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Ghent University

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# The spatial and community context of dispersal and life history evolution in the spider mite *Tetranychus urticae*

Annelies De Roissart

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**Supervisor:**

Prof. Dr. Dries Bonte - Ghent University, Belgium

**Co-supervisor:**

Dr. Thomas Van Leeuwen - University of Amsterdam, the Netherlands

**Reading committee:**

Prof. Dr. Caroline Nieberding
Université catholique de Louvain, Belgium
Prof. Dr. Frederik Hendrickx
KBIN, Belgium
Ghent University, Belgium
Dr. Emanuel Fronhofer
EAWAG, Switzerland

**Other members of the examination committee:**

Prof. Dr. Luc Lens (chairman) - Ghent University, Belgium
Prof. Dr. Wim Vyverman - Ghent University, Belgium

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# 1. GENERAL INTRODUCTION

Annelies De Roissart

# HETEROGENEITY: CAUSES, TYPESAND CONSEQUENCES

## CAUSES OF HETEROGENEITY

Although some species obtain a continuous distribution in a more or less homogeneous landscape, innumerable others have populations with some kind of spatial structure (Hanski et al. 2004). Human-induced changes of the landscape often lead to the fragmentation or reduction of quality of the habitat and forces many organisms to live in a heterogeneous environment conform to a metapopulation structure (Hanski 2011). In a heterogeneous landscape, differences among and within habitat patches can arise through variable (natural or human-induced) **biotic** (e.g. interactions with inter- and intraspecifics) and **abiotic** (e.g. landscape configuration) characteristics (Strevens 2011).

## TYPES OF HETEROGENEITY

If patches differ in quality, size and connectivity, this will generate **spatial heterogeneity** (Hanski 1994, Fahrig 2007). Additionally, spatial structure can also fluctuate in time and **temporal heterogeneity** can be considered an equally important aspect in spatial dynamical research. Temporal heterogeneity defines the changes in patial structure over time due to periodically stochastic variable conditions (Strevens 2011).

## CONSEQUENCES OF HETEROGENEITY

Spatial as well as temporal variation in habitat structure and/or resources affect both within- and among patch dynamics and result in a range of ecological consequences that have been thoroughly studied. The prevalence of for instance changes in **colonization-extinction dynamics, population regulation, and synchrony**, due to heterogeneity, are well documented (Abbott 2011, Benton et al. 2001, Capucino 1995, Strevens 2011).

### EXTINCTION-COLONISATION

Heterogeneity generates typical disequilibrium conditions in populations leading to high levels of demographic fluctuations. These fluctuations may arise by variation in the number of immigrants (colonization probability) or by variation in extinction probability (Olivieri et al. 1990).

### POPULATION REGULATION - DENSITY

Populations in heterogeneous habitats are expected to be regulated by external density-independent processes (e.g. weather, fire regimes, habitat management) but also by internal density-dependent feedbacks (Turchin 1999). The relation between population growth and density (density-dependence) can be negative as well as positive (Murdoch 1994, Turchin 1999, Sibly et al. 2005, Brook & Bradshaw 2006, Bonenfant et al. 2009). In case of negative density-dependence of population growth, the regulation of population change is attained through the presence of natural enemies or through the impact of inter- and/or intraspecific competition on resource availability (Royama 1992, Dooley 2013) hereby adversely affecting the fecundity of the next generation (Turchin 1999). In the opposite case of positive density-dependence, mechanisms associated with reproduction (e.g. mate-finding ability, reproductive facilitation, sperm limitation and cooperative breeding) or survival (e.g. aggregation, vulnerability to predators, cooperative behavior) can lead to the positive relationship between growth and density (Liebhold & Bascompte 2003, Courchamp et al. 2008, Dooley 2013). Below a threshold population density, the mechanisms leading to positive density-dependence can force Allee effects to occur, and might drive a population to extinction, while above this threshold negative density-dependent processes take over (Drake & Griffin 2010).

Dispersal can also be regarded as a density-dependent mechanism that regulates population dynamics (see further 'Density-dependent dispersal'). Density impacts on dispersal, changing the colonization-extinction balance. Local density-dependent processes have the ability to impact on population dynamics at the metapopulation-level and rescue populations at the brick of extinction (Cappuccino 1995). Most studies assume that the type of influential density-independent factors and density-dependent processes for population regulation are consistent across the range of a species (Rothery et al. 1997). However, some studies have shown that population regulation may differ across space (Dooley 2013).

### SYNCHRONY

Finally , regionalized disturbances can force local populations to fluctuate in synchrony (Moran 1953, Benton et al. 2001), thereby

increasing the spatial stability but reducing the temporal stability of a metapopulation (Wang & Loreau 2014). Next to these regionalized disturbances (the so-called Moran-effect), trophic interactions and dispersal movements have been identified as two other main causes of population synchrony (Benton et al. 2001, Ims & Andreassen 2005). Trophic interactions (e.g. predator-prey or parasite-host) can lead to synchrony if the interacting species differ in mobility (like nomadic predators switching between areas containing prey inducing synchronous fluctuations of prey populations) (Bjornstad et al. 1999, Krebs, 1995, Koenig 1999) while dispersal can cause synchrony by coupling local populations (spatial coupling) allowing a rapid response to changing resource conditions (Amarasekare 2008).

| Type | Cause | Consequence | Main findings | Approach | Reference |
|---|---|---|---|---|---|
| Spatial | Habitat fragmentation | Evolution of dispersal | Depends on immigration and extinction rates | Theoretical | Hanski 2011 |
| Spatial | Habitat fragmentation | Extinction risk, population dynamics | Environmental heterogeneity increases temporal metapopulation variability<br>Population persistence was maximized at intermedi-ate levels of habitat fragmentation | Empirical | Drake & Griffin 2010 |
| Spatial | Resource availability – presence predator | Density | Food addition positive effect on density<br>Predatore exclosure positive effect on density | Empirical | Krebs 1995 |
| Spatial, temporal and spatio-temporal | Resource availability | Population dynamics and dispersal | Different types of heterogeneity have different effects on populatiodynamics and dispersal | Theoretical and empirical | Strevens 2011 |
| Temporal | Resource availability | Synchrony | Environmental synchrony is an important determinate of population synchrony | Empirical | Benton et al. 2001 |

***Table 1.1 – Studies on the causes and consequences of heterogeneity***

## DISPERSAL IN RESPONSE TO HETEROGENEITY

Moving away from a bad quality habitat patch in an attempt to maximize fitness (dispersal) is one way of coping with heterogeneity. Dispersal is known to be a multicausal process with a number of reasons contributing to the complexity of it. A first aspect that adds to the complexity of dispersal lies in the fact that dispersal is driven by the interaction of an individual organism with its environment (Bowler & Benton 2005, Benard & McCauley 2008) (see **condition-dependent dispersal**). A second factor lies in the phenotype-dependence of dispersal, where every individual responds differently to cues about its environment and/or its own state (Bowler & Benton 2005, Clobert et al. 2009) (see **phenotype-dependent dispersal**). Thirdly, dispersal is a spatial process influenced by a variety of factors acting at different spatial scales including both biotic and abiotic components (Clobert et al. 2009) (see **dispersal in a metapopulation context**). Finally, the mechanisms of dispersal may contribute differentially to the three stages of the dispersal process (emigration, transfer and immigration – see further) and may affect long distance and short distance dispersal differently (Ronce 2007).

### THE DEFINITION OF DISPERSAL

Dispersal is defined as the movement of an individual from its natal site to the site of reproduction (natal dispersal) or the movement between successive sites of reproduction irrespective of the distance between them (breeding dispersal). The dispersal process leads to gene flow within and between populations (Ronce 2007; Clobert et al. 2009) and consists of three stages: emigration, transfer and immigration (often referred to as departure, transience and settlement). It is a key process in ecology, evolution and conservation biology that affects population dynamics and persistence, species distribution and abundance, and community structure (Clobert et al. 2009). Since dispersal allows the exploitation of spatially and temporally variable resources, it became essential for the persistence of species in a spatial and/or temporal heterogeneous environment (Ferriere et al. 2000).

The dispersal process is highly variable and can range from a single movement (e.g. a seed falling from a plant) to much more complex processes like sequences of dispersal sessions sometimes even including pre-dispersal behavior as exploration and social interactions (Mathysen 2012). In general, dispersal can be either active or passive. When actively

dispersing, an organism takes control over its own movement. This often includes the utilization of information gathered in their physical and social environment during the three dispersal stages (informed dispersal) (Clobert et al. 2009). The dispersal process is called uninformed when no information is used. Variation in dispersal capacity, motivation or propensity will impact on the active dispersal process (Benard & Mc Cauley 2008). As opposed to active dispersers, passive dispersers are not able to control their own locomotion. The movement they make will depend on external forces like gravity, wind, water, and animals (Mathysen 2012).

## DISPERSAL TO MAXIMIZE FITNESS

The inclusive fitness of an individual can be defined as the sum of its direct reproduction (the number of offspring it produces) and its indirect reproduction (the number of equivalents of its own offspring contributing to the next generation through the support of others) (Hamilton 1964). According to the ideal free distribution theory, individuals should only disperse when they can compensate for the potential costs, and when they expect an increased fitness by leaving the natal habitat (the principle of fitness optimization) (Lin & Batzli 2004, Bonte et al. 2012).

Many studies on dispersal focus on the balance between the benefits and costs of dispersal (Bonte et al. 2012). Costs associated with dispersal can be categorized into risk, opportunity, energetic and time costs and may arise during departure, transfer or settlement or even before the actual dispersal event (Bonte et al. 2012). Many costs of dispersal can arise during earlier life by investing in a special morphology or physiology which leaves fewer resources available for reproduction (e.g. the development of locomotory structures) (Bonte et al. 2011). The dispersal-phase itself also entails risks that are often invoked by moving through unsuitable, hostile habitat, exposed to predators and with a risk of starvation (Bowler & Benton 2005, Gros et al. 2006, Benard & Mc Cauley 2008). Benefits of dispersal generally comprise a reduction of competition with kin or non-kin by settling in low density environments (Bitume et al. 2013).

