered that by assessing the effects of road fragmentation for these model species, and by delineating mitigation measures based on this information, the number of species benefiting from the road mitigation would be in fact much larger. Nevertheless, generalizations to other species should be carefully acknowledged since predicting fragmentation effects can be hampered by the uniqueness of species (Wiegand *et al.* 2005). In the following section I present a brief characterization of both species.

2.1.1. WOOD MOUSE

This rodent has a mean weight of 25.5±6.5 g (data from 253 adult individuals captured in southern Portugal, *own unpublished data*), with a large distribution range that extends throughout Europe and parts of North Africa (Montgomery 1999). It is abundant in the Iberian Peninsula where populations can reach mean densities of as many as 35 individuals per hectare (Rosário & Mathias 2007). Radio-tracking data from a study performed in a *montado* landscape in southern Portugal reported a high variability in home range size, reaching a mean value of 1.124±0.925 ha (mean±SD), with no inter-sexual differences (Rosalino *et al.* 2011). Breeding usually occurs between March and October. A maximum lifespan is estimated to 18–20 months (Macdonald & Barrett 1993). This rodent is mainly a granivorous species, also consuming fruits and insects (Montgomery & Montgomery 1990).

From a conservation perspective, the wood mouse is an important study organism in the context of the Mediterranean agrosilvopastoral system (*montado*), particularly in southern Iberia Peninsula. In fact, the wood mouse is a key item within cycles of vegetative regeneration (Watts 1968), e.g. an important seed disperser of oak acorns (Gómez *et al.* 2008), being also a common prey for many top predators (Korpimaki & Norrdahl 1991; Sarmento 1996; Moleón & Gil-Sánchez 2003; Pineiro & Barja 2011).

2.1.2. STONE MARTEN

This medium-sized carnivore has a mean weight of 1420±330 g (data from 20 individuals captured in southern Portugal, *own unpublished data*), being widespread throughout Asia and Europe (Proulx *et al.* 2005). Is a carnivore very adaptable to human presence (Herr *et al.* 2009), but in southern Iberia its northern sympatric competitor, the pine marten (*Martes martes*), is absent and therefore stone marten is more associated to cork-oak woodlands (*montado*, see further) (Mortelliti & Boitani 2008).

The stone marten is a nocturnal, solitary and territorial species (except for mating season), with home ranges reaching 2 to 3 km^2. The breeding period occurs in summer, but sometimes can have a second heat in February. Once fertilized, the zygote develops to the blastocyst stage and then a pause occurs in the embryonic development (delayed implantation). This pause last until February, when the viable blastocysts finally is implanted into the uterus to develop for approximately 55-60 days (Canivenc *et al.* 1981). Its diet has a wide spectrum and include small mammals, birds, fruits, insects and reptiles, due to its ability in adapting to the local food availability and its character of an opportunistic predator (Serafini & Lovari 1993; Clevenger 1994; Genovesi *et al.* 1996; Mortelliti & Boitani 2008). Nevertheless, although in Mediterranean environments the fruits probable constitute a main item in stone marten diet, small mammals including wood mouse are also an important food item (Serafini & Lovari 1993), particularly in winter when fruits are less available.

Regarding its conservation, recent research documented that stone marten is particularly sensitive to forest fragmentation in cork oak woodlands (*montado*) (Virgós *et al.* 2002; Santos-Reis *et al.* 2004; Mortelliti & Boitani 2008; Grilo *et al.* 2011). In particular, this species is known to be vulnerable to road traffic, being the second most frequently killed carnivore on roads in southern Portugal (Grilo *et al.* 2009).

2.1.3. WHY FOCUS ON SPECIES ASSOCIATED TO MONTADO?

As referred, both model species (wood mouse and stone marten) are forest-dwelling species, typically associated to *montado* system in southern Iberian Peninsula. In this section I briefly present this system and its value for biodiversity, as well its major threats. I chose to focus on species associated to *montado* given that this system is today recognized by its high conservation value in the Mediterranean basin context (Myers *et al.* 2000; Blondel 2006). In fact, the *montado* system is one of the best examples of a managed agro-forestry-pastoral system that balances social and economic development with nature conservation (e.g. Bugalho *et al.* 2011). Representative of its importance for biodiversity, a large extent of *montado* areas is under the protection of the Natura 2000 network (EEA 2012).

The high biodiversity associated to this system is commonly attributed to the low-intensity of use, the long-term stability of habitat conditions, its extension, and the large variety and complex structure of habitats that co-exist within the cork oak woodlands matrix (Tellería 2001). The richness of the biodiversity associated to the *montado* has been widely documented (Blondel *et al.* 2010). Adapting a list provided by The Millennium Ecosystem Assessment Portuguese Report (Belo *et al.* 2009) and references therein, I resume the reasons that originate such biodiversity richness in *montado* areas:

i) The tree layer is dominated by native and long-lived species;

ii) Trees are left to grow until senescence, with regular pruning, thus ensuring its long life;

iii) The extensive and traditional exploration of resources (wood, cork) and livestock production, results in low disturbance;

iv) The vegetation structure has several layers, including herbaceous, shrubs and trees, and there is a cyclical rotation of crops and fallow and pastures in the understory;

v) Pruning and cork extraction result in numerous tree holes, which in turn provide shelter for numerous species;

vi) The existence of vast areas with low or no fragmentation or converted to other land uses;

vii) The native invertebrate fauna is very diversified and abundant, functioning as prey for many species.

This management context resulted in a vast community of species inhabiting *montado* patches, including invertebrates (e.g. da Silva *et al.* 2009), reptiles (Godinho *et al.* 2011), birds (Leal *et al.* 2011) or mammals (Santon-Reis & Correia 1999). From this latter group, it should be highlighted the importance of *montado* patches to endangered species as the Iberian lynx (*Lynx pardinus*) (Fernandez & Palomares 2000).

Nevertheless, although shaped by human traditional agricultural practices for centuries, mediterranean ecosystems and particularly the *montado* system have experienced increasing loss of habitat quality in recent years, mostly due to the intensification of livestock production (Meeus 1993; Donald *et al.* 2002; Stoate *et al.* 2009; Blondel *et al.* 2010; Reino *et al.* 2010). In fact, changes in agriculture and cattle production in Portugal, experienced since 1930s, reduced substantially the amount of good quality habitat. From this period on, either due to extreme political situation (from totalitarianism to revolution' agrarian reform) or market pressure, the traditional management system begun to be converted into a more intensified farming process closely bound to improving production quantity and profitability, which resulted in soil erosion and deforestation of large areas for crop production (Ferreira 2000).

More recently, after Portugal and Spain joined the EU in 1986, subsidies encouraged an increase of stocking rates, often leading to overexploitation of forage resources (Gaspar *et al.* 2007; Gaspar *et al.* 2009). This intensification of livestock endangered the preservation of the whole *montado* system: when exceeding the carrying capacity, the animals eat the young tree shoots, thereby preventing natural regeneration of the tree cover. As an indicator, the bovine density in Alentejo (livestock units per utilized agricultural hectare) grew from 0.15 in 2000 to 0.19 in the last 10 years, while for Estremadura this increase was from 0.14 to 0.18. Such increment rates were only overcame by seventeen out of over 400 European regions, for the same considered period (Eurostat 2010, source URL: http://nui.epp.eurostat.ec.europa.eu). It should be referred that the latest Common Agricultural Policy (CAP) reform intends to revert the situation, and the vast majority of subsidies will be paid independently from the volume of production, promoting extensive livestock rates, although its positive effects are yet to come.

The main consequence of the intensification of livestock production was the soil degradation due to trampling and overgrazing (Pinto-Correia & Mascarenhas 1999; Ferreira 2000; Torre *et al.* 2007), where the understory structure is often reduced to bare ground devoid of vegetative cover (Puerto *et al.* 1990; Ibáñez *et al.* 2007; Castro & Freitas 2009). Overgrazing and soil trampling have a significant effect over small mammal communities (Gibson *et al.* 1992; Milchunas *et al.* 1998; Eccard *et al.* 2000; Gonçalves *et al.* 2011) and therefore all trophic chain is expected to be affected. Therefore, by focusing on species associated to *montado*, I further intend to contribute to knowledge related to the conservation requirements of this system and its biodiversity.

2.2. STUDY AREAS

Most of the field work was performed in the Alentejo province of southern Portugal, within 38°38’N, 8°34’W and 37°55’N, 8°12’W, including the two studied highways - A2 and A6 (study areas 1, 2a and 3 in Fig. 2.1). For chapter 3, a collaboration between the Conservation Ecology research Group of the ‘Centro de Biologia Ambiental’ (Universidade de Lisboa) and the Terrestrial Ecology Group of the ‘Departamento de Ecología (Universidad Autónoma de Madrid) allowed the sampling of two highways in Spain – AP6 and AP51 (study area 2b in Fig. 2.1) In all cases, highways have four lanes, with a median strip of ca. 5 m, and are fenced in all its length. Study areas 1, 2a and 3 have elevations ranging from approximately 200 to a maximum of 250 m. Elevation in study area 2b ranges between 1000 and 1500 m. Land use across the study areas is mainly represented by *montado* and open habitats. To represent the spatial distribution of the potential habitat for wood mouse and stone marten, I used the CORINE land cover data (source URL: www.eea.europa.eu), selecting the land use classes described in Table 2.1.

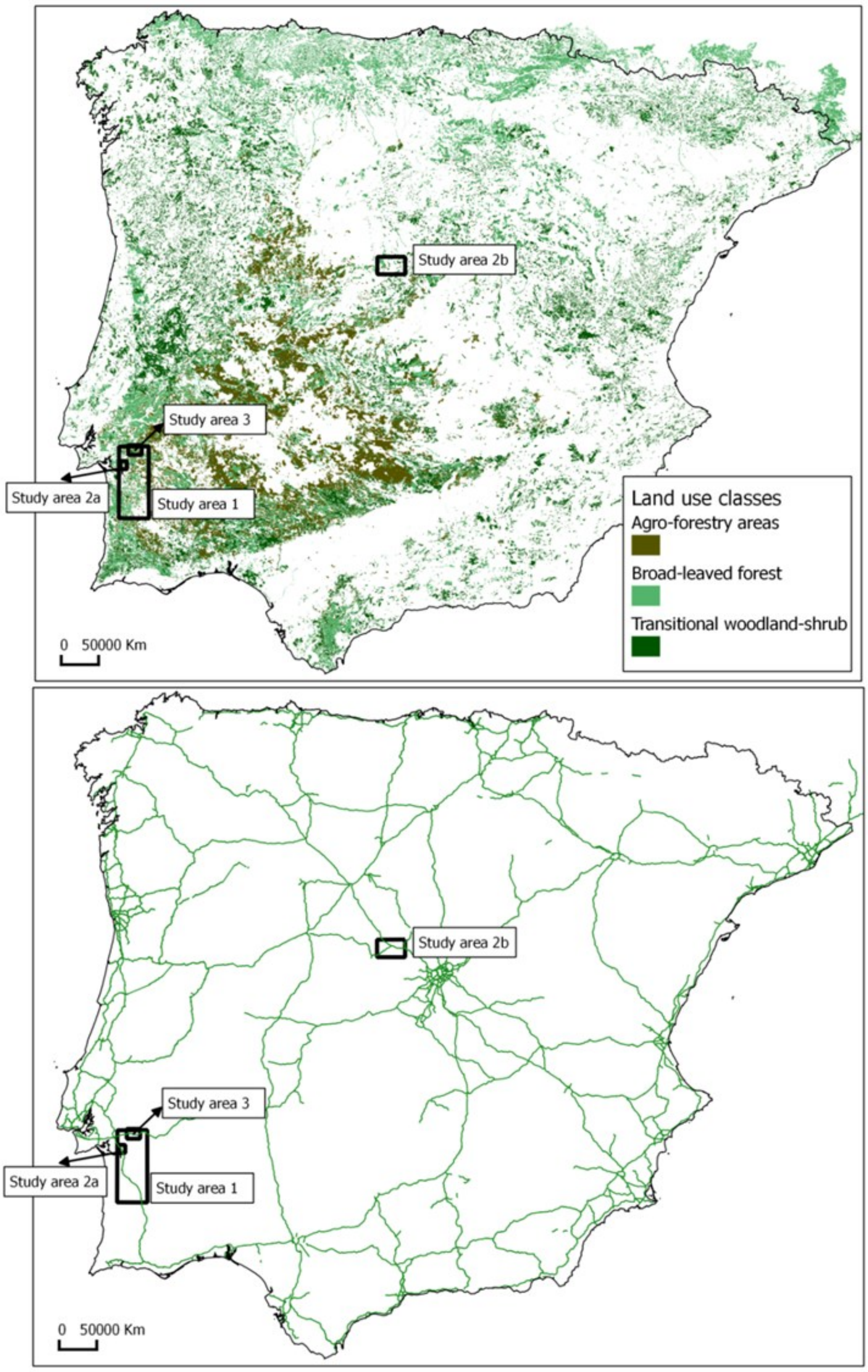

Fig. 2.1 - Location of the study areas in the Iberian Peninsula: *study area 1* - small mammal sampling area from chapter 2; *study area 2a* and *2b* - capture areas from chapter 3; *study area 3* - stone marten tracking area from chapter 4. Picture on the top shows the land cover classes from CORINE land cover (source: www.eea.europa.eu) that represent potential habitat for wood mouse and stone marten. Bottom picture shows the highway network in Iberian Peninsula (adapted from Open Street Map, source: www.openstreetmap.org)

Table 2.1 - Land use classes from CORINE 2006 used to map suitable habitat for stone marten and their cover area within Iberian Peninsula.

Class	Description	Area (ha)
244	Agro-forestry areas	3.12E+06
311	Broad-leaved forest	5.99E+06
324	Transitional woodland-shrub	4.77E+06
	Total	1.39E+07

According to the Iberian Climate Atlas (AEMET-IM 2011), in all study areas the climate is temperate (the coldest months are between 0 and 18 °C), with a dry period in summer which is classified as *hot* (average temperature in the hottest month above 22 °C, label *csa* in Fig. 2.2) or *temperate* (average temperature in the hottest month below or equal to 22 °C, and with four months or more with average temperatures above 10 °C, label *csb* in Fig. 2.2).

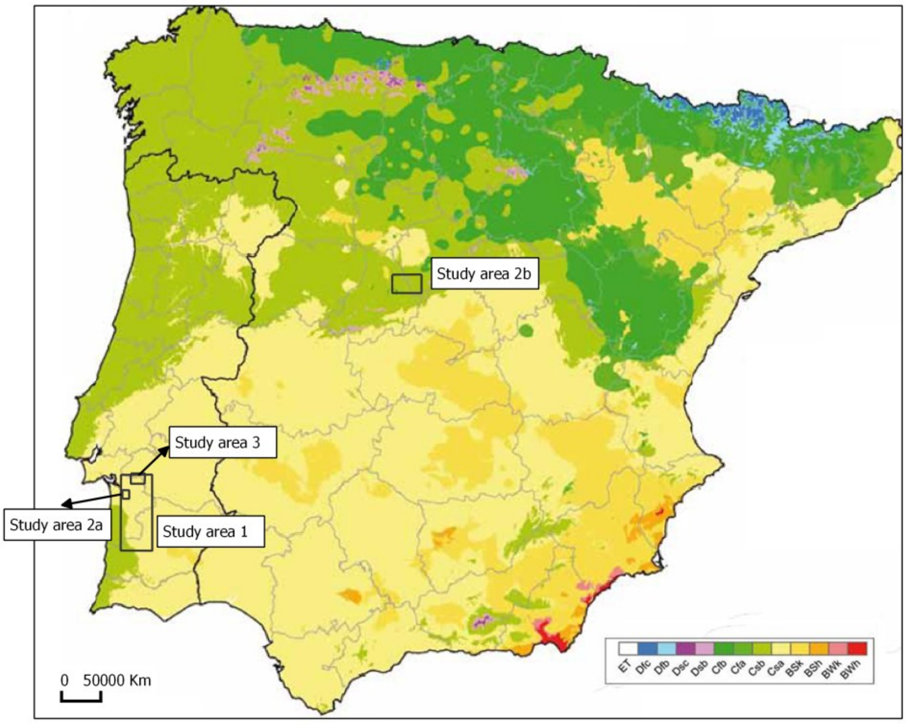

Fig. 2.2 - Köppen-Geiger Climate Classification for the Iberian Peninsula. Picture adapted from the Iberian Climate Atlas (AEMET-IM 2011).

3. Highway verges as habitat providers for small mammals in agrosilvopastoral environments

HIGHWAY VERGES AS HABITAT PROVIDERS FOR SMALL MAMMALS IN AGROSILVOPASTORAL ENVIRONMENTS

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ABSTRACT

The Mediterranean basin has an important conservation value given its high biodiversity and high number of endemic species, which have co-existed with human traditional practices for centuries. However, northern areas as the Iberian Peninsula have experienced intensification in livestock production in recent past, with consequent reduction in habitat quality. In this study we assessed the importance of fenced highway verges as habitat for small mammals in Mediterranean agrosilvopastoral landscapes. More specifically, we compared small mammal abundance between highway verges and the adjacent two main land uses (montado and open areas); compared the vegetative structure among these land uses; and addressed how vegetation structure influences species occupancy. Thirty-six sites were sampled in agrosilvopastoral system areas in southern Portugal (sampling effort 8840 trap-nights). A total of 351 individuals from target species were captured: 157 wood mice (*Apodemus sylvaticus*), 95 Western Mediterranean mice (*Mus* spretus) and 99 greater white-toothed shrews (*Crocidura russula*). Capture-mark-recapture analyses were performed to estimate population size. Our data suggests that fenced highway verges promote better vegetative structure conditions which in turn favor a higher animal abundance therein. We suggest the adoption of management practices to increase the height and cover of herbaceous and shrub layers in road verges, together with creating grazing controlled areas in highway vicinity, particularly in montado patches, linked by vegetated linear features. This would increase habitat and refuge for a large numbers of species, including small mammals, and thus benefiting the trophic chain and the whole agrosilvopastoral system.

Keywords: Mediterranean; Iberia Peninsula; montado woodlands; road ecology; capture-mark-recapture; *Apodemus sylvaticus*; *Mus spretus*; *Crocidura russula*

3.1. INTRODUCTION

The Mediterranean Basin has a worldwide recognized conservation value, mostly due to exceptionally high floristic species richness and a high proportion of endemic species (Myers *et al.* 2000). Although having successfully co-existed with human traditional practices for centuries, Mediterranean ecosystems are experiencing increasing reduction in habitat quality in recent years due to the intensification of livestock production (Blondel *et al.* 2010). This is particularly true in the Iberian Peninsula (Meeus 1993; Donald *et al.* 2002; Reino *et al.* 2010).

The main effects of intensive livestock production is overgrazing and soil degradation due to trampling (Pinto-Correia & Mascarenhas 1999; Ferreira 2000; Torre *et al.* 2007), where the understory structure is often reduced to bare ground devoid of vegetation (Puerto *et al.* 1990; Ibáñez *et al.* 2007; Castro & Freitas 2009). Overgrazing and soil trampling may affect small mammal communities (Gibson *et al.* 1992; Milchunas *et al.* 1998; Eccard *et al.* 2000; Gonçalves *et al.* 2011), by reducing food resources and making soils more compact and less suitable for building and maintaining burrow systems (Bilotta *et al.* 2007). Additionally, overgrazing can limit vegetation cover, which in turn will increase the exposure to predators (e.g. Torre *et al.* 2007).

It has been argued that within intensive agricultural landscapes, the conservation of linear features such as hedgerows and field margins play a critical role in maintaining biodiversity. Due to their spatial and structural characteristics, these linear vegetative features provide habitat and refuge for large numbers of mammal species

(Stoate *et al.* 2001; Tattersall *et al.* 2002; Alain *et al.* 2006; Gelling *et al.* 2007). Moreover, as they function as natural corridors, hedgerows and similar habitat elements improve the structural connectivity within landscapes (Bennett 1990) for these species.

The potential conservation value of road verges has also been recognized for decades (e.g Way 1977). Similar to hedgerows and field margins, road verges are known to be important refuge habitats for many small-sized vertebrate and invertebrate species, including ants (Tshiguvho *et al.* 1999), butterflies (Saarinen *et al.* 2005), bees (Hopwood 2008), beetles (Noordijk *et al.* 2009a); birds (Meunier *et al.* 2000) and small mammals (Oxley *et al.* 1974; Bellamy *et al.* 2000; Sabino-Marques & Mira 2011). Road verges may also function as corridors linking habitat patches for many wildlife species (Bennett 1990; Doncaster *et al.* 2001; Huijser & Clevenger 2006).

In the Iberian Peninsula highway verges may play an important role as habitat providers for small mammals in agrosilvopastoral habitats. In both Portugal and Spain highways are fenced to mark the boundary between highway right-of-way and adjacent lands and to prevent livestock from accessing the highway proper. This separation often results in roadside verges with lush ungrazed vegetation that may offer cover from predators. Because highways are widespread and expanding, verge management and conservation may help improving population connectivity and long-term viability in human-dominated landscapes (Huijser & Clevenger 2006). However, there is a lack of information regarding the importance of fenced highway verges for small mammal species, including for agrosilvopastoral landscapes in the Mediterranean region.

Highway verge management may be particularly important for small mammals and thus for local biodiversity. Small mammals are situated mid-way along the food chain (e.g. Golley 1960), playing an important role in seed dispersal and controlling invertebrates (Watts 1968; Montgomery & Montgomery 1990). Further, they are an important food source for birds of prey and carnivores (Virgós *et al.* 1999; Askew *et al.* 2007) including threatened species such as European wildcat (*Felis silvestris*) (Sarmento 1996; Moleón & Gil-Sánchez 2003). Hence, small mammals are key organisms in the trophic chain and their abundance and distribution can influence the population dynamics of both prey and predators along transportation corridors (Meunier *et al.* 2000; Hanski *et al.* 2001; Mortelliti & Boitani 2008).

The purpose of our study was to assess the importance of fenced highway verges as habitat providers for small mammals. Specifically we addressed i) whether there were differences between the relative abundance of small mammals living in highway verge habitat and two distinct but predominant adjacent habitat types (*montado* and open areas); ii) whether small mammal abundance in highway adjacent habitat types (*montado* and open areas) is similar to the one found in verge areas; iii) whether vegetative structure (height and cover) in highway verges differed from the two main habitat types; and iv) what effect vegetative structure may have on species occupancy. We focused on the three most abundant surface-dwelling species in our study area: wood mouse (*Apodemus sylvaticus* Linnaeus, 1758), western Mediterranean mouse (*Mus spretus* Lataste, 1883) and greater white-toothed shrew (*Crocidura russula* Hermann, 1870). For simplicity, greater white-toothed shrew will be designated as "shrew" hereafter.

We expect highway verges to be an important habitat for these species, expressed by higher abundances in highway verges relative to non-highway sites. Moreover, we expect higher vegetation structure in highway verges, which may be positively related with species occupancy. Our study should therefore be of interest for conservation biologists and transportation planners seeking to understand species-roadway interactions and to find solutions for conservation and protection of wildlife communities in increasingly developed mediterranean landscapes.

3.2. MATERIALS AND METHODS

3.2.1. STUDY AREA

The study area was located in the Alentejo region of southern Portugal, within 38°38′N, 8°34′W and 37°55′N, 8°12′W (Fig. 3.1).

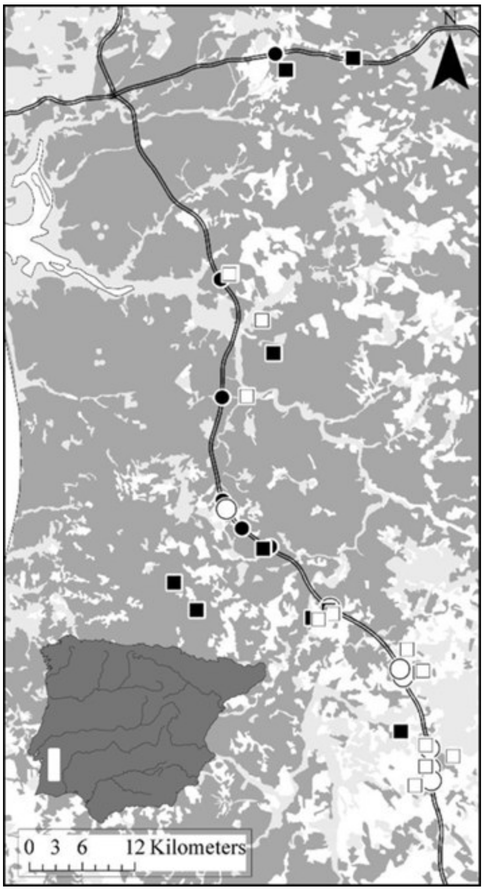

Fig. 3.1 - Location of the study area in the Iberian Peninsula and the sampling sites in the study area. Site types are represented as follows: black circles are highway sites surrounded by *montado* areas; black squares are sites in "montado" areas; white circles are highway sites surrounded by open areas; and white squares are sites in open areas. *Montado* and open areas are represented by dark grey and light grey, respectively. Other land uses are represented in white. Black lines are highways

Highway verge habitat was studied in two 4-lane highways: A2 and A6; opened to traffic in 1998 and 1995, respectively. Within the highway verge we differentiated the vegetated central median located between highway lanes and the vegetated highway margin. Central median width was ca. 5 m while the highway margin width ranged from 16 to 30 m. The fencing follows the outermost side of margin. During the sampling period (March to May of 2008), the mean nighttime traffic volume (2100 to 0600 h) was 242±301 (A2) and 163±163 (A6) vehicles per hour^{-} (BRISA highway enterprise database).

Land use across study area is mainly represented by *montado* and open habitats. *Montado* is a traditional Mediterranean agrosilvopastoral system (Aronson *et al.* 2009; Blondel *et al.* 2010), mostly concentrated in Portugal and Spain (where it is called *dehesa*), covering over three million ha in the Iberian Peninsula (Moreno &

Pulido 2008; Gaspar *et al.* 2009; Blondel *et al.* 2010). These areas are covered by scattered trees in varying densities, mainly dominated by cork oak (*Quercus suber*) and holm oak (*Q. ilex*), superimposed to a mosaic of crops, grassland, fallow lands, shrubland, and extensive animal grazing and cultivation (Pinto-Correia & Mascarenhas 1999). Shrubs are dominated by *Cistus, Erica, Lavandula,* and *Ulex* sp.. Amid *montado* patches there are areas devoid of trees that are used for crops or left fallow, hereafter referred as open habitats. According to the Iberian Climate Atlas (AEMET-IM 2011), across the entire study area the climate is temperate (the coldest months are between 0 and 18 °C), with a dry period in summer which is classified as *hot* (average temperature in the hottest month above 22 °C) or *temperate* (average temperature in the hottest month below or equal to 22 °C, and with four months or more with average temperatures above 10 °C).

3.2.2. SMALL MAMMAL SAMPLING

We quantified the target species abundance in highway verges within pastured *montado* and open habitats and in non-highway sites (pure *montado* and open areas). Our sampling design consisted of four different sampling site types: 1) highway verges surrounded by *montado* (HWY_MONT; n=7); 2) highway verges surrounded by open areas (HWY_OPEN; n=7); 3) pure *montado* areas (MONT; n=11); and 4) homogeneous open areas (OPEN; n=11). Prior to sampling, we identify 30 potential sampling sites for each site type. For non-highway sites, we only considered areas exhibiting frequent overgrazing signs: livestock manure, patches of bare ground without plant regeneration and, where present, young trees broken. The sampling sites were then randomly chosen within this set of locations. To minimize spatial autocorrelation sites were located at least 500 m apart (neighborhood distance: mean = 1702±1654 m; max = 7110 m).

Highway sites were sampled using three parallel trap-lines: one along the highway central median (MEDIAN), one along the highway verge (MARGIN) and one outside the highway area (OUTER). OUTER trap-lines were set parallel to the highway and at a distance equivalent to the highway pavement width (ca. 25 m) (Fig. 3.2). Data from this trap line was used to infer if the expected positive verge effect extends to bordering areas. Highway fencing is located in the outermost margin side. Between margin and main land use (*montado* or open habitats), there is a mowed strip with ca. 5 m, for fire prevention. For non-highway sites (MONT and OPEN), we set two parallel trap-lines, also ca. 25 m apart. In all cases, each trap-line contained 20 baited Sherman™ live traps, 10 m apart.

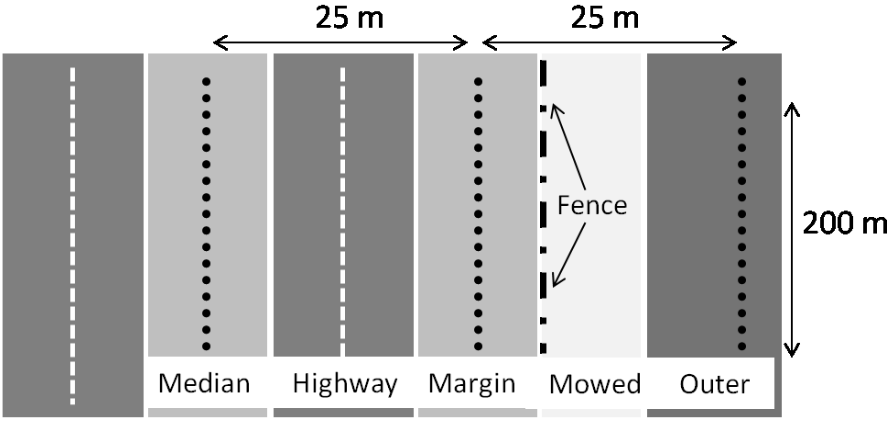

Fig. 3.2 - Trap-lines location in highway sites (median, margin and outer). Outer line was set at the same distance as median-margin line distance (ca. 25 m). Adjacent to the highway limit there is a mowed strip for fire prevention. Highway fence is placed between Margin and Mowed areas. Distances are not equally scaled.

The first four sampled sites were surveyed for six consecutive nights, but for logistical reasons, remaining sites were sampled for five nights. This was supported by inspecting the capture-recapture history, in which the majority of new captures occurred within the first three nights (see Supplementary material, Fig. 3.6). Site survey order was randomly assigned and four sites where sampled simultaneously.

Captured animals were marked with a fur cut mark, sexed, weighed and released at capture site. This procedure took approximately five minutes per individual. Capture procedures and animal handling were in conformity with Portuguese Biodiversity Conservation Institute directives (ICNB, Instituto da Conservação da Natureza e Biodiversidade).

3.2.3. VEGETATIVE STRUCTURE CHARACTERIZATION

Species of herbaceous and shrub layers in verges may often be similar to ones found in adjacent areas (Tanghe & Godefroid 2000; Santos *et al.* 2007), although several exotic species are regularly planted in highway verges (Forman & Alexander

1998). We were mainly concerned with vegetative structure (height and cover), as this may be a limiting factor for small mammals' persistence (Torre *et al.* 2007; Muñoz *et al.* 2009), even if provided by exotic species. Therefore, vegetation height and cover of both the herbaceous and shrub layers were quantified. These four variables were measured at all four site types by the same two observers. Around each trap location (one meter radius) we visually estimated the mean height and cover of each vegetation layer. The estimation for each trap was a consensus between the two observers. Previous to site categorization, several trials were performed and results discussed within the fieldwork team. Height estimates were categorized in 10 cm classes. For cover we classified the layers at each trap location as one of four classes (0-25%; 25-50%, 50-75% and 75-100%).

3.3. DATA ANALYSIS

3.3.1. SMALL MAMMALS' POPULATION SIZE

In order to evaluate if our sampling design could provide an accurate representation of population size (N), and hence reliable species abundances, we used capture-mark-recapture (CMR) analyses to estimate the population size (N_{est}) of each species. For sampling sites with two or more captured individuals (per species), we estimated N_{est} using the 'Rcapture' R package (Baillargeon & Rivest 2009). This package uses Poisson regressions to estimate parameters in a CMR experiments and produces maximum likelihood estimates of the loglinear models' parameters. N_{est} is then derived from these loglinear parameters (Baillargeon & Rivest 2009).

We assumed that all populations in sampled sites were "closed", which indicates that neither mortality nor immigration occurred during the sample period. Hence, the size of a closed population does not vary during the experiment. This assumption is reasonable for capture-recapture experiments held over a short period of time. We used a bias correction (function 'closedp.bc') for small samples to improve estimations and stabilize the standard errors estimates (Baillargeon & Rivest 2009); and assumed equal probabilities of capture during the experiment. Because small samples often lead to large standard errors, we considered only estimates where the coefficient of variation (standard deviation of N_{est} divided by N_{est}) was lower than 30% (L.-P. Rivest, *pers. comm.*). One limitation of 'Rcapture' is that it does not handle trap deaths. Therefore we remove from analysis all death records (wood mouse n=3; western Mediterranean mice n=2; and shrew n=4). The number of captures and N_{est} were compared by Wilcoxon paired test.

3.3.2. SMALL MAMMALS' ABUNDANCE AND VEGETATIVE STRUCTURE COMPARISONS AMONG SITES

We used the minimum number of live small mammals per sampling effort as an index of abundance. We compared small mammal abundance and the vegetation structure for each of the four sampling site types. We did not consider the data from the outer trap-line of highway sites in these comparisons, as we were primarily interested in relating the species information and the vegetation structure within the highway right-of-way with the two land use types. We then compared the abundance among the three trap-lines for highway sites (median, margin and outer).

We used a χ^2 contingency test to compare the number of small mammals trapped in each sampling site type by sex. Because of the small sample size we pooled all trap line data, except for wood mouse in HWY_MONT and for shrew in HWY_OPEN. For these, comparisons for each trap line were also performed. In all cases, p-values were computed by Monte Carlo simulations using 2000 replicates (Hope 1968).

In all comparisons, and due to non-normality of data and small sample size, we used Kruskal-Wallis tests and subsequent nonparametric multiple comparisons using the R package 'agricolae' (Mendiburu 2010). Because sequential Bonferroni correction of significance levels (Holm 1979) can inflate the Type I error rate (Moran 2003), we report unadjusted significance values, highlighting those that remain significant after correction (see Table 3.7 in Supplementary material).

3.3.3. RELATING SMALL MAMMALS OCCUPANCY WITH VEGETATIVE STRUCTURE

We modeled species occupancy according to vegetative structure at the trap level. For each species, all traps were coded as 'presence' if at least one individual was trapped during the sampling period and 'absence' otherwise, which was used as the response variable. Generalized linear mixed models (GLMM, Breslow & Clayton 1993) with logistic link, a binomial error distribution and Laplace approximation were built using the R package 'lme4' (Bates *et al.* 2010). The four vegetation variables (cover and height of herbaceous and shrub layers) were used in models as fixed effects and site identification nested in site type was used as random effect. Vegetation cover variables were treated as equally spaced ordinal variables.

Before model building, we checked for collinearity between vegetation variables as it may inflate the variances of the parameter estimates, by verifying the variance inflator factor (Graham 2003). We also checked for spatial autocorrelation by plotting the residuals of each GLMM models containing all uncorrelated explanatory variables in spline correlograms (Bjørnstad & Falck 2001; Bjørnstad 2009).

Alternative models using all possible combinations of variables were built and ranked according to the Akaike Information Criterion adjusted for small sample sizes (AIC_c) (Burnham & Anderson 2002). The goal of building models using all variables' combinations was to infer the relative importance of each vegetation predictor on species presence. We computed the Akaike weights (w_i) of all models and for each variable we summed the weights of those models where the variable was included (Burnham & Anderson 2002). AIC_c and w_i were computed using the R package 'MuMIn' (Barton 2011). Because cover attributes were treated as ordinal variables, 'MuMIn' does not report the models' coefficients for these variables. Instead, the package highlight the use of these variables in the models with a "+". Therefore, *ad-hoc* inspections were performed to verify the trend effect of herbaceous and shrub cover in species presence: all models with $\Delta AICc < 3$ containing these variables were rerun and the coefficients for the ordinal variables were checked. No interaction terms were included due lack of model convergence. All statistical computation and plots were made in R environment (R Development Core Team 2011).

3.4. RESULTS

3.4.1. POPULATION SIZE

We captured 355 different individuals comprising 157 wood mice, 95 western Mediterranean mice and 99 shrews in a total sampling effort of 8840 trap-nights. Incidental records included three Cabrera's voles (*Microtus cabrerae*) at highway sites and one black rat (*Rattus rattus*) at one open site. We were able to estimate population size at 19 sites, of which 10 were for wood mouse, five for western Mediterranean mouse and four for shrew. Estimates ranged from two to 29 individuals per site for wood mouse; four to 39 individuals for western Mediterranean mouse; and four to 20 individuals for shrew (Table 2.1). Estimated values were not significantly different than the observed results (Wilcoxon pair test, W=0, p-value <0.001). Hence, we consider that nearly all individuals in sampled sites were captured and thus computed abundances were reliable.

Table 3.1 - Estimates of population size from capture-mark-recapture data by species and sampling site type, assuming closed population scenario. Sampling sites consisted in highway sites surrounded by *montado* areas (HWY_MONT), highway sites surrounded by open areas (HWY_OPEN), pure *montado* areas (MONT) and pure open areas (OPEN). N – Minimum number alive; N_{est} - estimated population size for each site; SE - standard error of N_{est}.

Species	Site type	Site	N	N_{est}	SE
Wood mouse	HWY_MONT	B1	25	30.1	3.2
		B2	25	27.5	2.0
		B101	17	20.6	2.8
		B102	19	20.0	1.2
		B4	22	23.6	1.5
	HWY_OPEN	B3	9	10.7	1.9
	MONT	M1	10	14.3	3.7
		M100	4	4.2	0.4
Western Mediterranean mouse	HWY_MONT	B4	3	3.6	1.1
	HWY_OPEN	B5	19	27.8	5.6
	OPEN	E5	14	21.2	5.3
		E8	31	38.7	4.2
Greater white-toothed shrew	HWY_MONT	B4	3	3.6	1.1
	HWY_OPEN	B5	19	27.8	5.6
	OPEN	E5	14	21.2	5.3
		E8	31	38.7	4.2

3.4.2. ABUNDANCE AND VEGETATION STRUCTURE COMPARISONS AMONG SITES

Overall there was a higher abundance of small mammals in highway sites compared to non-highway sites; with highway sites surrounded by *montado* (HWY_MONT) having the highest abundance values (Fig. 3.3, Table 3.4 in Supplementary material). Wood mice were mainly captured in HWY_MONT, whereas few captures occurred in the other sites. We captured a low number of wood mice and recorded no captures of western Mediterranean mice at *montado* sites (MONT), although this latter species was captured in HWY_MONT. As for shrews, we found a higher abundance in both HWY_MONT and highway sites surrounded by open areas (HWY_OPEN).

Fig. 3.3 – Boxplots of small mammal abundance for each of the four sampling site types. Box upper and lower limits represent the interquartile range (IQR), thick line is the median and "whiskers" are the ± 1.5 * IQR. Circles are outliers. Sampling sites consisted in highway sites surrounded by *montado* areas (HWY_MONT), highway sites surrounded by open areas (HWY_OPEN), *montado* areas (MONT) and open areas (OPEN). Abundance refers to minimum number of individuals alive per sampling effort. Same letters indicate no significant differences ($p < 0.05$) between sites. Adjusted p-values from sequential Bonferroni correction are available in Supplementary material Table 3.4.

Regarding the number of captures by trap-line at the highway sites, the majority of individuals were captured in the margin line followed by the median trap line (Fig. 3.4, Table 3.5 in Supplementary material).

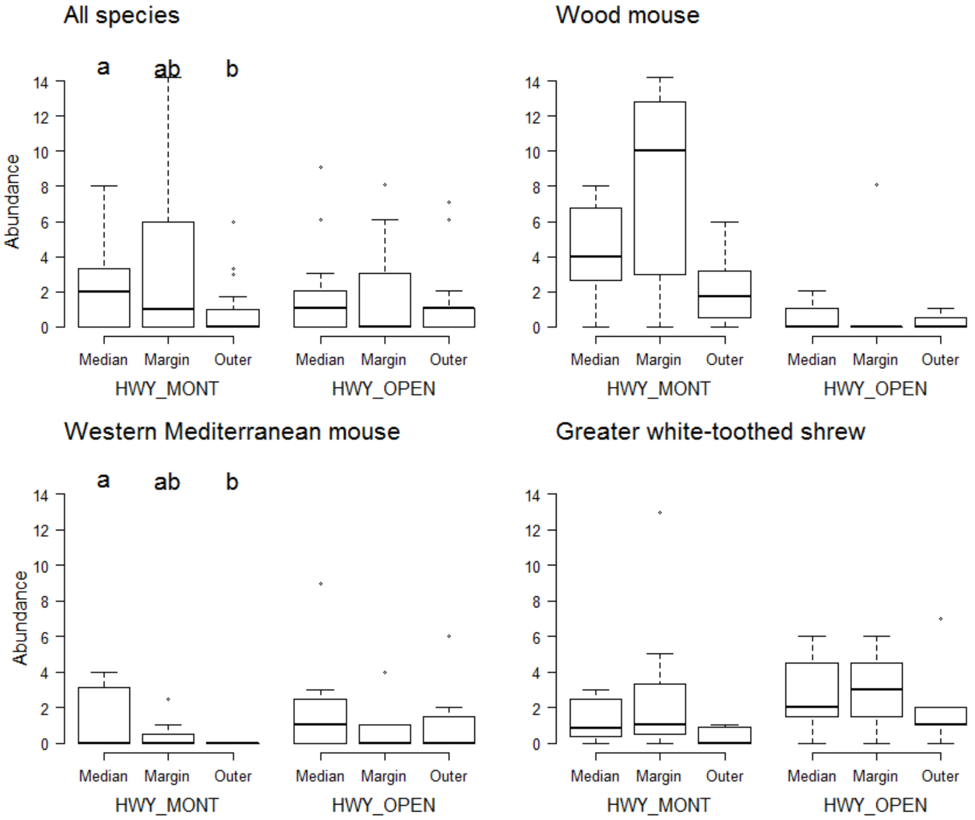

Fig. 3.4 – Boxplots of small mammal abundance in highway sites by trap line (median, margin and outer). Box upper and lower limits represent the interquartile range (IQR), thick line is the median and “whiskers” are the ± 1.5 * IQR. Circles are outliers. Highway sites were surrounded by *montado* areas (HWY_MONT) or open areas (HWY_OPEN). Abundance refers to minimum number of individuals alive per sampling effort. Where indicated, same letters indicate no significant differences ($p < 0.05$) between sites. None of the p-values remained significant after sequential Bonferroni correction (Supplementary material Table 3.5).

We recorded a small number of captures in the outer trap line in HWY_MONT. The abundance of the three species combined was significantly lower in the outer line than the median line ($K = 11.2$, p-value = 0.035). As for wood mouse only, its abundance in the outer line was marginally significantly lower from the verge line ($K = 6.5$, p-value = 0.051), while for western Mediterranean mouse it was marginally significantly lower from the median line ($K = 4.9$, p-value = 0.049). Nevertheless, all these comparisons are not significant if the sequential Bonferroni correction is applied (alpha = 0.017). Therefore, the species abundance in highway bordering areas seem to be similar than the one found in verges.

We found no significant differences in sex ratios, except for wood mouse in MONT where a higher number of males were captured ($\chi^2 = 6.5$, $n_1 = 5$, $n_2 = 17$, p-value = 0.020), and for shrew in HWY_OPEN where a higher number of females were captured ($\chi^2 = 5.3$, $n_1 = 32$, $n_2 = 16$, p-value = 0.032). This latter was due a higher number of captured females in the median line ($\chi^2 = 7.1$, $n_1 = 14$, $n_2 = 3$, p-value < 0.015).

Vegetative structure differed significantly among site types (Fig. 3.5, Table 3.6 in Supplementary material). The only pairs of site types for which we found no significant differences after the sequential Bonferroni correction were in HWY_OPEN–MONT (shrub height); HWY_OPEN–MONT (herbaceous height), and HWY_MONT–MONT (herbaceous height). Overall we found taller and denser shrub layers in highway sites, while herbaceous cover and height were higher in non-highway open sites (Fig. 3.5).

3.4.3. RELATING SMALL MAMMALS' OCCUPANCY WITH VEGETATIVE STRUCTURE

All variables considered in the analysis showed an inflation factor below 2.0, suggesting that collinearity was negligible (Chatterjee and Price 1991), and therefore the four predictors were retained for further analyses. We found no signs of autocorrelation for any species after fitting full models' residuals in correlograms (Fig. 3.7, in Supplementary material).

The most parsimonious model for explaining variations in wood mouse presence at the trap level was one in which shrub height and cover were the predictor variables. This model accounted for 35% of the Akaike weights (w_i) in the model set. According to ΔAICc ranking, the next four models had ΔAICc < 3, indicating their similarity with the best model, all including shrub height. These five models had a cumulative w_i of 0.93 (Table 2.2).

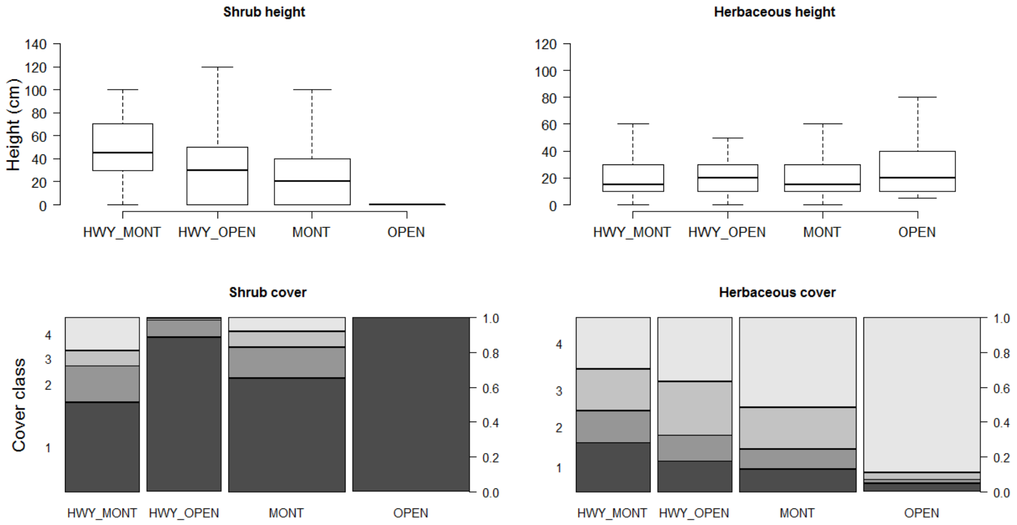

Fig. 3.5 – Vegetation structure characteristics. Upper graphs: boxplots of shrub and herbaceous height. Boxplot upper and lower limits represent the interquartile range (IQR), thick line is the median and “whiskers” are the ± 1.5 * IQR. Lower graphs: mosaic plots of shrubs and herbaceous layers, by site type. Cover is expressed in four classes where 1 represents a low cover and 4 represents high cover. The heights of the bars in cover plots correspond to the relative frequencies of each class in each site type. Secondary y axis represents the cumulative proportion of each class.

For western Mediterranean mouse we obtained three models with ΔAICc < 3, where the most parsimonious included herbaceous height only and had w_i of 0.47. The three models accounted for 85% of w_i. As for shrew, the most parsimonious model was also the only one with ΔAICc < 3, and included shrub height and herbaceous height, accounting for 70% of w_i. Coefficients of ordinal variables revealed a linear trend between cover and the presence of species in all cases. From all model combination we obtained the AICc weight for each vegetation cover attribute. Accordingly, the presence of wood mice was mainly related to tall and dense shrubs; western Mediterranean mice were associated with sites with tall herbaceous vegetation, while shrews were associated with tall shrubs and herbaceous vegetation (Table 2.3).

Table 3.2 - Generalized linear mixed models describing target species presence with shrub and herbaceous height and density (fixed effects), at the trap level. Cover variables were treated as ordinal. Shown are model estimates for each variable, log-likelihood (LL), number of parameters (k), change in Akaike information criterion with correction for small sample sizes (ΔAICc), AICc weight (w_i). Models are ranked according to the ΔAICc. Only models with ΔAICc < 3 are shown, together with the null model. All models for the three species are listed in Supplementary material (Table 3.7).

Intercept	Shrub height	Herbaceous height	Shrub cover	Herbaceous cover	LL	k	ΔAICc	w_i
Wood mouse								
-4.1	0.023		0.764		-359.4	7	0.0	0.35
-4.6	0.027				-362.9	4	1.0	0.21
-4.3	0.023	0.007	0.793		-359.1	8	1.4	0.17
-4.2	0.024		0.476	0.990	-357.6	10	2.5	0.10
-4.7	0.027	0.006			-362.7	5	2.6	0.10
-3.8	(null)				-384.5	3	42.3	0.00
Western editerranean mouse								
-5.6		0.029			-234.7	4	0.0	0.47
-5.7	0.004	0.029			-234.5	5	1.7	0.20
-5.8		0.026		0.841	-232.6	7	1.9	0.18
-5.1	(null)				-241.9	3	12.4	0.00
Greater white-toothed shrew								
5.5	0.011	0.042			-313.6	5	0.00	0.70
-3.9	(null)				-326.5	3	21.73	0.00

Table 3.3 - Relative importance of vegetation variable to explain species presence at the trap level. For each variable, the Akaike weights (w_i) of the models where the variable was present were summed. Higher values represent higher importance.

Species	Shrub height	Herbaceous height	Shrub cover	Herbaceous cover
Wood mouse	1.00	0.31	0.67	0.16
Western Mediterranean mouse	0.31	1.00	0.05	0.29
Greater white-toothed shrew	0.91	1.00	0.19	0.07

3.5. DISCUSSION

Our findings show that highway verges can be important habitat for small mammals within intensively livestock used Mediterranean landscapes. We confirmed that highway verges are capable of supporting a relatively high abundance of small mammals, in most cases higher than pure *montado* and open sites. This was particularly true for wood mice and shrews. Our results are in agreement with previous studies that have reported higher abundances of small mammals in highway verges compared to surrounding areas (e.g. Adams & Geis 1983; Meunier *et al.* 1999; Bellamy *et al.* 2000). Also, together with observed captures of the endangered Cabrera vole in our study sites, we were able to confirm that verges are not only used by multiple species, but also by those of high conservation concern (Santos *et al.* 2007).

We found no differences in sex ratios for highway sites except for shrews. However, we take this result with caution, as it can be difficult to identify correctly the sex of shrews due the lack of sexual dimorphism (see Searle 1985). Thus, considering only the results from wood mouse and western Mediterranean mouse, the presence of the highway does not induce changes in sex ratios. The data further shows that within intensively used areas, highway verges should be included in management plans toward conservation of biodiversity and the whole agrosilvopastoral system.

Being the first study in a Mediterranean environment regarding highway verge importance for small mammals, our results suggest that the higher abundance here found was related to the presence of fences. Fencing converts verges into grazing exclosure areas, allowing good vegetative structure to develop. In turn, the vegetation provides cover protection from predators and important food and shelter resources for small mammals. There is an extensive literature showing that cover is a limiting factor for small mammals and required mainly for predator avoidance (Longland & Price 1991; Fitzgibbon 1997; Kotler 1997; Torre *et al.* 2007; Muñoz *et al.* 2009). Our results are in agreement with this previous research. All models showed that a higher probability of occupancy of wood mice was related to taller and denser shrub cover. In fact, this species is often found in well conserved and extensively managed *montado* patches (Rosalino *et al.* 2011) and, according to our surveys, the shrub layer was significantly taller and denser in verges surrounded by *montado* areas, relatively to pure areas themselves. This may explain the higher abundance of this species in highway verges. Likewise, the absence of western Mediterranean mice in *montado* sites and the low number of captures of shrews in *montado* and open areas is probably related to the low vegetative structure there found. Shrews are known to respond positively to grassland cover (Rodríguez & Peris 2007) and its highest abundance was recorded in highway verges surrounded by open areas where the herbaceous layer was taller.

Therefore, fenced highways probably make the highway verges an attractive habitat for small mammals, particularly in areas of higher livestock pressure. In fact, vegetation in verges is usually planted or managed in a way to allow their growth for artificial landscaping reasons, with occasional mowing, while in *montado* areas the livestock grazing may significantly reduce the vegetative structure. Traditionally, extensive livestock stocking rates permitted maintaining the shrub and herbaceous layers stable (Moreno & Pulido 2008). However, after Portugal and Spain joined the European Union, the Common Agricultural Policy (CAP) led to a significant increase in stocking rates (Moreno & Pulido 2008). The livestock numbers reached similar densities to those found in highly productive regions in northern Europe, in spite of the Mediterranean environments being less productive (Campos 2004).

When in high densities, livestock grazing and trampling result in a decrease of vegetative complexity, and thus in a reduction of cover and protection for small mammals. For example, Torre *et al.* (2007) measured the small mammal abundance, species composition, vegetation structure and soil compaction in 22 plots pertaining to two grazing levels (grazed areas and cattle exclosures) in a Mediterranean landscape. They found that in areas where cattle had been excluded, the vegetation height and density increased and soil compaction decreased, which led to higher small mammal abundance and species richness. The authors concluded that the effect of grazing on small mammal communities was mainly due to reduced food availability and by negative effects of trampling on the suitability of soils for building burrow systems. Other authors also described the negative effects of trampling in soil compaction, with consequent reduction of the available area for building and maintaining stable burrows for small mammals (e.g. Bilotta *et al.* 2007).

These facts may also explain the different abundance detected in the trap lines of highway sites. Overall we found a higher abundance of small mammals in the median line and a lower abundance in the outer line, but no pair trap-line comparison remained significant after sequential Bonferroni correction. Although located nearby the verge, outer trap line traps were within the grazing area, and therefore the vegetative structure could probably offer a low protection for predators. Thus, the median line might provide an extra protection due to its location in between traffic lanes which may limit the incursions of predators such as carnivores (Fahrig & Rytwinski 2009), and therefore allowing for higher small mammal abundances in this line.

Given our results, we suggest that the proper management of highway verge vegetation and the creation of grazing controlled areas nearby the highway might significantly influence the abundance of small mammals, and therefore constitute two important actions aimed to conserve biodiversity and habitats. Transportation agencies generally mow highway verges regardless of the distance from highway edge. We recommend verges continue to be managed for motorist safety objectives, but also that verge margins outside of the "clear zone" (ca. 10-12 m from pavement edge) should be managed to allow taller and denser vegetative structure. Although our target species responded positively to higher vegetative structure, we detected some differences in the relative importance of different height and cover of herbaceous and shrub layers. Therefore, verge management should try to create vegetative structure that is variable in these attributes in order to favor greater biodiversity of small mammal species living there (e.g. creating some spots with taller shrubs and others without shrubs but with a higher and denser grass layer).

Where applicable, verge management should also be included in broader scale conservation actions away from the highway for protecting connectivity of small mammal populations and their habitat. Several studies demonstrated the positive effects of managed grazing areas in vegetative structure and soil quality, which in turn favored higher small mammal abundance (Fitzgibbon 1997; Hayward *et al.* 1997; Eccard *et al.* 2000; Todd *et al.* 2000; Gelling *et al.* 2007; Gonçalves *et al.* 2011). Thus, we suggest creating fenced areas, with very low rates of livestock grazing, within intensive sylviculture production areas. These managed grazed areas could be linked to highway verges through other habitat elements such as vegetated linear features, e.g. hedgerows or riparian areas. The creation of these managed grazing areas and restoration of vegetative linear features could take advantage of European Union agri-environment schemes (Kleijn & Sutherland 2003; Donald & Evans 2006; Whittingham 2007). In subsidy programs, farmers are paid to modify their farming practices to provide environmental benefits, including reduction of livestock farming.

By implementing these practices, we would expect the improvement of the overall landscape connectivity and population persistence of small mammals. In turn, by contributing to the population persistence of these species, these measures will help maintain the equilibrium of the trophic chain and the regeneration of the agrosilvopastoral system. For example, wood mice are an important prey species for top predators, including endangered species such as wildcat (Sarmento 1996; Moleón & Gil-Sánchez 2003), and also several raptor species (Korpimaki & Norrdahl 1991). Wood mice are also important seed dispersers of acorns (Gómez *et al.* 2008).

In order to implement these two conservation actions, it is important to have an inventory of distribution and quality of vegetative structure and habitat along highway networks in Mediterranean regions of the Iberian Peninsula. This inventory should consider the location and the quality of the vegetation structure of verge habitats occurring in highway corridor and lands adjacent to it, including hedgerows, field margins and riparian areas. A large-scale inventory could be performed with aerial images in a geographic information system, although site-specific management actions would require field verification. With this information, potential sites to implement managed grazing areas could be identified and selected based on proximity and connectivity to highway sections of conservation value.

It should be noted that by increasing the prey density in highway verges, these may attract more predators to the highway corridor. This will in turn increase the risk of predator-vehicle collision, as reported for polecat (*Mustela putorius*) by Barrientos & Bolonio (2009). However, as suggested by these authors and other studies (e.g. Grilo *et al.* 2009), by reducing the mesh size of existing highway fencing, particularly in locations where is likely to occur mortality hot-spots (Ramp et al. 2005), it is possible to reduce the access of predators to verge area.

Further study of fenced highway verges, together with grazing exclosure areas connected by natural habitat elements, will help identify the role of managed verges in the conservation of small mammal populations and predators in the Mediterranean agrosilvopastoral system.

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SUPPLEMENTARY MATERIAL

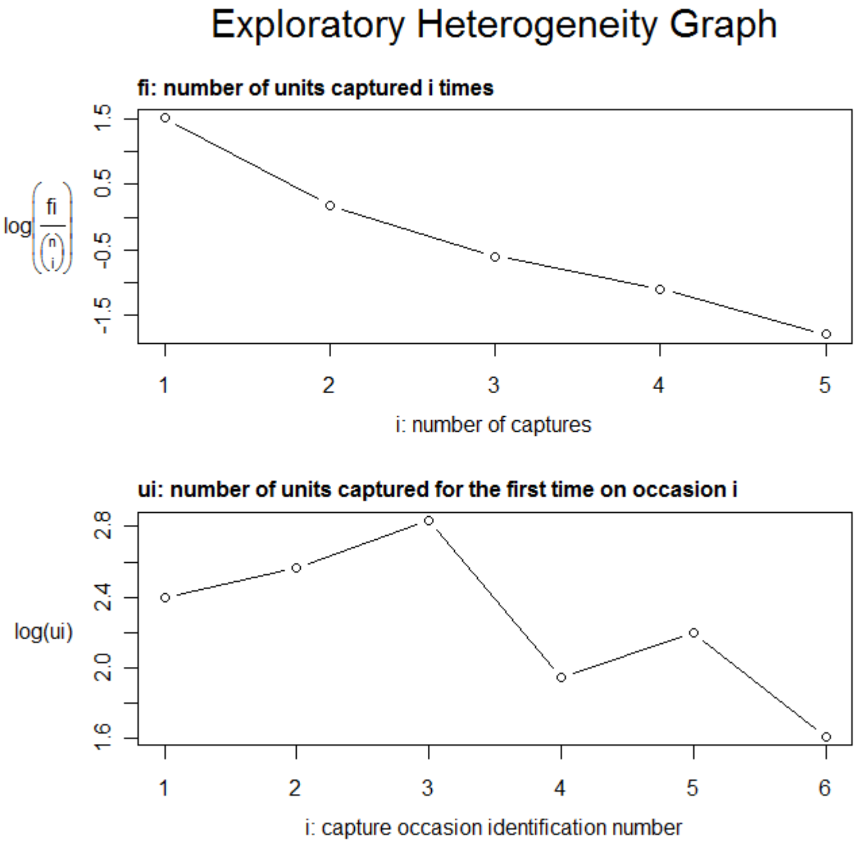

Fig. 3.6 - Output from 'descriptive' function in Rcapture package (Baillargeon & Rivest 2009) using the capture-mark-recature data from sites with 6 nights of: number of units captured i times (fi) and the number of units captured for the first time on occasion i (ui).

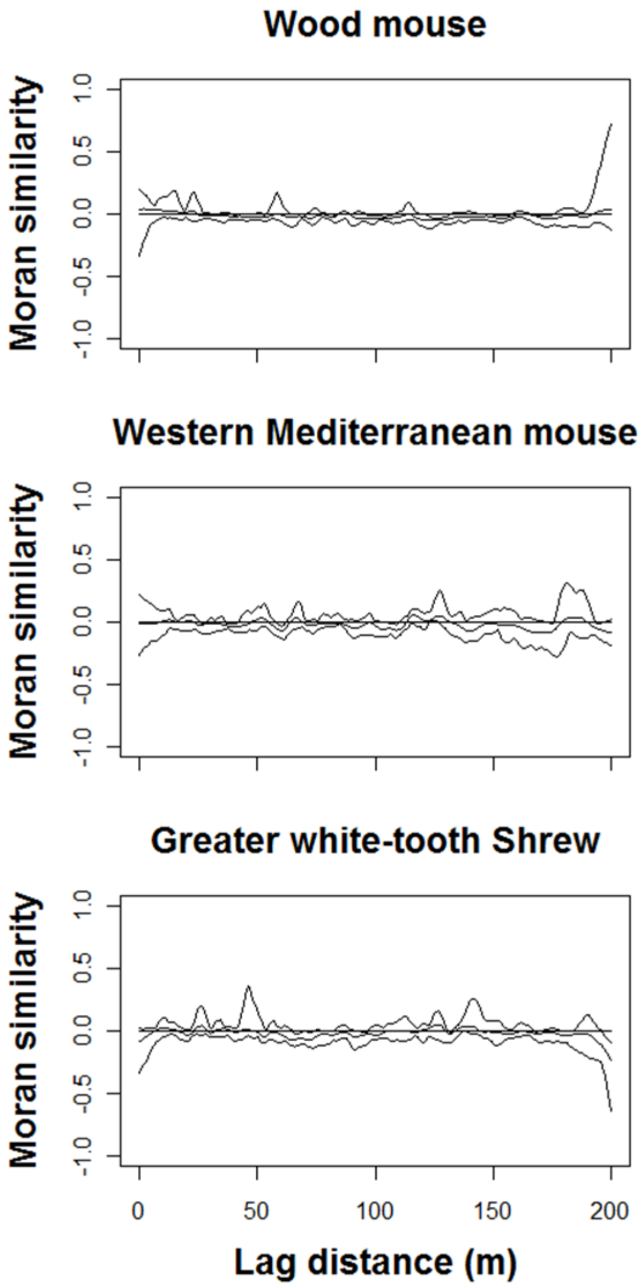

Fig. 3.7 - Spline correlogram with 95% pointwise bootstrap confidence intervals (1000 resamples for bootstrap confidence intervals) of the residuals from the GLMM including all the vegetation variables fitted to the species presence data. The intercept of the spline correlogram in xx-axis is the distance at which data are no more similar than that expected by-chance-alone across the defined region.

Table 3.4 - Pair site comparisons of abundance in the four sampling site types, performed by Kruskal-Wallis. Sampling sites consisted in highway sites surrounded by montado areas (HWY_MONT), highway sites surrounded by open areas (HWY_OPEN), *montado* areas (MONT) and open areas (OPEN). Abundance refers to minimum number of individuals alive per sampling effort. LCL and UCL are lower and upper confidence limits. The p-values in bold remained significant after sequential Bonferroni correction.

Pair site	All species				Wood mouse				Western Mediterranean mouse				Greater white-tooth Shrew			
	Test statistic	p-value	LCL	UCL	Test statistic	p-value	LCL	UCL	Test statistic	p-value	LCL	UCL	Test statistic	p-value	LCL	UCL
HWY_MONT - HWY_OPEN	5.4	0.220	-3.4	14.3	13.3	**0.010**	3.4	23.2	0.9	0.826	-7.6	9.5	2.9	0.382	-3.8	9.7
HWY_MONT - MONT	17.7	**<0.001**	9.7	25.7	10.3	0.025	1.4	19.3	13.1	**0.002**	5.3	20.8	14.2	**<0.001**	8.1	20.3
HWY_MONT - OPEN	15.1	**<0.001**	7.1	23.1	14.9	**0.002**	5.9	23.8	5.8	0.140	-2.0	13.5	15.0	**<0.001**	9.0	21.1
HWY_OPEN - MONT	12.3	**0.004**	4.3	20.3	3.0	0.506	-6.0	11.9	14.0	**0.001**	6.3	21.7	17.1	**<0.001**	11.0	23.2
HWY_OPEN - OPEN	9.6	0.020	1.6	17.6	1.6	0.719	-7.3	10.5	6.7	0.088	-1.1	14.4	18.0	**<0.001**	11.9	24.1
MONT - OPEN	2.6	0.452	-4.4	9.7	4.5	0.249	-3.3	12.4	7.3	0.036	0.5	14.1	0.9	0.745	-4.5	6.2

Table 3.5 - Pair trap-line comparisons of abundance in the highway sampling sites, performed by Kruskal-Wallis. Sampling sites consisted in highway sites surrounded by *montado* areas (HWY_MONT) and highway sites surrounded by open areas (HWY_OPEN). Abundance refers to minimum number of individuals alive per sampling effort. LCL and UCL are lower and upper confidence limits. None of the p-values remained significant after sequential Bonferroni correction.

Site tipe	Trap lines	All species				Wood mouse				Western Mediterranean mouse				Greater white-tooth Shrew			
		Test statistic	pvalue	LCL	UCL	Test statistic	pvalue	LCL	UCL	Test statistic	pvalue	LCL	UCL	Test statistic	pvalue	LCL	UCL
HWY_MONT	Margin – Outer	10.3	0.054	-0.2	20.7	6.5	0.050	0.0	13.0	0.8	0.754	-4.8	6.5	5.4	0.09	-1.1	11.9
	Median – Margin	0.9	0.852	-9.4	11.4	2.5	0.431	-4.0	9.0	2.5	0.354	-3.1	8.2	1.4	0.65	-5.1	7.9
	Median – Outer	11.2	0.035	0.8	21.6	4.0	0.213	-2.5	10.5	1.7	0.534	-3.9	7.3	4.0	0.21	-2.5	10.5
HWY_OPEN	Margin – Outer	1.5	0.778	-9.2	12.3	0.8	0.754	-4.8	6.5	0.5	0.850	-6.1	7.2	3.2	0.348	-3.8	10.2
	Median – Margin	3.6	0.500	-7.1	14.4	2.5	0.354	-3.1	8.2	2.4	0.450	-4.2	9.0	0.4	0.899	-6.5	7.4
	Median – Outer	5.1	0.339	-5.6	15.9	1.7	0.534	-3.9	7.3	1.8	0.560	-4.7	8.5	2.7	0.415	-4.2	9.8

Table 3.6 - Pair site comparisons of vegetation structure in the four sampling site types, performed by Kruskal-Wallis. Sampling sites consisted in highway sites surrounded by *montado* areas (HWY_MONT), highway sites surrounded by open areas (HWY_OPEN), montado areas (MONT) and open areas (OPEN). LCL and UCL are lower and upper confidence limits. The p-values in bold remained significant after sequential Bonferroni correction.

	Shrub height				Herbaceous height				Shrub cover				Herbaceous cover			
Pair site	Test statistic	pvalue	LCL	UCL	Test statistic	pvalue	LCL	UCL	Test statistic	pvalue	LCL	UCL	Test statistic	pvalue	LCL	UCL
HWY_MONT-HWY_OPEN	201.1	**<0.001**	151.0	251.1	85.8	**0.012**	18.6	153.0	283.3	**<0.001**	238.7	327.9	87.1	**0.002**	14.0	160.1
HWY_MONT-MONT	242.9	**<0.001**	197.6	288.1	26.1	0.399	-34.6	86.9	109.2	**<0.001**	68.8	149.5	194.7	**<0.001**	128.6	260.7
HWY_MONT-OPEN	620.8	**<0.001**	575.6	666.0	183.9	**<0.001**	123.1	244.7	358.5	**<0.001**	318.2	398.8	447.1	**<0.001**	381.0	513.1
HWY_OPEN-MONT	41.8	0.07	-3.4	87.0	59.7	0.054	-1.1	120.5	174.1	**<0.001**	133.8	214.4	107.6	**<0.001**	41.5	173.7
HWY_OPEN-OPEN	419.7	**<0.001**	374.5	465.0	98.0	**0.002**	37.3	158.8	75.2	**<0.001**	34.9	115.5	360.0	**<0.001**	293.9	426.0
MONT-OPEN	377.9	**<0.001**	338.0	417.8	157.7	**<0.001**	104.1	211.3	249.3	**<0.001**	213.7	284.9	252.4	**<0.001**	194.1	310.6

Table 3.7 - Generalized linear mixed models describing target species presence with shrub and herbaceous height and density (fixed effects), at the trap level. Cover variables were treated as ordinal. For each model it is shown the variables' estimates, log-likelihood (LL), number of parameters (k), change in Akaike information criterion with correction for small sample sizes (ΔAICc), AICc weight (w_i). Models are ranked according to the ΔAICc. Estimates for ordinal variables are shown only for models with AICc < 3.

Model	Intercept	Shrub height	Herbaceous height	Shrub cover	Herbaceous cover	LL	k	ΔAICc	w_i
Wood mouse									
1	-4.1	0.023		0.764		-359.4	7	0.0	0.35
2	-4.6	0.027				-362.9	4	1.0	0.21
3	-4.3	0.023	0.007	0.793		-359.1	8	1.4	0.17
4	-4.2	0.024		0.476	0.990	-357.6	10	2.5	0.10
5	-4.7	0.027	0.006			-362.7	5	2.6	0.10
6	-4.2	0.024	0.001	+	+	-357.6	11	4.5	0.04
7	-4.7	0.028			+	-362.4	7	6.0	0.02
8	-4.7	0.028	0.003		+	-362.3	8	7.9	0.01
9	-3.2			+		-371.8	6	22.8	0.00
10	-3.5		0.01	+		-371.1	7	23.6	0.00
11	-3.3			+	+	-370.4	9	26.1	0.00
12	-3.4		0.005	+	+	-370.3	10	27.9	0.00
13	-3.9					-384.5	3	42.3	0.00
14	-4.1		0.01			-383.9	4	43.1	0.00
15	-3.8				+	-384.4	6	48.1	0.00
16	-4.1		0.012		+	-383.7	7	48.6	0.00
Western Mediterranean mouse									
1	-5.6		0.029			-234.7	4	0.0	0.47
2	-5.7	0.004	0.029			-234.5	5	1.7	0.20
3	-5.8		0.026		0.841	-232.6	7	1.9	0.18
4	-6.0	0.006	0.026		+	-232.3	8	3.2	0.09
5	-5.7		0.029	+		-234.6	7	5.9	0.02
6	-5.9	0.005	0.029	+		-234.4	8	7.5	0.01
7	-5.8		0.026	+	+	-232.5	10	7.7	0.01
8	-6.0	0.006	0.026	+	+	-232.1	11	9.0	0.01
9	-5.5				+	-238.3	6	11.3	0.00
10	-5.1					-241.9	3	12.4	0.00
11	-5.7	0.006			+	-237.9	7	12.5	0.00
12	-5.1	0.002				-241.8	4	14.3	0.00
13	-5.4			+	+	-238.1	9	16.9	0.00

Model	Intercept	Shrub height	Herbaceous height	Shrub cover	Herbaceous cover	LL	k	ΔAICc	w_i
14	-5.6	0.006		+	+	-237.8	10	18.3	0.00
15	-5.2			+		-241.8	6	18.3	0.00
16	-5.3	0.003		+		-241.7	7	20.1	0.00
Greater white-toothed shrew									
1	-5.5	0.011	0.042			-313.6	5	0.00	0.70
2	-5.7	0.011	0.045	+		-312.1	8	3.10	0.15
3	-5.2		0.039			-317.0	4	4.71	0.07
4	-5.5	0.012	0.04		+	-313.3	8	5.44	0.05
5	-5.2		0.042	+		-315.0	7	6.89	0.02
6	-5.6	0.012	0.041	+	+	-311.3	11	7.45	0.02
7	-5.2		0.039		+	-317.0	7	10.74	0.00
8	-5.1		0.04	+	+	-314.5	10	11.97	0.00
9	-4.4	0.012			+	-321.4	7	19.71	0.00
10	-4.1	0.008				-324.7	4	20.11	0.00
11	-3.9					-326.5	3	21.73	0.00
12	-4.4	0.012		+	+	-319.8	10	22.45	0.00
13	-4.2	0.008		+		-324.0	7	24.76	0.00
14	-4.1				+	-325.1	6	24.99	0.00
15	-3.9			+		-325.8	6	26.36	0.00
16	-4.0			+	+	-322.9	9	26.63	0.00

4. Highways as barriers to gene flow for wood mouse: a genetic and agent-based modeling approach

HIGHWAYS AS BARRIERS TO GENE FLOW IN WOOD MICE (*APODEMUS SYLVATICUS*): A GENETIC AND AGENT-BASED MODELING APPROACH

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Preliminary note: the sampling design of this study involved two replicates for each of the three surveyed highways. Due to logistical constrains however, it was not possible to have the entire genetic datasets completed by the time this manuscript was written. Therefore, the data presented and the manuscript structure considers the existence of a single replicate per highway.

ABSTRACT

Roads are known to induce a barrier effect, a process by which roadside subpopulations may become isolated and therefore more prone to lose genetic variability by random drift, to inbreeding, and ultimately to extinction. To the best of our knowledge, no study has yet examined the relative importance of traffic volume and road age for the level of road barrier effect, although this information is crucial to guide and prioritize mitigation efforts. The main goal of our study was to investigate how genetic differentiation among roadside populations of small mammals may be influenced by road age and traffic volume, using the wood mouse (*Apodemus sylvaticus*) as a model species. We collected genetic samples of wood mice from both roadsides of three highways with similar width but different age and traffic volumes. A total of 155 samples were genotyped for nine microsatellite loci. We assessed genetic structure between roadsides by quantification of population differentiation, number of migrants, pairwise relatedness and clustering methods. We then compared observed genetic parameters with the results from a spatially-explicit simulation model to test inferences on the degree of barrier effect of each highway. The genetic differentiation and pairwise relatedness were similar for the three highways (e.g. F_{st}: 0.015, 0.011 and 0.09) and indicated some degree of isolation between roadsides. Comparisons between the observed data and simulations suggest that the time since the road was built has a stronger effect driving the reduction of connectivity between roadside sub-populations. We suggest the retrofitting of crossing structures (e.g. culverts), in older highways and in areas where verges have a well-developed vegetation structure, to prevent the negative consequences of increased genetic drift in road-bisected populations of small mammals.

Keywords: road barrier effect; genetic differentiation; relatedness; spatially-explicit simulation model; microsatellites

4.1. INTRODUCTION

Roads have become ubiquitous features in worldwide contemporary landscapes causing negative impacts in numerous species (Forman *et al.* 2003). Among the most important impacts, roads are known to induce a barrier effect (Forman & Alexander 1998). This can result from roads functioning as a physical obstacle hampering animal displacements (Marsh *et al.* 2005), or by promoting animal-vehicle collisions and thus eliminating potential road-crossers (Carr & Fahrig 2001; Grilo *et al.* 2009). Animals may also exhibit an avoidance behavior toward roads, thereby decreasing individual exchange across them (Ford & Fahrig 2008; McGregor *et al.* 2008), including wood mouse. For example, Macherson *et al.* (2011) performed a capture-recapture study of individuals inhabiting verges of two roads with less than 6 m and 3 m wide (both with less than 20 vehicles per hour), and detected that only 4.4% of the 363 movements equivalent to the road width involved actual road crossings. Moreover, road verges may be used as home range boundaries and this behavioral barrier can inhibit the reproduction of individuals that successfully cross roads (Burnett 1992; Riley *et al.* 2006).