## PROXIMATE AND ULTIMATE CAUSES OF DISPERSAL

A whole range of factors has the ability to impact on the cost-benefit balance of dispersal and can lead to the evolution of it. The different causes of dispersal are classically divided in proximate and ultimate causes. Proximate causes of dispersal entail the stimuli that elicit the

behavior including population density, sex-ratio, resource availability, interspecific interactions, and habitat quality and structure (see condition-dependent dispersal) (Bowler & Benton 2005, Clobert 2009). Ultimate causes including kin competition (Hamilton & May 1977), habitat stochasticity (Olivieri et al. 1995) and inbreeding avoidance (Gandon 1999), are factors that favor the evolution of dispersal traits. These ultimate factors explain unconditional dispersal strategies and can select for dispersal on an evolutionary timescale, irrespective of the environment (Mc Peek & Holt 1992).

## PHENOTYPE- AND CONDITION-DEPENDENT DISPERSAL

### PHENOTYPES AS THE RESULT OF GENOTYPE BY ENVIRONMENT INTERACTIONS

Typically, individuals from the same population vary in their morphological, physiological or behavioral state (phenotype) due to both genetic and environmental factors (GxE interaction). Recent studies demonstrate that dispersing individuals are no random subset of the population and have a different phenotype compared to philopatric individuals (Cote et al. 2010). The dispersal process is therefore often phenotype-dependent and correlates with a variety of physiological, morphological and other life history characteristics (e.g. body size, sex, developmental stage) rendering differences between dispersing versus philopatric individuals (Clobert et al. 2009). These phenotypic differences may or may not involve consistent suites of traits (e.g dispersal syndromes) (Clobert et al. 2009, Cote et al. 2010). An active metabolism combined with strong wing muscles and an appropriate body shape is an example of a dispersal syndrome in insects that rely on wings to disperse long distances (Zera & Denno 1997). Another example of a dispersal syndrome is the trade-off between dispersal and competitive ability in insects or mammals (Roff & Fairbairn 2001, Ebenhard 1990).

The dispersal phenotype can be either genetically determined or plastic. A genetically determined phenotype is strongly linked to the genotype, as for instance is the case for personalities, while a plastic phenotype occurs due to developmental contexts generating variation in body condition (Bonte and de la Peña 2009) or settling behavior (Bonte et al. 2011). Variation in the E-component of the GxE interaction leads to phenotypic plasticity while the GxE interaction itself indicates that different genotypes can have different reaction norms and that with its genetic basis, plasticity is able to evolve. Allelic variation in the genes coding for dispersal is the outcome of the G-component.

### CONDITION-DEPENDENCE

Dispersal theory has developed from dispersal as a fixed trait with individuals having an equal chance of dispersing to dispersal as a conditional context-dependent trait (Bowler & Benton 2009, Bitume et al. 2011, Travis et al. 1999). In conditional dispersal, the decision to disperse is not taken at random but depends on the habitat and physiological conditions that an organism experiences (proximate factors) (Bowler & Benton 2005, Clobert 2009, Gros et al. 2008). Conditional dispersal enables organisms to prospect actively for more favourable conditions (Mathysen 2012), allows organisms to escape local unfavorable conditions over the short term and causes the movement of individuals to make adaptive sense (Starrfelt & Kokko 2012). Some of the proximate conditions that trigger dispersal are listed below.

**Density and habitat quality**

A broad spectrum of empirical studies indicates an increase of dispersal propensity with density in order to avoid local overpopulation (Li & Margolies 1993, Demeester & Bonte 2010, Bitume et al. 2011). Exploitative (the effect of density on the *per capita* amount of resources) as well as interference (the effect of density itself) competitive interactions can reduce individual fitness under high densities and hereby enforce the dispersal propensity (Bowler & Benton 2005).

Nevertheless some studies found dispersal to be negatively dependent of density (Andreassen & Ims 2001, Mathysen 2005). This trend might occur if an increase in density renders dispersal more difficult or costly. Additionally, if density is positively correlated with habitat quality, mating opportunities or lower settlement costs, occupied habitats can be preferred (Clobert et al. 2009). Negative density-dependence of dispersal may also occur when the benefits of group-living exceed the costs of competition or through the presence of Allee effects (Courchamp et al. 1999, Le Goff et al. 2010). The benefits of living in a group include foraging facilitation and a diluted risk of predation. Allee effects on the other hand cause fitness to decline at low population density due to for example mate-finding difficulties. Above a certain threshold density, density dependence of dispersal should be positive leading to a u-shaped reaction norm (Kim et al. 2009). Additional to the wide variety of empirical studies a range of theoretical studies investigates the effects of density on different aspects of the dispersal process (e.g. emigration rates, population persistence,

and even invasion rates) (Travis et al.1999, Hovestadt & Poethke 2006, Hovestadt et al. 2010).

Since density provides diverse information including the number of potential mates, habitat quality and the level of inter- and intraspecific resource competition (Matthysen 2005), it is closely linked to other proximate cues like for instance habitat quality, interspecific interactions and food availability) triggering dispersal.

**Food availability**

In general food is a limited resource for many animals. As a logical consequence, lowered levels of food availability are reported to induce dispersal (Hanski et al. 2002). Since the amount of resources available for an organism will depend on the food level itself as well as on the number of competitors, effects of food availability are strongly linked with those of density.

**Interspecific interactions**

Additional to competition with conspecifics, interactions with other species or even other guilds can determine the suitability of a habitat patch and the dispersal propensity of an individual (e.g. predator-induced prey dispersal, above-belowground interactions) (Bezemer & van Dam 2005, Bowler & Benton 2005). The presence of predators in particular is known to affect the dispersal decisions and population stability of several prey species (Janssen & Sabelis 1992). Spider mites, for example, have been demonstrated to increase their emigration rate in the presence of predatory mites (Bernstein 1984).

**Sex ratio**

Dispersal propensity can be conditional to the sex of an individual leading to sex-specific dispersal. A bias towards males as well as towards females has empirically been reported (Gros et al. 2008). The avoidance of inbreeding can lead to sex-specific dispersal, since the negative consequences of inbreeding can be avoided if one of the sexes disperses. Asymmetrical competition for resources or asymmetrical costs of dispersal can also explain sex-specific dispersal. The sex suffering the most of competition is most likely to disperse (Clarke et al. 1997, Prugnolle & de Meeus 2002, Lawson et al. 2007). If the mating success varies in space due to differences in the number

of available mates sex-ratio may as well influence dispersal (Bowler & Benton 2005).

**Relatedness**
If interacting individuals possess the ability to recognize kin, a high relatedness with conspecifics can trigger dispersal as a means to avoid inbreeding or kin competition (Ronce et al. 1998, Bitume et al. 2013).

**Patch size & isolation**
Patch size and carrying capacity has been confirmed in numerous empirical and theoretical studies to display a negative correlation with emigration rate as a result of the edge to size ratios (Baguette et al. 2000, Poethke & Hovestadt 2002). Individuals living in smaller patches with a greater edge to size ratio are more likely to encounter the edge which increases the likelihood of leaving the patch (emigration). Next to emigration, patch size can also affect immigration as a result of patch area. The area of a patch determines the probability that a patch will be located by randomly dispersing individuals (Kindvall & Peterson 2000).

Several factors that covary with patch size (e.g. density (Hambäck & Englund 2005), relatedness (Léna et al. 1998)) have been identified. Whether it is the size of the patch per se that impacts on dispersal or the associated factors is difficult to disentangle. Additionally, the isolation of a patch can determine the chance on successful movement due to the accumulating costs of dispersal with distance moved (Bowler & Benton 2005).

**Matrix habitat**
The level of hospitality and resistence to movement of the matrix will determine the emigration propensity and the overall successful movement between patches (transfer-phase of dispersal) (Haynes et al. 2007).

**Habitat cues**
The use of cues to detect suitable surrounding habitat can reduce searching time and increase dispersal success (Bowler & Benton 2005). Habitat cues can also be used during the immigration process.

# THE METAPOPULATION CONCEPT

## GENERAL

In the metapopulation concept as developed by Levins, a metapopulation is defined as an assemblage of spatially delimited local populations linked by some degree of dispersal (Levins 1969). In this concept, metapopulation dynamics are solely driven by the extinction-colonization balance among local populations. Ever since the development of the metapopulation context, the awareness of the significance of spatial structure for population biology grew. Expansion of the theory by Hanski (2004) highlighted the fundamental role of dispersal in metapopulation dynamics and triggered the research on dispersal. Simultaneous consideration of landscape structure as a driving force for population persistence by Fahrig (2007) accentuated the role of spatial structure and provided new insights in conservation biology.

Spatially structured populations can be classified into different classes of 'metapopulations' (Harrison & Taylor 1997) ranging from classical metapopulations to mainland-island and patchy metapopulation systems, depending on the level of connectivity among the different discrete populations and their respective sizes. Classical metapopulations are characterized by a pronounced population turnover among (not necessarily equally sized) patches and by an intermediate occupancy. The omnipresence in nature of classical metapopulations was recently questioned and most spatially structured populations can be classified as patchy or mainland-island metapopulations (Baguette 2004, Fronhofer et al. 2012). Mainland-island systems are characterized by high levels of variation in patch size and asymmetrical connectivity while patchy metapopulations possess high levels of connectivity that prevent patch extinction.

Although it is tempting to classify different spatially structured populations in to different metapopulation types for the ease of use (Harrison & Taylor 1997), the existence of an enormous diversity of landscapes most probably leads to a huge diversity of "metapopulation structures" (Hanski & Gaggiotti 2004). Thinking in terms of metapopulation types with delineated patches can be conceptually useful but should be considered a simplification.

Where metapopulation theory mainly focuses on single species dynamics, the challenge of extension to multiple species lies in

metacommunity theory. In two-species systems or more complex multiple species systems, the presence of other species will impact on the distribution and amount of resources available for the focal species (Amarasekare 2008). In these metacommunities, colonization and extinction rates of the focal species become functions of both habitat characteristics and the presence of other species (Holt & Gilpin 1997), adding an important level of depth and realism.