In any of its multiple forms, the road barrier effect may lead to a genetic subdivision of animal populations (see reviews by Balkenhol & Waits 2009; Holderegger & Di Giulio 2010), a process known to accelerate the loss of genetic variability due to random drift (Hartl & Clark 1997). In turn, inbreeding may lower demographic rates and increase the probability of local extinctions (Saccheri *et al.* 1998; Westemeier *et al.* 1998; Reed *et al.* 2007). Therefore, the study of the road barrier effect and its consequences for wildlife population genetics has become a major topic in road ecology (Forman *et al.* 2003; Roedenbeck *et al.* 2007; Balkenhol & Waits 2009).

Two road characteristics have been referred as major factors influencing the barrier effect and consequently the level of genetic subdivision they may cause: traffic volume and road age (e.g. Keller & Largiadèr 2003; Riley *et al.* 2006; Marsh *et al.* 2008). However, to date no study examined the relative importance of these factors in genetic differentiation (Balkenhol & Waits 2009; Holderegger & Di Giulio 2010). This information is crucial to guide and prioritize mitigation efforts, but disentangling confounding effects in road ecology studies can be complex (Clevenger & Waltho 2005), particularly when dealing with road age and traffic volume. For instance, because spatial genetic structure takes several generations to build up, the barrier effect can be difficult to perceive in recently built highways (Murphy *et al.* 2008). Also, as roads are rarely complete barriers to animal movement (Holderegger & Di Giulio 2010), some migration is expected to occur between roadsides and hence populations may be only weakly differentiated. However, the success in road crossing is expected to vary with traffic volume (Jaarsma *et al.* 2006), thus resulting in different levels of road barrier effect.

Recently built roads and variable recurrent migration can therefore pose a challenge for the ascertainment of genetic subdivision. A possible strategy to deal with this problem is to use several replicates for each analyzed road, but collecting such amount of data may be cost and time prohibitive (Holderegger & Di Giulio 2010). A potentially useful approach, which can be used synergistically with field replication, is agent-based model simulation (ABM, DeAngelis & Mooij 2005; Epperson *et al.* 2010; Railsback & Grimm 2011). Unlike traditional approaches used to correlate genetic differentiation patterns with landscape features, agent-based modeling allows specifying landscape and demographic factors that shape gene flow, while controlling for several sources of uncertainty such as habitat heterogeneity, ecological interactions (diseases, competition, or predation), or historical effects (Landguth & Cushman 2010; Shirk *et al.* 2012). The most probable conditions in the real world can then be inferred by determining which parameters lead to simulated patterns of genetic differentiation that best match the observed ones (e.g. Landguth *et al.* 2010; Grosman *et al.* 2011;

Shirk *et al.* 2012). This process is usually referred to as inverse modeling (Hartig *et al.* 2011) and can be used to independently validate a road ecology genetic model.

Rodents are useful organisms to evaluate the effects of fragmentation on small terrestrial vertebrates because their biology is well known and they comprise a large number of species with different dispersal abilities and habitat preferences (Macdonald & Barrett 1993). Moreover, as demonstrated by several studies using capture-mark-recapture and translocation techniques (Goosem 2001; McGregor *et al.* 2008), movement tracking (Oxley *et al.* 1974; McDonald & Clair 2004; McLaren *et al.* 2011) or genetic approaches (Gerlach & Musolf 2000), small mammals are known to be particularly affected by the road barrier effect, with low crossing rates, inhibition of crossing movements, or population genetic structuring reported in different species.

Here we focused on the wood mouse (*Apodemus sylvaticus*) as a model species. This species apparently benefits from highway verge proximity in Mediterranean agrosilvopastoral landscapes, due to a higher vegetation cover close to highways (Ascensão *et al.* 2012; Ruiz-Capillas *et al. in press*), but there are no data regarding the genetic consequences of the road barrier effect for this species. From a conservation perspective, the wood mouse is an important study organism in the context of the Mediterranean agrosilvopastoral system (*montado*), particularly in southern Iberia Peninsula. In fact, the wood mouse is a key item within cycles of vegetative regeneration (Watts 1968), e.g. an important seed disperser of oak acorns (Gómez *et al.* 2008), being also a common prey for many top predators (Korpimaki & Norrdahl 1991; Sarmento 1996; Moleón & Gil-Sánchez 2003; Pineiro & Barja 2011). Therefore, the knowledge on how roads may affect the population genetics of this species may be also valuable for conservation management plans involving top predators and the agrosilvopastoral system.

The main goal of our study was to assess how genetic differentiation is related to road age and to traffic volume. We studied wood mice roadside populations in three highways with similar width but different age and traffic volumes. For each highway, we estimated the genetic differentiation, structure, and relatedness within and between roadsides. We then compared observed genetic parameters with the results from ABM simulations, to test inferences on the degree of barrier effect in each highway. We expected to obtain evidence for higher population fragmentation in the oldest and with more traffic volume highways, but the relative effects of traffic and age were unknown.

4.2. MATERIALS AND METHODS

4.2.1. STUDY SITES AND SAMPLING DESIGN

One highway in Portugal and two in Spain were selected for sampling. The three highways are similar in width (ca. 50 m) including a median strip and grassy verges, but have different ages and traffic volumes (Table 3.1). The sampling sites were located in areas in which the highways bisected oak woodlands, a preferred habitat for wood mice in Iberia (Rosalino *et al.* 2011). We avoided sites with any obvious signs of human disturbance (e.g. urban proximity).

Table 4.1 - Location (country and coordinates) and characteristics of the studied highways classified according to their age (when sampling was performed) and traffic volume (number of vehicles per day). Traffic refers to nighttime period, from 2000 to 0600, as the wood mouse is a crepuscular-active species (Corp *et al.* 1997).

Highway	Site location	Age / Class		Traffic / Class	
AP6	Spain (40°45'8, 4°20'55)	35	Old	2397	High
A2	Portugal (38°25'24, 8°30'57)	13	New	2200	High
AP51	Spain (40°43'22, 4°31'39)	10	New	612	Low

We trapped wood mice in both roadsides of each highway up to 50 m from the pavement. Wood mice were captured using Sherman™ live traps (size 23 x 8 x 9 cm; baited with a mixture of sardine in oil and oatmeal, and cotton as nesting material). We sampled at least 25 individuals from each roadside, which was normally achieved in less than 15 consecutive days. From each captured animal, we collected approximately 1 cm of the tip of the tail or an ear biopsy for DNA extraction, and the cuts were disinfected with antiseptic solution. The high frequency of recaptures confirmed our assumption that such sampling procedure was safe for the animals. Each individual was sexed and marked with a fur-clipping code denoting capture's roadside and released at capture site. Individual marking aimed at detecting road crossings and avoid resampling of individuals. The whole procedure took approximately five minutes per individual. Capture procedures and animal handling were in conformity with

Portuguese and Spanish nature conservation directives (Instituto da Conservação da Natureza e das Florestas in Portugal; Dirección General de Medio Natural in Spain), and in compliance with the European Communities Council Directive 86/609/EEC for animal experiments.

4.2.2. MICROSATELLITE GENOTYPING

We amplified nine microsatellites TNF(CA) (Makova *et al.* 1998); As11, As20, As34 (Harr *et al.* 2000); SFM2 (Wu *et al.* 2008); and SCFM2, SCFM6, SCFM9 (Gu *et al.* 2009). See *Supplementary Material S1* for laboratory procedures on DNA extraction and microsatellite genotyping.

4.2.3. GENERAL STATISTICS

For each road and roadside, we tested for linkage disequilibrium and analyzed deviations from Hardy–Weinberg equilibrium (HWE) using the exact probability and heterozygote deficiency tests, as implemented in the program GENEPOP 4.1.4 (Rousset 2008). P-values were adjusted by sequential Bonferroni correction for multiple tests (Rice 1989). We also estimated the inbreeding coefficient (F_{is}) for each road and roadside, using this same software. Allelic richness and private allelic richness were determined using HP-RARE 1.0 (Kalinowski 2005), with a sample size of 50 genes (25 diploid individuals), which corresponded to the fewest individuals sampled from a single roadside (see Results). We used MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.* 2004) to check for evidence of null alleles, allelic dropouts or mis-scoring.

4.2.4. GENETIC DIFFERENTIATION

In order to test for a barrier effect, we estimated and analyzed the genetic differentiation, population structuring, and relatedness of individuals between roadsides. We assessed the degree of genetic differentiation using F_{st} (Weir & Cockerham 1984). Because F_{st} can be influenced by the level of heterozygosity (Meirmans & Hedrick 2011), we also estimated Nei's G_{st} (Nei 1973), Hedrick's G'_{st} (Hedrick 2005) and Jost's D (Jost 2008). For each estimator we tested if it significantly differed from zero, the situation where both roadside populations belong to a single random breeding population, using bootstrap procedures (1000 replicates). A value of an estimator was deemed significant when the confidence interval around its mean did not contain zero. Estimators and bootstrap were calculated using R package 'diveRsity' (Keenan 2011).

4.2.5. ASSIGNMENT ANALYSIS

We conducted an assignment analysis to detect directly possible immigrants into each roadside. Assignment tests were performed using GENECLASS 2.0 (Piry *et al.* 2004). This software computes a likelihood ratio from the population where the individual was sampled over the highest likelihood value among all population samples, including the population where the individual was sampled. We used a frequency-based method (Paetkau *et al.* 1995) to calculate the likelihood ratios. Probability values were determined using the Monte Carlo resampling method, simulating 1000 individuals and with a threshold of 0.05 (Paetkau *et al.* 2004).

4.2.6. RELATEDNESS

We estimated the pairwise relatedness using four different moment estimators: Queller & Goodnight (1989), Li *et al.* (1993), Lynch & Ritland (1999) and Wang (2002). We calculated the average within-population relatedness for dyads in which both individuals come from the same roadside, and the average between-population relatedness for dyads in which individuals come from opposite roadsides. We tested for differences in relatedness of these two groups using 1000 bootstrap replicates as implemented in COANCESTRY 1.0 (Wang 2011). Moment estimators were preferred over maximum-likelihood estimators (e.g. Milligan 2003; Wang 2007) because the ideal properties of maximum-likelihood estimators are only achieved asymptotically, i.e. when the number of loci is large (Lynch & Ritland 1999; Wang 2002; Milligan 2003).

We also quantified the number of related pairs of individuals within and among roadsides in each highway. Relatedness values range from -1 to 1, indicating the percentage of alleles shared among individuals. Theoretically, a value of 1 means that genotypes are identical, a value of 0.5 indicates that 50% of the alleles are shared (e.g. parent/offspring or full-sibling relationship), and unrelated individuals have relatedness values ranging from -1 to 0. To define a threshold value over which a pair of individuals was considered related (Rollins *et al.* 2012), we simulated 10,000 genotypes of unrelated dyads from road-specific allele frequencies in COANCESTRY 1.0 (Wang 2011). We then estimated the pairwise relatedness for every pair of simulated individuals. The 99% percentile value of relatedness from the simulated data sets was used as the threshold over which any pair of individuals in the empirical data set was considered related.

4.2.7. DETECTING GENETIC CLUSTERS

The spatial population structure was investigated to detect clusters of genetically related individuals coinciding with the roadsides, using the Bayesian clustering method (Pritchard *et al.* 2000) implemented in STRUCTURE, version 2.3.4, and the discriminant analysis of principal components (DAPC; Jombart *et al.* 2010) available in the R package 'adegenet' (Jombart 2008). An important difference exists in the analytical approach between the two methods: STRUCTURE works by grouping individuals into clusters such that HWE is maximized within clusters, whereas the multivariate analysis used in DAPC does not make any assumption on the population genetic models (Jombart *et al.* 2010). This dissimilar approach may lead to different results (e.g. Latch *et al.* 2006; Frantz *et al.* 2009), and therefore both were used.

Regarding STRUCTURE, for each highway we conducted 10 independent runs for each value of *K* (the number of inferred clusters) with $K= 1$ and $K = 2$. Each run consisted of 1,000,000 Markov Chain Monte Carlo (MCMC) iterations, after a burn-in of 100,000 steps. We used a model allowing admixture and correlated gene frequencies (Hubisz *et al.* 2009). We calculated the average log-likelihood of all replicates for each value of *K* and estimated the posterior probability of each *K* using the formula given by Pritchard & Wen (2003). Evanno *et al.*'s (2005) ad hoc ΔK statistic is commonly used to infer the most plausible number of genetic clusters, but because it is based on the second-order rate of change of the likelihood function with respect to *K*, it cannot be estimated when testing is limited to $K = 1$ and $K = 2$. As for DAPC, *dapc* function was executed using the roadside as grouping variable of individuals (two groups). The number of PCA axes to be retained was inferred with the function *optim.a.score*, which measures the trade-off between power of discrimination and over-fitting. For both analyzes we inspected the probabilities of membership of individuals to the roadsides' clusters (*Q* values in STRUCTURE). If there is no genetic subdivision between roadside populations, we expect to find probability values close to 0.5 (Evanno *et al.* 2005).

4.2.8. AGENT-BASED MODEL SIMULATIONS

We developed an agent-based spatially explicit model to simulate the evolution of microsatellite-like loci in a population of territorial animals across a landscape. The landscape is composed of 30 x 40 patches representing 'territories', all of them with equal habitat conditions. The total simulated area is seen as large enough to mimic the population dynamics found in real landscapes. Gene flow is modeled as the result of dispersal and mating events, and follows an isolation by distance pattern (Wright 1943): dispersal and breeding are more likely among adjacent territories.

The landscape is bisected by a highway that imposes a variable barrier effect, hereafter referred as 'permeability'. This parameter varies between zero and 0.5, where zero corresponds to an impermeable barrier to gene flow, values between zero and 0.5 represent different permeability due to traffic volume and/or the road itself, and 0.5 represents no barrier effect, i.e., dispersal and mating are not influenced by the road. Four permeability values were tested in the simulations: 0.0, 0.1, 0.3 and 0.5. The model included four subroutines: mating, breeding, dispersal and death.

Mating: females mate with the nearest male. Males can mate with several females. For simplicity, we considered only one mating season per year. The probability of females mating with males in the opposite roadside is dependent on the permeability level.

Breeding: for each roadside, if there are available territories, fecundity is set to three young per mother, randomly decreasing to one or two young per mother when the number of adults surpasses the number of available territories. Density dependence has been proposed as a main regulatory mechanism of population size in wood mice, where females produce an average of four to seven young (Montgomery 1989a,b; Montgomery *et al.* 1991). We limited fecundity to a maximum of three to accommodate other causes of death, particularly predation. Moreover, it was our aim to simulate a population in equilibrium, so that we could assess the road barrier effect on population genetic differentiation.

Dispersal: given the scarcity of data on dispersal distances for rodents, and particularly for wood mice, we followed equation 3 in Bowman *et al.* (2002, equation 3). According to these authors, the mean dispersal distance in mammals has a direct relationship with home range area, expressed by the relation *mean dispersal distance = 7 x √home range*. Because cells in our model are squares that encompass one home range, we set dispersal to follow a Poisson distribution with mean 7.

Death: our model assumes discrete generations, and therefore individuals older than one year are removed from the simulation.

Simulations were run for 100 years. The first 30 years of each run were without road barrier effect (permeability = 0.5), to allow the establishment of isolation by distance (IBD) patterns, after which the permeability was changed if required. For genetic data analysis, the model sampled individuals from the four patch lines closer to the road, two in each roadside. For each simulation, we sampled 25 individuals from each roadside, a sample size similar to our empirical data sets. Individuals were sampled after dispersal. We used nine loci, again as in the empirical data set, with a mutation probability per generation of 4.5×10^{-5} (value referred for mouse by Dietrich *et al.* 1992) and assuming a stepwise mutation model (Kimura & Ohta 1978).

For each permeability value we ran 1,250 replicates, in a total of 5,000 replicates. We estimated F_{st} (Weir & Cockerham 1984) and relatedness (*r*) using Wang's (2002) estimator for all replicates and years to examine the evolution of these parameters with simulation time.

Previous to using our model, we compared it with EASYPOP 2.0.1 (Balloux 2001) to verify that they produced similar results under a Wright–Fisher population process. Details about the comparison between our ABM and EASYPOP can be found in *Supplementary Material S2*. Our ABM was developed in Netlogo 5.0.3, a free cross-platform multi-agent programmable modeling environment developed at Northwestern University (Wilensky 1999). The source code is available at http//roadgenetics.fc.ul.pt. Except when specified otherwise, all data analyses and plots were done using the R package (R Development Core Team, 2011).

4.3. RESULTS

4.3.1. SAMPLING

We sampled 155 wood mice in the roadsides of the three highways (n= 54 in AP6, n = 50 in A2, n = 51 in AP51) with a minimum of 24 samples in one roadside of AP6. We recaptured at least once 44 wood mice (total number of recaptures = 103), but there were no recaptures in opposite roadsides (i.e., no road crossings were detected).

4.3.2. GENERAL STATISTICS

No allelic dropout, stuttering or null alleles were detected. Of the 54 roadside/locus combinations, 43 and 42 were in HWE respectively for the probability and exact test (Table 4.4). The locus *as11* was involved in five of the disequilibria for each test, but since we found no evidence for null alleles we did not exclude it for the subsequent data analyses. Similar result was found when considering the roadsides together, for each road (Table 4.4 in *Supplementary Material S3*). We found no evidence of linkage disequilibrium among loci. Mean allelic richness at each roadside ranged from 15 in AP6 to 20 in A2, and the mean private allelic richness ranged between 0.386 in AP6 and 1.073 in A2 (Table 4.5 in *Supplementary Material S3*).

4.3.3. GENETIC DIFFERENTIATION

Results for the four genetic differentiation estimators (F_{st}, G_{st}, G'_{st} and D) were similar for the three highways, although AP6 had a stronger signal of differentiation, as revealed by the highest values of the four estimators and their confidence intervals (not containing 0, Table 4.2).

Table 4.2 - Genetic divergence among roadsides of observed data. Divergence was measured using F_{st} (Weir & Cockerham 1984), Nei's G_{st} (Nei 1973), Hedrick's G'_{st} (Hedrick 2005) and Jost's D (Jost 2008).

HW	F_{st}	G_{st_Nei}	G_{st_Hed}	D
AP6	0.015 (0.001-0.029)	0.009 (0.001-0.016)	0.202 (0.093-0.311)	0.136 (0.019-0.254)
A2	0.009 (0.000-0.021)	0.006 (0.000-0.013)	0.159 (0.061-0.257)	0.074 (0.000-0.177)
AP51	0.011 (0.000-0.026)	0.007 (0.002-0.015)	0.168 (0.063-0.272)	0.084 (0.000-0.188)

4.3.4. ASSIGNMENT ANALYSIS

Analyses with GENECLASS suggested that the overall proportion of recent migrants between roadsides was high, with the lowest being for highway AP6: eight individuals in AP6 (15%), 10 individuals in A2 (20%) and 17 in AP51 (34%).

4.3.5. RELATEDNESS

The pairwise relatedness analyses showed similar results among the three highways (Table 4.3). For the three highways, we found significant differences in relatedness, at the 95% confidence level, between individuals sampled in the same roadside relatively to individuals sampled in opposite roadsides. However, we detected a few pairs of individuals that could be considered related in opposite roadsides of highways A2 and AP51, but not in highway AP6 (Table 4.3).

Table 4.3 - Mean relatedness of individuals captured within the same and in opposite roadsides (bold) according to four estimators: *QG*, Queller and Goodnight (1989); *Li*, Li et al. (1993); *LR*, Lynch and Ritland (1999); and *W*, Wang (2002). Between brackets is the inferred number of related dyads between the roadsides. Number of dyad combinations for roadside 1, roadside 2 and between roadsides are as follows: AP6 300, 406 and 725; A2 300, 350 and 650; AP51 300, 300 and 625.

Estimator		AP6	A2	AP51
QG	Roadside 1	-0.003 (14)	-0.009 (10)	-0.009 (6)
	Roadside 2	-0.007 (17)	-0.012 (9)	-0.011 (14)
	Opposite	-0.032 (0)	-0.029 (9)	-0.030 (4)
Li	Roadside 1	-0.011 (17)	-0.023 (7)	-0.018 (6)
	Roadside 2	-0.018 (22)	-0.024 (7)	-0.025 (15)
	Opposite	-0.043 (0)	-0.041 (5)	-0.040 (3)
LR	Roadside 1	-0.005 (10)	-0.013 (7)	-0.013 (6)
	Roadside 2	-0.011 (9)	-0.015 (9)	-0.014 (6)
	Opposite	-0.029 (0)	-0.026 (1)	-0.027 (2)
W	Roadside 1	-0.011 (13)	-0.022 (8)	-0.023 (6)
	Roadside 2	-0.018 (14)	-0.027 (5)	-0.025 (12)
	Opposite	-0.042 (0)	-0.041 (3)	-0.042 (4)

4.3.6. DETECTING GENETIC CLUSTERS

For the three highways, STRUCTURE's cluster solution of highest likelihood was obtained for $K = 1$ and the posterior probability of having one population was equal to 1. Nevertheless, we observed a higher variability in probability membership values for AP6 when compared with A2 and AP51, for which we obtained values close to 0.5 (Fig. 4.1). This indicates higher genetic heterogeneity across AP6 than across A2 and AP51. As for DACP, the PCA components retained were 16, 11 and 14, which explained 60.5%, 59.2% and 60.7% of the total variance observed, respectively for AP6, A2 and AP51. This analysis revealed a higher population structuring, again with a similar pattern across roads, and assigned the population membership in with higher accordance to individuals' roadside of capture. For example, the mean membership probability of individuals caught in *roadside 1* belonging to cluster of *roadside 1* was 0.89±0.22 for AP6; 0.85±0.24 for A2 and 0.80±0.24 for AP51.

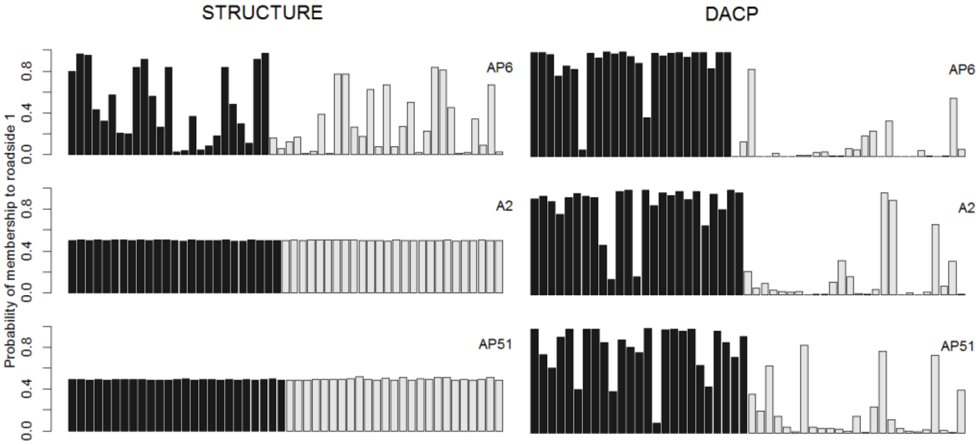

Fig. 4.1 - Bar plots showing individual genotype membership to *roadside 1* according to STRUCTURE and DACP methods (for STRUCTURE the plot presented for each road is a sample of the multiple runs). Each vertical bar represents an individual, colored according to the roadside where it was caught.

4.3.7. AGENT-BASED MODEL SIMULATIONS

The four relatedness estimators gave almost identical results with the simulated data sets, thus we only present results for Wang's (2002) estimator (Fig. 4.2). As expected, when the permeability level is reduced, F_{ST} increases and r decreases with simulation time. However, even for a low level of permeability (0.1), F_{ST} and r tend to change slowly and eventually stabilize a few generations after the highway is introduced in the simulations. The comparison of the observed F_{ST} and r with their respective distributions from the simulations suggests that the three highways, despite their differences in age and traffic volume, have caused a roughly similar reduction in gene flow between roadsides. Interestingly, F_{ST} comparisons would suggest a permeability level for the studied highways of about 0.3, whereas *r* comparisons would suggest road permeability of the highways to be around 0.1 (Fig. 4.2).

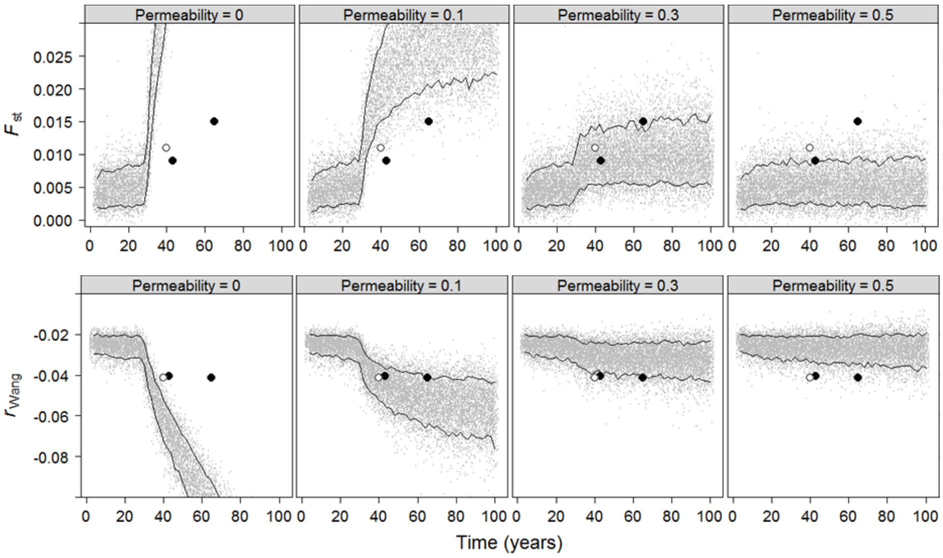

Fig. 4.2 - F_{st} and *r* (Wang 2002) from simulations (grey dots) with simulation time and road 'permeability' level. Black lines delimit the 10-90 percentil envelope of simulated data. Simulations were performed for 'permeability' varying between 0.0 (complete isolation) and 0.5 (no barrier effect). All simulations were replicated 1000 times. In all simulations a road is placed in the middle of the landscape at time = 30 years. Both F_{st} and *r* are measured from the individuals inhabiting the road vicinity cells. Observed data values are represented by the circles: AP6 and A2 are black dots (the former is the oldest road) and AP51 is the white dot. Remain relatedness estimators had similar results to Wang's *r.*

4.4. DISCUSSION

The fine-scaled genetic analysis applied in this study, replicated over three highways from two distinct geographic regions, demonstrated that highways can reduce the gene flow between roadside populations of wood mice. We aimed to quantify the relative importance of road age and of traffic volume in driving genetic isolation between roadside populations. Unexpectedly, several analyses showed evidences of a similar barrier effect among the three highways. This was the case for F_{st} and related estimators, for the relatedness estimators and for clustering analysis using DACP.

If there was a strong relation between the genetic isolation and traffic volume, the migration rate between roadsides in AP6 and A2 would be similar and lower than the migration rate in AP51. This would result in higher F_{st} and lower *r* in those highways relatively to AP51. On the other hand, if there was a strong relation between the genetic outputs and the time since highway was built, the migration rate in A2 and AP51 would be similar and higher than the migration rate of AP6. This would result in lower F_{st} and higher r in former highway relatively to AP6.

Hence, the obtained similar genetic patterns across highways may have resulted from two distinct causes: either none of the studied highways represent a barrier and had no effect on genetics of wood mice inhabiting its vicinity; or the road presence represent in fact a barrier that leads to a change in the genetic patterns, but those genetic patterns level shortly after the road is built. The former explanation is unlikely to be the correct one, given the low inferred number of related dyads between roadsides, particular for AP6 for which no related dyads were detected between roadsides. Also, the structuring pattern obtained with DACP for the three roads (and with STRUCTURE for AP6) is not concordant with a fully permeable highway scenario.

The second explanation implies that the population genetic patterns suffer some modification in first generations following the road is built as a result of the decreased permeability, but then it evolves gradually slowly with time. This second explanation is supported by the spatially explicit model simulations: we tested our observed data against a set of simulated scenarios where the migration rate between road sides varied, to determine the most probable level of road permeability. According to simulation results, the road permeability should decrease significantly relatively to the situation where the highway is either not present or has a null effect and both roadside populations belong to a single random breeding population. This caused the F_{st} and r to change steeply in first generations after the road was installed, and then to level or evolve in a slow rate.

An important road related genetic differentiation agent is the road-killing (Jackson & Fahrig 2011). In this study we did not account for this effect in driving population genetics, since the number of crossing attempts is likely to be small, as reported by a vast number of publications focusing on small mammals' crossing rates (Oxley *et al.* 1974; Goosem 2001; McDonald & Clair 2004; Rico *et al.* 2007; McGregor *et al.* 2008; Macpherson *et al.* 2011; McLaren *et al.* 2011). Also, during the present study we recorded no crossings from recapture data. In line with this, a preceding study using capture-recapture data also suggested that highways impose a barrier effect on wood mice populations: after surveying 14 highway segments, with 300 trap.night^{-1} each, only five of the 74 wood mice captured (7%) were recorded as having crossed a 2-lane highway, although the nighttime traffic volume was lower than 250 vehicles per hour (*authors' unpublished data*). Therefore, as bibliography suggests, the low crossing rates by small mammals are more likely to be due to a low number of crossing attempts irrespective of road traffic variations (Ford & Fahrig 2008; McGregor *et al.* 2008).

Hence, the most probable response of wood mouse to highway construction is a decline in the daily movement rates between roadside locations, regardless the traffic volume. However, albeit the decline in connectivity, some individuals inhabiting opposite roadsides still breed, and these events may be sufficient to prevent the complete isolation of roadside sub-populations. Hence, these scarce crossing movements are likely to be concentrated in time. In fact, movement rates of mammals, and of wood mouse in particular, increase during breeding and dispersal periods (Montgomery *et al.* 1991).

Nevertheless, in all analysis a stronger effect was detected for the oldest highway, AP6. This suggests that, with time, the decrease in connectivity may end up promoting a more severe isolation between roadside sub-populations. Therefore, the time since the road was built has an overall stronger effect promoting the partial isolation of the roadside sub-populations. This would explain why clustering analysis using STRUCTURE failed to detect the population differentiation, particularly for the younger highways: probably the time lag between road construction and surveys was short.

This result was not unexpected, as STRUCTURE does not always detect distinct subpopulations in cases where genetic differentiation between them is weak (Latch *et al.* 2006). Evanno et al. (2005) investigated the impact of sample size (samples of individuals and samples of loci) on the performance of STRUCTURE and noticed a decrease in performance when smaller sample sizes were applied. Consequently, a post-doc analysis was performed using STRUCTURE, with the data from 10 randomly chosen simulations with 'permeability' set to 0.1 (and the same number of loci and remain settings). This analysis suggested $K = 2$ for both timeframes. This suggests that the sample size was not an issue, but instead the current gene flow is such that the resulting genetic patterns prevent the software from capturing the decrease in permeability. This also reinforces that the probable road permeability in studied highways was not lower than 0.1.

On the other hand, the recently described method DAPC was able to capture the putative decrease in connectivity for all highways. The use of DAPC was important for analyzing genetic clusters according to the individuals' roadside, as the standard application of STRUCTURE analysis missed any sign of clustering in younger highways. In fact, the complement of using STRUCTURE and DAPC has been recommended for achieving less biased assessment of data (e.g. Kanno *et al.* 2011).

The stronger signal detected in relatedness analysis relatively to the F_{st} (and its relatives) is consistent with population genetic expectations. In fact, Palsbøll et al. (2010) recently suggested that kinship-based methods can be useful to estimate population structure when genetic divergence is low, such as when migration rates have suffered a recent reduction due to a road barrier effect. In fact, the differences in relatedness between individuals inhabiting the same roadside and in opposite sides, and the low number of related dyads captured in opposite roadsides support the evidence that roads are imposing a barrier to animal movement.

Although matching simulated genetic patterns to observed genetic patterns can subject to false inferences, error in simulations is largely independent of error in the empirical analysis approach. Thus, analysis using both methods on the same data would represent a more robust means of landscape genetic inference. In fact, agent-based model simulations have been successfully applied in landscape and barrier detecting genetic studies (e.g. Landguth *et al.* 2010; Shirk *et al.* 2012). Our study supports the importance of complementing empirical data with simulation models, as we were able to detect the probable level of barrier effect. Therefore, deducing that roads impose a connectivity breakdown only from capture-recapture observations could have led to inaccurate perception of complete barrier in our study system. Our approach is supported by previous research highlighting the need of combining field observations with genetic data when assessing population connectivity (e.g. Riley *et al.* 2006; Fedy *et al.* 2008; Finnegan *et al.* 2012).

Nevertheless, the question remains if the inferred road permeability is enough to counteract fragmentation or genetic drift and secure the genetic coherence of roadside populations of small mammals. Despite the accumulated ecological and behavioral data from decades of studies of natural populations, still little is known about the importance of roads as barriers to gene flow in small mammals (see reviews by Balkenhol & Waits 2009; Holderegger & Di Giulio 2010). Our study highlights that wood mouse and probably species with similar ecological traits may be partially isolated by roads. Given the high density of the road network in developed countries, some cumulative effects are expected to emerge and therefore management is required to mitigate the population isolation. Moreover, the effective barrier level can be higher, as the genetic outputs can be masked by several confounding effects, including by the population size (Gauffre *et al.* 2008; Marsh *et al.* 2008).

One form of mitigating the barrier effect could be the retrofitting of crossing structures such as culverts, in order to make them attractable for wood mice to use for crossings. The use of culverts by wood mice and other small mammals has been reported in previous research (Mata *et al.* 2005), and these crossing structures remain one of the few viable options for mitigating the effects of roads on smaller species (Clark *et al.* 2010). The retrofitting of road crossing structures should ideally be conducted in areas where verges have a lush vegetation structure. In fact, these areas can play a critical role for small mammals' persistence, providing refuge habitats and corridors (Bennett 1990; Bellamy *et al.* 2000; Doncaster *et al.* 2001; Huijser & Clevenger 2006; Ascensão *et al.* 2012). Moreover, according to our findings, older highways should be targeted first in mitigating the barrier effect for small mammals.

Nevertheless, road crossing structures might not mitigate the population structuring in all situations (Corlatti *et al.* 2009), particularly where the spatial arrangement of roads may strongly affect both the equilibrium density and the spatial distribution of populations (Borda-de-Água *et al.* 2011). We therefore suggest adopting a precautionary approach when road density is expected to disrupt the minimum size of habitat patches to sustain a viable population.

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SUPPLEMENTARY MATERIAL

S1–DNA EXTRACTION AND MICROSATELLITE GENOTYPING

We amplified microsatellites singly in 10 µL PCRs containing: 2 µL of genomic DNA extract, 0.8 mM dNTP (Bioline), 6.25 µg Bovine Serum Albumin (New England Biolabs), 2.5 mM MgCl2, 1X PCR buffer, 0.5 U SURF HotTaq DNA polymerase (StabVida) and 0.5 µM of a primer mix containing locus-specific forward and reverse primers and a fluorescently labeled (HEX or FAM) M13 primer tail (Schuelke 2000).

Two different PCR protocols were used for genotyping: a two-step PCR or a touchdown PCR. The two-step protocol consisted of an initial denaturation step of 95 °C for 15 min followed by 10 cycles of 94 °C for 30 s, 65 °C for 30 s and 72 °C for 45 s, then 30 cycles of 94 °C for 30 s, 60 °C (As20, SFM2, As11, TNF, SCFM2) or 55 °C (SCFM6) for 30 s and 72 °C for 45 s, with a final extension of 20 min at 72 °C. The touchdown procedure also included the 15 min initial denaturation, followed by 30 s at 94 °C, 30 s at 65 °C (dropping 1 °C every cycle for 12 cycles), 72 °C for 45 s, finishing with another 25 cycles of 94 °C for 30 s, 53 °C (As34 and SCFM9) for 30 s, 72 °C for 45 s, and a final extension for 20 min at 72 °C.

PCR amplifications were performed in a GeneAmp 2700 thermocycler (Applied Biosystems) and loci were combined in post-PCR multiplexes (1 µL of each PCR) for fragment analysis. Fragments were either analyzed in-house (ABI310 Sequencer) or sent to Macrogen Korea (www.macrogen.com) in 96-well plates (3730XL ABI Prism Sequencer). We ran three samples in-house for every plate sent to Macrogen, to merge the results from different machines under different run conditions.

We used GENEMAPPER 3.7 (Applied Biosystems) to visualize and size the peaks against a GS500 ROX-labelled size standard. Alleles were scored using bins created in TANDEM (Matschiner and Salzburger 2009) from sized alleles of a subset of 88-94 individuals for each locus. All extractions and amplifications were carried out in a UV cabinet with dedicated pipettes, filter tips and negative controls were used to monitor for contamination.

S2–COMPARISONS WITH EASYPOP

Because EASYPOP does not include IBD patterns, we set our model to allow random mating (any female could mate with any male in the landscape) and random dispersal (newborns could disperse to any patch). For both softwares we set migration rate ('permeability' in our model) to 0.50 and 0.10. Both programs were run for 100 generations, with population size of 500 and 1000 individuals, 9 loci, initiated with maximal alleles possible per locus of 30, allele mutation rate of 5×10^{-4} and equal sex-ratio. For each time step of each simulation we used observed heterozygosity (Ho), number of alleles (A), genetic differentiation between 'roadsides' (G_{ST}), and inbreeding coefficient (F_{is}). (outputs from EASYPOP), to compare the models.

Outputs from REPoP were equivalent to EASYPOP when simulating Wright–Fisher populations. The comparisons showed that the two models produced identical outcomes for all genetic summary statistics as function of time in generations (Fig. 4.3). These results validated the use of the REPoP model for the simulations in this study.

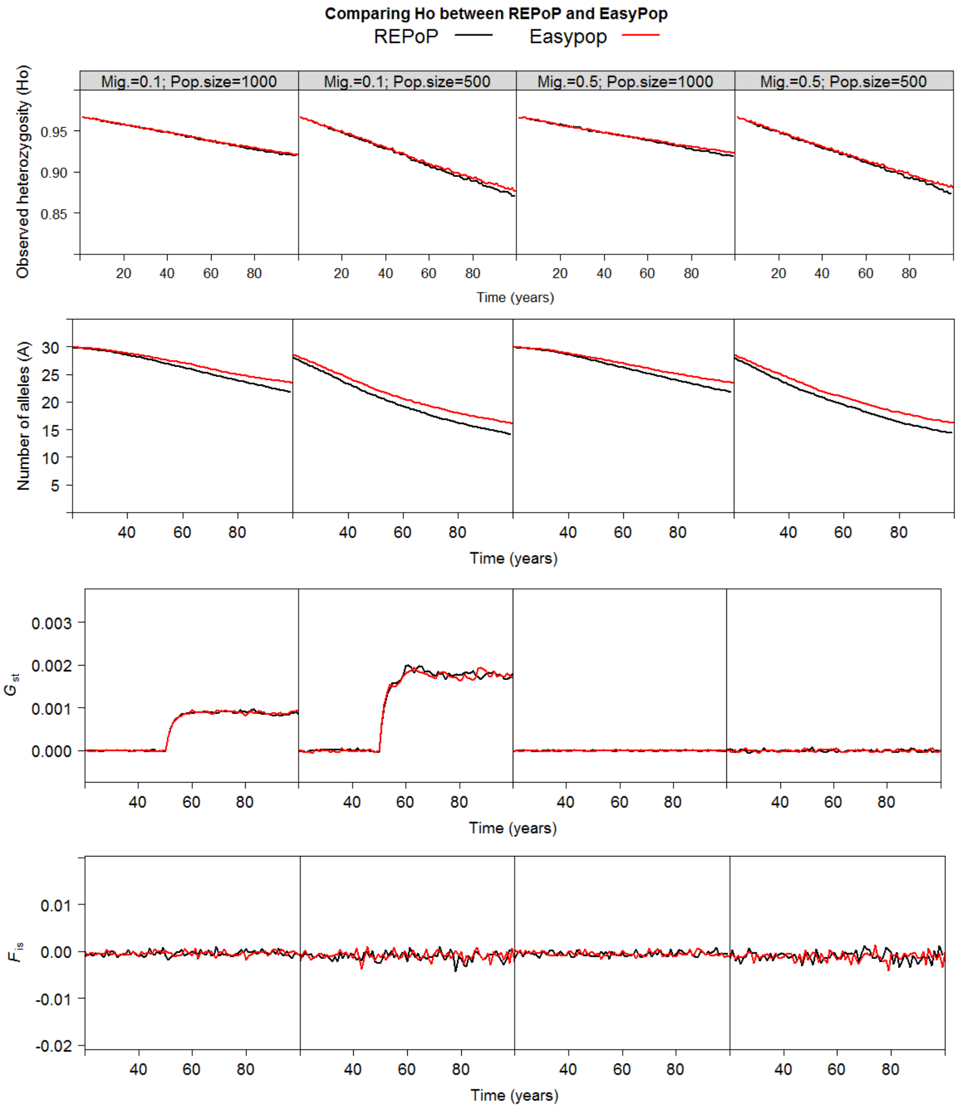

Fig. 4.3 – Comparing REPoP and EASYPOP models regarding genetic outputs: Observed heterozygosity (Ho), number of alleles (A), genetic differentiation between 'roadsides' (G_{ST}), and inbreeding coefficient (F_{is}). Comparisons were made simulating two populations sizes (500 and 1000 individuals) and two migration (permeability in our model) rates (10% and 50%). Lines represent the median values for 30 replicates.

S3–GENERAL STATISTICS

Table 4.4 - Hardy-Weinberg tests for roadside datasets separately and for both sides jointly. Significant tests are signed with * (after Bonferroni corrections, alpha = 0.0009 and alpha = 0.0018, respectively).

	AP6			A2			AP51		
Allele	side 1	side 2	Both	side 1	side 2	Both	side 1	side 2	Both
Hardy-Weinberg exact probability tests									
as20	0.002	0.079	0.004	0.954	0.931	0.9545	0.214	0.050	0.000*
sfm2	0.000*	0.854	0.008	0.814	0.737	0.7215	0.034	0.000*	0.002
as7	0.233	0.062	0.026	0.442	0.562	0.3816	0.000	0.005	0.000*
as34	0.233	0.016	0.061	0.014	0.000	0.0000*	0.037	0.000*	0.000*
as11	0.000*	0.000*	0.000*	0.000*	0.000*	0.0000*	0.003	0.000*	0.000*
tnf	0.200	0.038	0.001*	0.474	0.103	0.0198	0.717	0.332	0.664
scfm6	0.344	0.007	0.027	0.000*	0.028	0.0000*	0.340	0.597	0.126
scfm2	0.984	0.109	0.220	0.187	0.015	0.0000*	0.000*	0.112	0.000*
scfm9	0.396	0.035	0.016	0.000*	0.024	0.0000*	0.235	0.004	0.051
Hardy-Weinberg heterozygote deficiency test									
as20	0.052	0.310	0.019	1.000	1.000	1.0000	0.863	0.839	0.934
sfm2	0.000*	0.721	0.000*	0.185	0.169	0.1275	0.009	0.077	0.000*
as7	0.036	0.248	0.028	0.296	0.467	0.2727	0.000*	0.070	0.000*
as34	0.084	0.079	0.003	0.000*	0.008	0.0000*	0.000*	0.000*	0.000*
as11	0.000*	0.000*	0.000*	0.000*	0.000*	0.0000*	0.011	0.000*	0.000*
tnf	0.702	0.044	0.102	0.425	0.127	0.0428	0.765	1.000	0.957
scfm6	0.442	0.010	0.043	0.000*	0.016	0.0000*	0.007	0.281	0.077
scfm2	0.999	0.000	0.050	0.139	0.002	0.0510	0.000*	0.002	0.000*
scfm9	0.101	0.354	0.107	0.004	0.005	0.0000*	0.466	0.049	0.122

Table 4.5 – Allelic richness and Private Allelic richness for roadside datasets separately and for both sides jointly.

	AP6			A2			AP51		
	side 1	side 2	both	side 1	side 2	both	side 1	side 2	both
Allelic richness									
as20	14	14	17	17	15	19	13	15	17
sfm2	12	14	16	13	15	16	14	14	16
as7	15	16	19	18	17	19	14	18	20
as34	20	21	24	19	18	22	17	15	20
as11	17	25	30	17	21	26	21	22	29
tnf	13	13	16	15	15	20	13	13	16
scfm6	14	16	18	13	15	17	17	17	19
scfm2	19	19	24	20	25	31	22	21	28
scfm9	11	15	16	14	13	16	15	13	16
Average	15	17	20.0	16.2	17.1	20.7	16.2	16.4	20.1
Private Allelic richness									
as20	1.960	0.033	1.939	1.177	2.000	3.127	0.960	1.041	2.094
sfm2	1.002	0.000	0.943	0.000	0.000	0.000	0.000	0.000	0.000
as7	0.007	0.001	0.020	0.045	0.923	0.985	0.960	0.038	1.020
as34	0.001	0.857	0.944	0.006	0.040	1.062	0.000	0.000	0.000
as11	0.174	3.496	4.773	0.085	0.997	2.054	1.103	2.042	3.128
tnf	0.010	0.002	0.000	1.927	2.811	5.976	0.999	0.020	1.006
scfm6	0.000	0.828	0.926	0.960	0.001	0.980	0.999	0.960	2.074
scfm2	0.247	0.033	1.018	2.883	2.846	5.955	1.138	0.960	2.150
scfm9	0.074	0.828	0.944	0.027	0.037	0.078	0.172	0.960	1.074
Average	0.386	0.675	1.279	0.790	1.073	2.246	0.703	0.669	1.394
F_{is}									
as20	0.118	0.045	0.085	-0.081	-0.069	-0.079	-0.085	-0.040	-0.060
sfm2	0.458	-0.010	0.212	0.069	0.025	0.054	0.092	0.191	0.146
as7	0.071	0.047	0.076	0.023	0.004	0.019	0.332	0.126	0.244
as34	0.077	0.092	0.087	0.155	0.223	0.192	0.151	0.352	0.259
as11	0.192	0.177	0.189	0.442	0.359	0.406	0.164	0.375	0.268
tnf	-0.020	0.177	0.102	0.058	0.065	0.077	-0.038	-0.090	-0.057
scfm6	0.034	0.111	0.077	0.306	0.162	0.231	0.133	0.015	0.075
scfm2	-0.058	0.153	0.061	0.107	0.124	0.118	0.332	0.110	0.226
scfm9	0.144	0.048	0.093	0.268	0.195	0.233	-0.016	0.146	0.066
Over loci	0.111	0.093	0.109	0.150	0.121	0.140	0.121	0.132	0.132

5. Road effects on marten space use and movement: combining radio-tracking with agent-based simulation models

ROAD EFFECTS ON MARTEN SPACE USE AND MOVEMENT: COMBINING RADIO-TRACKING WITH AGENT-BASED SIMULATION MODELS

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ABSTRACT

Mammalian carnivores can be highly vulnerable to wildlife-vehicle collisions due to the high probability of encountering a road during their displacements. Thus there is a growing need to better understand how individual responses toward the highways may influence the overall effect of roads at the population level. Here we applied an individual-based analytical framework to assess the influence of a highway on stone marten (*Martes foina*). We analyzed radio-tracking data from seven individuals using biased random bridges to estimate their utilization distributions and quantified modeled their movement response angles near the highway. We compared the observed data with simulations from a spatially-explicit model to test for a highway influence on marten space use and movement patterns. Three null hypotheses were tested for each individual: i) marten space use is not influenced by the presence of the highway nor the structure of the verge vegetation, ii) the highway does not significantly affect marten response angles, and iii) the highway is not a physical barrier to marten movements. Individual martens varied in their behavioral responses toward the highway. All tracked marten exploited resources in close proximity to the highway and none marten demonstrated a preference for verge vegetation quality. Further four marten routinely crossed the highway and in fact the highway passed through these marten home ranges. We suggest that the use of road passages is governed by the activity type an individual is performing; the distance to the nearest passage; and whether the marten was resident or transient. Given their willingness to cross the highway, our findings suggest that marten are highly susceptible to traffic induced mortality. We suggest that effective mitigation measures should include exclusionary fencing, with a smaller mesh size, linking to existing passages.

Keywords: wildlife-vehicle collision; biased random bridges; response angles; *Martes foina*; crossing structures; road ecology

5.1. INTRODUCTION

Roads and their associated traffic have long been recognized as having major negative impacts on wildlife (Forman *et al.* 2003). Wildlife-vehicle collisions (WVCs) are a significant non-natural mortality source that can severely deplete populations and disrupt landscape connectivity (Fahrig & Rytwinski 2009; Benítez-López *et al.* 2010; Jackson & Fahrig 2011). In turn, these reduced populations suffer fitness consequences (e.g., due to reduced genetic diversity) that can further reduce long-term population viability (Riley *et al.* 2006; Eigenbrod *et al.* 2008; Holderegger & Di Giulio 2010).

Mammalian carnivores are highly vulnerable to WVCs as they are territorial, typically have large home ranges, and regularly travel long distances (Noss *et al.* 1996), thereby increasing their probability of encountering roads, particularly in regions where the road network is extensive. In Europe for example, medium-sized carnivores experience high road-kill rates (Clarke *et al.* 1998; Philcox *et al.* 1999; Hauer *et al.* 2002; Grilo *et al.* 2009; Klar *et al.* 2009), especially stone marten (*Martes foina*, hereafter referred to as 'marten') a species that is highly affected by road mortality, particularly in the southern Iberian Peninsula (Grilo *et al.* 2009). Grilo *et al.* (2012) investigated marten road mortality and their movement behavior along a highway, finding that martens established home-ranges adjacent to the highway, with some individuals occupying both sides of the highway. Their study suggested that marten preferred to cross the highway at locations with greater vegetation structure in road verges, irrespective of the presence of crossing structures (e.g. drainage culverts or underpasses).

The population-based approach of Grilo *et al.* (2012, analyzes were performed using individuals as random effect in GLMM) however limited their ability to infer individual-based variation. Also, their reliance on marten movement directionality (i.e. toward/away from the highway), prohibited them from considering the range of movement angles and potential differences between individual's responses as the animal approaches the highway. Thus their ability to capture the complex individual responses toward the highway was hindered. Moreover, the time the animals spent in their movements was not accounted for, thus inferences on resource use intensity, particularly those related to verges (e.g. Barrientos & Bolonio 2009), were not possible.

In this paper, we attempt to address several of the potential shortcomings of Grilo *et al.* (2012). We applied an individual-based analytical framework to better understand the highway influence on individual martens. We used biased random bridges (BRB, Benhamou & Adler 2011) to provide a conservative utilization distribution (UD, Worton 1989) per individual. We also quantitatively analyzed the movement responses of martens toward the highway by modeling the response angles of individuals in proximity to the highway (Tracey *et al.* 2005). We used agent-based modeling simulations (ABMs, DeAngelis & Mooij 2005; Railsback & Grimm 2011) to test for a highway influence on marten space use and movement patterns, by comparing recorded marten movements to simulation data where the highway was removed. With this approach, we are able to clarify whether marten space use and movements are indeed influenced by the highway and its verge habitat quality, or whether potential patterns could emerge solely as a result of the small size of marten home ranges that overlapped the highway, as suggested by Grilo *et al.* (2012).

We tested three null hypotheses per individual: i) marten space use is not influenced by the presence of the highway or vegetation structure in the verges, ii) the presence of the highway has no significant effect on marten response angles, and iii) the number and location of marten highway crossings are not constrained by the highway. We predicted significant differences between observed and simulated data, with observed tracks showing: i) an overall lower space use intensity adjacent to the highway, except when verges have a lush vegetation structure; ii) a road avoidance behavior with predominantly negative response angles; and iii) fewer highway crossing events and that these are randomly located. Our individual-based approach improved our ability to infer biological relevance of recorded locations, thereby providing a better understanding of marten-highway interactions. Our study will be of interest to road planners, ecologists, and conservation biologists who seek to understand the impacts of roads on carnivore populations and their potential mitigation strategies.

5.2. MATERIAL AND METHODS

5.2.1. STUDY AREA

Field work was conducted in the Mediterranean region of the Iberian Peninsula, southern Portugal, in an area dominated by savannah-like forests of cork oak (*Quercus suber*) and holm oak (*Q. ilex*), a preferred habitat for stone marten (Santos-Reis *et al.* 2004; Santos & Santos-Reis 2010; Grilo *et al.* 2011). Our study area included an approximately 10 km stretch of the four-lane A6 highway and its adjacent surroundings (38°36′ 8°22′ | 38°39′ 8°15′) (Fig. 5.1A).

This highway was built in 1995, has a speed limit of 120 km/h and receives on average 169 ± 159 vehicles/hour between 2000 and 0800hr (source: BRISA, highway enterprise). It has livestock exclusion fencing on both sides, whose mesh size (15 x 15 cm) is too large to impede martens from accessing the highway surface. This highway segment has 21 crossing structures: 13 culverts (1.0 - 1.5 m in diameter) for water flow, seven larger under-passages, and one over-passage for cars and agricultural machinery (5 m width). This section of highway passes through elevations ranging from 200 m to 250 m (Portuguese altimetry vectorial database, available at URL: www.igeo.pt).

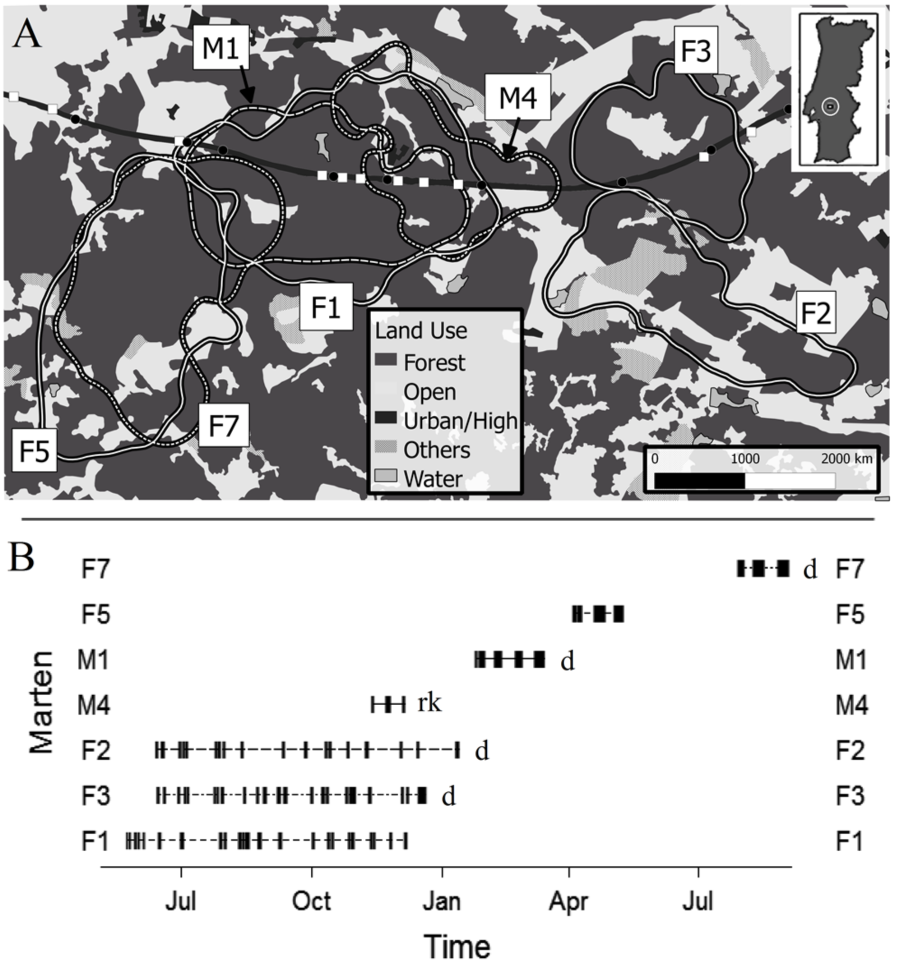

Fig. 5.1 - A: highway A6 and its road passages (squares - culverts, circles – under/over passages), main land uses and marten home range areas (white lines). B: temporal distribution of tracking nights for each marten (each bar is one night). "d": marten disappeared; "rk": confirmed WVC (corpse recovered). Apparent home range overlap of F1 with M1 and M4, and F5 with F7 correspond to distinct periods.

5.2.2. DATA COLLECTION

The marten location data used in the present study are a subset of the data previously collected and presented in Grilo *et al.* (2012). In their work, 11 adult stone marten (five males and six females) were captured during 6551 trap-nights. For our analyses, we used data on seven of these individuals (two males and five females). Data on the remaining four marten were insufficient for our analyzes. Individual marten identification hereafter is that of Grilo *et al.* (2012), i.e. F1, F2, F3, F5 and F7 for females, and M1 and M4 for males.

Martens were captured using box-traps (Tomahawk Live Trap Co., Wisconsin, USA) baited with meat or fish, and set at a maximum of 1500 m from the highway, the approximate diameter of a marten home range (Santos-Reis *et al.* 2004). Traps were checked daily at dawn as the species is strictly nocturnal (Santos-Reis *et al.* 2004). Trapped martens were weighed and immobilized (Imalgene 1000, 10 mg/kg; Midazolan, 0.1 mg/kg), sexed, aged, and fitted with a VHF tracking collar (Telonics, MOD-080, weight **ca.** 36 g). Animal trapping and handling procedures were approved by Portuguese Environment Institute (ICNB/CEMPA Licenses105/2008, 39/2009 and 168/2009).

Fieldwork was performed between April 2008 and September 2009 (Fig. 5.1B). Due to the intensity of our tracking efforts, we focused on one marten per night, tracking it from dusk to dawn. Martens were tracked by two observers simultaneously, each moving in separate cars and in permanent radio contact, using telemetry receivers (Telonics Inc., Mesa, Arizona, USA) and two element Yagi antennas to estimate the marten location. To minimize the position error, we only considered fixes valid if observers and the animal completed an angle within 60° and 120° (White & Garrott 1990). We continuously tracked individuals and attempted to locate the marten every 30 minutes, locating their daytime resting site the following day. In addition to the location data, we also recorded the height and proportion of shrub and tree cover of the highway verge, for each highway segment of 100 m.

5.2.3. AGENT-BASED SIMULATION MODEL

We simulated martens moving within their observed home ranges to determine the influence of the highway on marten movement and space use patterns. We created a spatially-explicit agent-based model simulation (ABM, DeAngelis & Mooij 2005; Railsback & Grimm 2011), set to replicate the empirical movement characteristics of tracked individuals, except our simulations were naïve to the presence of the highway. The model runs in a virtual landscape with dimensions similar to our study area, covering 430 x 300 cells each 30 x 30 m (over 16,100 ha). *A priori*, we found no evidence for marten preference of forests or open patches (main land cover classes) within their home ranges (see *Supplementary Material S1*). Therefore, we did not include land cover preference in our ABM and it was assumed that martens would primarily respond to their home range boundaries.

Each simulation per marten was parameterized with the observed number of tracking sessions and locations, and the same step lengths (i.e., the distance between successive locations), as well as its home range boundary (indicated by the utilization distribution data as described below). For each tracking session, an agent (i.e., simulated marten) starts from an observed resting site, chosen at random, and then moves the same number of steps whose length follows the observed step lengths' sequence. The agent's successive location is governed by the home range boundary (i.e., the location must fall within the observed home range), and a random direction. This process is repeated for each tracking session and per marten, at the end of which the coordinates for each simulated location are saved for further analysis.

We replicated the model for 1000 iterations per marten and used the results to estimate the confidence intervals for simulated distributions regarding the space use (Utilization Distribution, UD), response angles, and the number and location of highway crossings (see Data analysis and Supplementary Material S2 for 'response angle' description). Based on likelihood significance tests, the highway has an effect if the observed parameter ranked outside the 5-95% percentile envelope of the simulated parameter distributions (see Hartig *et al.* 2011). All simulations were conducted within NetLogo 5.0.2 (Wilensky 1999).

5.2.4. DATA ANALYSIS

5.2.4.1. Marten space use in the vicinity of the highway

We estimated marten home ranges with biased random bridges (BRB), a conservative yet realistic method for quantifying animal utilization distributions (Benhamou & Adler 2011). The BRB model inserts interpolated locations at regular intervals between each observed location, and then uses classical kernel estimation, with a variable smoothing parameter dependent on the time between successive relocations, to estimate a UD (Benhamou & Adler 2011). Rather than requiring independence between successive locations, as other home range estimators require (Mohr 1947; Swihart & Slade 1985; Worton 1989), the BRB model uses the time between successive locations to parameterize the biased random walks between each location (Benhamou & Adler 2011). Thus, as the time between successive locations decreases, the width of the bridges (i.e., the size of the area within which the individual may have passed through between successive fixes) decreases, thereby producing a more realistic probability of the true path (Horne *et al.* 2007).

We computed the BRB per marten with the function *BRB* available within the 'adehabitatHR' package (Calenge 2006, 2011) for program R (R Core Development Team 2012). The BRB produces a raster that can be used to estimate a UD for each animal, whose cell values suggest the probability of relocating the animal at said cell within the home range area (Marzluff *et al.* 2004; Benhamou & Adler 2011). These cell values can then be related to space use intensity and hence how individuals spatially exploit their available resources (Marzluff *et al.* 2004; Donovan *et al.* 2011). We used these utilization distributions to delineate marten home ranges as the area in which the probability of relocating the marten was higher than 1% (i.e., we used the 99% isopleth as the home range boundary). We assessed the stability of the home range by sequentially including data from consecutive tracking nights to the BRB model (starting with data from the first five tracking nights) and checking the resulting area from the 99% isopleth.

If the highway had a null effect in marten space use, we would expect the observed space use intensity to be unrelated to highway proximity. For each marten, the UD from all replicates were summed (overlaid rasters) and we calculated the 5 and 95 percentiles for each cell. We then identified areas where the observed space use was lower or higher than from the simulated 5 and 95 percentiles, respectively.

We used the amount of time an individual spent within an area as a surrogate for its attractiveness, thus if martens preferred verges with a higher vegetation quality, these areas should have higher UD values. We created a buffer of 50 m around the locations where verge vegetation was characterized and then intersected these buffers with each marten utilization distribution. Due to small sample sizes (i.e., the number of descriptive points of verge vegetation structure, per individual), we used Spearman's rank correlation to investigate associations between marten usage (mean values at descriptive points' buffer) and the verge vegetation structure.

5.2.4.2. Marten response angles toward the highway

We used a set of nonlinear regression models previously described by Tracey *et al.* (2005) to model marten movements when approaching the highway. These models allow us to infer from the model parameters the qualitative response of an individual (e.g., attraction or avoidance) to a landscape feature, i.e., the highway. We analyzed marten response angles to measure the highway influence on marten movement behavior. Response angles are defined as the change in direction between two consecutive locations in relation to the highway position (see Supplementary Material S2 – Response angle description). Response angles vary between π and $-\pi$ radians, with negative or positive values denoting movements away from or toward the highway, respectively, and values close to zero representing a neutral effect.

These models use the von Mises distribution which is characterized by both the mean and a concentration parameter, analogous to the precision of a normal distribution. A constant mean response angle is applied, but the concentration parameter varies as a function of the individual distance to the highway (Tracey *et al.* 2005). For example, if a marten strongly avoids the highway, we would expect a negative mean response angle with a narrow concentration that decays as the marten's distance to the highway increases. The concentration parameter can follow an exponential or logistic function, producing two response models (Tracey *et al.* 2005), hereafter referred as 'Resp.Exp' and 'Resp.Log', respectively. The two responsive models are then compared to the 'Basic' (i.e., null) model that does not consider the distance from the highway (i.e., it is naïve to the presence of the highway).

Model statistical inference is likelihood based, similar to those for generalized linear models to obtain the maximum likelihood estimates (Tracey *et al.* 2005). We used the Akaike information criterion (AIC) for model selection. If the highway does not influence marten movement, the 'Basic' model will best fit the empirical data from our martens. We also ran these models on the simulated response angles and also compared the mean turning angles between the empirical and simulated martens. We excluded data from stationary martens (i.e., resting or active but not moving) for this analysis.

5.2.4.3. Marten highway crossing patterns

We identified highway crossings as pairs of consecutive marten locations during the same tracking session recorded on opposite sides of the highway. To test whether marten crossed the highway more or less often than expected, we compared the number of observed crossings for each marten with the number of simulated crossings. We considered a significant highway effect if the number of observed crossings was lower than 5% of the simulated crossings (negative effect) or higher than 95% of the simulated crossings (positive effect).

For each marten, we calculated a utilization distribution of their crossing locations (UD_{cross}) using the same procedure described above (BRB), to calculate the crossing probability at each highway segment (Horne *et al.* 2007; Lewis *et al.* 2011). The UD_{cross} was intersected with points displayed along the highway every 50 m. For each point, we also summed the UD_{cross} of all simulated replicates and calculated the 5 and 95 percentiles. If marten favored certain road segments for crossing over others, the observed UD_{cross} of preferred highway segments should be higher than 95% of the simulated UD_{cross}. We then visually inspected the relation of the observed UD_{cross} with the passageway locations to assess whether marten tended to cross the highway near passageways.

5.3. RESULTS

We selected 136 tracking nights from the seven marten (mean 19±7 per marten), yielding 1489 locations (mean 10±4 locations per night per marten). The mean time between successive locations was 39±22 min with an estimated mean location error of 182 m. Marten home ranges averaged 532±149 ha. The relationship between the home range area and tracking effort reached the asymptote (an indication of range stability) for 5 of 7 martens (see *Supplementary Material S3*). Marten M4 yielded insufficient data to assess the stability of his home range as he was captured several months after the trapping was initiated in that area (Fig. 5.1B).

5.3.1. MARTEN SPACE USE IN THE VICINITY OF THE HIGHWAY

Martens often included the highway within their home range, suggesting the highway does not impede marten space use (Fig. 5.2A).

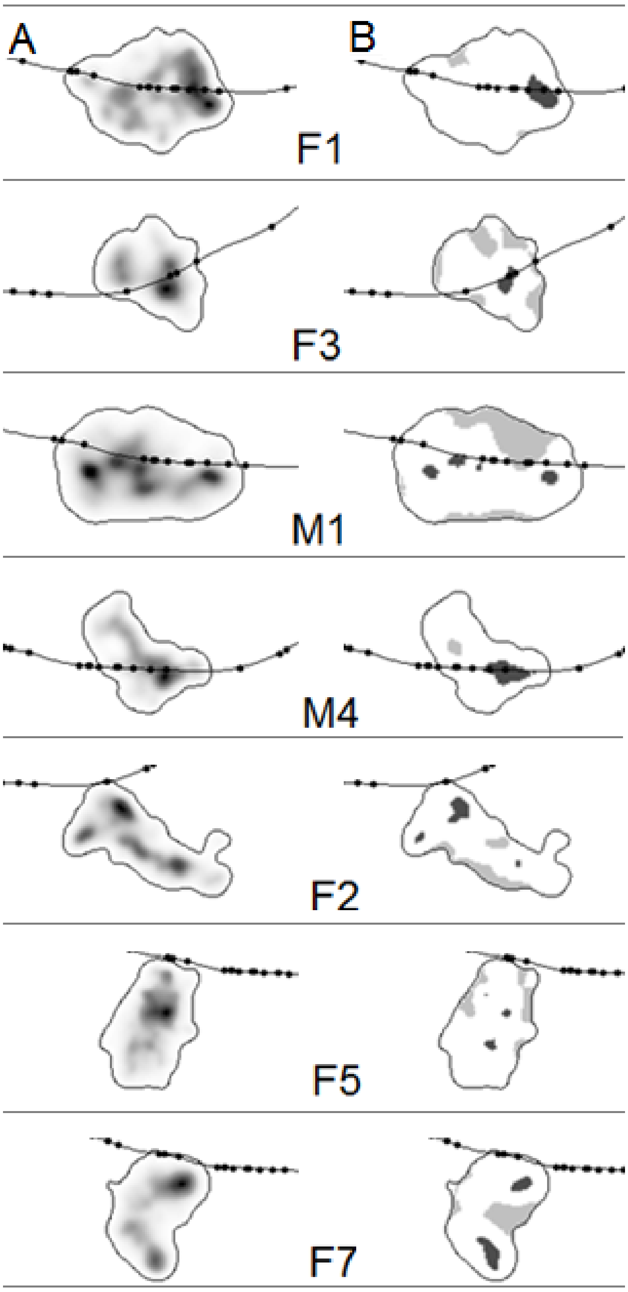

Fig. 5.2 - A: the utilization distributions (biased random bridges) of tracked martens with increasing shading indicating increasing use intensity. B: areas for which marten spent more (dark grey) or less (light grey) time than expected by chance (i.e., simulations). The highway and crossing structures are shown in each plot. Individuals are sorted by whether they crossed the highway (top four animals) or never crossed the highway (last three).

Further, comparisons between observed and simulated space use intensities indicate that marten do not avoid the highway, but rather one individual (M1) even used the area near the highway more than expected, despite spending most of its time in the southern highway side of its territory (Fig. 5.2B). No martens demonstrated a preference for highway verge areas with higher vegetation structure, and M1 even significantly avoided these verge areas (Fig. 5.3). Moreover, martens that regularly crossed the highway intensively used areas adjacent to the highway. Martens also demonstrated lower use intensity of their home range peripheries and of some open areas (Fig. 5.2B).

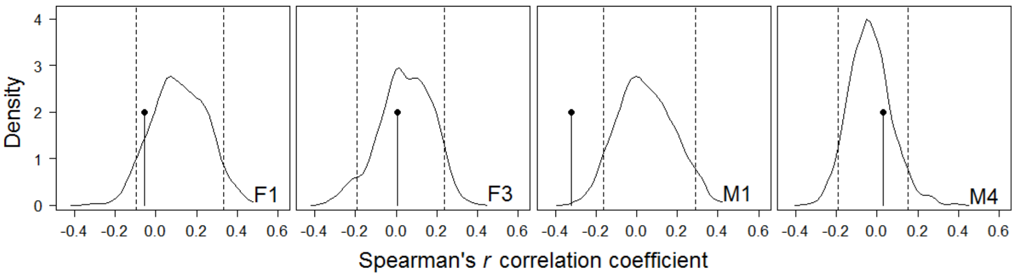

Fig. 5.3 - Spearman's correlation coefficients relating the UDs in highway vicinity and verge vegetation structure. For each marten the density plot refers to the distribution of the coefficient from all simulations. The correlation coefficient of the observed data is indicated by the black dot. Vertical dotted lines represent the 5 and 95 percentiles of the simulated datasets.

5.3.2. MARTEN RESPONSE ANGLES TOWARD THE HIGHWAY

For six individuals, the 'Resp.Exp' model best fitted the observed data, M4 being the single exception whose data was best fitted with the 'Resp.Log' model (Table 5.1).

Table 5.1 – Model parameters from non-linear regression models relating turning angles with highway proximity. The 'Basic' model is a null model, i.e., not accounting for highway proximity. Resp.Exp and Resp.Log are responsive models which the concentration parameter has an exponential or logistic decay with highway distance, respectively. Estimates for the mean angle (μ), log likelihood (LL) and AIC are presented. For each marten, models in bold had the lowest AIC. Individuals are sorted according if they crossed the highway (top four animals) or never crossed the highway (last three).

Marten	Model	μ	LL	AIC
F1	Basic	-0.07	-305.1	614.2
	Resp.Exp	-0.24	-300.7	607.4
	Resp.Log	-0.24	-300.7	609.4
F3	Basic	2.44	-474.2	952.3
	Resp.Exp	-1.16	-472.9	951.7
	Resp.Log	-2.72	-474.2	956.3
M1	Basic	-0.19	-338.2	680.3
	Resp.Exp	-2.30	-337.0	680.0
	Resp.Log	-3.10	-336.4	680.7

Marten	Model	μ	LL	AIC
M4	Basic	0.43	-102.9	209.8
	Resp.Exp	1.61	-100.1	206.3
	Resp.Log	1.73	-98.1	204.2
F2	Basic	1.28	-328.9	662.0
	Resp.Exp	-2.96	-325.8	657.7
	Resp.Log	0.07	-329.0	666.0
F5	Basic	-1.50	-338.2	680.3
	Resp.Exp	-3.08	-330.6	667.2
	Resp.Log	-3.11	-330.6	669.3
F7	Basic	-2.06	-340.0	684.0
	Resp.Exp	2.62	-335.4	676.7
	Resp.Log	1.51	-340.0	688.0

Interestingly however, response angles revealed a wide variety of response tendencies across individuals. For example, the mean negative response angles of five individuals (F1, F3, M1, F2, and F5) suggests they avoid the highway, whereas two individuals (M4 and F7) appeared to be attracted to the highway, based on their mean positive response angles. However, the mean observed response angle for each marten fell within the confidence intervals of the simulated estimates (Fig. 5.4), suggesting that these patterns may not originate from marten avoidance/preference behavior *per se*, but is a result of their home range size and shape, overlapping the highway.

5.3.3. MARTEN HIGHWAY CROSSING PATTERNS

We found no general pattern in marten crossing activity (Fig. 5.5). The disparity of results ranged from fewer crossings than expected (M1), to a higher number of crossings than expected (M4). Also, for F1 the number of crossings was only marginally higher for ca. 5% of simulations, while for F3 the number of crossings approximated the mean number from simulations.

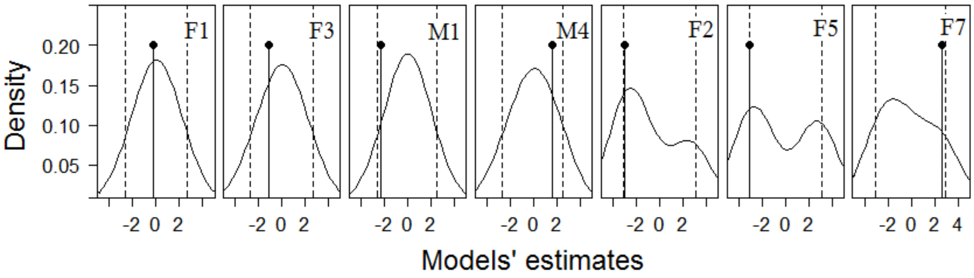

Fig. 5.4 - Distribution of the mean turning angles from the simulations of each marten. Turning angle distributions are derived from the respective model of the observed data with lowest AIC value (Resp.Exp or Resp.Log, see Table 1). Turning angles suggest the effect the highway is expected to have on marten movement: negative values -avoidance, positive values - attraction. The mean observed turning angle is indicated with a black dot. Vertical dotted lines represent the 5 and 95 percentiles of simulated datasets. Individuals are sorted by whether they crossed the highway (first four animals) or never crossed the highway (last three).