## DISPERSAL IN A METAPOPULATION CONTEXT

The movement of individuals or propagules among suitable habitat patches is an essential prerequisite of metapopulation dynamics (Clobert et al. 2004) and couples the local populations. Dispersal in a metapopulation context is considered to be condition and phenotype dependent and is found to be a function of a variety of patch and individual features like density, patch size, the distance between patches, sex or age (Hanski 1999, Clobert et al. 2004).

Through the translocation of an individual from one habitat patch to another, dispersal has an impact on individual fitness but also on the dynamics, genetics and the distribution of populations and species (Hanski 1999, Clobert et al. 2001). The persistence of spatially structured populations can be both positively as negatively influenced by the dispersal process. This emphasizes the importance of an explicit description of dispersal in metapopulation systems (Benton et al. 2004).

### THE IMPACT OF DISPERSAL ON POPULATION DYNAMICS

In a metapopulation system, dispersal influences local population dynamics through changes in the extinction and colonization dynamics (Levins 1969). This affects local densities and dynamics (Ives et al. 2004) and impacts on the stability of these dynamics (Hanski 1999, Hovestadt & Poethke 2006). In spatially structured populations, the mechanism of informed dispersal has the potential to generate complex movement patterns that lead towards an ideal free distribution of individuals leveling out the spatiotemporal variation in fitness (Clobert et al. 2009, Delgado et al. 2014).

The relation between dispersal and population dynamics is reciprocal in the sense that dispersal can act as a consequence as well as a cause of population dynamics through its effects on colonization and extinction rates (Clobert et al. 2009). Density-dependence of dispersal is the most evident way of feedback between dispersal and population dynamics (Clobert et al. 2004).

## THE IMPACT OF DISPERSAL ON METAPOPULATION DYNAMICS

The impact of dispersal on dynamics clearly moves beyond the boundaries of local populations. Through the impact on colonization-extinction dynamics, dispersal can have an effect on the metapopulation as a whole. In classical metapopulation systems, dynamics will depend on local extinction, colonization due to dispersal in low-density or extinct patches, and reinforcement in high-density patches (Hanski 1998, Clobert et al. 2009). Only if extinctions are in balance with colonizations due to asynchronous dynamics of local populations, the metapopulation can persist regionally because in this case the variance in metapopulation size and hence the risk of metapopulation extinction is reduced (Hanski 1998).

Another link between dispersal and metapopulation dynamics lies in the mechanism of synchrony. If local populations of a metapopulation are identically density dependent, their abundances can be forced to fluctuate in synchrony by correlated patterns of density-independent effects like for example regionalized disturbances (the so-called Moran-effect) or trophic interactions. Dispersal has however been identified as another main cause of population synchrony. High dispersal rates can increase the synchrony of local population dynamics and therefore elevate the probability of simultaneous extinctions (Heino et al. 1997). However, the extinction risk due to synchrony is further increased without dispersal as extinct patches cannot be recolonized (Hudson & Cattadori 1999). The role of dispersal in eliciting extinction due to synchrony is therefore considered dual. Since the degree to which local population abundances fluctuate in synchrony will determine the chance of global extinction of the metapopulation, dispersal hereby creates a link between local and regional population dynamics. (Benton et al. 2001, Ims & Andreassen 2005).

*Table 1.2 – Studies on the evolution of life history traits other than dispersal in a metapopulation context*

| Trait | Direction of evolution | Approach | Reference |
|---|---|---|---|
| Life span | - | Theoretical | Kirchner & Roy 1999 |
| Age at maturity | - | Theoretical and empirical | deJong et al. 2000 |
| Reproductive effort | + or - | Theoretical | Ronce et al. 2000 |
| | - | Theoretical | Pen 2000 |
| | + or - | Theoretical | Ronce & olivieri 1997 |

## EVOLUTION IN A METAPOPULATION CONTEXT

In 'Habitat, the template for ecological strategies' (Southwood 1977), a species habitat is expressed in terms of variability in space and time. Although, Southwood was the first to suggest that habitat functions as a template that shapes the evolution of life history traits, studies on the evolution of life history in a metapopulation context are still relatively scarce (Table 1.2).

Metapopulations are composed of different populations coupled by dispersal in which spatial positions of individuals and populations influence local and regional dynamics. The variation in spatial and genetic structure of metapopulations generates typical disequilibrium conditions characterised by population extinction and high levels of demographic fluctuations (Olivieri et al. 1990). Metapopulation theory suggests that these non-equilibrium conditions in metapopulations can affect the evolutionary dynamics (de Jong et al. 2000). Empirical work on the evolution of life history traits in a metapopulation context is a relatively unexplored domain in the field of evolutionary biology. Most of the research done has a theoretical background and does not include the extinction-(re)colonisation dynamics typical of metapopulations. Moreover, studies on evolution in a metapopulation context often focus on the evolution of dispersal propensity (Friedenberg 2003, Fjerdingstad et al. 2007) and aging (Ronce & Olivieri 1997, Ronce et al. 2000) neglecting other life history traits (Table 1.2).

### DISPERSAL EVOLUTION

Spatial and/or temporal environmental uncertainty strongly influences the evolution of life histories (including dispersal) of organisms (Wilbur & Rudolf, 2006) of which dispersal evidently is a part. Organisms throughout the world show wide variation in their life history to adapt to this uncertainty.

A large body of mainly theoretical work exists on the evolution of dispersal in a metapopulation context and in the presence of spatial and temporal heterogeneity (Ronce & Olivieri 1997, Ronce et al. 2000). Spatially heterogeneous metapopulations consist of local patches with variable size. In these metapopulations, a minority of individuals that live in the small, low quality patches benefit from dispersal. This should lead to the evolution of a decreased dispersal propensity (Hanski & Gaggiotti 2004). Temporal variability of the habitat in contrary, increases the extinction frequency and should lead to the evolution of an increased dispersal. In these metapopulations, where extinctions occur regularly,

increased dispersal is selected since long-term survival is only possible if genotypes are able to re-colonise patches from where they have become locally extinct. Dispersal offers in these metapopulations a possibility to escape the local certitude of extinction (Friedenberg 2003, Dytham & Travis 2006).

The evolutionarily stable conditional dispersal strategy (in function of density) in a metapopulation context corresponds with a U-shape rather than a linear one (Delgado et al. 2010). Above a certain threshold density dispersal will increase with density and tends to homogenize population density across patches (Poethke & Hovestadt 2002). Below the threshold density, in particular in small populations, relatedness among individuals is high and kin competition may lead to higher dispersal (Gandon & Michalakis 1999). This corresponds with kin-selection theory that predicts an increase in dispersal with increasing relatedness in local patches (Ronce et al. 2000).

Although counterintuitive, the U-shape indicates that when dispersal is increasingly risky in more fragmented metapopulations, it will be strongly selected for (Delgado et al. 2010). The lowered patch occupancy creates conditions in which the cost of dispersal is high due to low survival chance but is compensated as the rare successful dispersers land in a vacant habitat with good breeding prospects (Kokko 2007, Delgado et al. 2010).

### OTHER LIFE-HISTORY TRAITS

The evolution of reproductive effort and life span in metapopulations was investigated by Ronce & Olivieri (1997). Reproductive effort can be defined as the amount of resources allocated to reproduction rather than to growth or maintenance (Williams 1966). A high reproductive effort is typically coupled to a low survival rate and a high fecundity (Ronce et al. 2000). By using a metapopulation model, the results of Ronce & Olivieri (1997) indicate that a higher reproductive effort might evolve if disturbances in a metapopulation are more frequent and colonization opportunities are elevated (Ronce & Olivieri 1997). Additionally, an increase in fragmentation of the metapopulations could shorten life span (Ronce & Olivieri, 1997) and lead to the evolution in the direction of an r-strategy (Ronce & Olivieri, 1997). Several studie have been devoted to the combined evolution of life history traits, linked to each other in life history syndromes. This has been described for several times and several studies have been trying to relate these patterns to characteristics of the habitat (Ronce et al. 2000). An example of a life

history syndrome is the colonizer syndrome or syndrome of the fugitive species. This syndrome occurs in unstable or disturbed habitats and correlates high dispersal, high fecundity and short lifespan (Ronce 2000, Stevens et al. 2012).

### THE MOLECULAR GENETICS OF DISPERSAL EVOLUTION

The molecular genetics of dispersal for species that show continuous variation in dispersal behaviour, are only beginning to be understood (Roff & Fairbairn 2001). Recent work demonstrates that genetic variation linked to individual dispersal propensity scales up to correlate with dispersal differences among demes (Hanski 2011). Therefore, understanding the genomic background of dispersal variation can contribute to general insights in ecological and evolutionary dynamics (Wheat 2012).

The molecular genetic basis of dispersal evolution is mostly studied through the use of candidate genes. A few candidate genes have at this moment been shown to contribute to the genetic basis of dispersal and are involved in foraging behavior, metabolism, and dispersal propensity (Fitzpatrick et al. 2005). The most well studied examples are the *pgi* gene for dispersal and the *for* gene which is linked to foraging behavior (Hanski & Saccheri 2006, Wheat 2012). The *pgi* locus encodes for the glycolitic enzyme PGI and is known to correlate with dispersal rate and flight metabolic rate (Niitepõld et al. 2009) in the Glanville fritillary butterfly (*Melitaea cinxia*) (Hanski et al. 2002).

The *for* locus is another candidate-gene that encodes for cGMP-dependent protein kinase (PKG) and has been shown to influence foraging behavior of both larval and adult *Drosophila melanogaster* individuals (Osborne et al. 1997, Sokolowski 2001). Larvae that display 'roving' behavior (a higher propensity to leave a food patch and longer travel trails while feeding) express more of the PKG enzyme. Recently Edelsparre et al. (2014) uncovered through laboratory and field experiments that allelic variation in the *for* gene also influences the adult dispersal tendencies. Rover flies (higher foraging activity - $for^R$) are more likely to disperse and disperse over longer distances than sitter flies (lower foraging activity – $for^S$). Additionally, an increase of the *for* expression in the brain and nervous system increased the dispersal behavior of the sitter flies (Edelsparre et al. 2014).