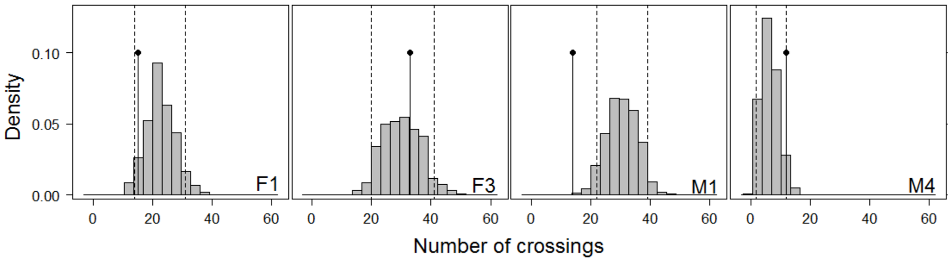

Fig. 5.5 – Histograms showing the predicted frequency of marten highway crossings (simulations; grey bars) and the observed number of crossings (black dot). Vertical dotted lines represent the 5 and 95 percentiles of the simulated datasets.

For the four martens that regularly cross the highway, the UD_{cross} values suggest that these individuals usually crossed in sections that have road passages. In fact, although most of their UD_{cross} are within the expected interval from the simulations, the road segments with higher use intensity also had passages (white arrows in Fig. 5.6). Interestingly, both F1 and M1 seem to avoid crossing the highway where a paved road intersects the highway (black arrows in Fig. 5.6), suggesting a possible behavioral preference for passage type (Fig. 5.6).

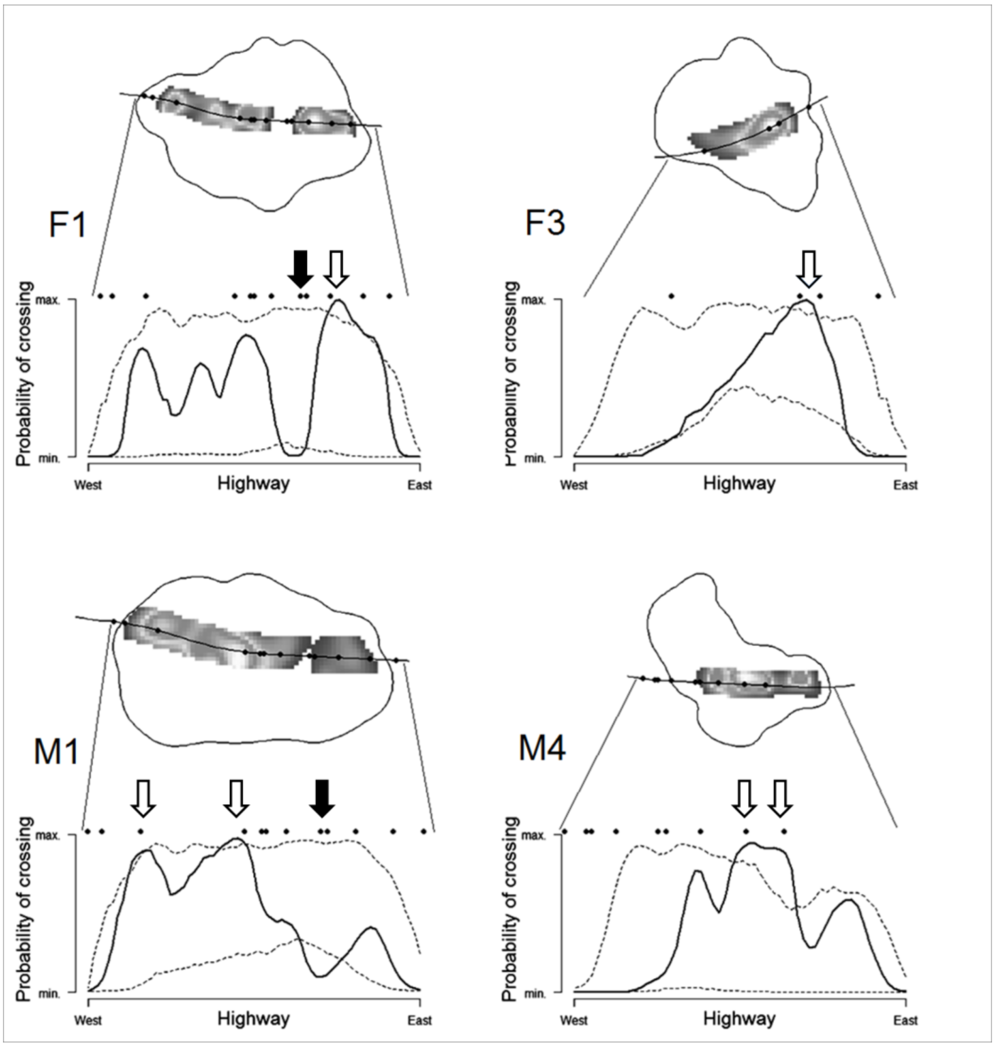

Fig. 5.6 – Per marten, top: the utilization distribution of marten highway crossing locations with a 200 m buffer. Bottom: probability of crossing the highway at each road segment. Observed probability (solid line) and 5 and 95 percentiles of the simulated data from our agent-based model (dotted line). Dots indicate highway passages and white (black) arrows indicate a passage with higher (lower) use than expected.. The highway segment in upper pictures is projected in the X axis from the bottom picture.

5.4. DISCUSSION

Our findings highlight the complexity of individual responses towards roads, complementing the findings from Grilo *et al* (2012). The time-based BRB method allowed us to conservatively estimate individual utilization distributions, which suggested that martens exploited resources regardless of the highway proximity or verge vegetation structure. In fact, some areas along the highway were used more intensively than expected. By applying an individual movement based analysis, focusing on response angles to the highway, we found a high amount of variability in marten movement behaviors relative to the highway, including a lack of evidence for highway avoidance behavior.

Grilo *et al.* (2012) suggested that marten movement patterns to be positively related with the vegetation suitability in the road verges (tree and shrubs). Authors also detected movement avoidance behavior from the highway when individuals were in its close vicinity. According to our results however, these described patterns likely resulted from spurious effects originating from the statistical method used to estimate habitat use (non-correlated locations) and the configuration of the home ranges relative to the highway.

Marten highway crossings appear to be governed by individual preferences. Each individual seemed to select one passage and use it regularly, suggesting that martens may search for specific passageways for safely crossing the highway or that individuals preclude others from accessing the crossing structures. This result improves the previous understanding of the apparent lack of relation between crossings and passage location reported by Grilo *et al.* (2012), as therein 'crossing structures' were grouped for analysis. Interestingly however, some highway sections lacking crossing structures also had high probability of being used for crossings. This suggests that their use may be governed by individual preferences, but also by how far away the individual is when deciding whether to cross though a passage or to cross over highway, as referred by Grilo *et al.* (2012).

Previous work on southern Portuguese roads (including our study site) found that marten were frequently killed on the highway (Grilo *et al.* 2009), while their use of existing crossing structure was also high (Grilo *et al.* 2008). Our work provides insight to this apparently contradicting pattern. We suggest that marten activity, energy trade-offs, and behaviors are influencing marten highway crossing locations and these three are not necessarily mutually exclusive.

First, our martens were likely using the verges for purposes other than simply for crossings, such as foraging or seeking shelter (*pers. obs.*), similar to behaviors reported for polecats (*Mustela putorius*) (Barrientos & Bolonio 2009). Although not a main food item, small mammals constitute an important part of martens diet when available (Clevenger 1994; Genovesi *et al.* 1996) and small mammals occur at higher densities in road verges than in surrounding areas (Ascensão *et al.* 2012). Secondly, the choice of where to cross the highway and whether to do so via a passage or not, depends on how far the animal is from a passage. Our data suggest that if the nearest passage is not close enough, martens may prefer to cross over the surface of the highway (see F1 in Fig.6). These decisions may be based on an energy-risk balance assessment. Lastly, whether martens are residents or roamers/dispersers will likely influence their perception of road-kill risk and their preferences for certain passages.

Resident individuals most probably learn to use the crossing structures to safely cross the highway. In contrast, non-resident individuals might be less aware of the mortality risks of the highway, the location of the crossing structures, are precluded by resident marten from using those passages, or a combination of these factors, thus tending to cross over the surface of the highway. Long-term research on crossing structure use and learning process by large mammals has shown that an adaptation period of animals to existing structures, therefore a learning process, is unequivocal (Dodd *et al.* 2007; Clevenger *et al.* 2009; Gagnon *et al.* 2011). The martens F1 and F3 were most probably residents (stable home range area at least for 6 months) and they most likely regularly used the road passages. Conversely, M4 was apparently a non-resident and crossed the highway more often than expected and in several locations. This supports our 'behavioral' effect hypothesis. Further, M4 was a confirmed WVC a short time after he was trapped (Fig. 1, B), suggesting that he was not a resident at the start of our study and highlights the increased mortality risks experienced by non-resident individuals.

When combined, these three effects (i.e., activities near the highway, marten proximity to available passageways, and knowledge of the available passageways) may influence the risk for WVCs, despite the passages being regularly used. This may explain the road-kill and passage use patterns previously described (Grilo *et al.* 2008; Grilo *et al.* 2009). In fact, even low crossing rates associated with low mortality risk can have high WVCs rates (Hels & Buchwald 2001; Row *et al.* 2007). This has important implications for conservation. As the mesh size of the existing highway fence is not small enough to prevent marten from entering the highway right-of-way, we suggest that effective mitigation measures should include exclusionary fencing, with a smaller mesh size and a "L" shape, linking to existing passages, as described in Klar *et* *al.* (2009). Recently, Ascensão and colleagues (*under revision*) suggested that partial fencing (ca. 75%) of the road is the most effective mitigation measure for stone marten, both to prevent population size depletion and genetic differentiation. Therefore we suggest investigating the effectiveness of partial fencing in our study area and other highway segments where high WVCs are likely to occur.

Understanding how animal behavior is influenced by linear features is a major challenge for ecologists (Forman *et al.* 2003). Although the utility of inferences based upon individual responses to local environments might be limited (Gill *et al.* 2001; Jaeger *et al.* 2005), to properly mitigate the negative effects of roads at the population level it is necessary to understand the processes that affect the movements of individuals (Klar *et al.* 2009). This information in turn provides a spatio-temporal bridge between the individual and its population (Patterson *et al.* 2008; Schick *et al.* 2008). However, the study of behavioral responses is constrained in part by the need to capture and track multiple individuals. This can be very challenging, especially for carnivore species that occur at low densities. In our case, martens are likely to have experienced a significant reduction in population density since the construction of the highway, given the considerable higher trapping effort between our study (see Grilo *et al.* 2012) and a previous study in a roadless area (Santos & Santos-Reis 2010). Nevertheless, the variability between individual marten responses that we observed and, most importantly, the differences from some of the patterns previously described when focusing on a population-based approach improved our knowledge of marten behavior and movement patterns near roads. This highlights the importance of multi-level approaches combining a population-level approach (Grilo *et al.* 2012) and the methodology described here.

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SUPPLEMENTARY MATERIAL

S1 - WEIGHTED COMPOSITIONAL ANALYSIS OF HABITAT USE

We tested whether marten demonstrated a preference for forest patches and/or avoidance of open areas. For that, we used a Weighted Compositional Analysis of Habitat Use as described by Millspaugh *et al.* (2006), relating the spatial distributions of UDs with land cover classes. For each marten we assessed the proportion of UDs within each main land cover class (forest and open), which equaled the sum of the probability values of all cells of the UD, per land use class, and considered these proportions to be the 'available' habitat. The 'used' habitat was assessed by calculating the proportion of each land cover class in all radio-tracking locations. The test was performed using the function *compana* in the R package 'adehabitatHS' (Calenge 2006) with a randomization test (1000 permutations). We found no evidence for a significant difference between the use and availability for any land cover class ($\Lambda = 0.72$, $p = 0.18$).

S2 - RESPONSE ANGLE DESCRIPTION

Following Tracey *et al.* (2005), we define the response angle A_i as $A_i = (B_i - C_i)$, where B_i is the i_{th} move angle and C_i is the i_{th} animal-to-object angle (Fig. 5.7).

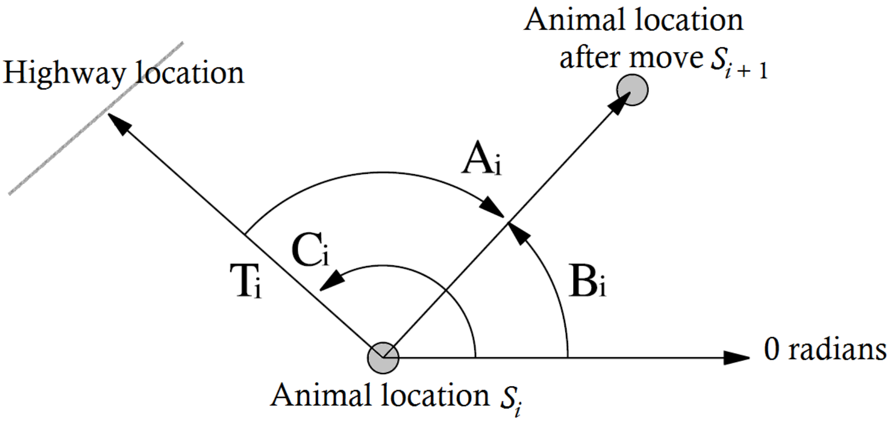

Fig. 5.7 – Diagram illustrating the response angle in relation to the highway location. The animal moves from S_i to S_{i+1}. The highway is the grey line. The animal-to-highway angle in radians is C_i and the animal-to-highway distance is T_i. The move angle is B_i and the response angle is A_i. Adapted from Tracey *et al.* (2005).

S3 – HOME RANGE AREAS

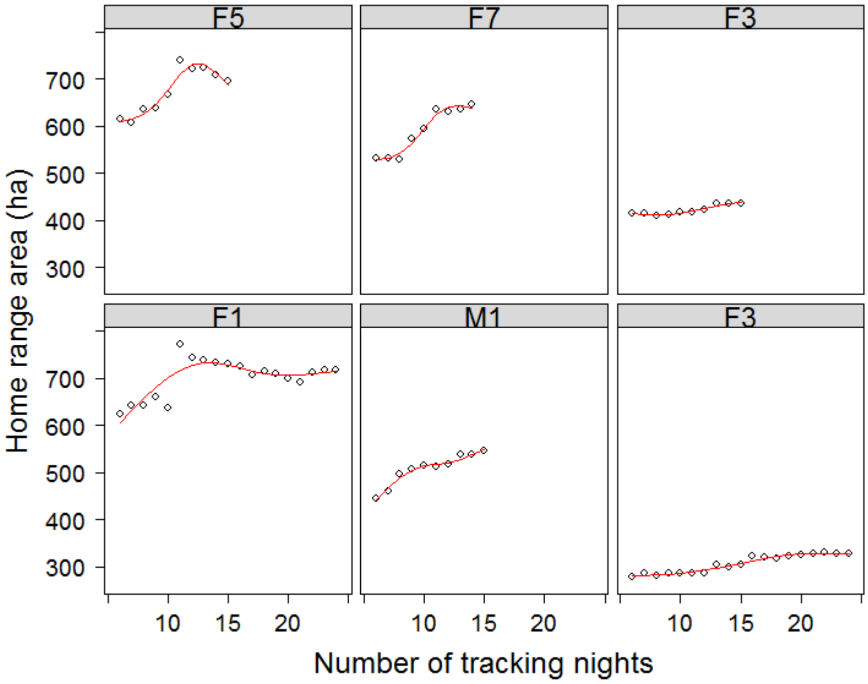

Fig. 5.8 – Home range areas relative to tracking effort. For each marten the tracking data for computing the BRB was sequentially added, by night, starting in five tracking nights. The 'home range area' refers to the area in which the probability of relocating the marten was higher than 1% (i.e. the 99% isopleth). Red line indicates the polynomial line (third order). M4 was tracked for five nights and thus data is not shown.

6. Wildlife-vehicle collision mitigation: partial fencing is an answer? An agent-based model approach.

WILDLIFE-VEHICLE COLLISION MITIGATION: IS PARTIAL FENCING THE ANSWER? AN AGENT-BASED MODEL APPROACH

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ABSTRACT

Evaluating management options for mitigating the impacts of wildlife-vehicle collisions (WVCs) is a major goal for road ecology. Fencing along roads in conjunction with the construction of wildlife road passages has been widely accepted as the most effective way to minimize WVCs. However, limited resources often require wildlife managers to focus on a single method of mitigation, yet the relative effectiveness of fences and passages for reducing road mortality and restoring population connectivity is unclear. Using the stone marten (*Martes foina*, Erxleben, 1777) as a model species, we developed an individual-based, spatially explicit simulation model to develop predictions concerning the relative performance of fencing and passage construction under different rates of road mortality. For five levels each, we varied probability of road mortality, fencing extent, and number of passages in a full factorial design, for a total of 125 management scenarios. We then compared the relative impact of these two mitigation approaches on population abundance (N) and genetic differentiation (F_{st}) using linear regression. Our results predict that fences are much more effective than passages at mitigating the effects of road mortality on abundance. Moreover, we show that under most circumstances, fences are also more effective than passages at reducing genetic differentiation. This is likely driven by the ability of fencing to eliminate road mortality, which in turn increases genetic diversity, thereby slowing differentiation across the road. Partial fencing can reduce road mortality nearly as well as full fencing while still allowing adequate population connectivity across roads. Thus, we argue that partial fencing of roads alone may often be the best and most cost-effective management option for road mitigation.

Key words: road permeability; population persistence; genetic differentiation; landscape connectivity; medium-sized carnivores; *Martes foina*.

6.1. INTRODUCTION

Roads and associated traffic negatively impact a vast number of species, with mortality due to wildlife-vehicle collisions (WVCs) being among the most important effects (Forman *et al.* 2003). WVCs impact populations beyond the road vicinity (Forman 2000) and may be responsible for highly reduced population sizes, increased demographic structure, and decreased landscape connectivity (Mumme *et al.* 2000; Steen & Gibbs 2004; Nielsen *et al.* 2006). Reduced population abundance and connectivity due to WVCs can in turn result in inbreeding and loss of genetic variability through genetic drift (Wright 1931; Miller & Waits 2003). Taken together, these impacts are expected to reduce individual fitness and the probability of long-term population survival (see Forman & Alexander 1998; Hanski 1998; Frair *et al.* 2008). How to effectively mitigate the effects of WVCs on wildlife populations thus merits further study.

The primary aim of WVC mitigation currently is to reduce the access of animals to road pavement while maintaining the permeability of roads to animal movement, in an attempt to retain population connectivity (Forman *et al.* 2003). Several studies suggest that fencing in combination with wildlife passages is the most effective way to minimize WVCs (Clevenger *et al.* 2001b; Bissonette & Cramer 2008; Huijser *et al.* 2009). However, given the expense of building these mitigating structures, it may not always be possible or desirable to do both, and the question remains as to which of these methods is more effective. That is, what is the relative impact of building fences versus building wildlife passages versus building both on the mitigation of important population impacts due to WVCs Moreover, there is little empirical data concerning whether passages can effectively restore population connectivity and thus decrease genetic differentiation due to roads (Corlatti *et al.* 2009). Likewise, although complete exclusionary fencing of roads will likely decrease population connectivity, it is unclear what the impacts of partial fencing of roads will be on population connectivity or on the mitigation of reduced abundance due to road mortality. Thus, road and population managers will benefit from an exploration of the relative impacts of differing amounts of fence and passage construction on the mitigation of populations experiencing varying levels of road mortality.

To investigate this question empirically would be logistically challenging. It would require gathering demographic and genetic data from populations near a large number of roads of similar ages, while controlling for external factors that might be correlated with roads (such as urbanness, habitat structure, or population history). These roads would also need to be furnished with varying levels and combinations of fencing and passages, constructed at similar time periods. Collecting such data would be extremely costly and time consuming (Holderegger & Di Giulio 2010), and likely impossible for most species. One solution is to use agent-based model simulations (ABM, DeAngelis & Mooij 2005; Railsback & Grimm 2011). ABM simulations have several advantages in that they allow for the control of several sources of uncertainty, such as habitat heterogeneity, non-road mortality (e.g., due to disease, competition, or predation), or historical effects (which may particularly affect patterns of genetic structure). In addition, simulations allow for a sufficient number of replicates in order to account for stochastic effects.

In this study, we developed the Road Effects on Population Persistence (REPoP) model, a spatially explicit simulation model that can be adjusted and parameterized to capture the specific life-history and landscape characteristics associated with a variety of species and spatial extents. Previous research throughout Europe has shown high road-kill rates for medium-sized carnivores (Ferreras *et al.* 1992; Clarke *et al.* 1998; Philcox *et al.* 1999; Hauer *et al.* 2002; Grilo *et al.* 2009), suggesting that this group will benefit from studies that investigate how to mitigate road-kill effects in natural populations. However, because road-kill events involving medium-sized carnivores rarely represent a threat to human safety, mitigation efforts directed at these species have seldom been implemented or studied (but see Ferreras *et al.* 2001; Klar *et al.* 2009).

Here we investigate the relative effectiveness of two road mitigation measures (fences and passages) in the face of varying road mortality using simulated populations of stone martens (*Martes foina* Erxleben, 1777; hereafter referred to as 'martens'), a territorial mustelid widely distributed throughout Europe (Proulx *et al.* 2005). Although this species is capable of living in deforested and human-altered environments (Rondinini & Boitani 2002; Herr *et al.* 2009), martens are known to be sensitive to the effects of fragmentation due to road presence (Grilo *et al.* 2009; Grilo *et al.* 2011). Moreover, research on stone marten movement near highways has shown that this species exhibits low highway avoidance (Grilo *et al.* 2012), and is thus likely very susceptible to mortality due to WVCs (Jaeger and Fahrig 2004).We anticipate that this study will be useful both to road planners interested in mitigation as well to ecologists and conservation biologists who seek to understand the effects of roads on important population processes.

6.2. METHODS

6.2.1. MODEL DESCRIPTION

Our model description follows the ODD (Overview, Design concepts, Detail) protocol for describing agent-based models (Grimm *et al.* 2006; 2010) and is based on previous model descriptions (Railsback & Johnson 2011). The model was implemented in NetLogo (Wilensky 1999), and therefore we use some of its conventions (e.g., variable names).

6.2.1.1. Purpose

The purpose of the REPoP model is to investigate the relative effectiveness of two road mitigation measures—fences and passages—under varying degrees of road mortality. This model is parameterized using basic life history traits of martens.

6.2.1.2. Entities, state variables and scales

This section describes the model's structure. Entities are what in NetLogo are called 'turtles' and 'patches', state variables are the attributes that characterize the entities, and scales are the temporal and spatial resolutions and extents of the model.

6.2.1.2.1. *Entities*

The model is a spatially explicit individual-based system, consisting of a landscape with reflecting borders, not toroidal (individuals at one edge of the space cannot jump to cells on the opposite edge), and occupied solely by marten individuals. There are three types of entities: martens, territories, and road passages. Martens are the main entity in the model, and are represented as mobile individuals with *state variables* related to their identity, location and biology (Table 6.1).

Table 6.1 – Attributes for the entities used in the model: Martens, territories and culverts. Each entity is characterized by different parameters.

Entities	Parameters	Values/range and unit
Marten	Identifier	Unique number
	Sex	M/F
	Location	Spatial coordinates
	Territoriality	Y/N
	Territory	Patch identity (Netlogo convention)
	Life stage (age in days)	Juveniles (0-120), sub-adults (121-510) and adults (>511)
	Pregnancy condition (for females)	Y/N
	Movement steps	150 m each
	Annual death rate	0.26
	Genotype (diploid)	Microsatellite length
Territories	Inhabited	Y/N
	Roadside	Left, right
	Fenced	Y/N
Culverts	Funnel distance	100 m

All territories are considered to have equal habitat quality. Territories are designated as 'left' or 'right' according to their position relatively to the road. When required, the patches adjacent to the road can be furnished with road passages and/or fencing. When encountering a fenced section, simulated martens are not able to cross the road at these patches unless a passage is within it. When the selected management option includes passages, martens always use the nearest passage if one is available (< 220 m). Different numbers of road passages and fenced sections are located along the road, according to the simulated management scenario (Fig. 6.1).

Both road passages and fenced sections are randomly assigned to patches along the road. When the number of fence sections and passages differ, the model first assigns a location to the maximum number of patches with both mitigation measures, and then randomly assigns locations to the remaining mitigation measures.

Marten identity is used to link juveniles to their mother, and to compute the genotype of juveniles. Marten coordinates are used to track the position of martens in respect to the road and road passages, and to link adult males to their territory. The timing of life history events (which we call "life stages") such as mating, birth, dispersal, and death follows the known marten annual cycle.

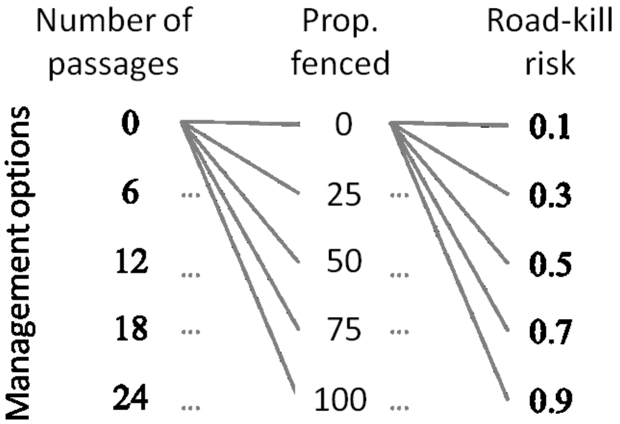

Fig. 6.1 –Simulation experiment treatments using the REPoP model to assess the relative effectiveness of WVC mitigation management options. We simulated 125 scenarios involving a factorial combination of *Number of passages*, *Proportion of road fenced* and *Road-kill risk* (five levels each) . All scenarios were run for 1000 replicates. Population size (N) and F_{st} were calculated at the end of each simulation run.

6.2.1.2.2. *Scales*

The spatial extent of the model is 20 x 24 patches (n = 480). We considered patches to be 2200 x 2200 m in size (529 ha), representing territories inhabited by a single individual. This cell size was based on previous research on organisms tracked near highways (Grilo *et al.* 2012). The total area is therefore over 250,000 ha and is assumed to be large enough to capture large-scale population dynamics. For simplicity, there's no overlap among territories.

Each time step equals one day. Each simulation is first run for 25 years without a road present to allow time for populations to reach a steady state. Then a vertical road is added which evenly bisects the landscape. Thirty additional years of population dynamics are then simulated in the presence of the road. In addition to simulations that include a road ("treatment" scenarios), we simulated a control scenario without roads (which we refer to as the "null" scenario) in order to obtain an expected "baseline" population outcome with which to compare outcomes from the treatment simulations. The null scenario was run the same number of generations as the treatments.

6.2.1.3. Process overview and scheduling

At each daily time step a given list of actions is performed by martens, depending on the life stage and time of the year (Fig. 6.2). These actions consist of mating, birth, dispersal and death. For each action, the order in which martens are called to execute them is randomly assigned.

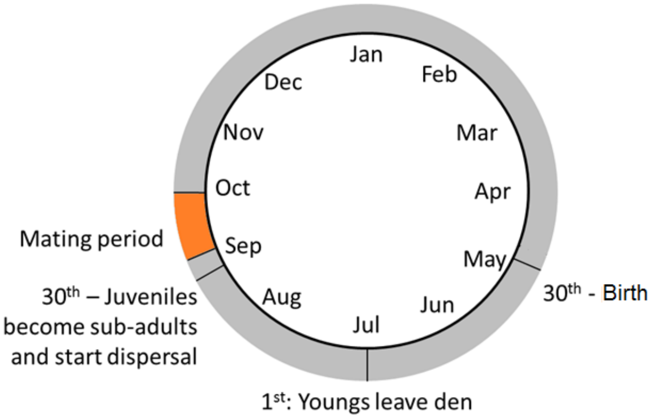

Fig. 6.2 – Annual calendar of all marten life-history events in the model.

Mating: during the month of September adult males try to find non-pregnant adult females in nearby territories (section 6.2.1.7.1). Males can mate with more than one female. Interactions with roads, fences, and road passages can occur during mating.

Birth: on the last day of April pregnant females give birth. Litter size is dependent on population density (section 6.2.1.7.2).

Dispersal: when juveniles reach the sub-adult life stage, August 30th in the model, they disperse from the mother's home range, searching for an empty territory. While dispersing, sub-adults are susceptible to road-kill events and may also interact with fences and road passages (section 6.2.1.7.3).

Death: martens can die by natural death, road-kill events or absence of a vacant territory. Natural death becomes more likely with aging (section 6.2.1.7.4).

6.2.1.4. Design concepts

This section describes the model at a conceptual level, using the eleven design concepts of the ODD protocol (Grimm et al., 2010).

Basic principles: in this section we describe the ecological parameters used when modeling marten life cycle and biology/behavior. These parameters include animal movement, population growth and fecundity, and probability of being killed while crossing a road. Where empirically derived parameter estimates are lacking for martens, we instead use accepted estimates from other carnivores or closely related mammals.

Animal movement: two types of movement were simulated: 1) males searching for female mates and 2) sub-adults dispersing. For mate search, we used a simple random walk, where searching was constrained to the eight neighboring patches of a male's territory (section 6.2.1.7.1). For dispersal events, dispersers follow a highly correlated path, i.e., they make few large turns (see Palmer *et al.* 2011 and citations therein). We implemented this constraint by limiting the movement directionality to a 60° cone centered in the previous step azimuth (section 6.2.1.7.3). Each movement step has a length equal to one tenth of the linear home range size (LHRS, the square root of home range area), which is 220 m in this model. We chose to relate the movement to the LHRS as this approach has been successfully applied previously (Bissonette & Adair 2008). For each time step (day), martens can perform 20 movement steps, summing 4400 m per night. This conforms to the estimated path length per night that martens patrol territory (Genovesi *et al.* 1997; Grilo *et al.* 2012).

Population growth and fecundity: in REPoP, population growth is density dependent, which has been shown in closely related species including *M. americana* (Fryxell *et al.* 1999) and *M. martes* (Zalewski & Jedrzejewski 2006). For simplicity, in

REPoP, density dependence is imposed on birth rate (where three pups per female is the maximum litter size; section 6.2.1.7.2).

Probability of being killed while crossing a four-lane highway: in the real world, the probability of WVCs may vary according to traffic volume, species/vehicle velocities, animal/driver ability to perceive the car/animal approaching, or road configuration (tortuosity) (e.g. Malo *et al.* 2004; Grilo *et al.* 2011). These different sources of variability are not modeled because they are not relevant to our main question which regards the relative impact of WVCs on population size and genetic differentiation, and how to mitigate them, regardless the mechanistic details.

Interaction: There are several interactions occurring in the model among agents. Female adults reproduce if their territory is visited by an adult male during mating. Otherwise, females will not give birth. Offspring inherit half of their diploid genotype from each parent. Sub-adults can only settle in vacant territories. When a road is present, individuals interact with the road, fences, and passages.

Stochasticity: Stochasticity is used in initializing the model, to randomly assign territories, sex and genotypes to adult martens. During simulations, stochasticity is also used to (1) assign directionality to animal movements (during both mating and dispersal); (2) determine whether martens die when crossing the road; 3) assign number of offspring to each female; 4) assign parental alleles to offspring; and 5) determine whether martens succumb to natural death

Observation: For each simulated scenario, the population size, genotypes of settled individuals, and total number of road-kills are recorded at the end of each simulation for use in analysis (see section 6.2.2).

Emergence: Population dynamics emerge from the life cycle of individuals and, in cases where a road is present, from the interaction of individuals with roads, fences, and passages.

Adaptation: There is no individual adaptation or learning in the model.

Objectives: As the model does not include any adaptive traits, there are no objectives to achieve by individuals.

Learning and **Prediction** are not represented in the model.

Sensing: When crossing a road, marten are able to perceive any passages within 220 m.

Collectives: There are no collectives in this model.

6.2.1.5. Initialization

The model is initialized by randomly assigning age, sex, and territory to martens. Simulations start on September 1st, and in the following 30 days adult males search for a mate; all simulations initialize without a road, and hence there is no mitigation. All individuals possess 20 diploid unlinked microsatellite loci (30 possible alleles per locus), which evolve under a strict stepwise mutation model (mutation probability = 10^{-4}) without recombination (Bhargava & Fuentes 2010).

6.2.1.6. Input data

The model does not use input data to represent time-varying processes.

6.2.1.7. Sub-models

The following subsections provide full detail on how model processes are simulated according to marten life cycle.

6.2.1.7.1. *Mating*

During the mating period (Fig. 6.2), adult males search for mating females (procedure *find-a-female*). Within this period, males search within the eight neighboring cells. If a male steps into the territory of a non-pregnant female, they mate. One male can mate with several females but not vice-versa. Each male is allowed to perform ten movement steps per day, after which the procedure *go-home* is invoked, which sends males back to their territory. In both *find-a-female* and *go-home* procedures, road-kills can occur if a male crosses the road without using a passage. When an adult male encounters a fence, it changes its direction toward a randomly chosen patch on the same roadside. An exception to this is when a fence is approached while responding to the *go-home* command. In this case, if a fence is approached, a marten will attempt to return to its territory using the nearest road passage or unfenced patch.

6.2.1.7.2. *Birth*

Pregnant females give birth to between one and three pups, depending on animal density: for each roadside, if there are available territories during mating, then litter size is set to three. Otherwise, it decreases randomly to one or two pups. This litter size estimate is based on marten body size (see Buskirk & Ruggiero 1994). Note that although martens normally produce two to four pups, we restrain the number to a maximum of three to accommodate other causes of death that likely occur at or near birth in natural populations.

Births occur at day 120 (marten females undergo winter embryonic diapause). For each of the 20 diploid microsatellite loci, offspring randomly inherit one allele from each parent. Offspring remain in the mother's territory until the age of four months. We do not assume male parental care.

6.2.1.7.3. *Dispersal*

Juveniles become sub-adults at four months and initiate dispersal. Every unsettled sub-adult searches the landscape for an empty territory. At each movement-step, if an individual enters into an unoccupied territory, it occupies it (and stops moving). Dispersal lasts for four months, after which unsettled sub-adults die. All settled sub-adults become adults on the last day of August the following year, at the age of one year.

6.2.1.7.4. *Death*

Animals may die from natural death or from WVCs. Natural death becomes more likely as animals age. There are two exceptions: 1) if a mother dies, her juveniles also die; 2) unsettled sub-adults die at the end of the dispersal period. To parameterize natural death rates, we used annual death rate estimates based on American martens from Bull and Heater (2001; 37%) and McCann et al. (2010; 19%), and on pine martens from Zalewski and Jedrzejewski (2006; 38%). We fixed the daily probability of natural death for all individuals at 0.07% (based on an annual probability of 26%) as this yielded optimum stability in population dynamics. Thus, the probability of any individual reaching the sub-adult stage, adult stage, or maximum life span is 90%, 74% and 22%, respectively. WVCs may kill dispersing sub-adults or male adults searching for a mate.

6.2.2. DATA ANALYSIS

We first ran REPoP for 1000 iterations under "null" conditions (i.e., with no road present) which allowed us to obtain a "baseline" distribution of expected population outcomes (population size and genetic differentiation) with which to compare outcomes emerging from the various road and mitigation treatments. We then simulated five levels each of WVC probabilities (ranging from 0.1 to 0.9), fencing proportions (ranging from 0 to 100 percent road fencing), and passage numbers (ranging from 0 to 24 passages) in a full factorial design (for a total of 125 treatment scenarios; Fig. 6.1). Each scenario was performed for 1000 replicates.

The maximum number of passages was chosen based on Bissonette and Adair (2008), who used allometric methods to infer optimal spacing between wildlife crossings such that population connectivity is maintained. They found that setting the distance between passages to the linear home range distance (LHRD, square root of home range area) could provide adequate road permeability. At the end of each simulation run (at year 55), we recorded the population size (N) and genetic differentiation (F_{st})(Weir & Cockerham 1984). F_{st} was calculated between groups of individuals bisected by the road (this same grouping was done for the "null" condition, despite no road being present).

To determine the relative effectiveness of passages and fences for mitigating WVC effects, we performed multiple regressions where response variables were population size and F_{st} (in separate analyses) and predictor variables were numbers of passages (*Pass*) and proportions of fencing (*Fenc*), both treated as categorical data. For each response, we repeated three regressions where *Pass* and *Fenc* were included separately and jointly in the model. This was repeated for the five levels of road-kill risk. Akaike information criterion (AIC) values were calculated to determine the information value of each variable (*Pass* and *Fenc*) when predicting population size and F_{st} outcomes. R^2 values were calculated to compare model fit. Variable coefficients from the full model were used to determine the influence of road passages and fences at each mortality level. We carried out analyses using the statistical package R 2.13.1 (R core team, 2011).

6.3. RESULTS

As expected, 'Null' conditions led to stable population sizes (*N*) over time and a lack of genetic differentiation (F_{st}) throughout all simulations (Fig. 6.3). These outcomes were similar to those from treatment scenarios with no mitigation (e.g., number of passages = 0 and proportion of fencing = 0%) and low probability of road mortality (0.1). This is evidenced by a large proportion of outcomes from this treatment (from > 90% of replicates) falling within the 99% confidence intervals of the "null" model outcomes (Fig. 6.4). Regarding the relative effectiveness of the mitigation measures, the majority of variation in *N* was governed by the proportion of road fenced (*Fenc*) rather than the number of passages (*Pass*) (Table 6.2).

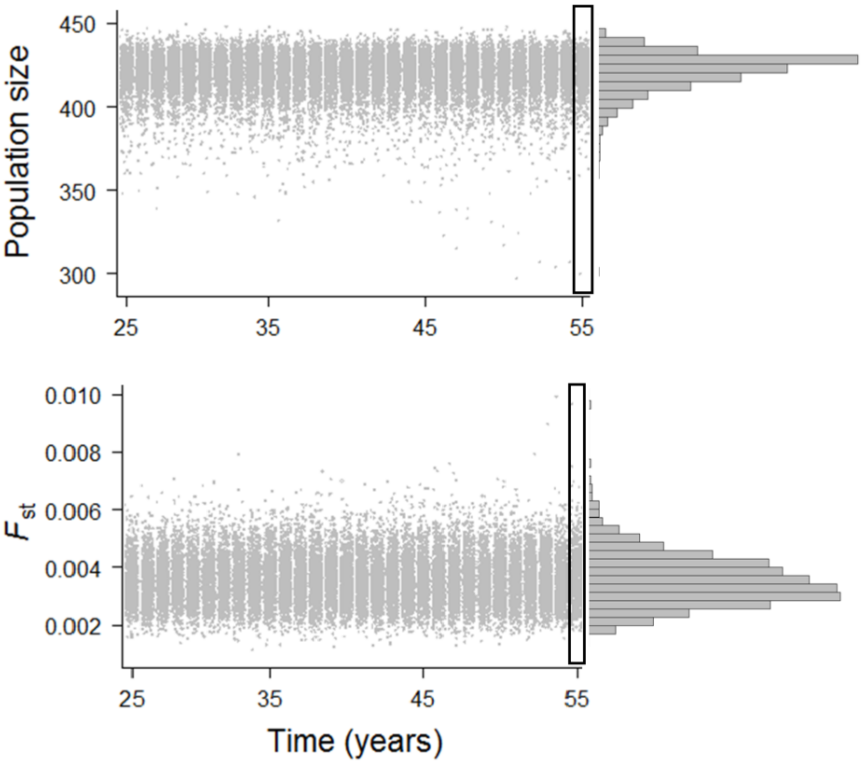

Fig. 6.3 – Population size (*N*) and F_{st} for roadless simulations (scenario 'Null', 1000 replicates). For year = 55 (highlighted) the histograms with the distributions of *N* and F_{st} are shown.

Table 6.2 – Changes in AIC score and R2 values for all linear regression models relating population size (N) and genetic differentiation (Fst) between roadsides. Regressions were repeated across five levels of road-kill risk (RK). Model weights (i.e., probability a model is the ''true'' model) are 1.0 for the full model (Pass + Fenc) in all cases.

		RK = 0.1		RK = 0.3		RK = 0.5		RK = 0.7		RK = 0.9	
Resp.	Model	ΔAIC	R^2	ΔAIC	R^2	ΔAIC	R^2	ΔAIC	R^2	ΔAIC	R^2
	Pass	37	0.01	1013	0.02	2337	0.02	3326	0.02	4187	0.02
N	Fenc	10	0.02	62	0.33	143	0.59	152	0.72	215	0.80
	Pass + Fenc	0	0.02	0	0.35	0	0.62	0	0.74	0	0.82
	Pass	2651	0.09	1551	0.16	639	0.19	1245	0.16	1831	0.12
F_{st}	Fenc	635	0.60	742	0.39	660	0.18	688	0.34	823	0.47
	Pass + Fenc	0	0.69	0	0.55	0	0.38	0	0.51	0	0.65

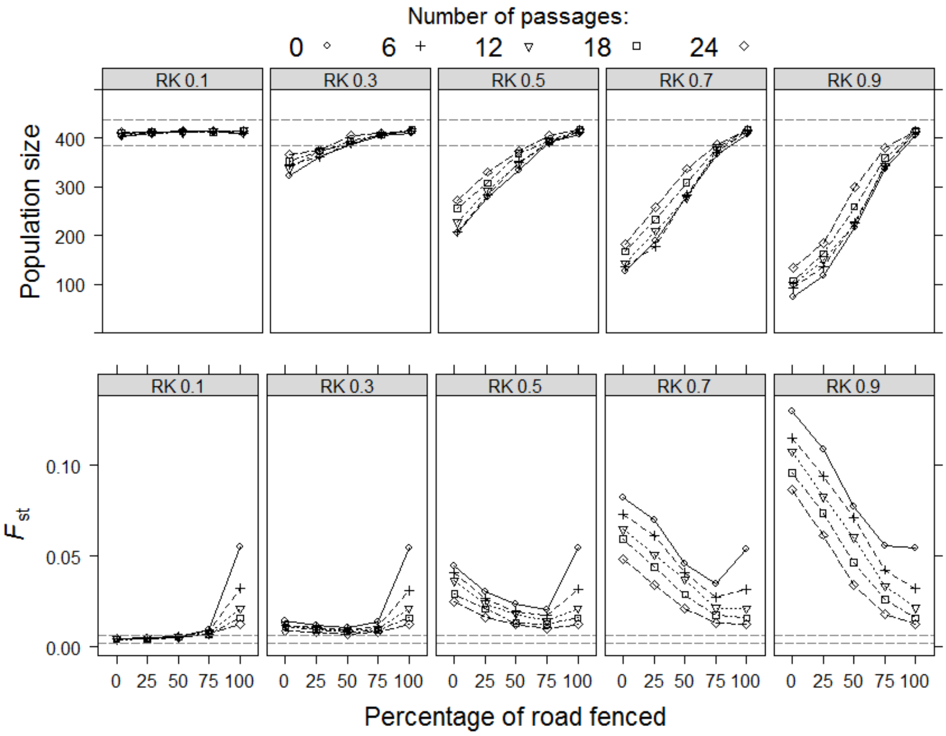

Fig. 6.4 – Results from the simulation of 125 scenarios involving different combinations of road passages (dot symbols), percentage of road fenced (xx axis) and road-kill risk (RK, panels from left to right). Outcomes shown are the mean population size (top) and mean F_{st} (bottom). Dotted grey lines represent the 99 percentile envelope from the 1000 replicates of the 'Null' scenario (no road involved).

Except for the lowest road-kill level (RK = 0.1; in which case neither fences nor passages improved abundance), *Fenc* explained nearly all of the non-random variation in *N* (*Fenc:* R^2 = 0.33-0.80; *Pass:* R^2 = 0.01-0.02), as expected. In fact, increasing the proportion of road fenced always led to higher N when compared to increasing the number of passages, in any level of comparison (Fig. 6.4 and Table 6.3). This was particularly visible at higher road-kill levels. For example, for RK = 0.7 the expected population size was 133 individuals when no road mitigation was in place. Installing between six and 24 passages on average added 10 (7.3%) to 42 (31.6%) individuals to the population. Fencing 25-100% of the road on average added between 68 (51.3%) and 261(195.4%) individuals (Table 6.3).

Table 6.3 – Coefficient values for the number of passages (*Pass*) and proportion of fenced road (*Fenc*) for full models regarding each mortality risk level (RK).

Resp.	Coefficients	RK = 0.1	RK = 0.3	RK = 0.5	RK = 0.7	RK = 0.9
N	Intercept	407	334	218	133	78
	Pass: 6	3	3	9	10	4
	Pass: 12	3	8	13	13	16
	Pass: 18	3	12	25	28	29
	Pass: 24	4	17	38	42	48
	Fenc: 25%	2	28	61	68	55
	Fenc: 50%	4	51	118	152	150
	Fenc: 75%	6	66	161	225	257
	Fenc: 100%	4	71	179	261	318
Fst	Intercept	0.011	0.020	0.045	0.085	0.128
	Pass: 6	-0.005	-0.006	-0.008	-0.012	-0.015
	Pass: 12	-0.008	-0.009	-0.012	-0.018	-0.025
	Pass: 18	-0.009	-0.011	-0.017	-0.026	-0.034
	Pass: 24	-0.010	-0.012	-0.019	-0.032	-0.044
	Fenc: 25%	0.000	-0.003	-0.009	-0.018	-0.022
	Fenc: 50%	0.001	-0.003	-0.016	-0.035	-0.047
	Fenc: 75%	0.004	-0.002	-0.019	-0.044	-0.068
	Fenc: 100%	0.023	0.015	-0.007	-0.040	-0.077

Interestingly, *Fenc* also explained the majority of the non-random variation in F_{st} (*Fence:* R^2 = 0.18-0.60; *Pass:* R^2 = 0.09-0.19). Only in the case of intermediate road mortality risk (RK = 0.5) did fencing and passages explain similar amounts of variation in F_{st} (Table 6.2). Fencing generally leads to either neutral or negative effects on F_{st}. At low road kill levels (RK ≤ 0.3) fencing up to 75% of the road length could be achieved without increasing genetic differentiation above fenceless levels (Table 6.3). At higher road-kill levels (RK ≥ 0.5), increased fencing did lead to reduced F_{st} , although this effect generally leveled off or was reversed at 100% fencing (Fig. 6.4). Furthermore, the slope of this relationship increased as the probability of road mortality increased (Fig. 6.4 and Table 6.3).

As expected, installing passages generally decreased F_{st}, although this reduction was only evident for higher road-kill risk scenarios or when complete fencing was applied (Fig. 6.4). That is, the slope of this relationship increased as the probability of road mortality increased, as found for *Fenc*, but at a lower rate (Fig. 6.4 and Table 6.3). For example, for RK = 0.7, the expected F_{st} was 0.085 under zero mitigation, and adding between six and 24 passages on average reduced F_{st} by between -0.012 and -0.032, respectively. Applying fencing to between 25 to 75% of the road on average reduced F_{st} by -0.018 and -0.044 (Table 6.3). This difference was even more pronounced for the higher road-kill level, where complete fencing actually lead to lower F_{st} values than installing 24 passages (Fig. 6.4 and Table 6.3).

Models including both fences and passages always led to higher population size and lower F_{st} (Table 6.2). However, when one mitigation option must be selected over the other, building fences is always equally good or better at increasing abundance and decreasing genetic differentiation than building an equivalent number of passages (Fig. 6.4 and Table 6.3).

6.4. DISCUSSION

Roads represent an important source of mortality for many species which can severely reduce the abundance and connectivity of natural populations. In this study we developed a stochastic, spatially explicit, individual-based model for stone martens in order to evaluate the relative performance of two approaches commonly adopted to mitigate the negative impacts of roads.

As expected, when any form of mitigation was absent, the population size (N) decreased with increasing road-kill risk. Road-kills are known to be a major contributor to population declines (Ramp & Ben-Ami 2006; Row *et al.* 2007; Chambers & Bencini 2011), and our model shows that once the probability of road mortality is 30% or higher, populations are no longer able to compensate increased road mortality with decreased competition for resources. Also as expected, the genetic differentiation between roadsides (F_{st}) increased with increasing road-kill risk, due to the combined effects of mortality and barrier effects, since potential crossers are killed during dispersal. This effect of mortality on genetic structure has been demonstrated previously (Riley *et al.* 2006; Jackson & Fahrig 2011), and is well supported by population genetic expectations.

Our results suggest that fences are much more effective at mitigating the effects of roads than are passages for our model species. As expected, fencing is an effective way to mitigate reduced abundance due to road mortality (e.g. Clevenger *et al.* 2001b). Even incomplete fencing (75%) can effectively return population size to near that expected under roadless conditions when road mortality is extremely high. The mitigating capacity of passages is very weak by comparison. Implementing the highest number of passages in our model (24) is not as effective at mitigating abundance as is implementing the lowest proportion of fencing (25%; Table 3).

More surprising is how effectively fencing can also reduce genetic differentiation, a relationship opposite to that often assumed (Hepenstrick *et al.* 2012). When mortality risk is low to medium and the entire road is fenced, fencing does increase differentiation over the course of 30 years. However, when fencing is done partially, or when mortality risk is high, the more fencing implemented, the slower that differentiation will develop. Thus, fencing often appears to effectively promote rather than impede connectivity.

This relationship makes sense in light of population genetic expectations: by promoting larger population sizes, fencing also promotes higher genetic diversity, thus diminishing the rate at which genetic drift differentiates populations. Conversely, many more passages would need to be constructed than we have simulated to equal the ability of fences at reducing genetic differentiation. Passage building is more effective at slowing differentiation than fence building only when mortality risk is low or when fencing is 100%.

Assessing the relative effect of fences and passages at mitigating road mortality effects requires the study of different populations inhabiting areas near roads with different levels of fencing and road passages. To our knowledge such a study has yet to be conducted and would be difficult to achieve in an empirical framework. Simulations are thus an ideal way to investigate the relative effectiveness of these two common mitigation techniques and our study is the first to quantitatively investigate this question while independently varying levels of fencing, passage construction, and road mortality. Our results suggest that fencing is much better at recouping lost abundance and genetic diversity (and under most conditions, reducing genetic differentiation) than passage building.

A second clear outcome from our simulations was that populations are predicted to be resilient to WVC events when risk of mortality is low (≤10%). This resilience effect likely resulted from reduced competition for resources in response to increased road mortality, an effect facilitated by density dependent growth. This suggests that mitigation efforts may be wasteful and even harmful when road mortality is expected to be low. This point was previously made by Jaeger and Fahrig (2004), who predicted that fences could reduce population persistence when organisms are able to effectively avoid roads or traffic. Although we predict that populations may be robust to small levels of road mortality, extreme caution should be taken because WVCs may have a cumulative effect with other sources of mortality, and may thus inflict a larger negative effect than expected (Forman 2000).

Taken as a whole, our results demonstrate that for roads with medium to high WVC risk, both mitigation approaches can improve abundance and connectivity, but that fences are more effective in most cases. We suggest that partial fencing (particularly at ~75%) is a promising and underappreciated management option for mitigating negative road effects. Fencing 75% of the road achieved nearly all the improvements in abundance obtained by fencing 100% of the road. Yet fencing at 75% also appears to have allowed for enough gene flow across the road to generally prevent the increased differentiation that can develop when dispersal is completely severed by 100% fencing. Surprisingly, the potential improvement in N or reduction in F_{st} by adding passages on top of partial fencing is apparently very small, which challenges previous claims (Jaeger & Fahrig 2004). To our knowledge, the merits of partial fencing have not before been tested and our results highlight this as a good management option.

Our model may underestimate the pervasiveness of mortality risk from roads on populations. For example, in our model road mortality only affects dispersing sub-adults and male adults searching for females. Mortality effects are likely higher in nature where settled adults may also be vulnerable to roads that bisect their territories. In fact, recent studies have shown that large sections of highway often bisect marten home ranges (Grilo *et al.* 2012). In addition, our model assumed that martens cross roads over a minimum time period. However, there is evidence that some carnivores often hunt in highway verges where prey is abundant (Barrientos & Bolonio 2009), and thus may linger near roads for long periods. This behavior would likely heighten the mortality risk for carnivores beyond that seen in our model under a given level of simulated risk.

The Netlogo code for REPoP is freely available (URL: https://sites.google.com /site/roadmitigation) and can be easily adapted to other species or management scenarios. Thus, the REPoP model can be a valuable tool when planning road mitigation actions, not only for martens and other small sized carnivores, but for a variety of species. We stress that our model described here was based on martens and our results are most applicable to martens and other medium-sized carnivores. Extrapolation to other species with different behaviors - such as road avoidance (McGregor *et al.* 2008), high sensitivity to road passage characteristics (Clevenger *et al.* 2001a), or seasonal migrations, which may require massive numbers of individuals (e.g., ungulates or amphibians) to cross roads over short periods - should be carefully pondered.

ACKNOWLEDGEMENTS

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7. General Discussion

7.1. THESIS OVERVIEW AND MAIN FINDINGS

In this thesis I analyzed the responses of two model species – wood mouse and stone marten – to the road barrier effect, considering both the potential benefits and negative influences for species persistence resulting from the road presence. The major goal was to contribute for a sound scientific basis of road impacts and generate advice for improving existing management practices that could benefit the maximum number of species, striving towards a better conciliation of road networks with wildlife conservation.

The responses of the two model species were assessed by looking at different animal-road interaction levels, each discussed in previous chapters, namely road verges as habitat providers for small mammals (Chapter 2); roads as agents of wood mouse population differentiation (Chapter 3); roads as agents shaping movement of individual stone martens (Chapter 4); and which measures better mitigate medium-sized carnivores-vehicle collisions (Chapter 5).

Higher abundances of small mammals were detected in highway verges, particularly of wood mouse, relatively to the main land uses in the region (Chapter 2). This effect was a result of the highways fencing, which creates a 'grazing exclosure' effect in verges, contrasting with the high grazing rates found in montado and agricultural areas and the resulting overgrazing and trampling effects. Nevertheless, despite the benefit of verges functioning as good habitat for small mammals, roads seem to impede wood mouse movement leading to population structuring; this was supported by evidences of genetic differentiation between individuals captured in opposite roadsides in three highways from two distinct regions in the Iberian Peninsula (Chapter 3).

Inversely, stone marten movement and space use were not strongly hindered by the presence of a highway although individual responses were observed, probably related to a combination of effects including different perceptions of the road-kill risk or residency status (Chapter 4). The apparent lack of road avoidance by the stone martens tracked in the vicinity of the highway may explain the high road-kill rates detected in previous research (Grilo *et al.* 2009). Therefore, a better understanding of the effectiveness of wildlife vehicle collision (WVC) mitigation measures usually applied for medium-sized carnivores was the logical follow-up of previous approaches in my thesis. The results suggest that partial fencing can have a greater success in diminishing both population depletion and genetic differentiation due to WVCs (Chapter 5).

The following sections of this chapter aim to integrate these major findings and discuss its implications towards the improvement of road management and wildlife conservation, offering recommendations for management and suggestions for future research in this field.

7.2. EVIDENCES SUPPORTING AN ECOLOGICALLY SCALED RESPONSE

Overall, the findings from this thesis support the hypothesis under study – the species biological and ecological traits are determinant in predicting the road effects on population persistence. I detected clear evidences of a preference for highway verges by wood mouse, which could indicate an attraction to roads and thus a positive effect for population density. In fact, fenced highways seem to benefit this smaller and less vagile species (comparatively to stone marten), particularly in landscapes where overgrazing lead to soil and vegetation degradation. Moreover, small mammal' populations are likely to be low impacted by road mortality when compared to larger species; although the number of records of smaller species may be high in road-kill surveys (e.g. Garriga *et al.* 2012), its effect on population density is most probably very low as this parameter, in general, is inversely correlated with the body size (Silva & Downing 1995). This relation is clearly visualized when relating the 'body mass' (a proxy of body size) and 'population density' from Jones et al. (2009) mammal dataset (Fig. 6.1).

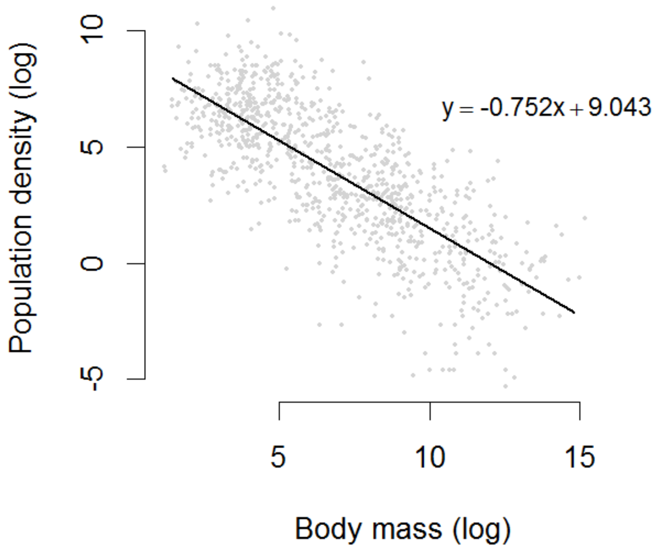

Fig. 6.1 – Relation between population density and body mass for mammals (here used as a proxy for body size). Data was taken from Jones (2009), for species for which the two variables '*X21.1_PopulationDensity_n.km2*' and '*X5.1_AdultBodyMass_g*' were available (n=922). Population density is expressed as number of individuals per square kilometer and body mass in grams (variables are log transformed). Slope of the linear model is concordant with the allometric relation (see Silva and Downing 1995). See Table 1.1 for variable description.

Therefore, the relevance of the number of road-kills for a given species is also ecologically scale dependent: the population effect of 10 critically endangered Iberian lynxes (*Lynx pardinus*) found dead in one year in a given road segment is different if they were 10 wild rabbits (*Oryctolagus cuniculus*). While for the first a significant decrease in the whole population size would occur, for the latter the impact is expected to be minimal.

On the other hand, roads apparently are inducing some structuring and isolation in the local wood mouse population (Chapter 3). This pattern has been reported for other small mammals (see reviews by Balkenhol & Waits 2009; Holderegger & Di Giulio 2010).

Regarding the research involving the stone marten, results clearly showed that the species' movements are not constrained by highway presence, and therefore WVC events are likely to occur (Chapter 4). Interestingly, however, I found that individuals may display different behavioral responses toward roads, probably because they may be more or less familiarized with the presence of the roads and incoming vehicles. Residents may be more aware of the road-kill risk and about road passages location, whereas non-residents are less knowledgeable about the terrain and road attributes.

Nonetheless, the fact that resident stone martens are more familiar with roads the need to frequently cross the road to explore its territory and make full use of resources therein, originates a high cumulative WVCs risk. For example, considering an individual inhabiting a territory bisected by a road, and for which the probability of being hit on each crossing is merely 0.2% [value used in Kramer-Schadt et al. (2004) for parameterizing the mortality probability on main roads per crossing for Eurasian lynx *Lynx lynx*], the chance of an individual surviving a year-round (with everything else being equal) decreases to less than 50% (99.8% ^ 365 ≈ 48.2% - probability of surviving all days of one year).

Hence, the main road impact for carnivores are WVCs, as described in several studies (Ferreras *et al.* 1992; Clarke *et al.* 1998; Philcox *et al.* 1999; Hauer *et al.* 2002; Grilo *et al.* 2009; Colino-Rabanal *et al.* 2011). However, despite WVCs being an agent of population differentiation (Jackson & Fahrig 2011), the high mobility of these species probably prevent roads from structuring populations (Kyle & Strobeck 2003; Van de Zande *et al.* 2007).

Being the aim of my thesis to contribute, as much as possible, to multi-species mitigation solutions, a third ecological model need to be considered in this discussion – the wild ungulates – as they are larger-sized and may be particularly affected by fenced roads. In fact, the height and mesh size of Iberian highway fences is currently a deterrent for animal movement of such size (Fig. 6.2). Moreover, these fences are installed in all highway length (obligatory by law) to avoid large animals to access the road pavement and cause human injuries due to collision. Therefore, its populations may become heavily fragmented as the fenced highway network proliferates (e.g. Hepenstrick *et al.* 2012). Although the study of fencing effect in these larger species in Iberian Peninsula is still in its early steps, preliminary results already confirm their vulnerability to road-induced isolation (Malo & Mata 2010).

Fig. 6.2 – Example of a fence installed in the highway A6 verge to avoid livestock and wild boar (*Sus scrofa*) to enter the road area. Fence height is ca. 1.5 m.

It should be noted that where fences are absent, wild ungulates are also vulnerable to WVC, as medium-sized carnivores (Bruinderink & Hazebroek 1996; Seiler 2005; Colino Rabanal 2011). For example, Malo et al. (2004) used an official traffic database, from Jefatura Provincial de Tráfico de Soria (Spain), to relate the locations of WVCs with road and environmental variables. This database is naturally biased toward larger animals as these cause traffic accidents. From a total record of 2067 records during a 13 year period, three species were involved in more than 98% of the accidents, roe deer *Capreolus capreolus* (38%), wild boar *Sus scrofa* (35%) and red deer *Cervus elaphus* (25%). Considering that the road network had 3212 km not fenced, these represents a WVC rate of 4.85 individuals per 100 km per year.

These general patterns are conceptually illustrated in Fig. 6.3, based on the exploratory analysis from Chapter 1 and the data gathered in Chapters 2 to 5.

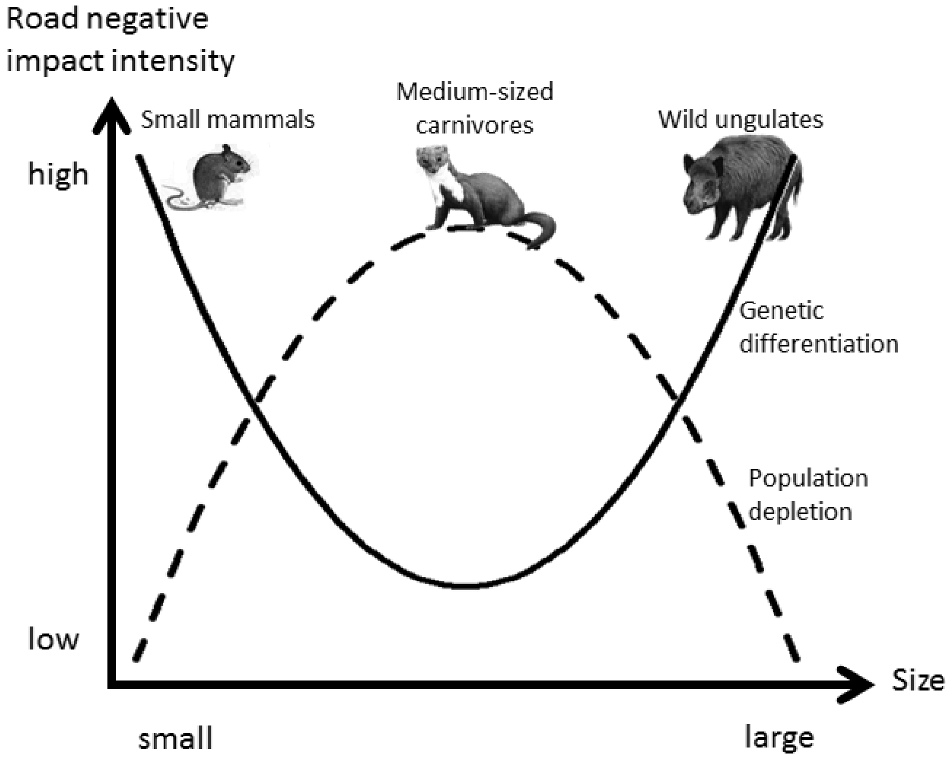

Fig. 6.3 – Conceptual framework of the relation between species ecological profiles (here represented by body size) and the two main effects of fenced roads: population depletion (dashed line) and genetic differentiation (continuous line). Three ecological models are represented: small mammals, subject to low road mortality but more affected by population isolation due to repulse for road pavement; medium-sized carnivores, for which roads do not represent a physical obstacle and thus are unlikely to structure populations, but represent a significant source of mortality; and ungulates, not prone to road-kills due to fencing which in turn is likely to isolate populations. See text for further details.

This conceptualization refers to (ground dwelling) mammals, which are generally faster than other species with similar size (e.g. small mammals vs. frogs), and for which it is not described any behavior implying a static position in road pavement, as thermoregulation for reptiles. Also, I'm considering that the traffic volume is below the threshold over which most individuals do not attempt to cross the roads (Seiler 2003). Over this threshold, the most probable representation in figure 6.3 would be two parallel lines, indicating a low effect of population depletion and high genetic differentiation also for medium-sized carnivores. This would be a consequence of the expected low crossing attempts that would lead to a population structuring.

7.3. DIFFERENT ECOLOGICAL PROFILES, DIFFERENT EFFECTS: ROAD MITIGATION FOR ALL?

As referred in Chapter 1 and throughout the thesis, population depletion and genetic structuring are probably the main road negative impacts in wildlife (Forman *et al.* 2003). Both effects promote an interruption of the normal flow of individuals and ultimately of their gametes through the landscape. The data obtained in this thesis supports the hypothesis that species biological and ecological traits are related with these main road effects. Their magnitude seem to follow an ecological scale where small mammals (and wild ungulates in fenced roads) are probably more prone to genetic structuring, while medium-sized carnivores are more vulnerable to population depletion.

As so, it is unlikely that any species fully benefits from the presence of road in landscape, with the exception for invasive species (Hulme 2009). This leads to the first main outcome of this thesis: when planning a new road, a first management option to consider for biodiversity conservation should be *not* to construct the road, as it will inevitably lead to negative consequences for the wildlife. However, if the construction is inevitable, road mitigation plans should target the most vulnerable species to the above mentioned road effects.

Considering the results from Chapter 5, it is clear that mitigation should primarily focus in diminishing the WVCs events, to maintain a viable population size and also prevent the genetic differentiation. This means that lowering the number of road-kills for the most vulnerable species should constitute the priority. According to this thesis' results and available literature, medium-sized carnivores may be seriously vulnerable to road-killing and therefore mitigation management of Iberian highways should chiefly target this group.

The most effective mitigation measure to diminish the WVCs is installing fences in conjunction with wildlife crossings (Fahrig & Rytwinski 2009; Lesbarreres & Fahrig 2012). This fencing could be similar to the one applied for the wildcat in Germany (Klar *et al.* 2009, see Fig. 6.4): 2 m high, with a 5x5 cm mesh size to prevent animals from jumping or passing over, with a 50 cm wide metal sheet in the top to avoid climbers from overpassing the fence, and a board made of recycled material dug 30 cm deep in the soil, to impede species as badgers (***Meles meles***) and foxes (***Vulpes vulpes***) from digging under the fence.

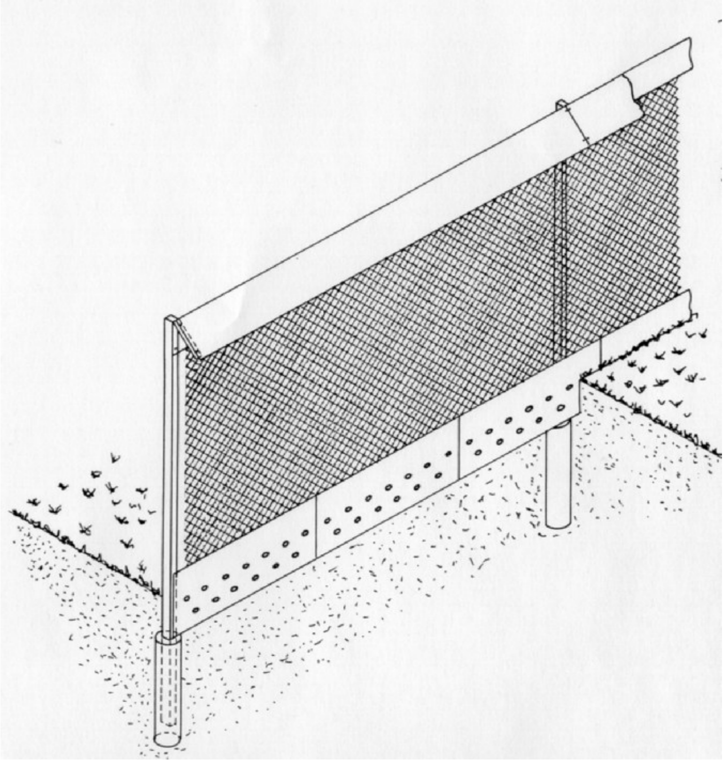

Fig. 6.4. – Fence installed in a highway that crosses an area occupied by wild cat (*Felis silvestris*). The height is 2 m with a mesh size of 5x5 cm. The top of the fence has a metallic anti-climbing structure with 50 cm width (vertical arrow). Main structure is highlighted. Figure adapted from a Klar et al. 2009).

By impeding medium-sized carnivores from crossing the road, individuals become risk-free from being hit in significant numbers. However, as suggested by the results from Chapter 5, it is not be necessary to apply this improved fence version in all highway length leaving openings to allow some individuals that are not aware of the location of road passages or avoid using them, to cross the road. Inevitably, some individuals may be hit and eventually killed in these fence-free road segments but in a significantly lower rate than in the current situation.

In turn, installing this fence type may also provide benefits for small mammals. By preventing the access of carnivores to verges, the predatory stress on small mammals decrease, and therefore their abundance is likely to increase. Moreover, as suggested in Chapter 2, fenced road verges could be incorporated in regional conservation plans by managing the vegetative structure of highway verges in conjunction with the implementation of livestock exclusionary areas located within the landscape and connected to the road verges by other linear features (such as riparian areas or hedgerows). These actions are expected improve the habitat conditions for small mammals, and their abundance. In turn, increasing the small mammal abundance in the exclusion areas (outside verges) is likely to increase the prey availability, thus favoring the persistence of predators (Torre *et al.* 2007; Bush *et al.* 2012).

Road fencing should be linked to existing road passages, such as culverts. Fortunately, in Iberian Peninsula roads, and particularly highways, are required to have an efficient transversal drainage system to avoid the flooding of the platform and surrounding areas. This is because in the Mediterranean region the rainfall is concentrated in short periods over the year (Ceballos *et al.* 2004). This implies that highways have one or more culverts every two kilometers, most of which over one meter wide and numerous authors have demonstrated the use of these structures by Mediterranean carnivores for road crossings, although its use is dependent on several road- and landscape-related attributes (e.g. Yanes *et al.* 1995; Mata *et al.* 2005; Grilo *et al.* 2008; Mateus *et al.* 2011). Installing the above mentioned fence in highway segments, linked to existing road passages, is therefore the best solution to significantly diminish the WVC events involving medium-sized carnivores (Grilo *et al.* 2009).

However, as discussed in Chapter 3 some individual variability is expected by medium-sized carnivores in response to existing road passages. This was also reported for vulnerable species such as the wildcat (Klar *et al.* 2009) or the wolf (*Canis lupus*) (Blanco *et al.* 2005). Therefore, installing fauna passages may be also required, particularly where highways bisect regions that host endangered species' populations, such as Iberian lynx, wildcat or wolf. These eco-passages do not necessarily represent a significant extra cost in road mitigation. As suggested by Lesbarreres and Fahrig (2012), wider extended stream crossings are most effective in restoring road permeability (Fig. 6.5).

Fig 6.5 – Example of an eco-passage in A6: an extended stream crossing. Vegetation at the entrance and along the passages could be planted to minimize human presence and favor animal use. Large banks provide best conditions for animal use.

These elongated, open-span structure over a natural stream, including wide banks on both sides wider passages can be used by all ground dwelling animals, including wild ungulates, and even birds and bats (Bach *et al.* 2004). Similar structures are already implemented when highways cross streams, and only few improvements are necessary, namely the inclusion of wider and dry banks, and dense vegetation to restrain human use (Fig. 6.5).

Bissonete and Adair (2008) used allometric methods to infer the best spacing between wildlife crossings, in order to maintain habitat connectivity. They found that using the linear home range distance (square root of home range area), as spacing distance between passages could provide a good level of road permeability. This spacing represents a high number of passages along the highway. However, as shown in Chapter 5, this density of passages might be overestimated as the key point in road mitigation should be to maintain a viable population size (Reed *et al.* 2003; Reed 2004), while guaranteeing that some crossings occur to insure that all population dynamics' processes are not segmented or blocked.

In my perspective, this is the critical aspect of road management plans. Mitigation should be framed within a landscape context, where special attention should be paid to the relation between the road density and the availability of suitable habitat, particularly for endangered medium-sized carnivores. In fact, by restricting the access of individuals to the road via exclusionary fencing, we will be also reducing (intentionally) the movement rate between road sides. While the open-span passages may alleviate the 'physical' barrier effect, it is necessary that the road density never exceed the threshold over which the access to resources (including mates) is hampered, lowering the chances to allow the persistence of viable populations (Pereira *et al.* 2004; Borda-de-Água *et al.* 2011). In other words, there is a minimum area devoid of roads, the critical patch size, below which a population will decline to extinction (Pereira *et al.* 2004), and this should be undoubtedly avoided.

Again, larger species are more vulnerable to this area constrain effect. Despite the minimum viable population size being generally higher for smaller animals, and therefore requiring a higher number of territories; the minimum number of individuals necessary to allow a viable population of larger species demand larger landscape extents (Biedermann 2003). This is easily perceived when relating the minimum area required for a population to persist and their body size. I used the data from Reed (2004), who estimated the minimum viable adult population size (MVP) for many vertebrates. Following Reed (2004), MVP is the minimum number of adults required in a population of size equal to the area's carriyng capacity, assuming a stable age distribution. This value allow to define a minimum habitat area for the establishment and persistence of a population. I considered only the estimates for mammals from this dataset. For each species I added the values of body mass from Jones et al. (2009) (variable code '*5-1_AdultBodyMass*', see Table 1.1). These values were then related using linear regression (Fig. 6.6).

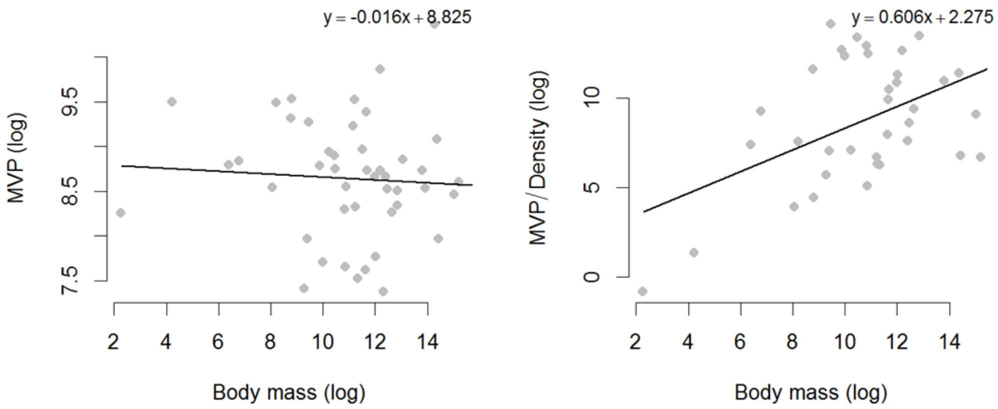

Fig. 6.6 - Relation between minimum viable population size (MVP) and body mass for mammals (here used as a proxy for body size) (A); and MVP/Density with Body mass (B). MVP data was taken from Reed (2004) only for mammals (n=50 species), and refers to the values corrected for sample size (MVP_C in their work). Species' body mass and density data was taken from Jones (2009). Body mass is expressed in grams and Population density as the number of individuals per square kilometer (variables are log transformed).

As expected, smaller species require a larger population size to persist. However, when padronizing the MVP with the species density, the slope becomes positive, denoting that larger species' populations require larger habitat extents to persist (Fig. 6.6). Therefore, road mitigation managers should focus not only on preventing WVCs, but also consider the 'big picture' by assessing how the highway may influence the overal population dynamics. Although the effect of one motorway may be minimal for the persistence of a given population, the concurrence of several such roads may affect population persistence in the long run (Roedenbeck *et al.* 2007; Klar *et al.* 2009).