## ECO-EVOLUTIONARY DYNAMICS

The fundamental interest in biological diversity is central to both ecological and evolutionary research (Post & Palkovacs 2009). Evolutionary research largely focuses on the processes that generate this diversity while ecological studies concentrate on the maintenance and implications of biological diversity (Post & Palkovacs 2009). The connection between both lies in eco-evolutionary research (Hairston et al. 2005, Kinnison & Hairston 2007).

Three types of interactions between ecological and evolutionary dynamics exist: ecological change that influences evolutionary change, evolutionary change that influences ecological change and reciprocal influences between ecological and evolutionary changes called eco-evolutionary feedbacks (Hanski 2012).

**Impact of ecological change on evolutionary change**

The impact of ecological change on evolutionary change is founded on the basic principle of natural selection where under particular ecological conditions, certain genotypes will have a higher fitness than others and increase in frequency. If the ecological conditions change, an evolutionary change will most likely take place and populations will become locally adapted (Hanski 2012)

**Impact of evolutionary change on ecological change**

This type of interaction between ecology and evolution is concerned with situations where the genotypic composition of a population influences ecological change (Hanski 2012). Historically, evolution is considered to act too slowly and is herefore considered of no importance for ecological processes. However, numerous studies have documented the occurrence of rapid evolution in the past few decades (Thompson 1999, Hendry & Kinnison 1999, Saccheri & Hanski 2006) and demonstrations of the impact of evolutionary change on ecological change, are accumulating. In these interactions evolution has the ability to affect population demography, community structure and ecosystem functioning (Hairston et al. 2005, Bull et al. 2007, Kokko & López-Sepulcre 2007).

**Reciprocal eco-evolutionary dynamics**

Despite the presence of ecological as well as evolutionary studies, the present body of research generally lacks explicit consideration of reciprocal ecological and evolutionary effects. Studies on the reciprocal influences between ecological and evolutionary changes are scarce since they are more challenging to demonstrate than unidirectional changes (Hanski 2012). Eco-evolutionary feedbacks have mainly been theoretically analyzed, while empirical studies are less common (Hairston et al. 2005, Hanski & Saccheri 2006, Ezard et al.2009).

In metapopulations, eco-evolutionary dynamics can prevail since dispersal determines the genetic constitutions of different populations, which affects local growth and dispersal rates. Typical examples include the Glanville fritillary butterfly on the Aland archipelago (Hanski & Mononen 2011) or stick insect metapopulations (Farkas et al. 2013). In the study of Hanski & Mononen (2011), an eco-evolutionary model for dispersal was constructed that aimed to analyze spatial variation in the long-term frequency of fast-dispersing individuals among local populations in a metapopulation with explicit spatial structure. The model was validated with data on spatial variation in the *Pgi*-allele, which is strongly linked to the dispersal rate in the Glanville fritillary butterfly. The results of this study indicate a close coupling between ecological and evolutionary dynamics.

Farkas et al. 2013 investigated how local adaptation (evolution) in the stick insect *Tinema cristinae* structures metapopulations and (multitrophic) communities (ecology). Observations and manipulations in the field indicate that imperfect camouflage (maladaptation) reduces population size comparable to ecological factors like patch size and host-plant identity. Maladaptation reduced population size through an increase in bird predation and ultimately lead to a decrease in herbivory on host plants. This study proves that ongoing evolution can impact on population and community dynamics.

## OBJECTIVES

The goal of this thesis was to gain insight in the link between spatial structure, heterogeneity, population dynamics and the evolution of life history traits including dispersal by using artificial (meta)populations. Understanding dynamics in spatially structured systems is currently considered as one of the major challenges within the field of evolutionary biology, epidemiology and conservation biology. Biotic as well as abiotic interactions may drive spatial as well as temporal heterogeneity and interfere with the ecology and evolution of population dynamics. Acquiring insights in how the eco-evolutionary feedbacks between population dynamics, dispersal, life history evolution and the spatial structure of the landscape affect the distribution of species is essential for population conservation (see Fig.1.1).

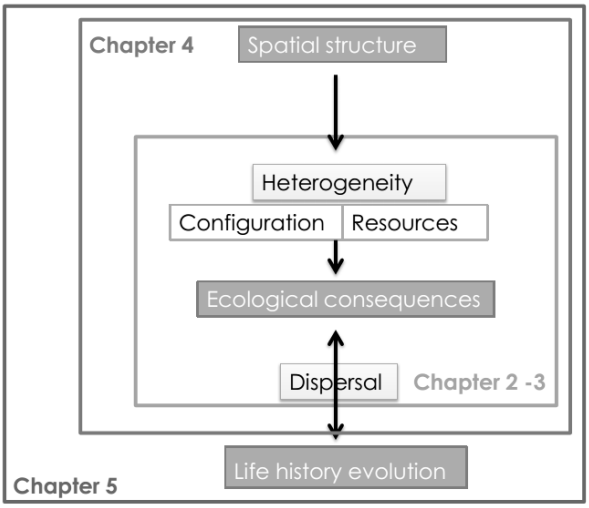



***Fig. 1.1 – Overview of the research chapters.*** *Spatial structure has an impact on the level of heterogeneity that an individual experiences. Heterogeneity can occur through differences in landscape configuration or through differences in the amount of resources (habitat quality). The level of heterogeneity of the habitat has consequences for the ecology of organisms which than impacts on the evolution of life history traits including dispersal.*

## THE MODEL SPECIES

The spider mite *Tetranychus urticae* (Acarina: Tetranichidae) is a phytofagous aboveground herbivore, feeding on plant cell fluids. It is considered a pest species on a wide variety of crops and plants in general (Grbic et al. 2007) because of its high fecundity and short generation time. Spider mite populations are characterized by an explosive growth and have the ability to grow 40% each day. This growth is stopped abruptly when their host is overexploited, forcing the mites to disperse. Mites colonizing new hosts form new strongly growing populations (Krips et.al 1998; Yano & Takafuji 2002). Generally, spider mite populations follow a logistic growth curve. Since they are longer in the exponential growth phase than in the phase where the carrying capacity is approached, *T.urticae* is considered to be a r-selected species (Krips et al. 2008). The combination of rapid population growth, evolutionary potential and easy laboratory maintenance makes *Tetranychus urticae* an ideal organism for eco-evolutionary experiments (Grbic et al. 2007) and is the reason why we chose *Tetranychus urticae* as a model organism in our experiments.

### LIFE HISTORY

The species is characterized by a haplodiploid life cycle with unmated females producing unfertilized eggs that develop into males and mated females producing unfertilized as well as fertilized eggs, the latter developing into females. Mite sex ratio is commonly female-biased ( 3:1-females: males) and is known to evolve in response to local mate competition (Macke et al. 2011). Female mites lay eggs which develop into larvae, protonymphs, deutonymphs and finally into adults. Each of these intermediate lifestages is followed by an immobile quiescent stage. Developmental time (from egg to adult) fluctuates between 10 and 14 days depending on external conditions like temperature, host plant and light regime (Hance & Van Impe 1999). Mites can live for more than 15 days as adults in favorable conditions (Helle & Sabelis 1985).

### DISPERSAL

*T. urticae* has the ability to disperse over short distances by walking but is particularly known for its well-developed long distance dispersal capacity by aerial dispersal (Osakabe et al. 2008). Spider mites have evolved a well-developed dispersal technique of aerial (long distance) dispersal (Osakabe et al., 2008) that is easily quantified under laboratory conditions as it is initiated by a unique pre-dispersal behavior, termed

"*rearing*" (Fig.1.2) (Li and Margolies, 1993a, 1994). Rearing entails orientating away from light sources and stretching of the forelegs (Osakabe et al., 2008). Young inseminated females are in both dispersal modes the dominant dispersers. Previous studies demonstrated a strong underlying genetic component of the aerial dispersal (Li & Margolies 1993a; Li & Margolies 1994) and proximate control in response to host plant senescence, population density and aerial humidity (Li & Margolies 1993b).

## STRAINES USED DURING EXPERIMENTS

Two genetically diverse strains of *Tetranychus urticae* were used in our experiments: LS-VL and BR-VL. The LS-VL strain of *T. urticae* was collected in October 2000 from roses in Ghent, Belgium, where pesticides had not been used for at least 10 years (Van Leeuwen et al. 2008). The BR-VL (bifenazate resistant strain) was selected from LS-VL by successively applying bifenazate at concentrations that killed 90% of individuals (Van Leeuwen et al. 2006). In the laboratory, stock populations of both strains of two-spotted mites were reared on bean plants (*Phaseolus vulgarisL.*), a preferred host plant (Yano et al. 1998). Stock breeding populations were maintained in a climate room at 23°C and a photoperiod of L16:D8.

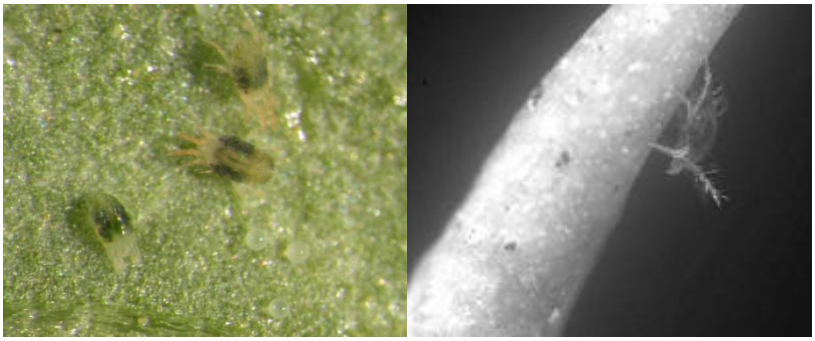


***Fig. 1.2 – Tetranychus urticae.*** *Tetranychus urticae individuals feeding and performing its pre-dispersal behavior (rearing) (Osakabe et al. 2008).*

# Thesis outline

This thesis contains a general introduction, 4 research chapters and a general discussion. In **chapter 2** we focus on the influence of heterogeneity caused by interspecific interactions on the evolution of the dispersal behavior of a phytophagous mite (*Tetranychus urticae*). Plant quality and aboveground herbivore performance are influenced either directly or indirectly by the soil community. Since herbivore dispersal is a conditional strategy relative to plant quality, we examined whether belowground biotic interactions (the presence of root-feeding nematodes or arbuscular mycorrhizal fungi) affect aerial dispersal of a phytophagous mite (*Tetranychus urticae*) through changes in performance of their host plant (*Phaseolus vulgaris*). Aerial dispersal strategies of mites were analyzed in wind-tunnel experiments, in which a unique mite pre-dispersal behavior (rearing) was assessed in relation to the presence of belowground biota for the host plant of the mites.