To highlight the importance of integrating the road mitigation planning into a landscape framework, I analyzed how the available habitat in my study area may be segmented by the highway network. Using the CORINE land cover data (source URL: www.eea.europa.eu), I selected the land use classes described in Table 6.1 as the habitat preferential for stone marten and the estimated MVP (n = 1867 individuals) for the ecologically similar American marten (*Martes americana*) (Reed *et al.* 2003). Considering a conservative home range area for stone marten of 200 ha (Grilo *et al.* 2012, Chapter 4), the viable population size would require ca. 373,400 ha.

Table 6.1 – Land use classes from CORINE 2006 used to map suitable habitat for stone marten, and their cover area (ha) the two areas (A and B) highlighted in Fig. 6.6.

Class	Description	Area A	Area B
244	Agro-forestry areas	5.55E+04	1.80E+05
311	Broad-leaved forest	1.12E+05	4.47E+04
324	Transitional woodland-shrub	1.71E+05	1.57E+04
	Total	3.38E+05	2.40E+05

Observing the Figure 6.7, we can see that a great proportion of the Iberian landscape contains suitable habitat for the stone marten. However, when overlaying the highway network, some resulting polygons have a total habitat area bellow the estimated threshold to maintain a viable population. For example, areas A and B from Fig. 6.6 (left) have a total habitat area of ca. 340,000 and 240,000 ha, respectively (see Table 6.1).

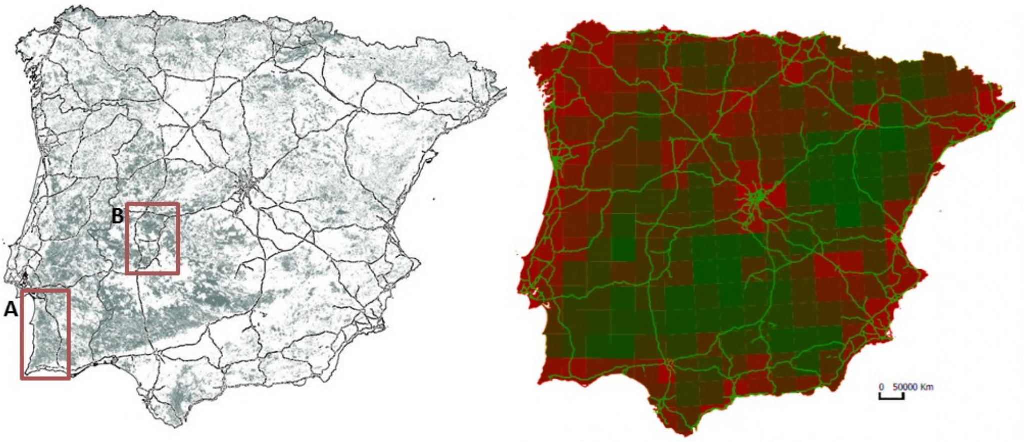

Fig. 6.7 – Left: distribution of the suitable habitat for stone marten in Iberian Peninsula. Habitat was obtained by merging the CORINE land use classes described in Table 6.1. Square areas (A and B) highlight polygons with a total habitat amount lower than the required to sustain a viable population (Reed et al. 2003). Right: colored grid stand for total road density. Light green to darker red colors represent lower to higher road density. Values range between 0.2-16.5 Km / Km^2. Road network data was adapted from *open street map* (source URL: www.openstreetmap.org).

Therefore, although the overall amount of suitable habitat is still present in vast areas, its fragmentation due to the highway network and other linear infrastructures may jeopardize the connectivity of the landscape, resulting into potentially not sustainable areas to host viable MVPs. Moreover, when mapping the total road network in the Iberian Peninsula (Fig. 6.6 – right), it is visible that large extensions of the landscape hold high road densities (and consequently human pressure). Therefore, road mitigation must also acknowledge the cumulative effect of secondary roads, which themselves may constitute a severe source of WVCs (Grilo *et al.* 2009).

It should be noted that these impacts in stone marten population are expected to be much more exacerbated if we consider vulnerable species as Iberian lynx, wildcat or wolf, which home ranges are much larger and therefore the total amount of non-fragmented habitat, necessary to host a viable population, is consequently larger. Hence, when planning a new highway, the mitigation planning ought to include a sufficient number of crossing structures accounting for the overall effect of the network in the wildlife, and not just focusing in mitigating the effect over local populations.

7.4. CONCLUDING REMARKS AND FURTHER RESEARCH

With this thesis I aimed to demonstrate that the road fragmentation effect is a major threat for wildlife conservation. By focusing on forest dwelling mammals as ecological models, I intended to infer the potential road effects on an overarching assembly of species. Consistent evidences were obtained that the species' responses to roads and traffic follow an ecological scale, where small mammals are more vulnerable to isolation and medium-sized carnivores more prone to population depletion. I concluded that this latter group should be the focus of road mitigation plans. Based on these findings I suggested that the main road network should be upgraded with exclusionary fence, impermeable to carnivores and larger species in the most part of the road length, combined with a sufficient number of open-span structures to allow the access of individuals to resources. The mitigation management, particularly the number of open-span passages to be installed, should consider not only the local scale but also the landscape perspective, in order to guarantee that the accessible habitat area is sufficient to host viable populations.

With this thesis, I hope to have contributed to an improvement of our understanding of species' responses variability to road fragmentation and the different processes involved. Despite the fact that much is yet to be done within this research area, namely by testing the study hypothesis with other ecological models and in different environments, I believe that the study of the ecologically scaled responses to road effects should continue integrating two main research areas.

The first was already mentioned, and refers to the need of incorporating the landscape scale into road ecology studies. Even the simplest study of road passage use has cause-effect with the surrounding landscape. Almost a decade ago, Forman (2000) estimated that one fifth of the US area was affected by the road network and this estimate is likely to be higher for Europe and particularly for the Iberian Peninsula given the higher road network densities herein. Nevertheless, despite this concern was highlighted in road ecology literature (Roedenbeck *et al.* 2007; van der Ree *et al.* 2007; Thorne *et al.* 2009; van der Ree *et al.* 2011), still few studies addressed the landscape scale in their approaches.

The second research area, also linked to the previous one, is the need of assessing the impact of road fragmentation considering climate change scenarios. Although roads impact primarily the individuals inhabiting in its vicinity, as demonstrated throughout the thesis, the road effects may spread over larger distances. Likewise, landscape dynamics involving species' range shifts resulting from climate change conditions, might be disrupted when encountering major roads (Mantyka-Pringle *et al.* 2012). Therefore, road managers and road ecologists must join forces when conceiving mitigation plans. This task force ought to consider the potential impacts at the local and landscape scale, but also for current and future environmental conditions.

A final word dedicated to emerging countries. As referred, the current knowledge on road ecology is vast, but is biased toward landscapes and organisms from developed countries. Most of peer-reviewed publications focusing road ecology issues were performed in these countries, with clear preponderance from North America (US and Canada). I believe that this knowledge must embrace and be adapted to provide valuable insights for emerging countries challenges. These regions, in my perspective, should concentrate most efforts for wildlife conservation while there are still conditions to allow a sustainable human development. While some important research is being held in these countries (e.g. Li *et al.* 2010; Seshadri & Ganesh 2011), the knowledge on road ecology should flow to these as a world responsibility. This should be guaranteed by improving the collaboration protocols between academia and road engineers from developed and emerging countries.

References

Adams, L. W. , Geis, A. D. (1983). Effects of roads on small mammals. *Journal of Applied Ecology,* **20:** 403-415.

AEMET-IM (2011). Iberian Climate Atlas - Air Temperature and Precipitation (1971-2000).). AEMET: Agencia Estatal de Meteorología and Instituto de Meteorologia. Spain, Closas-Orcoyen.

Alain, B., Gilles, P. , Yannick, D. (2006). Factors driving small rodents assemblages from field boundaries in agricultural landscapes of western France. *Landscape Ecology,* **21:** 449-461.

Andrén, H. (1994). Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. *Oikos,* **71:** 355-366.

Araujo, M. B., Alagador, D., Cabeza, M., Nogues-Bravo, D.*, et al.* (2011). Climate change threatens European conservation areas. *Ecology Letters,* **14:** 484-492.

Aronson, J., Pereira, J. S. , Pausas, J. G. (2009). *Cork oak woodlands on the edge: ecology, adaptive management, and restoration*. Island. Washington, DC.

Ascensão, F., Clevenger, A. P., Grilo, C., Filipe, J.*, et al.* (2012). Highway verges as habitat providers for small mammals in agrosilvopastoral environments. *Biodiversity and Conservation,* **21:** 3681-3697.

Askew, N. P., Searle, J. B. , Moore, N. P. (2007). Agri-environment schemes and foraging of barn owls *Tyto alba*. *Agriculture, Ecosystems & Environment,* **118:** 109-114.

Bach, L., Burkhardt, P. , Limpens, H. J. G. A. (2004). Tunnels as a possibility to connect bat habitats. *Mammalia,* **68:** 411-420.

Baillargeon, S. , Rivest, L.-P. (2009). Rcapture: Loglinear Models for Capture-Recapture Experiments. *URL http://CRAN.R-project.org/package=Rcapture, R package version 1.2-0. Access date 1 January 2012*.

Balkenhol, N. , Waits, L. P. (2009). Molecular road ecology: exploring the potential of genetics for investigating transportation impacts on wildlife. *Molecular Ecology,* **18:** 4151-4164.

Balloux, F. (2001). EASYPOP (version 1.7): a computer program for population genetics simulations. *Journal of Heredity,* **92:** 301-302.

Barrientos, R. , Bolonio, L. (2009). The presence of rabbits adjacent to roads increases polecat road mortality. *Biodiversity and Conservation,* **18:** 405-418.

Barton, K. (2011). MuMIn: Multi-model inference. *URL http://CRAN.R-project.org/package=MuMIn, R package version 0.13.21. Access date 1 January 2012*.

Barton, K. A., Phillips, B. L., Morales, J. M. , Travis, J. M. J. (2009). The evolution of an 'intelligent' dispersal strategy: biased, correlated random walks in patchy landscapes. *Oikos,* **118:** 309-319.

Bates, D., Maechler, M. , Bolker, B. (2010). lme4: Linear mixed-effects models using S4 classes. URL http://CRAN.R-project.org/package=lme4, R package version 0.999375-37. Access date 1 January 2012.

Bellamy, P. E., Shore, R. F., Ardeshir, D., Treweek, J. R.*, et al.* (2000). Road verges as habitat for small mammals in Britain. *Mammal Review,* **30:** 131-139.

Belo, C. C., Pereira, M. S., Moreira, A. C., Coelho, I. S.*, et al.* (2009). Montado. In: *Ecossistemas e Bem-estar Humano: Avaliação para Portugal do Millennium Ecosystem Assessment.* Pereira, H. M., Domingos, T., Vicente, L.*, et al.* (Eds.). Escolar Editora.Lisboa.

Benhamou, S. , Adler, F. R. (2011). Dynamic Approach to Space and Habitat Use Based on Biased Random Bridges. *PLoS ONE,* **6:** 324-336.

Benítez-López, A., Alkemade, R. , Verweij, P. A. (2010). The impacts of roads and other infrastructure on mammal and bird populations: a meta-analysis. *Biological Conservation,* **143:** 1307-1316.

Bennett, A. F. (1990). Habitat corridors and the conservation of small mammals in a fragmented forest environment. *Landscape Ecology,* **4:** 109-122.

Bhargava, A. , Fuentes, F. F. (2010). Mutational dynamics of microsatellites. *Molecular biotechnology,* **44:** 250-266.

Biedermann, R. (2003). Body size and area-incidence relationships: is there a general pattern? *Global Ecology and Biogeography,* **12:** 381-387.

Bilotta, G. S., Brazier, R. E. , Haygarth, P. M. (2007). The impacts of grazing animals on the quality of soils, vegetation, and surface waters in intensively managed grasslands. *Advances in Agronomy,* **94:** 237-280.

Bissonette, J. A. , Adair, W. (2008). Restoring habitat permeability to roaded landscapes with isometrically-scaled wildlife crossings. *Biological Conservation,* **141:** 482-488.

Bissonette, J. A. , Cramer, P. C. (2008). *Evaluation of the use and effectiveness of wildlife crossings*. Transportation Research Board. Report #615. Washington, D.C.

Bissonette, J. A. , Storch, I. (2002). Fragmentation: Is the message clear? *Conservation Ecology,* **6:** 14 [Online] URL: http://www.consecol.org/vol6/iss2/art14. .

Bjørnstad, O. N. (2009). ncf: spatial nonparametric covariance functions. URL http://CRAN.R-project.org/package=ncf, R package version 1.1-3. Access date 1 January 2012.

Bjørnstad, O. N. , Falck, W. (2001). Nonparametric spatial covariance functions: estimation and testing. *Environmental and Ecological Statistics,* **8:** 53-70.

Blanco, J. C., Cortes, Y. , Virgos, E. (2005). Wolf response to two kinds of barriers in an agricultural habitat in Spain. *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* **83:** 312-323.

Blondel, J. (2006). The 'Design' of Mediterranean Landscapes: A Millennial Story of Humans and Ecological Systems during the Historic Period. *Human Ecology,* **34:** 713-729.

Blondel, J., Aronson, J. , Bodiou, J.-Y. (2010). *The Mediterranean region. Biological diversity in space and time*. Oxford University Press. New York.

Borda-de-Água, L., Navarro, L., Gavinhos, C. , Pereira, H. M. (2011). Spatio-temporal impacts of roads on the persistence of populations: analytic and numerical approaches. *Landscape Ecology,* **26:** 1-13.

Boswell, G. P., Britton, N. F. , Franks, N. R. (1998). Habitat fragmentation, percolation theory and the conservation of a keystone species. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* **265:** 1921-1925.

Bowman, J., Jaeger, J. A. G. , Fahrig, L. (2002). Dispersal distance of mammals is proportional to home range size. *Ecology,* **83:** 2049-2055.

Breslow, N. E. , Clayton, D. G. (1993). Approximate inference in generalized linear mixed models. *Journal of the American Statistical Association,* **88:** 9-25.

Bruinderink, G. W. T. A. G. , Hazebroek, E. (1996). Ungulate traffic collisions in Europe. *Conservation Biology,* **10:** 1059-1067.

Bugalho, M. N., Caldeira, M. C., Pereira, J. S., Aronson, J.*, et al.* (2011). Mediterranean cork oak savannas require human use to sustain biodiversity and ecosystem services. *Frontiers in Ecology and the Environment,* **9:** 278-286.

Bull, E. L. , Heater, T. W. (2001). Survival, causes of mortality, and reproduction in the American marten in northeastern Oregon. *Northwestern Naturalist*: 1-6.

Burnett, S. E. (1992). Effects of a rainforest road on movements of small mammals: mechanisms and implications. *Wildlife Research,* **19:** 95-104.

Burnham, K. P. , Anderson, D. R. (2002). *Model Selection and Multimodel Inference.* 2nd edn. Springer. New York.

Bush, E. R., Buesching, C. D., Slade, E. M. , Macdonald, D. W. (2012). Woodland Recovery after Suppression of Deer: Cascade effects for Small Mammals, Wood Mice (Apodemus sylvaticus) and Bank Voles (Myodes glareolus). *PLoS ONE,* **7**.

Buskirk, S. W. , Ruggiero, L. F. (1994). American marten. In: *The scientific basis for conserving forest carnivores: American marten, fisher, lynx, and wolverine in the western United States*: 7-37. Ruggiero, L. F., Aubry, K. B., Buskirk, S. W.*, et al.* (Eds.). Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. Fort Collins, CO, U.S.

Butchart, S. H. M., Walpole, M., Collen, B., van Strien, A.*, et al.* (2010). Global Biodiversity: Indicators of Recent Declines. *Science,* **328:** 1164-1168.

Calenge, C. (2006). The package “adehabitat” for the R software: A tool for the analysis of space and habitat use by animals. *Ecological modelling,* **197:** 516-519.

Calenge, C. (2011). Home Range Estimation in R: the adehabitatHR Package.). Office national de la classe et de la faune sauvage. Auffargis.

Campos, P. (2004). Towards a sustainable global economics approach for Mediterranean agroforestry systems. In: *Sustainability of agrosilvopastoral systems*. Schnabel, S. , Ferreira , A. (Eds.). Catena Verlag, Reiskirchen, Germany.

Canivenc, R., Mauget, C., Bonnin, M. , Aitken, R. J. (1981). Delayed Implantation in the Beech Marten (*Martes Foina*). *Journal of Zoology,* **193:** 325-332.

Carr, L. W. , Fahrig, L. (2001). Effect of road traffic on two amphibian species of differing vagility. *Conservation Biology,* **15:** 1071-1078.

Castro, H. , Freitas, H. (2009). Above-ground biomass and productivity in the Montado: From herbaceous to shrub dominated communities. *Journal of Arid Environments,* **73:** 506-511.

Ceballos, A., Martínez-Fernández, J. , Luengo-Ugidos, M. Ã. (2004). Analysis of rainfall trends and dry periods on a pluviometric gradient representative of Mediterranean climate in the Duero Basin, Spain. *Journal of Arid Environments,* **58:** 215-233.

Chambers, B. , Bencini, R. (2011). Road mortality reduces survival and population growth rates of tammar wallabies on Garden Island, Western Australia. *Wildlife Research,* **37:** 588-596.

Chan, K., Pringle, R. M., Ranganathan, J., Boggs, C. L*., et al.* (2006). When agendas collide: human welfare and biological conservation. *Conservation Biology,* **21:** 59-68.

Clark, R. W., Brown, W. S., Stechert, R. , Zamudio, K. R. (2010). Roads, interrupted dispersal, and genetic diversity in timber rattlesnakes. *Conservation Biology,* **24:** 1059-1069.

Clarke, G. P., White, P. C. L. , Harris, S. (1998). Effects of roads on badger *Meles meles* populations in south-west England. *Biological Conservation,* **86:** 117-124.

Clevenger, A. P. (1994). Feeding ecology of the Eurasian pine marten *Martes martes* and stone marten *Martes foina* in Europe. In: *Martens, sables, and fishers: biology and conservation*: 326-340. Buskirk, S. W., Harestad, A. S., Raphael, M. G*., et al.* (Eds.). Cornell University Press.Ithaca, NY.

Clevenger, A. P., Chruszcz, B. , Gunson, K. E. (2001a). Drainage culverts as habitat linkages and factors affecting passage by mammals. *Journal of Applied Ecology***:** 1340-1349.

Clevenger, A. P., Chruszcz, B. , Gunson, K. E. (2001b). Highway mitigation fencing reduces wildlife-vehicle collisions. *Wildlife Society Bulletin***:** 646-653.

Clevenger, A. P., Ford, A. T. , Sawaya, M. A. (2009). Banff wildlife crossings project: Integrating science and education in restoring population connectivity across transportation corridors. Final report to Parks Canada Agency, Radium Hot Springs, British Columbia, Canada. 165.).

Clevenger, A. P. , Waltho, N. (2005). Performance indices to identify attributes of highway crossing structures facilitating movement of large mammals. *Biological Conservation,* **121:** 453-464.

Coffin, A. W. (2007). From roadkill to road ecology: A review of the ecological effects of roads. *Journal of Transport Geography,* **15:** 396-406.

Colino-Rabanal, V. J., Lizana, M. , Peris, S. J. (2011). Factors influencing wolf Canis lupus roadkills in Northwest Spain. *European Journal of Wildlife Research,* **57:** 399-409.

Colino Rabanal, V. (2011). *Contribuciones al análise de mortalidad de vertebrados en carreteras. Doctoral thesis*. Universidad de Salamanca. Salamanca.

Corlatti, L., HacklaeNder, K. , Frey Roos, F. (2009). Ability of wildlife overpasses to provide connectivity and prevent genetic isolation. *Conservation Biology,* **23:** 548-556.

Corp, N., Gorman, M. L. , Speakman, J. R. (1997). Ranging behaviour and time budgets of male wood mice Apodemus sylvaticus in different habitats and seasons. *Oecologia,* **109:** 242-250.

Curtis, J. T. (1956). The modification of mid-latitude grasslands and forests by man. In: *Man's Role in Changing the Face of the Earth*: 721-736. Thomas, W. L. J. (Ed.). University of Chicago Press. Chicago, USA.

da Silva, P. M., Aguiar, C. A. S., Niemela, J., Sousa, J. P.*, et al.* (2009). Cork-oak woodlands as key-habitats for biodiversity conservation in Mediterranean landscapes: a case study using rove and ground beetles (Coleoptera: Staphylinidae, Carabidae). *Biodiversity and Conservation,* **18:** 605-619.

Dahle, B., Reimers, E. , Colman, J. E. (2008). Reindeer (Rangifer tarandus) avoidance of a highway as revealed by lichen measurements. *European Journal of Wildlife Research,* **54:** 27-35.

DeAngelis, D. L. , Mooij, W. M. (2005). Individual-based modeling of ecological and evolutionary processes. *Annual Review of Ecology Evolution and Systematics,* **36:** 147-168.

Debinski, D. M. , Holt, R. D. (2000). A survey and overview of habitat fragmentation experiments. *Conservation Biology,* **14:** 342-355.

Delibes, M. (1983). Interspecific competition and the habitat of the stone marten *Martes foina* (Erxleben 1777) in Europe. *Acta Zoologica Fennica,* **174:** 229-231.

Diaz, M., Santos, T. , Telleria, J. L. (1999). Effects of forest fragmentation on the winter body condition and population parameters of an habitat generalist, the wood mouse *Apodemus sylvaticus*: a test of hypotheses. *Acta Oecologica,* **20:** 39-49.

Didham, R. K., Kapos, V. , Ewers, R. M. (2012). Rethinking the conceptual foundations of habitat fragmentation research. *Oikos,* **121:** 161-170.

Dietrich, W., Katz, H., Lincoln, S. E., Shin, H. S.*, et al.* (1992). A Genetic-Map of the Mouse Suitable for Typing Intraspecific Crosses. *Genetics,* **131:** 423-447.

Dodd, N. L., Gagnon, J. W., Manzo, A. L. , Schweinsburg, R. E. (2007). Video surveillance to assess highway underpass use by elk in Arizona. *Journal of Wildlife Management,* **71:** 637-645.

Donald, P. F. , Evans, A. D. (2006). Habitat connectivity and matrix restoration: the wider implications of agri-environment schemes. *Journal of Applied Ecology,* **43:** 209-218.

Donald, P. F., Pisano, G., Rayment, M. D. , Pain, D. J. (2002). The Common Agricultural Policy, EU enlargement and the conservation of Europe's farmland birds. *Agriculture, Ecosystems & Environment,* **89:** 167-182.

Doncaster, C. P., Rondinini, C. , Johnson, P. C. D. (2001). Field test for environmental correlates of dispersal in hedgehogs *Erinaceus europaeus*. *Ecology,* **70:** 33-46.

Donovan, T. M., Freeman, M., Abouelezz, H., Royar, K.*, et al.* (2011). Quantifying home range habitat requirements for bobcats (*Lynx rufus*) in Vermont, USA. *Biological Conservation,* **144:** 2799-2809.

Dray, S. , Dufour, A. B. (2007). The ade4 package: Implementing the duality diagram for ecologists. *Journal of Statistical Software,* **22:** 1-20.

Eccard, J. A., Walther, R. B. , Milton, S. J. (2000). How livestock grazing affects vegetation structures and small mammal distribution in the semi-arid Karoo. *Journal of Arid Environments,* **46:** 103-106.

EEA (2012). Environmental European Agency. Available at: http://www.eea.europa.eu/data-and-maps/data/natura-2000 Access Date 07-08-2012.

Eigenbrod, F., Hecnar, S. , Fahrig, L. (2008). Accessible habitat: an improved measure of the effects of habitat loss and roads on wildlife populations. *Landscape Ecology,* **23:** 159-168.

Epperson, B. K., McRae, B. H., Scribner, K. I. M., Cushman, S. A.*, et al.* (2010). Utility of computer simulations in landscape genetics. *Molecular Ecology,* **19:** 3549-3564.

Evanno, G., Regnaut, S. , Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology,* **14:** 2611-2620.

Ewers, R. M. , Didham, R. K. (2007). Confounding factors in the detection of species responses to habitat fragmentation. *Biological Reviews,* **81:** 117-142.

Ewers, R. M., Thorpe, S. , Didham, R. K. (2007). Synergistic interactions between edge and area effects in a heavily fragmented landscape. *Ecology,* **88:** 96-106.

Fahrig, L. (1997). Relative effects of habitat loss and fragmentation on population extinction. *Journal of Wildlife Management,* **61:** 603-610.

Fahrig, L. (2003). Effects of habitat fragmentation on biodiversity. *Annual Review of Ecology, Evolution, and Systematics,* **34:** 487-515.

Fahrig, L. , Rytwinski, T. (2009). Effects of roads on animal abundance: an empirical review and synthesis. *Ecology and Society,* **14:** 21.

Fedy, B. C., Martin, K., Ritland, C. , Young, J. (2008). Genetic and ecological data provide incongruent interpretations of population structure and dispersal in naturally subdivided populations of white-tailed ptarmigan (Lagopus leucura). *Molecular Ecology,* **17:** 1905-1917.

Fernandez, N. , Palomares, F. (2000). The selection of breeding dens by the endangered Iberian lynx (*Lynx pardinus*): implications for its conservation. *Biological Conservation,* **94:** 51-61.

Ferreira, D. B. (2000). Environmental impact of land use change in the inner Alentejo (Portugal) in the 20th century. In: *Geomorphology and Human Activity: Their Role in Global Environmental Change*: 249-267. Slaymaker, O. (Ed.). J. Wiley & Sons.Chichester.

Ferreras, P., Aldama, J. J., Beltran, J. F. , Delibes, M. (1992). Rates and causes of mortality in a fragmented population of Iberian lynx *Felis pardina* Temminck, 1824. *Biological Conservation,* **61:** 197-202.

Ferreras, P., Gaona, P., Palomares, F. , Delibes, M. (2001). Restore habitat or reduce mortality? Implications from a population viability analysis of the Iberian lynx. *Animal Conservation,* **4:** 265-274.

Finnegan, L. A., Wilson, P. J., Price, G. N., Lowe, S. J.*, et al.* (2012). The complimentary role of genetic and ecological data in understanding population structure: a case study using moose (Alces alces). *European Journal of Wildlife Research,* **58:** 415-423.

Fischer, J. , Lindenmayer, D. B. (2007). Landscape modification and habitat fragmentation: a synthesis. *Global Ecology and Biogeography,* **16:** 265-280.

Fitzgibbon, C. D. (1997). Small Mammals in Farm Woodlands: The Effects of Habitat, Isolation and Surrounding Land-Use Patterns. *Journal of Applied Ecology,* **34:** 530-539.

Ford, A. T. , Fahrig, L. (2008). Movement patterns of eastern chipmunks (*Tamias striatus*) near roads. *Journal of Mammalogy,* **89:** 895-903.

Forman, R. T. T. (1995). Some General-Principles of Landscape and Regional Ecology. *Landscape Ecology,* **10:** 133-142.

Forman, R. T. T. (2000). Estimate of the area affected ecologically by the road system in the United States. *Conservation Biology,* **14:** 31-35.

Forman, R. T. T. , Alexander, L. E. (1998). Roads and their major ecological effects. *Annual Review of Ecology and Systematics,* **29:** 207-231.

Forman, R. T. T., Sperling, D., Bissonette, J., Clevenger, A.*, et al.* (2003). *Road ecology: science and solutions*. Island Press. Washington, DC.

Frair, J. L., Merrill, E. H., Beyer, H. L. , Morales, J. M. (2008). Thresholds in landscape connectivity and mortality risks in response to growing road networks. *Journal of Applied Ecology,* **45:** 1504-1513.

Franklin, A. B., Noon, B. R. , George, T. L. (2002). What is habitat fragmentation? In: *Effects of habitat fragmentation on birds in western landscapes : contrasts with paradigms from the eastern United States. Studies in Avian Biology Nº 25*: 20-29. George, T. L. , Dobkin, D. S. (Eds.). Cooper ornithological society.

Frantz, A. C., Cellina, S., Krier, A., Schley, L.*, et al.* (2009). Using spatial Bayesian methods to determine the genetic structure of a continuously distributed population: clusters or isolation by distance? *Journal of Applied Ecology,* **46:** 493-505.

Fryxell, J. M., Falls, J. B., Falls, E. A., Brooks, R. J.*, et al.* (1999). Density dependence, prey dependence, and population dynamics of martens in Ontario. *Ecology,* **80:** 1311-1321.

Gagnon, J. W., Dodd, N. L., Ogren, K. S. , Schweinsburg, R. E. (2011). Factors associated with use of wildlife underpasses and importance of long-term monitoring. *The Journal of Wildlife Management,* **75:** 1477-1487.

Garriga, N., Santos, X., Montori, A., Richter-Boix, A.*, et al.* (2012). Are protected areas truly protected? The impact of road traffic on vertebrate fauna. *Biodiversity and Conservation,* **21:** 2761-2774.

Gaspar, P., Mesías, F. J., Escribano, M. , Pulido, F. (2009). Assessing the technical efficiency of extensive livestock farming systems in Extremadura, Spain. *Livestock Science,* **121:** 7-14.

Gaspar, P., Mesías, F. J., Escribano, M., Rodriguez de Ledesma, A.*, et al.* (2007). Economic and management characterization of dehesa farms: implications for their sustainability. *Agroforestry Systems,* **71:** 151-162.

Gauffre, B., Estoup, A., Bretagnolle, V. , Cosson, J. F. (2008). Spatial genetic structure of a small rodent in a heterogeneous landscape. *Molecular Ecology,* **17:** 4619-4629.

Gehring, T. , Swihart, R. (2003). Body size, niche breadth, and ecologically scaled responses to habitat fragmentation: mammalian predators in an agricultural landscape. *Biological Conservation,* **109:** 283-295.

Gelling, M., Macdonald, D. W. , Mathews, F. (2007). Are hedgerows the route to increased farmland small mammal density? Use of hedgerows in British pastoral habitats. *Landscape Ecology,* **22:** 1019-1032.

Genovesi, P., Secchi, M. , Boitani, L. (1996). Diet of stone martens: an example of ecological flexibility. *Journal of Zoology,* **238:** 545-555.

Genovesi, P., Sinibaldi, I. , Boitani, L. (1997). Spacing patterns and territoriality of the stone marten. *Canadian Journal of Zoology,* **75:** 1966-1971.

Gerlach, G. , Musolf, K. (2000). Fragmentation of landscape as a cause for genetic subdivision in bank voles. *Conservation Biology,* **14:** 1066-1074.

Gibbs, J. P. , Shriver, W. G. (2005). Can road mortality limit populations of pool-breeding amphibians? *Wetlands Ecology and Management,* **13:** 281-289.

Gibson, C. W. D., Brown, V. K., Losito, L. , McGavin, G. C. (1992). The response of invertebrate assemblies to grazing. *Ecography,* **15:** 166-176.

Gill, J. A., Norris, K. , Sutherland, W. J. (2001). Why behavioural responses may not reflect the population consequences of human disturbance. *Biological Conservation,* **97:** 265-268.

Godinho, S., Santos, A. P. , Sa-Sousa, P. (2011). Montado management effects on the abundance and conservation of reptiles in Alentejo, Southern Portugal. *Agroforestry Systems,* **82:** 197-207.

Golley, F. B. (1960). Energy dynamics of a food chain of an old-field community. *Ecological Monographs,* **30:** 187-206.

Gómez, J. M., Puerta-Piñero, C. , Schupp, E. W. (2008). Effectiveness of rodents as local seed dispersers of Holm oaks. *Oecologia,* **155:** 529-537.

Gonçalves, P., Alcobia, S., Simões, L. , Santos-Reis, M. (2011). Effects of management options on mammal richness in a Mediterranean agro-silvo-pastoral system. *Agroforestry Systems,* **85:** 383-395.

Goodwin, B. J. , Fahrig, L. (2002). How does landscape structure influence landscape connectivity? *Oikos,* **99:** 552-570.

Goosem, M. (2001). Effects of tropical rainforest roads on small mammals: inhibition of crossing movements. *Wildlife Research,* **28:** 351-364.

Graham, M. H. (2003). Confronting multicollinearity in ecological multiple regression. *Ecology,* **84:** 2809-2815.

Grilo, C. (2012). A rede viária e a fauna - Impactos, mitigação e implicações para conservação das espécies em Portugal. In: *Ecologia de estradas: tendências e pesquisas*. Bager, A. (Ed.). UFLA. Lavras, Brasil.

Grilo, C., Ascensão, F., Santos-Reis, M. , Bissonette, J. A. (2011). Do well-connected landscapes promote road-related mortality? *European Journal of Wildlife Research,* **57:** 707-716.

Grilo, C., Bissonette, J. A. , Santos-Reis, M. (2008). Response of carnivores to existing highway culverts and underpasses: implications for road planning and mitigation. *Biodiversity and Conservation,* **17:** 1685-1699.

Grilo, C., Bissonette, J. A. , Santos-Reis, M. (2009). Spatial-temporal patterns in Mediterranean carnivore road casualties: Consequences for mitigation. *Biological Conservation,* **142:** 301-313.

Grilo, C., Sousa, J., Ascensão, F., Matos, H.*, et al.* (2012). Individual Spatial Responses towards Roads: Implications for Mortality Risk. *PLoS ONE,* **7:** e43811.

Grimm, V., Berger, U., Bastiansen, F., Eliassen, S.*, et al.* (2006). A standard protocol for describing individual-based and agent-based models. *Ecological modelling,* **198:** 115-126.

Grimm, V., Berger, U., DeAngelis, D. L., Polhill, J. G.*, et al.* (2010). The ODD protocol: A review and first update. *Ecological modelling*.

Grimm, V. , Railsback, S. F. (2012). Pattern-oriented modelling: a 'multi-scope'for predictive systems ecology. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **367:** 298-310.

Grimm, V., Revilla, E., Berger, U., Jeltsch, F.*, et al.* (2005). Pattern-oriented modeling of agent-based complex systems: lessons from ecology. *Science,* **310:** 987-991.

Grosman, P. D., Jaeger, J. A. G., Biron, P. M., Dussault, C.*, et al.* (2011). Trade-off between road avoidance and attraction by roadside salt pools in moose: An agent-based model to assess measures for reducing moose-vehicle collisions. *Ecological modelling,* **222:** 1423-1435.

Gu, X. D., Liu, S. Y., Wang, Y. Z. , Wu, H. (2009). Development and characterization of eleven polymorphic microsatellite loci from South China field mouse (*Apodemus draco*). *Conservation Genetics,* **10:** 1961-1963.

Haila, Y. (2002). A conceptual genealogy of fragmentation research: from island biogeography to landscape ecology. *Ecological Applications,* **12:** 321-334.

Hanski, I. (1994). A Practical Model of Metapopulation Dynamics. *Journal of Animal Ecology,* **63:** 151-162.

Hanski, I. (1998). Metapopulation dynamics. *Nature,* **396:** 41-49.

Hanski, I., Henttonen, H., Korpimäki, E., Oksanen, L.*, et al.* (2001). Small-rodent dynamics and predation. *Ecology,* **82:** 1505-1520.

Harr, B., Musolf, K. , Gerlach, G. (2000). Characterization and isolation of DNA microsatellite primers in wood mice (*Apodemus sylvaticus*, Rodentia). *Molecular Ecology,* **9:** 1664-1665.

Harrison, S. , Bruna, E. (1999). Habitat fragmentation and large-scale conservation: What do we know for sure? *Ecography,* **22:** 225-232.

Hartig, F., Calabrese, J. M., Reineking, B., Wiegand, T.*, et al.* (2011). Statistical inference for stochastic simulation models–theory and application. *Ecology Letters,* **14:** 816–827.

Hartl, D. L. , Clark, A. G. (1997). *Principles of population genetics*. 3rd edn. Sinauer Associates Sunderland, Massachusetts.

Hauer, S., Ansorge, H. , Zinke, O. (2002). Mortality patterns of otters (*Lutra lutra*) from eastern Germany. *Journal of Zoology,* **256:** 361–368.

Hayward, B., Heske, E. J. , Painter, C. W. (1997). Effects of livestock grazing on small mammals at a desert cienaga. *The Journal of Wildlife Management,* **61:** 123-129.

Hedrick, P. W. (2005). A standardized genetic differentiation measure. *Evolution,* **59:** 1633-1638.

Heller, N. E. , Zavaleta, E. S. (2009). Biodiversity management in the face of climate change: a review of 22 years of recommendations. *Biological Conservation,* **142:** 14-32.

Hels, T. , Buchwald, E. (2001). The effect of road kills on amphibian populations. *Biological Conservation,* **99:** 331-340.

Hepenstrick, D., Thiel, D., Holderegger, R. , Gugerli, F. (2012). Genetic discontinuities in roe deer (*Capreolus capreolus*) coincide with fenced transportation infrastructure. *Basic and Applied Ecology,* **13:** 631-638.

Herr, J., Schley, L. , Roper, T. J. (2009). Stone martens (*Martes foina*) and cars: investigation of a common human–wildlife conflict. *European Journal of Wildlife Research,* **55:** 471-477.

Hodgson, J. A., Thomas, C. D., Dytham, C., Travis, J. M. J.*, et al.* (2012). The Speed of Range Shifts in Fragmented Landscapes. *PLoS ONE,* **7:** e47141.

Holderegger, R. , Di Giulio, M. (2010). The genetic effects of roads: A review of empirical evidence. *Basic and Applied Ecology,* **11:** 522-531.

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scandinavian journal of statistics,* **6:** 65-70.