In **chapter 3**, the fitness-effects of dispersal are empirically assessed. Dispersal is essential for population persistence in transient environments. While costs of dispersal are ubiquitous, individual advantages of dispersal remain poorly understood. Not all individuals from a population disperse, and individual heterogeneity in costs and benefits of dispersal underlie phenotype dependent dispersal strategies. Dispersing phenotypes are always expected to maximize their fitness through adaptive decision making relative to the alternative strategy of remaining residential. While this first principle is well acknowledged in theoretical ecology, empirical verification is extremely difficult due to a plethora of experimental constraints. We studied fitness prospects in a game theoretical context using the two-spotted spider mite *Tetranychus urticae* as a model species.

In **chapter 4** we assessed the influence of heterogeneity caused by spatial structure on dispersal and local and regional population dynamical parameters. Despite a variety of theoretical studies, empirical knowledge on how known variability affects population biology in metapopulation systems is scarce. By manipulating metapopulation structure of experimental metapopulations we studied the impact of metapopulation structure on local and regional demography, extinction and dispersal.

In **chapter 5** we focus on the influence of heterogeneity caused by spatial structure on evolutionary dynamics of *T. urticae* in experimental

metapopulations. The awareness of the importance of spatial structure for demographic and population dynamical processes and its ensuing interaction with evolutionary processes, is slowly, but steadily growing. By manipulating spatial structure in experimental metapopulations of the phytophagous spider mite *Tetranychus urticae*, we assess the influence of spatial structure on the evolution of life history, stoichiometry as well as gene expression.

In the 'general discussion' chapter, the obtained results concerning dispersal, population dynamics and eco-evolutionary feedbacks in spatially structured populations are evaluated. The importance of a comprehensive knowledge of the processes and mechanisms underlying metapopulation dynamics in order to prevent extinction and promote conservation of naturally fragmented landscapes is discussed.

# 2. THE PRESENCE OF ROOT-FEEDING NEMATODES AFFECTS AN HERBIVORE DISPERSAL STRATEGY

∗ Adapted from: De Roissart A., de la Pena E., Van Oyen L., Van Leeuwen T., Ballhorn D. & Bonte D. (2013) The presence of root-feeding nematodes, but not AMF affects an herbivore dispersal strategy. Acta Oecologica, 52, 38-44.

## ABSTRACT

Plant quality and aboveground herbivore performance are influenced either directly or indirectly by the soil community. As herbivore dispersal is a conditional strategy relative to plant quality, we examined whether belowground biotic interactions (the presence of root-feeding nematodes or arbuscular mycorrhizal fungi) affect aerial dispersal of a phytophagous mite (*Tetranychus urticae*) through changes in performance of their host plant (*Phaseolus vulgaris*). Aerial dispersal strategies of mites were analyzed in wind-tunnel experiments, in which a unique mite pre-dispersal behavior (rearing) was assessed in relation to the presence of belowground biota on the host plant on which mites developed. Spider mite pre-dispersal behavior significantly increased with the experienced mite density on the host during development. Additionally, plants infected with root-feeding nematodes induced an increase of spider mite aerial dispersal behavior. The results highlight that belowground herbivores can affect population dynamics of aboveground herbivores by altering dispersal strategies.

# INTRODUCTION

Dispersal, the movement of organisms away from their natal habitat, affects individual fitness, but also population dynamics, population genetics and species distribution (Bowler and Benton, 2005; Clobert et al., 2009). Consequently, dispersal is a key process in ecology as well as in evolutionary and conservation biology (Kokko and Lopez-Sepulcre 2006, Ronce 2007). Dispersal strategies are known to be shaped by selection pressures related to the spatial arrangement of habitat, kin competition and inbreeding avoidance (Bowler and Benton, 2005). Additionally, proximate factors related to, for instance, habitat quality and population density, are known to increase emigration from patches of adverse quality (Clobert et al., 2009; De Meester and Bonte, 2010). Environmental conditions experienced during development, not just those experienced during the dispersal phase, affect the body condition of an animal (Benard and McCauley, 2008) and as such the costs of dispersal (Bonte et al., 2012). Thus, such environmental conditions influence how far or how often individuals move away from their place of birth.

The habitat of small herbivores is mainly determined by the host plant on which they feed and live. Plants are known to respond to herbivore attack with the expression of various defense strategies. These traits range from chemical defenses, for instance alkaloids or herbivore-induced volatiles, to mechanical defenses such as trichomes or though cuticles (Baldwin, 1991; Bezemer and van Dam, 2005; Ballhorn et al., 2013a). Since plants are composed of inter-connected organs, biotic interactions at specific plant regions are expected to induce strong effects on plant quality and the subsequent trophic interactions throughout all regions of the plant (Bezemer and van Dam, 2005; Ohgushi, 2005; van Dam and Heil, 2011). A prominent example of these plant-mediated trophic interactions is the link between the aboveground herbivores and mutualistic or antagonistic biota associated with roots (Wardle et al., 2004; De Deyn and Van der Putten, 2005; Hartley and Gange, 2009; Koricheva et al., 2009; Thamer et al., 2011). Interactions between above- and belowground plant herbivores are known to be complex. Positive, negative and neutral effects in both directions have been demonstrated (Masters, 1995; Tindall and Stout, 2001; Blossey and Hunt-Joshi, 2003; De Deyn et al., 2007). Interactions between plant mutualists, such as arbuscular mycorrhizal fungi (AMF), and aboveground biota result in a positive plant response in most cases (Hoffmann et al., 2009). However, depending on the feeding guild and host specialization of the herbivore, AMF can also have a neutral or negative effect on the

plant (Graham and Abbott, 2000; Reynolds et al., 2006; Sudova and Vosatka, 2008; Hartley and Gange, 2009; Koricheva et al., 2009). Previous studies indicate that belowground-induced changes in host quality can affect population dynamics of foliar herbivores by altering individual growth rates and population sizes of such aboveground herbivores (Awmack and Leather, 2002; van Dam et al., 2003; Hoffmann et al., 2009; Bonte et al., 2010). Changes in plant quality and plant chemistry caused by the induction of direct and indirect defense mechanisms may additionally induce signaling through the production of volatiles and as such impact behavioral aspects of foliar herbivores (Dicke, 2000; Ballhorn et al., 2013b).

The suitability of host plants for herbivores does not only depend on the intrinsic nutritional quality of the plants, but also on the presence and density of con- and heterospecific herbivores (Ohgushi, 2005). When increased local densities of herbivores lead to an enhanced depletion of resources, exploitative competition will be strong (Klomp,1964) and local density may act as a source of information to adjust dispersal strategies (De Meester and Bonte, 2010). Previous studies have shown emigration propensity to increase with density for a variety of taxa (Bowler and Benton, 2005; De Meester and Bonte, 2010), including two-spotted spider mites (Li and Margolies, 1993b) that are the subject of this study.

Two-spotted spider mites (*Tetranychus urticae* Koch; Acari: Tetranychidae) are generalist cell-content sucking herbivores (Helle and Sabelis, 1985) that feed on leaf parenchyma of a wide variety of plant species belonging to many different families. Due to their wide host range, severe feeding damage and rapid population growth these herbivores are a pest to many crops (Yano and Takafuji, 2002; Van Leeuwen et al., 2010). Spider mites have evolved a well-developed dispersal technique of aerial (long distance) dispersal (Osakabe et al., 2008) that is easily quantified under laboratory conditions as it is initiated by a unique pre-dispersal behavior, termed "*rearing*" (Li and Margolies, 1993a, 1994). Rearing entails orientating away from light sources and stretching of the forelegs (Osakabe et al., 2008). Previous studies demonstrated a strong underlying genetic component of this dispersal strategy (Li and Margolies, 1993a; Li and Margolies, 1994 for *Tetranychus*; Jia et al., (2002) for a predatory mite). The controlling proximate factors for changes in dispersal were host plant senescence, population density and aerial humidity (Li and Margolies, 1993b).

In the present study, the impact of belowground biota on the density-dependent aerial dispersal strategies of aboveground spider mites was

examined. We applied two belowground treatments: herbivory by root-feeding nematodes (*Pratylenchus penetrans;* Tylenchida: Pratylenchidae) and the symbiosis with arbuscular mycorrhizal fungi (*Glomus* spp.; Glomerales: Glomaceae) (hereafter referred to as AMF). Dispersal strategies of spider mites were evaluated using wind-tunnel experiments. In a previous studies, in which the prevalence of local adaptation on spider mites was tested (Bonte et al., 2010), belowground nematodes induced water stress in host plants and induced a significant overall decrease in fitness (i.e. growth rate) in spider mites. Considering these results, increased levels of dispersal are expected, and more specifically an increased level of pre-dispersal behavior in these herbivores should be observed when mites are reared on plants infected with belowground living nematodes. A shift in the positive density-dependence of aerial dispersal is anticipated if nematodes induce changes in food quality and lower the overall carrying capacity on plant leaves. Since no effect of AMF on mite fitness was previously observed (Bonte et al. 2010), levels of dispersal in spider mites are not expected to change when reared on plants that have established this belowground symbiosis.

# MATERIAL AND METHODS

## THE MODEL SYSTEM

### PLANT TREATMENTS

We used common bean or snap bean (*Phaseolus vulgaris* L.; Fabales: Fabaceae) as host plant in our experiments. Beans were grown in 5 liter trays (15 x 15 x 35 cm; 15 plants/tray) under greenhouse conditions (25°C; 16:8 LD photoperiod) in commercial standard potting soil (Structural™ Type 0; containing 1.25g/$m^3$ of 14-16-18 N-P-K fertilizer) that was sterilized by autoclaving (120°C, 120 minutes, 1 Atm) as a control treatment. Two experimental treatments were applied, in which plant parasitic nematodes *Pratylenchus penetrans* (Tylenchida: Pratylenchidae) or a mixture of arbuscular mycorrhizal fungi (AMF) (*Glomus* spp.; Glomerales: Glomaceae) were added to the sterilized substrate (Fig.2.1). Around 5000 *Pratylenchus* individuals (commercial inoculum; hzpc research B.V.) were added per plant-tray and allowed to establish a population on the beans for one month. In the AMF treatment, plants were inoculated by watering plant-trays with 500 ml of demineralized water containing 1 g of spore blend of *Glomus spp.* (commercial mycorrhizal inoculums; MycoGrow™) and the symbiosis was allowed to establish for one month (according to the manufactures protocol). This resulted in consistent root colonization of the bean plants by *Glomus spp*.. All trays were watered twice per week with tap water. After one month, plants were transferred to growth chambers for inoculation with mites.

### VALIDATION OF TREATMENTS

Levels of infection by AMF and root nematodes were verified in experimental plants at the end of the experiment. The substrate was removed from the roots by washing with water. Roots of 27 plants per treatment were cut in 1cm fragments and nematodes were subsequently extracted using the Baermann funnel technique over a period of 96h. This resulted in on average 1.31±0.75 nematodes/g of soil and 44.3±12.2 nematodes/g root. For AMF colonization, root samples were processed the same way and fragments were stained following the technique of Vierheilig et al. (1998) after cutting. Root samples were investigated using the technique of Giovannetti & Mosse (1980) using an Olympus microscope. The AMF treatment resulted in an average root infection of (21±7%, n=27). No nematode infections were observed in plants from the sterile and AMF treatments and no hyphae were observed in plants from the sterile and the nematode treatments.

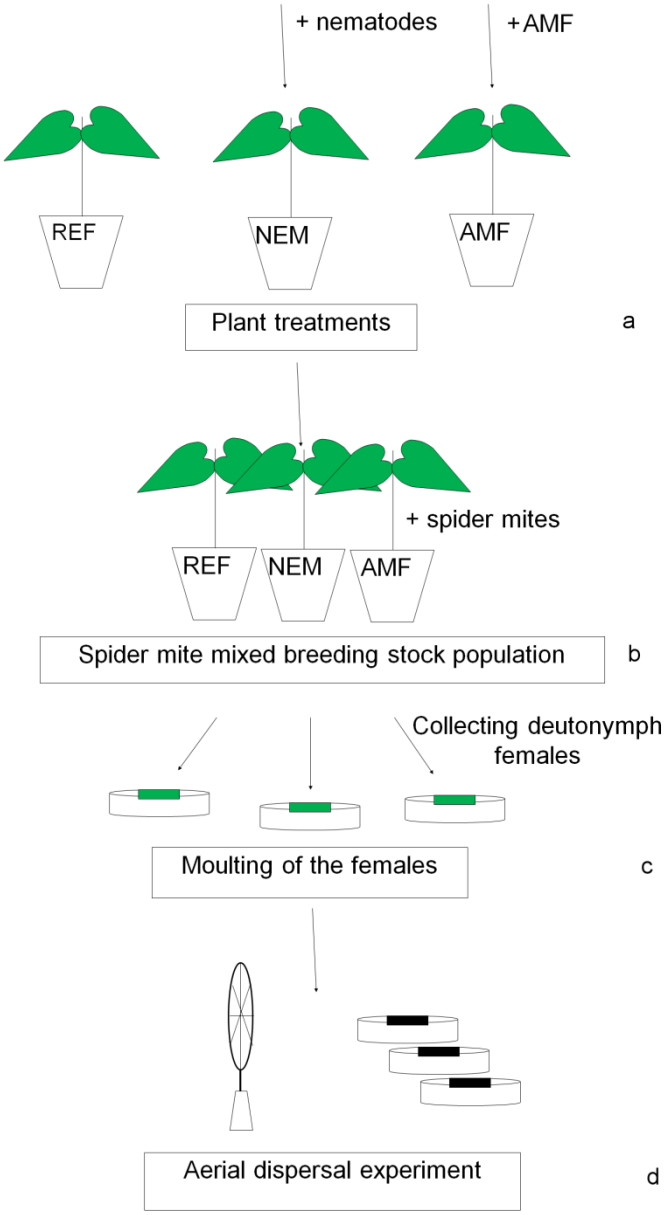



***Fig. 2.1 – Course of the experiments.*** *1a: plant treatment; 1b: set-up of the mite breeding stock population; 1c: moulting of the selected deutonymph females; 1d: aerial dispersal experiment using wind-tunnel.*

## ESTABLISHMENT OF AN EXPERIMENTAL POPULATION OF SPIDER MITES

A genetically diverse population of two-spotted spider mites (Van Leeuwen et al. 2008) that has been kept in stock culture on snap bean (*Phaseolus vulgaris* L.) since 2000 was used to establish an experimental mite population. In order to obtain mites from plants of all treatments, a mix of 30 bean plants, containing 10 plant individuals from each of the three treatments was used for the experimental population (Fig.2.1). Each plant individual was potted separately to avoid mixing of belowground treatments. Every third week, before all plants died due to herbivory, a random subsample of mites was used to inoculate the next mixture of bean plants. Local adaptation towards one of the three treatments was avoided by (i) the diffusive spread of the adult mites over the plants from the inoculation point, (ii) the heterogeneous bean stock with respect to belowground treatment and (iii) random inoculation events and the lack of any preference-performance correlation in relation to the considered plant types (Bonte et al. 2010, unpub. data). Mites for the dispersal experiments were sampled from the different plant types in this heterogeneous stock population.

## HOST PLANT PERFORMANCE

To analyze the impact of nematodes or AMF on plant performance, we used 10 two-week old bean plants from every belowground treatment (of the same growth stage as the plants provided to the mites). Above- and belowground biomass were measured after drying the plants for 40 hr in an air-flow oven at 70°C. For another three bean plants, nitrogen content was analyzed by ISO 5983-2 standards following the Kjelldahl method and measured with a continuous flow analyzer (Foss Fiastar 5000). Phosphorus-content was analyzed by colorimetry (EC L279/15 20.12.71) (A more detailed methodology is provided in the supplementary material S1). Three subsamples from each plant were taken for chemical analyses. Water content of shoots and roots was calculated from the relative difference between fresh and dry weight. Bean plants from the genus *Phaseolus* commonly show a variety of nitrogen- and carbon-based plant defenses. We tested whether cyanogenic precursors were present in plant tissue (Ballhorn et al. 2011), but all plant individuals tested were not cyanogenic (see also Bonte et al. 2010).

## AERIAL DISPERSAL PROTOCOL

### SELECTION OF DISPERSIVE FEMALES

One or two days after mating *Tetranychus urticae* females disperse aerially (Li & Margolies 1994). In order to obtain females of the same age, available quiescent deutonymph (1 day before adult emergence) females were transferred from each plant type of the breeding stock population to a mite-free 1 cm x 1cm bean leaf disc of the same plant type as from which they were collected. Spider mites do not move during development. Therefore the plant of collection resembles the plant of development (Fig.1). Three guarding males per female were added in order to guarantee mating immediately after molting of females to the adult stage. The leaf discs were placed with the abaxial face upwards in Petri dishes (diameter 4 cm) on wet cotton to avoid wilting and prevent mites from escaping. Leaf discs were stored in a growth chamber (16:8 day:night light regime, 60% RH , 25°C). Prior to mite transfer for the aerial dispersal assay, we recorded mite density (average number of individuals per $cm^2$) on the leaf from which the mites were collected. All mites collected from the same leaf were analyzed together and considered as one unit of replication in our statistical analyses.

### AERIAL DISPERSAL ASSAY

To evaluate the effect of our treatments and mite density on rearing dispersal behavior, mated females were transferred to test arenas. These dispersal tests were conducted from October 2008-April 2009 on a total of 1158 mites within a total of 28 test days comprising of more than 180 hours of observation. We applied the same setup as used by Li and Margolies (1993a; 1994). The test arenas consisted of 1 $cm^2$ black painted plastic discs placed on soaked cotton dishes in order to prevent mites from escaping. Depending on the number of available mites, between one and ten individuals from one leaf (from plants with one of the three belowground treatments) were placed on one $cm^2$ disc [no effects of mite densities on the test dishes were observed during test trials ($F_{1,135}$=0.95; $P$=0.3325)]. We applied an upward (30°) wind current of 1.5 m/s (produced by a fan) to the test arenas with and a strong light source (990 lumen) at the source of the air current. As mites perform rearing behavior before entering the air column, we were able to count and select the number of mites performing this behavior (for at least 5 seconds) in the subsequent 3 hours. Mites that showed this behavior were immediately removed in order to avoid pseudo-replication. We simultaneously tested mites derived from plants of all three treatments.

## STATISTICAL ANALYSES

All plant performance parameters were averaged per plant individual and subsequently analyzed using ANOVA with treatment as independent factor. Generalized linear mixed models (GLMM) for binomially distributed data with logit-link and correction for potential overdispersion were used to analyze the proportion of mites displaying rearing behavior. In these logistic regressions, the number of mites that showed the pre-dispersal behavior relative to the total number of tested mites from one leaf was the dependent factor. Independent factors were "total mite density on the leaf of development" (continuous factor) and the "treatment of the host plant". In order to control for possible correlation due to the common date of testing, we included "date" as a random effect. Because of this random error structure, we visualized the modeled regression instead of multiple regression lines for each day when tests were performed. In addition, rearing frequencies for subsamples were plotted when consisting of more than 10 tested mites in order to minimize scatter from small subsamples with low weight in the entire regression analysis. Effective degrees of freedom in all analyses were estimated by Satterthwaite procedure (Verbeke & Molenberghs 2000). All analyses were performed with SAS 9.1 (Proc Glimmix; SAS Institute 2001).