Hope, A. C. A. (1968). A Simplified Monte Carlo Significance Test Procedure. *Journal of the Royal Statistical Society Series B-Statistical Methodology,* **30:** 582-598.

Hopwood, J. L. (2008). The contribution of roadside grassland restorations to native bee conservation. *Biological Conservation,* **141:** 2632-2640.

Horne, J. S., Garton, E. O., Krone, S. M. , Lewis, J. S. (2007). Analyzing animal movements using Brownian bridges. *Ecology,* **88:** 2354-2363.

Hubisz, M. J., Falush, D., Stephens, M. , Pritchard, J. K. (2009). Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources,* **9:** 1322-1332.

Huijser, M. , Clevenger, A. (2006). Habitat and corridor function of rights-of-way. In: *The Ecology of Transportation: Managing Mobility for the Environment*: 233-254. Davenport, J. , Davenport, J. L. (Eds.).Springer, Dordrecht, The Netherlands.

Huijser, M. P., Duffield, J. W., Clevenger, A. P., Ament, R. J.*, et al.* (2009). Cost-benefit analyses of mitigation measures aimed at reducing collisions with large ungulates in the United States and Canada; a decision support tool. *Ecology and Society,* **14:** 15.

Hulme, P. E. (2009). Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology,* **46:** 10-18.

Ibáñez, J., Martínez, J. , Schnabel, S. (2007). Desertification due to overgrazing in a dynamic commercial livestock-grass-soil system. *Ecological modelling,* **205:** 277-288.

Jaarsma, C. F., van Langevelde, F. , Botma, H. (2006). Flattened fauna and mitigation: traffic victims related to road, traffic, vehicle, and species characteristics. *Transportation Research Part D: Transport and Environment,* **11:** 264-276.

Jackson, N. D. , Fahrig, L. (2011). Relative effects of road mortality and decreased connectivity on population genetic diversity. *Biological Conservation,* **144:** 3143–3148.

Jaeger, J. A. G., Bowman, J., Brennan, J., Fahrig, L.*, et al.* (2005). Predicting when animal populations are at risk from roads: an interactive model of road avoidance behavior. *Ecological modelling,* **185:** 329-348.

Jaeger, J. A. G. , Fahrig, L. (2004). Effects of road fencing on population persistence. *Conservation Biology,* **18:** 1651-1657.

Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics,* **24:** 1403-1405.

Jombart, T., Devillard, S. , Balloux, F. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *Bmc Genetics,* **11:** 94.

Jones, K. E., Bielby, J., Cardillo, M., Fritz, S. A.*, et al.* (2009). PanTHERIA: a species-level database of life history, ecology, and geography of extant and recently extinct mammals. *Ecology,* **90:** 2648-2648.

Jost, L. (2008). GST and its relatives do not measure differentiation. *Molecular Ecology,* **17:** 4015-4026.

Kalinowski, S. T. (2005). HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. *Molecular Ecology Notes,* **5:** 187-189.

Kanno, Y., Vokoun, J. C. , Letcher, B. H. (2011). Fine-scale population structure and riverscape genetics of brook trout (*Salvelinus fontinalis*) distributed continuously along headwater channel networks. *Molecular Ecology,* **20:** 3711-3729.

Keenan, K. (2011). diveRsity v1.3.2. Available at: http://CRAN.R-project.org/package=diveRsity. 20 November 2012.

Keller, I. , Largiadèr, C. R. (2003). Recent habitat fragmentation caused by major roads leads to reduction of gene flow and loss of genetic variability in ground beetles.

Proceedings of the Royal Society of London, Series B, Biological Sciences, **270:** 417–423.

Kimura, M. , Ohta, T. (1978). Stepwise mutation model and distribution of allelic frequencies in a finite population. *Proceedings of the National Academy of Sciences,* **75:** 2868-2872.

Klar, N., Herrmann, M. , Kramer-Schadt, S. (2009). Effects and Mitigation of Road Impacts on Individual Movement Behavior of Wildcats. *Journal of Wildlife Management,* **73:** 631-638.

Kleijn, D. , Sutherland, W. J. (2003). How effective are European agri-environment schemes in conserving and promoting biodiversity? *Journal of Applied Ecology,* **40:** 947-969.

Kokko, H. , Lopez-Sepulcre, A. (2006). From individual dispersal to species ranges: Perspectives for a changing world. *Science,* **313:** 789-791.

Korpimaki, E. , Norrdahl, K. (1991). Numerical and Functional-Responses of Kestrels, Short-Eared Owls, and Long-Eared Owls to Vole Densities. *Ecology,* **72:** 814-826.

Kotler, B. P. (1997). Patch use by gerbils in a risky environment: manipulating food and safety to test four models. *Oikos,* **78:** 274-282.

Kramer-Schadt, S., Revilla, E., Wiegand, T. , Breitenmoser, U. R. S. (2004). Fragmented landscapes, road mortality and patch connectivity: modelling influences on the dispersal of Eurasian lynx. *Journal of Applied Ecology,* **41:** 711-723.

Kyle, C. J. , Strobeck, C. (2003). Genetic homogeneity of Canadian mainland marten populations underscores the distinctiveness of Newfoundland pine martens (Martes americana atrata). *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* **81:** 57-66.

Landguth, E. L. , Cushman, S. A. (2010). CDPOP: A spatially explicit cost distance population genetics program. *Molecular Ecology Resources,* **10:** 156-161.

Landguth, E. L., Cushman, S. A., Murphy, M. A. , Luikart, G. (2010). Relationships between migration rates and landscape resistance assessed using individual-based simulations. *Molecular Ecology Resources,* **10:** 854-862.

Latch, E. K., Dharmarajan, G., Glaubitz, J. C. , Rhodes, O. E. (2006). Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics,* **7:** 295-302.

Laurance, W. F. (2008). Theory meets reality: how habitat fragmentation research has transcended island biogeographic theory. *Biological Conservation,* **141:** 1731-1744.

Leal, A. I., Correia, R. A., Granadeiro, J. P. , Palmeirim, J. M. (2011). Impact of cork extraction on birds: Relevance for conservation of Mediterranean biodiversity. *Biological Conservation,* **144:** 1655-1662.

Lesbarreres, D. , Fahrig, L. (2012). Measures to reduce population fragmentation by roads: what has worked and how do we know? *Trends in Ecology & Evolution,* **27:** 374-380.

Lewis, J. S., Rachlow, J. L., Horne, J. S., Garton, E. O.*, et al.* (2011). Identifying habitat characteristics to predict highway crossing areas for black bears within a human-modified landscape. *Landscape and Urban Planning,* **101:** 99-107.

Li, C. C., Weeks, D. E. , Chakravarti, A. (1993). Similarity of DNA fingerprints due to chance and relatedness. *Human Heredity,* **43:** 45-52.

Li, T. A., Shilling, F., Thorne, J., Li, F. M.*, et al.* (2010). Fragmentation of China's landscape by roads and urban areas. *Landscape Ecology,* **25:** 839-853.

Longland, W. S. , Price, M. V. (1991). Direct observations of owls and heteromyid rodents: can predation risk explain microhabitat use? *Ecology,* **72:** 2261-2273.

Lynch, M. , Ritland, K. (1999). Estimation of pairwise relatedness with molecular markers. *Genetics,* **152:** 1753.

MacArthur, R. H. , Wilson, E. O. (1963). An equilibrium theory of insular zoogeography. *Evolution,* **17:** 373-387.

MacArthur, R. H. , Wilson, E. O. (1967). *The theory of island biogeography*. Princeton University Press.

Macdonald, D. W. , Barrett, P. (1993). *Mammals of Britain & Europe*. Harper Collins Publishers. London.

Macpherson, D., Macpherson, J. L. , Morris, P. (2011). Rural Roads as Barriers to the Movements of Small Mammals. *Applied Ecology and Environmental Research,* **9:** 167-180.

Makova, K. D., Patton, J. C., Krysanov, E. Y., Chesser, R. K.*, et al.* (1998). Microsatellite markers in wood mouse and striped field mouse (genus Apodemus). *Molecular Ecology,* **7:** 247-249.

Malo, J. E. , Mata, C. (2010). Roads and railways split ungulate populations triggering their genetic differentiation. In: *IUFRO Landscape Ecology Working Group International Conference*. Azevedo, J. C., Feliciano, M., Castro, J.*, et al.* (Eds.). Instituto Politécnico de Bragança. Bragança, Portugal.

Malo, J. E., Suarez, F. , Diez, A. (2004). Can we mitigate animal-vehicle accidents using predictive models? *Journal of Applied Ecology,* **41:** 701-710.

Mantyka-Pringle, C. S., Martin, T. G. , Rhodes, J. R. (2012). Interactions between climate and habitat loss effects on biodiversity: a systematic review and meta-analysis. *Global Change Biology,* **18:** 1239–1252.

Marsh, D. M., Milam, G. S., Gorham, N. P. , Beckman, N. G. (2005). Forest Roads as Partial Barriers to Terrestrial Salamander Movement. *Conservation Biology,* **19**.

Marsh, D. M., Page, R. B., Hanlon, T. J., Corritone, R., *et al.* (2008). Effects of roads on patterns of genetic differentiation in red-backed salamanders, *Plethodon cinereus*. *Conservation Genetics,* **9:** 603-613.

Marzluff, J. M., Millspaugh, J. J., Hurvitz, P. , Handcock, M. S. (2004). Relating resources to a probabilistic measure of space use: forest fragments and Steller's jays. *Ecology,* **85:** 1411-1427.

Mata, C., Hervás, I., Herranz, J., Suárez, F., *et al.* (2005). Complementary use by vertebrates of crossing structures along a fenced Spanish motorway. *Biological Conservation,* **124:** 397-405.

Mateus, A. R. A., Grilo, C. , Santos-Reis, M. (2011). Surveying drainage culvert use by carnivores: sampling design and cost-benefit analyzes of track-pads vs. video-surveillance methods. *Environmental monitoring and assessment,* **181:** 101-109.

McCann, N. P., Zollner, P. A. , Gilbert, J. H. (2010). Survival of Adult Martens in Northern Wisconsin. *The Journal of Wildlife Management,* **74:** 1502-1507.

McDonald, W. R. , Clair, C. C. S. (2004). The effects of artificial and natural barriers on the movement of small mammals in Banff National Park, Canada. *Oikos,* **105:** 397-407.

McGarigal, K. , Cushman, S. A. (2002). Comparative evaluation of experimental approaches to the study of habitat fragmentation effects. *Ecological Applications,* **12:** 335-345.

McGregor, R. L., Bender, D. J. , Fahrig, L. (2008). Do small mammals avoid roads because of the traffic? *Journal of Applied Ecology,* **45:** 117-123.

McLane, A. J., Semeniuk, C., McDermid, G. J. , Marceau, D. J. (2011). The role of agent-based models in wildlife ecology and management. *Ecological modelling,* **222:** 1544-1556.

McLaren, A. A. D., Fahrig, L. , Waltho, N. (2011). Movement of small mammals across divided highways with vegetated medians. *Canadian Journal of Zoology,* **89:** 1214-1222.

McShane, T. O., Hirsch, P. D., Trung, T. C., Songorwa, A. N., *et al.* (2011). Hard choices: Making trade-offs between biodiversity conservation and human well-being. *Biological Conservation,* **144:** 966-972.

Meeus, J. H. A. (1993). The transformation of agricultural landscapes in Western Europe. *The Science of the Total Environment,* **129:** 171-190.

Meirmans, P. G. , Hedrick, P. W. (2011). Assessing population structure: FST and related measures. *Molecular Ecology Resources,* **11:** 5-18.

Mendiburu, F. d. (2010). Agricolae: Statistical procedures for agricultural research. R package version v1.0.9. Available at: http://CRAN.R-project.org/package=agricolae. 1 January 2012

Meunier, F. D., Corbin, J., Verheyden, C. , Jouventin, P. (1999). Effects of landscape type and extensive management on use of motorway roadsides by small mammals. *Canadian Journal of Zoology,* **77:** 108-117.

Meunier, F. D., Verheyden, C. , Jouventin, P. (2000). Use of roadsides by diurnal raptors in agricultural landscapes. *Biological Conservation,* **92:** 291-298.

Milchunas, D. G., Lauenroth, W. K. , Burke, I. C. (1998). Livestock grazing: animal and plant biodiversity of shortgrass steppe and the relationship to ecosystem function. *Oikos,* **83:** 65-74.

Miller, C. R. , Waits, L. P. (2003). The history of effective population size and genetic diversity in the Yellowstone grizzly (Ursus arctos): implications for conservation. *Proceedings of the National Academy of Sciences,* **100:** 4334.

Milligan, B. G. (2003). Maximum-likelihood estimation of relatedness. *Genetics,* **163:** 1153.

Millspaugh, J. J., Nielson, R. M., McDonald, L., Marzluff, J. M.*, et al.* (2006). Analysis of resource selection using utilization distributions. *Journal of Wildlife Management,* **70:** 384-395.

Mittermeier, R. A., Myers, N., Thomsen, J. B., da Fonseca, G. A. B.*, et al.* (1998). Biodiversity hotspots and major tropical wilderness areas: Approaches to setting conservation priorities. *Conservation Biology,* **12:** 516-520.

Mohr, C. O. (1947). Table of equivalent populations of North American small mammals. *American midland naturalist,* **37:** 223-249.

Moleón, M. , Gil-Sánchez, J. M. (2003). Food habits of the wildcat (*Felis silvestris*) in a peculiar habitat: the Mediterranean high mountain. *Journal of Zoology,* **260:** 17-22.

Montgomery, S. S. J. , Montgomery, W. I. (1990). Intrapopulation Variation in the Diet of the Wood Mouse *Apodemus sylvaticus*. *Journal of Zoology,* **222:** 641-651.

Montgomery, W. I. (1989a). Population regulation in the wood mouse, *Apodemus sylvaticus* I. Density dependence in the annual cycle of abundance. *The Journal of Animal Ecology,* **58:** 465-475.

Montgomery, W. I. (1989b). Population regulation in the wood mouse, *Apodemus sylvaticus* II. Density dependence in spatial distribution and reproduction. *The Journal of Animal Ecology,* **58:** 477-494.

Montgomery, W. I. (1999). *Apodemus sylvaticus*. In: *The atlas of European mammals*. Mitchell-Jones, A. J., Amori, G., Bogdanowicz, W.*, et al.* (Eds.). Academic Press, London, UK.

Montgomery, W. I., Wilson, W. L., Hamilton, R. , McCartney, P. (1991). Dispersion in the Wood Mouse, *Apodemus sylvaticus*: Variable Resources in Time and Space. *The Journal of Animal Ecology,* **60:** 179-192.

Morales, J. M. , Ellner, S. P. (2002). Scaling up animal movements in heterogeneous landscapes: The importance of behavior. *Ecology,* **83:** 2240-2247.

Moran, M. D. (2003). Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos,* **100:** 403-405.

Moreno, G. , Pulido, F. J. (2008). The Functioning, Management and Persistence of Dehesas. In: *Agroforestry in Europe Current Status and Future Prospects*: 127-160. Rigueiro-Rodróguez, A., Mcadam, J. , Mosquera-Losada, M. R. (Eds.). Springer Netherlands.

Mortelliti, A. , Boitani, L. (2008). Interaction of food resources and landscape structure in determining the probability of patch use by carnivores in fragmented landscapes. *Landscape Ecology,* **23:** 285-298.

Mumme, R. L., Schoech, S. J., Woolfenden, G. E. , Fitzpatrick, J. W. (2000). Life and Death in the Fast Lane: Demographic Consequences of Road Mortality in the Florida Scrub-Jay. *Conservation Biology,* **14**.

Muñoz, A., Bonal, R. , Díaz, M. (2009). Ungulates, rodents, shrubs: interactions in a diverse Mediterranean ecosystem. *Basic and Applied Ecology,* **10:** 151-160.

Murphy, M. A., Evans, J. S., Cushman, S. A. , Storfer, A. (2008). Representing genetic variation as continuous surfaces: an approach for identifying spatial dependency in landscape genetic studies. *Ecography,* **31:** 685-697.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B.*, et al.* (2000). Biodiversity hotspots for conservation priorities. *Nature,* **403:** 853-858.

Nei, M. (1973). Analysis of Gene Diversity in Subdivided Populations. *Proceedings of the National Academy of Sciences of the United States of America,* **70:** 3321-3323.

Nielsen, S. E., Stenhouse, G. B. , Boyce, M. S. (2006). A habitat-based framework for grizzly bear conservation in Alberta. *Biological Conservation,* **130:** 217-229.

Noordijk, J., Delille, K., Schaffers, A. P. , Sýkora, K. V. (2009a). Optimizing grassland management for flower-visiting insects in roadside verges. *Biological Conservation,* **142:** 2097-2103.

Noordijk, J., Raemakers, I. P., Schaffers, A. P. , Sýkora, K. V. (2009b). Arthropod richness in roadside verges in the Netherlands. *Terrestrial Arthropod Reviews,* **2:** 63-76.

Noss, R. F., Quigley, H. B., Hornocker, M. G., Merrill, T.*, et al.* (1996). Conservation biology and carnivore conservation in the Rocky Mountains. *Conservation Biology,* **10:** 949-963.

Nupp, T. E. , Swihart, R. K. (1998). Effects of forest fragmentation on population attributes of white-footed mice and eastern chipmunks. *Journal of Mammalogy,* **79:** 1234-1243.

Nupp, T. E. , Swihart, R. K. (2000). Landscape-level correlates of small-mammal assemblages in forest fragments of farmland. *Journal of Mammalogy,* **81:** 512-526.

Opdam, P. , Wascher, D. (2004). Climate change meets habitat fragmentation: linking landscape and biogeographical scale levels in research and conservation. *Biological Conservation,* **117:** 285-297.

Oxley, D. J., Fenton, M. B. , Carmody, G. R. (1974). The effects of roads on populations of small mammals. *Journal of Applied Ecology,* **11:** 51-59.

Paetkau, D., Calvert, W., Stirling, I. , Strobeck, C. (1995). Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology,* **4:** 347-354.

Paetkau, D., Slade, R., Burden, M. , Estoup, A. (2004). Genetic assignment methods for the direct, real time estimation of migration rate: a simulation based exploration of accuracy and power. *Molecular Ecology,* **13:** 55-65.

Palmer, S. C. F., Coulon, A. , Travis, J. M. J. (2011). Introducing a 'stochastic movement simulator' for estimating habitat connectivity. *Methods in Ecology and Evolution,* **2:** 258-268.

Palsbøll, P. J., Zachariah Peery, M. , Berube, M. (2010). Detecting populations in the 'ambiguous' zone: kinship-based estimation of population structure at low genetic divergence. *Molecular Ecology Resources,* **10:** 797-805.

Patterson, T. A., Thomas, L., Wilcox, C., Ovaskainen, O.*, et al.* (2008). State–space models of individual animal movement. *Trends in Ecology & Evolution,* **23:** 87-94.

Pe'er, G., Henle, K., Dislich, C. , Frank, K. (2011). Breaking Functional Connectivity into Components: A Novel Approach Using an Individual-Based Model, and First Outcomes. *PLoS ONE,* **6:** e22355.

Pereira, H. M. , Daily, G. C. (2006). Modeling biodiversity dynamics in countryside landscapes. *Ecology,* **87:** 1877-1885.

Pereira, H. M., Daily, G. C. , Roughgarden, J. (2004). A framework for assessing the relative vulnerability of species to land-use change. *Ecological Applications,* **14:** 730-742.

Philcox, C. K., Grogan, A. L. , Macdonald, D. W. (1999). Patterns of otter Lutra lutra road mortality in Britain. *Journal of Applied Ecology,* **36:** 748-761.

Pineiro, A. , Barja, I. (2011). Trophic strategy of the wildcat *Felis silvestris* in relation to seasonal variation in the availability and vulnerability to capture of *Apodemus* mice. *Mammalian Biology,* **76:** 302-307.

Pinto-Correia, T. , Mascarenhas, J. (1999). Contribution to the extensification/intensification debate: new trends in the Portuguese montado. *Landscape and Urban Planning,* **46:** 125-131.

Piry, S., Alapetite, A., Cornuet, J. M., Paetkau, D.*, et al.* (2004). GENECLASS2: a software for genetic assignment and first-generation migrant detection. *Journal of Heredity,* **95:** 536-539.

Pritchard, J. K., Stephens, M. , Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics,* **155:** 945.

Pritchard, J. K., Wen, W. , Falush, D. (2003). Documentation for STRUCTURE software: version 2. Available at: http: //pritch.bsd.uchicago.edu.

Proulx, G., Aubry, K., Birks, J., Buskirk, S*., et al.* (2005). World distribution and status of the genus Martes in 2000. In: *Martens and Fishers (Martes) in Human-Altered Environments*: 21-76. Harrison, D. J., Fuller, A. K. , Proulx, G. (Eds.). Springer.New York.

Prugh, L. R. (2009). An evaluation of patch connectivity measures. *Ecological Applications,* **19:** 1300-1310.

Puerto, A., Rico, M., Matias, M. D. , Garcia, J. A. (1990). Variation in structure and diversity in Mediterranean grasslands related to trophic status and grazing intensity. *Journal of Vegetation Science,* **1:** 445-452.

Pulliam, H. R. (1988). Sources, sinks, and population regulation. *American Naturalist,* **132:** 652-661.

Queller, D. C. , Goodnight, K. F. (1989). Estimating relatedness using genetic markers. *Evolution,* **43:** 258-275.

R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Railsback, S. F. , Grimm, V. (2011). *Agent-Based and Individual-Based Modeling: A Practical Introduction*. Princeton University Press.

Railsback, S. F. , Johnson, M. D. (2011). Pattern-oriented modeling of bird foraging and pest control in coffee farms. *Ecological modelling,* **222:** 3305-3319.

Ramp, D. , Ben-Ami, D. (2006). The effect of road-based fatalities on the viability of a peri-urban swamp wallaby population. *Journal of Wildlife Management,* **70:** 1615-1624.

Reed, D. H. (2004). Extinction risk in fragmented habitats. *Animal Conservation,* **7:** 181-191.

Reed, D. H., Nicholas, A. C. , Stratton, G. E. (2007). Genetic quality of individuals impacts population dynamics. *Animal Conservation,* **10:** 275-283.

Reed, D. H., O'Grady, J. J., Brook, B. W., Ballou, J. D*., et al.* (2003). Estimates of minimum viable population sizes for vertebrates and factors influencing those estimates. *Biological Conservation,* **113:** 23-34.

Reino, L., Porto, M., Morgado, R., Moreira, F*., et al.* (2010). Effects of changed grazing regimes and habitat fragmentation on Mediterranean grassland birds. *Agriculture Ecosystems & Environment,* **138:** 27-34.

Revilla, E. , Wiegand, T. (2008). Individual movement behavior, matrix heterogeneity, and the dynamics of spatially structured populations. *Proceedings of the National Academy of Sciences,* **105:** 19120-19125.

Revilla, E., Wiegand, T., Palomares, F., Ferreras, P.*, et al.* (2004). Effects of Matrix Heterogeneity on Animal Dispersal: From Individual Behavior to Metapopulation‐Level Parameters. *The American Naturalist,* **164:** 130-153.

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution,* **43:** 223-225.

Rico, A., Kindlmann, P. , Sedlacek, F. (2007). Barrier effects of roads on movements of small mammals. *Folia Zoologica,* **56:** 1-12.

Riley, S. P. D., Pollinger, J. P., Sauvajot, R. M., York, E. C.*, et al.* (2006). A southern California freeway is a physical and social barrier to gene flow in carnivores. *Molecular Ecology,* **15:** 1733-1741.

Rodríguez, C. , Peris, S. J. (2007). Habitat associations of small mammals in farmed landscapes: implications for agri-environmental schemes. *Animal Biology,* **57:** 301-314.

Roedenbeck, I. A., Fahrig, L., Findlay, C. S., Houlahan, J. E.*, et al.* (2007). The Rauischholzhausen agenda for road ecology. *Ecology and Society,* **12:** 11.

Rollins, L. A., Browning, L. E., Holleley, C. E., Savage, J. L.*, et al.* (2012). Building genetic networks using relatedness information: a novel approach for the estimation of dispersal and characterization of group structure in social animals. *Molecular Ecology,* **21:** 1727-1740.

Rondinini, C. , Boitani, L. (2002). Habitat use by beech martens in a fragmented landscape. *Ecography,* **25:** 257-264.

Rondinini, C. , Doncaster, C. P. (2002). Roads as barriers to movement for hedgehogs. *Functional Ecology***:** 504-509.

Rosalino, L. M., Ferreira, D., Leitão, I. , Santos-Reis, M. (2011). Usage patterns of Mediterranean agro-forest habitat components by wood mice *Apodemus sylvaticus*. *Mammalian Biology,* **76:** 268-273.

Rosário, I. T. , Mathias, M. L. (2007). Annual weight variation and reproductive cycle of the wood mouse (*Apodemus sylvaticus*) in a Mediterranean environment. *Mammalia mamm,* **68:** 133-140.

Rousset, F. (2008). Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources,* **8:** 103-106.

Row, J. R., Blouin-Demers, G. , Weatherhead, P. J. (2007). Demographic effects of road mortality in black ratsnakes (*Elaphe obsoleta*). *Biological Conservation,* **137:** 117-124.

Ruiz-Capillas, P., Mata, C. , Malo, J. E. (*in press*). Road verges are refuges for small mammal populations in extensively managed Mediterranean landscapes. *Biological Conservation*.

Rytwinski, T. , Fahrig, L. (2011). Reproductive rate and body size predict road impacts on mammal abundance. *Ecological Applications,* **21:** 589-600.

Rytwinski, T. , Fahrig, L. (2012). Do species life history traits explain population responses to roads? A meta-analysis. *Biological Conservation,* **147:** 87-98.

Saarinen, K., Valtonen, A., Jantunen, J. , Saarnio, S. (2005). Butterflies and diurnal moths along road verges: Does road type affect diversity and abundance? *Biological Conservation,* **123:** 403-412.

Sabino-Marques, H. , Mira, A. (2011). Living on the verge: are roads a more suitable refuge for small mammals than streams in Mediterranean pastureland? *Ecological Research,* **26:** 277-287.

Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P.*, et al.* (1998). Inbreeding and extinction in a butterfly metapopulation. *Nature,* **392:** 491-494.

Santon-Reis, M. , Correia, A. I. (1999). *A Flora e a Fauna do Montado da Herdade da Ribeira Abaixo (Grândola, Baixo Alentejo)*. Centro de Biologia Ambiental. Lisboa.

Santos-Reis, M., Santos, M., Lourenço, S., Marques, J.*, et al.* (2004). Relationships between Stone Martens, Genets and Cork Oak Woodlands in Portugal. In: *Martens and Fishers (Martes) in Human-Altered Environments. An International Perspective*: 147-172. Harrison, D. J., Fuller, A. K. , Proulx, G. (Eds.). Springer-Verlag.Massachusetts.

Santos, M. J. , Santos-Reis, M. (2010). Stone marten (*Martes foina*) habitat in a Mediterranean ecosystem: effects of scale, sex, and interspecific interactions. *European Journal of Wildlife Research,* **56:** 275-286.

Santos, S. M., Mathias, M., Mira, A. , Simoes, M. P. (2007). Vegetation structure and composition of road verge and meadow sites colonized by Cabrera vole (*Microrus cabrerae* Thomas). *Polish Journal of Ecology,* **55:** 481-493.

Sarmento, P. (1996). Feeding ecology of the European wildcat *Felis silvestris* in Portugal. *Acta theriologica,* **41:** 409-414.

Saunders, D. A., Hobbs, R. J. , Margules, C. R. (1991). Biological Consequences of Ecosystem Fragmentation: A Review. *Conservation Biology,* **5:** 18-32.

Schick, R. S., Loarie, S. R., Colchero, F., Best, B. D.*, et al.* (2008). Understanding movement data and movement processes: current and emerging directions. *Ecology Letters,* **11:** 1338-1350.

Searle, J. B. (1985). Methods for Determining the Sex of Common Shrews (Sorex-Araneus). *Journal of Zoology,* **206:** 279-282.

Seiler, A. (2001). Ecological effects of roads–a review. *Swedish University of Agricultural Sciences, Department of Conservation Biology, Introductory Research Essay,* **9**.

Seiler, A. (2003). *The toll of the automobile: wildlife and roads in Sweden*. Swedish University of Agricultural Sciences. Uppsala, Sweden.

Seiler, A. (2005). Predicting locations of moose-vehicle collisions in Sweden. *Journal of Applied Ecology,* **42:** 371-382.

Serafini, P. , Lovari, S. (1993). Food-Habits and Trophic Niche Overlap of the Red Fox and the Stone Marten in a Mediterranean Rural Area. *Acta theriologica,* **38:** 233-244.

Seshadri, K. S. , Ganesh, T. (2011). Faunal mortality on roads due to religious tourism across time and space in protected areas: A case study from south India. *Forest Ecology and Management,* **262:** 1713-1721.

Shirk, A. J., Cushman, S. A. , Landguth, E. L. (2012). Simulating pattern-process relationships to validate landscape genetic models. *International Journal of Ecology,* **2012:** Article ID 539109, 8 pages.

Silva, M. , Downing, J. A. (1995). The Allometric Scaling of Density and Body-Mass - a Nonlinear Relationship for Terrestrial Mammals. *American Naturalist,* **145:** 704-727.

Spellerberg, I. A. N. (1998). Ecological effects of roads and traffic: a literature review. *Global Ecology and Biogeography,* **7:** 317-333.

Steen, D. A. , Gibbs, J. P. (2004). Effects of roads on the structure of freshwater turtle populations. *Conservation Biology,* **18:** 1143-1148.

Stoate, C., Baldi, A., Beja, P., Boatman, N. D.*, et al.* (2009). Ecological impacts of early 21st century agricultural change in Europe - A review. *Journal of Environmental Management,* **91:** 22-46.

Stoate, C., Boatman, N. D., Borralho, R. J., Carvalho, C.*, et al.* (2001). Ecological impacts of arable intensification in Europe. *Journal of Environmental Management,* **63:** 337-365.

Stoner, D. (1925). The toll of the automobile. *Science,* **61:** 56-57.

Swihart, R. K. , Slade, N. A. (1985). Influence of Sampling Interval on Estimates of Home-Range Size. *Journal of Wildlife Management,* **49:** 1019-1025.

Tanghe, M. , Godefroid, S. (2000). Road verge grasslands in southern Belgium and their conservation value. *Fragmenta Floristica et Geobotanica,* **45:** 147-163.

Tattersall, F. H., Macdonald, D. W., Hart, B. J., Johnson, P.*, et al.* (2002). Is habitat linearity important for small mammal communities on farmland? *Journal of Applied Ecology,* **39:** 643-652.

Taylor, B. D. , Goldingay, R. L. (2010). Roads and wildlife: impacts, mitigation and implications for wildlife management in Australia. *Wildlife Research,* **37:** 320-331.

Taylor, P. D., Fahrig, L., Henein, K. , Merriam, G. (1993). Connectivity is a vital element of landscape structure. *Oikos,* **68:** 571-573.

Tellería, J. L. (2001). Passerine bird communities of Iberian dehesas: a review. *Animal biodiversity and conservation,* **24:** 67-78.

Thorne, J. H., Huber, P. R., Girvetz, E. H., Quinn, J., *et al.* (2009). Integration of Regional Mitigation Assessment and Conservation Planning. *Ecology and Society,* **14:** 47 [online] URL: http://www.ecologyandsociety.org/vol14/iss1/art47/.

Todd, I. A., Tew, T. E. , Macdonald, D. W. (2000). Arable habitat use by wood mice (*Apodemus sylvaticus*). 1. Macrohabitat. *Journal of Zoology,* **250:** 299-303.

Torre, I., Díaz, M., Martínez-Padilla, J., Bonal, R., *et al.* (2007). Cattle grazing, raptor abundance and small mammal communities in Mediterranean grasslands. *Basic and Applied Ecology,* **8:** 565-575.

Tracey, J., Zhu, J. , Crooks, K. (2005). A set of nonlinear regression models for animal movement in response to a single landscape feature. *Journal of agricultural, biological, and environmental statistics,* **10:** 1-18.

Tshiguvho, T. E., Dean, W. R. J. , Robertson, H. G. (1999). Conservation value of road verges in the semi-arid Karoo, South Africa: ants (Hymenoptera*:* Formicidae) as bio-indicators. *Biodiversity and Conservation,* **8:** 1683-1695.

Turner, M. G. (2005). Landscape ecology: What is the state of the science? *Annual Review of Ecology Evolution and Systematics,* **36:** 319-344.

Urban, D. , Keitt, T. (2001). Landscape connectivity: a graph-theoretic perspective. *Ecology,* **82:** 1205-1218.

Van de Zande, L., Van de Vliet, M., Pertoldi, C., Loeschcke, V., *et al.* (2007). Genetic structure within and among regional populations of the Eurasian badger (*Meles meles*) from Denmark and the Netherlands. *Journal of Zoology,* **271:** 302–309.

van der Ree, R., Jaeger, J. A. G., van der Grift, E. A. , Clevenger, A. P. (2011). Effects of Roads and Traffic on Wildlife Populations and Landscape Function: Road Ecology is Moving toward Larger Scales. *Ecology and Society,* **16:** 48 [online] URL: http://www.ecologyandsociety.org/vol16/iss1/art48/.

van der Ree, R., van der Grift, E., Gulle, N., Holland, K., *et al.* (2007). Overcoming the barrier effect of roads–how effective are mitigation strategies? An international review of the use and effectiveness of underpasses and overpasses designed to increase the permeability of roads for wildlife. In: *International Conference on Ecology and Transportation*: 423-433. Irwin, C. L., Nelson, D. , Mcdermott, K. P. (Eds.). Center for Transportation and Environment, North Carolina State University Raleigh, North Carolina, USA.

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M. , Shipley, P. (2004). Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes,* **4:** 535-538.

Vermeulen, H. J. W. , Opdam, P. F. M. (1995). Effectiveness of Roadside Verges as Dispersal Corridors for Small Ground-Dwelling Animals - a Simulation Study. *Landscape and Urban Planning,* **31:** 233-248.

Virgós, E. (2001). Role of isolation and habitat quality in shaping species abundance: a test with badgers (*Meles meles* L.) in a gradient of forest fragmentation. *Journal of Biogeography,* **28:** 381-389.

Virgós, E., Llorente, M. , Cortés, Y. (1999). Geographical variation in genet (*Genetta genetta* L.) diet: a literature review. *Mammal Review,* **29:** 117-126.

Virgós, E., Tellería, J. L. , Santos, T. (2002). A comparison on the response to forest fragmentation by medium-sized Iberian carnivores in central Spain. *Biodiversity and Conservation,* **11:** 1063-1079.

Vos, C. C., Verboom, J., Opdam, P. F. M. , Ter Braak, C. J. F. (2001). Toward ecologically scaled landscape indices. *American Naturalist,* **157:** 24-41.

Wang, J. (2002). An estimator for pairwise relatedness using molecular markers. *Genetics,* **160:** 1203.

Wang, J. (2007). Triadic IBD coefficients and applications to estimating pairwise relatedness. *Genetical Research,* **89:** 135.

Wang, J. (2011). COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Molecular Ecology Resources,* **11:** 141-145.

Watling, J. I., Nowakowski, A. J., Donnelly, M. A. , Orrock, J. L. (2011). Meta-analysis reveals the importance of matrix composition for animals in fragmented habitat. *Global Ecology and Biogeography,* **20:** 209-217.

Watts, C. H. S. (1968). The Foods Eaten by Wood Mice (*Apodemus sylvaticus*) and Bank Voles (*Clethrionomys glareolus*) in Wytham Woods, Berkshire. *Journal of Animal Ecology,* **37:** 25-41.

Way, J. M. (1977). Roadside verges and conservation in Britain: a review. *Biological Conservation,* **12:** 65-74.

Webb, T. J. , Raffaelli, D. (2008). Conversations in conservation: revealing and dealing with language differences in environmental conflicts. *Journal of Applied Ecology,* **45:** 1198-1204.

Weir, B. S. , Cockerham, C. C. (1984). Estimating F-Statistics for the Analysis of Population-Structure. *Evolution,* **38:** 1358-1370.

Westemeier, R. L., Brawn, J. D., Simpson, S. A., Esker, T. L.*, et al.* (1998). Tracking the long-term decline and recovery of an isolated population. *Science,* **282:** 1695-1698.

White, G. C. , Garrott, R. A. (1990). *Analysis of wildlife radiotracking data*. Academic Press, San Diego, California.

Whittingham, M. J. (2007). Will agri-environment schemes deliver substantial biodiversity gain, and if not why not? *Journal of Applied Ecology,* **44:** 1-5.

Wiegand, T., Revilla, E. , Moloney, K. (2005). Effects of Habitat Loss and Fragmentation on Population Dynamics. *Conservation Biology,* **19:** 108-121.

Wilensky, U. (1999). NetLogo: Center for connected learning and computer-based modeling.

Worton, B. J. (1989). Kernel methods for estimating the utilization distribution in home-range studies. *Ecology,* **70:** 164-168.

Wright, S. (1931). Evolution in Mendelian populations. *Genetics,* **16:** 97.

Wright, S. (1943). Isolation by distance. *Genetics,* **28:** 114-138.

Wu, H., Zhan, X. J., Yan, L., Liu, S. Y.*, et al.* (2008). Isolation and characterization of fourteen microsatellite loci for striped field mouse (*Apodemus agrarius*). *Conservation Genetics,* **9:** 1691-1693.

Yanes, M., Velasco, J. M. , Suárez, F. (1995). Permeability of roads and railways to vertebrates: the importance of culverts. *Biological Conservation,* **71:** 217-222.

Zalewski, A. , Jedrzejewski, W. (2006). Spatial organisation and dynamics of the pine marten *Martes martes* population in Bialowieza Forest (E Poland) compared with other European woodlands. *Ecography,* **29:** 31-43.

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