# RESULTS

## HOST PLANT PERFORMANCE

The belowground treatment had a significant effect on the plant biomass allocation and nutritional composition (Table 2.1). Nematodes and AMF had a detrimental effect on total and shoot biomass (Fig. 2a). Nematodes also decreased root biomass in comparison to plants that were grown with AMF and sterile substrate (Fig. 2.2a). Root biomass allocation (i.e. the root/shoot ratio) was higher in AMF plants (0.44 ± 0.08se) than in plants from the nematode (0.14 ± 0.02se) and the sterile-substrate (0.14 ± 0.03se) treatments. N and P concentration were lower in the control than in nematode or AMF treated plants (Fig. 2.2c). Water content for roots averaged 86.23% ± 0.60% se over all treatments (no significant differences among treatments were observed; $F_{2,27}$=2.18; $P$=0.138). Shoot water content was an average of 3% lower in plants treated with nematodes compared to plants from the sterile-substrate treatment (Fig. 2.2b) (Table 1).

## AERIAL DISPERSAL

The probability for an individual female mite to perform the rearing dispersal behavior increased with increasing mite density on the host ($\beta$=0.021±0.007; $F_{1,126}$=8.39; $P$<0.005). This positive density-dependence was independent of the treatment (interaction density x treatment $F_{3,123}$=1.21; $P$=0.310), but overall rearing probabilities depended on the host treatment ($F_{2,126}$=5.53; $P$=0.005; see Fig. 2.3). Mites that developed on plants inoculated with nematodes showed a higher rearing probability compared to mites from the sterile treatment (Fig. 2.4; *Tukey's* test: $t_{126}$=3.27; $P$=0.004). Treatment with AMF had no effect on the mite rearing dispersal behavior (differences in the two other pair-wise combinations were not significant; Tukey's test: $t_{126}$<1.81; $P$>0.05).

***Table 2.1 –*** *Results of ANOVA of the measured plant biomass and plant quality variables in relation to the belowground treatment*

| Dependent variable | Num df | Den df | F | P |
|---|---|---|---|---|
| Total biomass (g) | 2 | 27 | 12.44 | <0.0001 |
| Aboveground biomass (g) | 2 | 27 | 13.69 | <0.0001 |
| Belowground biomass (g) | 2 | 27 | 8.86 | <0.0001 |
| Root/shoot ratio | 2 | 27 | 11.26 | <0.0001 |
| Root water content (%) | 2 | 27 | 2.18 | 0.138 |
| Shoot water content (%) | 2 | 27 | 42.95 | <0.0001 |
| N-content (% dry weight) | 2 | 6 | 7.09 | 0.026 |
| P-content (% dry weight) | 2 | 6 | 17.81 | 0.003 |

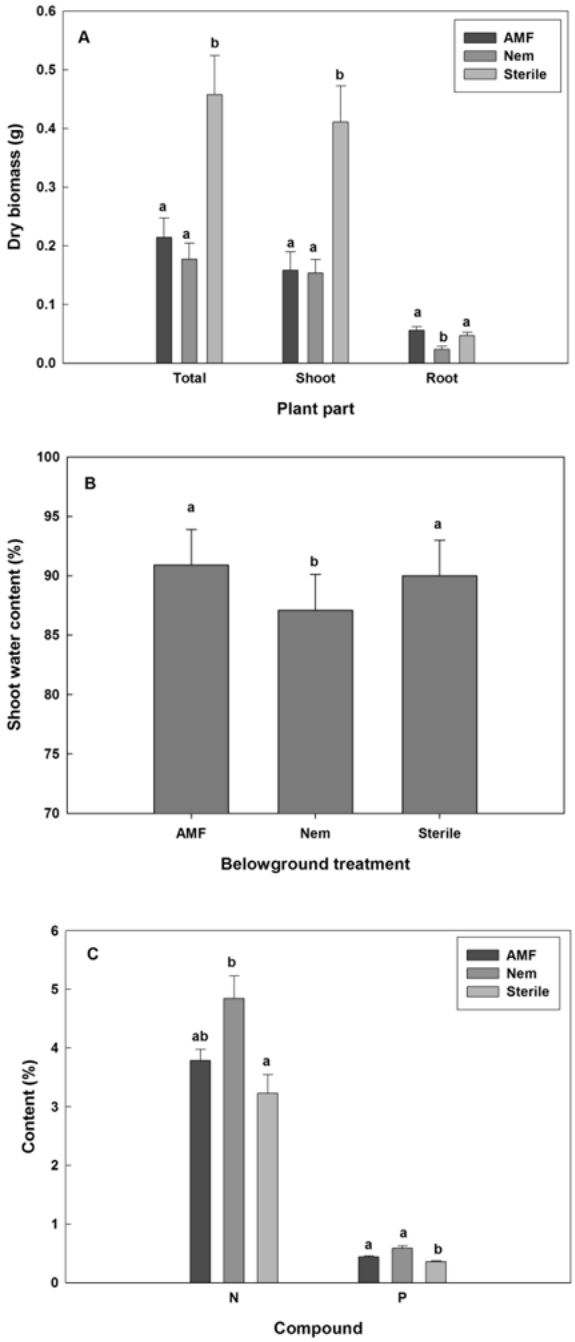



***Fig. 2.2 – Effects of the belowground biotic treatment on plant performance.*** *2a: plant dry biomass; 2b: shoot water content; 2c: N and P-content. Equal notations indicate non-significant contrast for the respective plant performance measurements. Error bars represent standard errors.*

***Fig. 2.3 – Effects of density and soil treatments on the aerial dispersal behavior.*** *Proportions of mites performing pre-aerial dispersal behavior in relation to its experienced density on the host and the host belowground treatment (AMF: green, NEMatode: red or STERile: black). Notice that frequencies are plotted in the graph as dots (independent of the total number of tested individuals) but that individual behavior (whether pre-dispersal behavior occurred or not) was modeled as a binomial variable. We omitted data points with less than ten tested mites according to density from the figure to enhance visibility of the observed pattern. Lines indicate predictions derived from the binomial mixed model.*

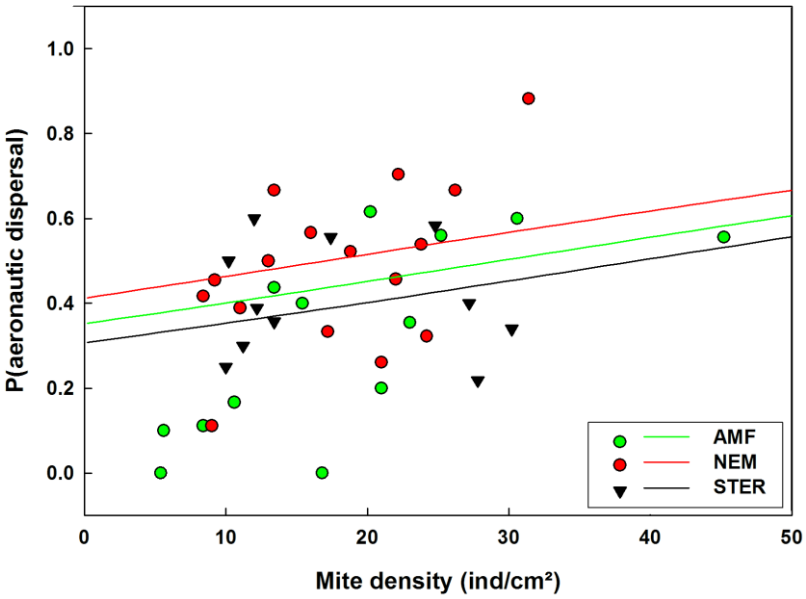
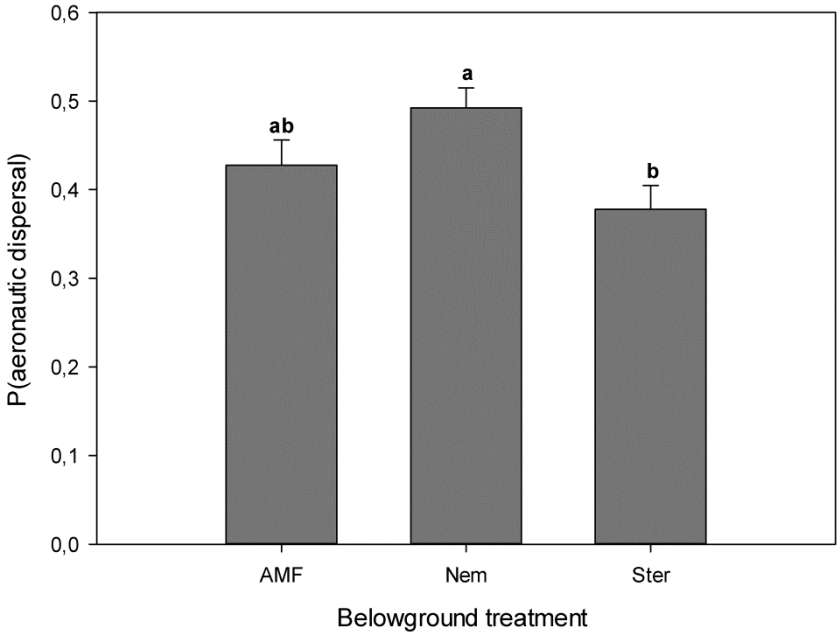


***Fig. 2.4 – Overall effects of soil treatments on the aerial dispersal behavior.*** *Proportions of mites performing pre-aerial dispersal behavior in relation to the host belowground treatment for the overall average density of 19.35 ± 0.66 SE mites. Equal notations indicate non-significant contrast. Error bars represent standard errors.*



# DISCUSSION

It is generally acknowledged that a change in the belowground community structure can affect plant performance and population growth of aboveground herbivores (Wardle et al. 2004; De Deyn & Van der Putten 2005; Kaplan et al. 2008; Hoffmann & Schausberger 2012), with cascading effects on higher trophic levels (Van der Putten et al. 2001). Prevailing mechanisms behind these plant-mediated multitrophic interactions are diverse, but these mechanisms are always mediated through changes in host plant quality and/or the up-regulation of plant defense strategies (Masters & Brown 1997; Van der Putten et al. 2001). The complexity of these interactions is, however, enormous and depends on variation in abiotic conditions at both temporal and spatial scales (Wardle et al. 2004; Vandegehuchte et al. 2010; Thamer et al. 2011). The impact of belowground biota on oviposition and feeding behavior of foliar herbivores has been demonstrated by Anderson et al. (2011). Here, we document the impact of belowground biota on the dispersal behavior of aboveground herbivores for the first time.

Rearing rates for aerial dispersal were positively associated with the mite population density on the source leaf. This corresponds to previous findings of positive density-dependent strategies in vertebrates (Matthysen 2005) and invertebrates (De Meester & Bonte 2010; Bitume et al. 2013) and stresses the need to consider the intraspecific competition as a proximate driver of individual dispersal (Clobert et al. 2009). In the case of passive dispersal strategies, costs of dispersal are very high (Bonte et al. 2012).However, if habitat quality decreases, costs of philopatry will exceed those of dispersal and an induction of high dispersal rates is expected (Travis et al. 1999; Hovestadt et al. 2001; Kun & Scheuring 2006).

No detectable effect of AMF on mite fitness (Bonte et al. 2010) and dispersal were observed despite a significant decrease of biomass in plants treated with AMF. The observed neutral to negative effect of AMF on plant performance might be due to the use of young 2-week old bean plants (Johnson et al. 1997). In the first weeks following germination, plants obtain their necessary resources from seed reserves. In this stage, the loss of carbon to the fungus decreases allocation to photosynthesis or defense and AMF can become parasitic (Johnson et al. 1997). Because dispersal evolves as an alternative behavioral strategy to philopatry, both strategies should have equal fitness expectations (Clobert et al. 2009; Bonte et al. 2012). As such, while some plant quality parameters were affected by this treatment, they appear not to impact the mite's future

fitness (Bonte et al. 2010) and therefore do not have a strong impact on the aerial dispersal strategy.

In nematode infected plants, dispersal propensity was anticipated to increase under high mite population densities due to a decrease of plant quality or an increased production of defensive compounds, thereby lowering the mite carrying capacity. Aerial dispersal was, however, higher in mites that developed on nematode-treated hosts. Increased dispersal of spider mites therefore appears to be caused by their development on plants with belowground nematode herbivory. This belowground interaction resulted in decreased water content and/or the production of unidentified defensive metabolites. Because no change in the density threshold has been observed, increased dispersal rates are regarded as a response to future fitness costs when staying on a host of subordinate quality (Bonte et al. 2010) due to, for instance, the production of secondary defense metabolites (van Dam et al. 2005). In our experiment, no declines in nutritional plant tissue quality were detected (Bonte et al. 2010) nor detectable levels of cyanogenic potential in nematode treated plants (Ballhorn et al. 2007). Total nitrogen-content is known to be a poor predictor of nutritional plant quality (Awmack & Leather 2002; Schoonhoven et al. 2005). The absence of cyanogenic glycosides does not rule out the prevalence of hitherto unidentified nitrogen-containing defensive metabolites (e.g. alkaloids or tannins) which in some cases reduce digestive efficiency of arthropod herbivores and may have caused the increased dispersal response.

Shoot water content was systematically lower in plants treated with nematodes. A decrease in shoot water content due to root herbivory has previously been described as mechanism negatively affecting aboveground herbivore presence and performance (Erb et al. 2009, 2011). Since plant structural and biochemical parameters changed in multiple and non-correlated ways after treatment with belowground biota, we were not able to assign one exact plant trait to be the driving force for the observed change in dispersal potential, and likely, the response is due to multiple, mutually interacting changes in plant quality.

In conclusion, our study demonstrates that plant growth is negatively affected by AMF and nematode infection of the roots. Since only nematode herbivory affected the overall level of aerial dispersal, without a shift in density dependence, we attribute specific changes in plant quality like water turgor, but potentially also other factors, as the proximate cause of the increased aerial dispersal rates. Because such effects were not found for AMF, our results demonstrate that at least

some specific belowground biotic interactions in the plant rhizosphere affect dispersal of aboveground herbivores. Soil biotic interactions may consequently affect the dispersal strategies of aboveground herbivores and their eventual spatial population dynamics (Sacket et al. 2010).

## ACKNOWLEDGEMENTS

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# SUPPLEMENTARY MATERIAL S1

## THE BELOWGROUND PLAYERS: GENERAL INFORMATION

*Pratylenchus penetrans* is an economically important migratory endoparasitic nematode with a wide host range. It enters the root cortex using a stylet, subsequently dissolving the connections between cortical cell walls by enzymes produced in the salivary glands (Zunke 1990, van Dam et al. 2005). *Pratylenchus* was inoculated (5000 nematodes/tray) and allowed to establish a population on the beans for one month. After this period, bean plants were carefully transferred to the growth chambers for inoculation with mites. AMF treatment was performed according to the manufactures protocol (with modifications). Plants were inoculated by watering plant-trays with 500 ml of demineralized water containing 1g of spore blend of *Glomus* spp. (*Glomus intraradices, Glomus mosseae, Glomus aggregatum* and *Glomus etunicatum;* commercial mycorrhizal inoculum, MycoGrow™). This resulted in consistent root colonization of the bean plants by *Glomus spp*.

## METHODOLOGY PHOSPHORUS AND NITROGEN ANALYSES

Nitrogen and phosphorus content were measured after drying the plants for 40 hr in an air-flow oven at 70°C. Nitrogen content was extracted according to ISO 5983-2 standards following the Kjelldahl method and measured with a continuous flow analyser (Foss Fiastar 5000).

Total phosphorus content was determined by emission spectrometry. Acccording to EC L279/15 20.12.71 standards 20 ml of sulphuric acid was added to 1 g of each plant sample. The mixture was shaken, heated and kept at boiling temperature for 10 minutes. 2 ml of nitric acid was added, gently heated and left to cool slightly. A little more nitric acid was added and the mixture was brought back to boiling point until a colorless solution was obtained. The solution was cooled and water was added. The liquid was decanted into a 500 ml flask, made up to volume with water, homogenized and filtered. An aliquot part of the filtrate was diluted to obtain a phosphorus concentration of not more than 40 µg/ml. 10 ml of this solution was placed in a test tube and 10 ml of molybdovanadate reagent was added. The mixture was homogenized and kept for 10 minutes at 20°C. Optical density was measured in a spectrophotometer at 430 nm against a solution obtained by adding 10 ml of the molybdovanadate reagent to 10 ml of water. From the standard solution we prepared solutions containing respectively 5, 10, 20,

30 and 40 µg of phosphorus per ml. Optical densities of all the solutions were plotted to trace the calibration curve.

## SUPPLEMENTARY TABLE S1

**Table S1:** Average densities of mites (number of individuals/$cm^2$) on the different hosts

| Treatment | Average density | SE |
|---|---|---|
| **REF** | 18,21 | 1,15 |
| **AMF** | 16,44 | 1,22 |
| **NEM** | 15,67 | 1,04 |

# 3. FITNESS MAXIMISATION BY DISPERSAL: EVIDENCE FROM AN INVASION EXPERIMENT

∗ Adapted from: Bonte D. °, De Roissart A. °, Wybouw, N. & Van Leeuwen T. (2014) Fitness maximization by dispersal: evidence from an invasion experiment. Ecology, in press.

°: joint first authorship

## ABSTRACT:

Dispersal is essential for population persistence in transient environments. While costs of dispersal are ubiquitous, individual advantages of dispersal remain poorly understood. Not all individuals from a population disperse, and individual heterogeneity in costs and benefits of dispersal underlie phenotype dependent dispersal strategies. Dispersing phenotypes are always expected to maximize their fitness by adaptive decision making relative to the alternative strategy of remaining philopatric. While this first principle is well acknowledged in theoretical ecology, empirical verification is extremely difficult due to a plethora of experimental constraints. We studied fitness prospects of dispersal in a game theoretical context using the two-spotted spider mite *Tetranychus urticae* as a model species. We demonstrate that dispersing phenotypes represent those individuals able to maximize their fitness in a novel, less populated environment reached after dispersal. In contrast to philopatric phenotypes, successful dispersers performed better in a low density post-dispersal context, but worse in a high density philopatric context. They increased fitness with about 450% relative to the strategy of remaining philopatric. The optimization of phenotype-dependent dispersal thus maximizes fitness.

## INTRODUCTION

Any active or passive attempt to move from a site of birth to another breeding site is referred to as dispersal (Clobert et al. 2009) and recognized as a key life history trait to minimize kin competition, crowding and any consequence of deteriorating habitat quality (Bowler and Benton 2005). As such, it allows organisms to survive increasing rates of habitat fragmentation and climate change (Kokko and Lopez-Sepulcre 2006, Berg et al. 2010) but selective advantages to dispersing individuals remain poorly understood (Ronce 2007). Understanding heterogeneity in dispersal is currently crucial given the consideration of new conservation strategies (Thomas 2011b, Thomas 2011a) that aim to translocate individuals from natal ranges into potential suitable habitat that cannot (anymore) be reached by natural dispersal. These strategies are likely to fail if the transferred individuals are not optimally adapted to the new context (Travis et al. 2013).

Advantages of dispersal generally comprise a reduction of competition with kin or non-kin by settling in low density environments (Bitume et al. 2013). There is currently a strong consensus that dispersal encompasses a three-stage process, including the effective departure, transfer and settlement (Bowler and Benton 2005). Dispersal decisions at each of these stages are taken in a conditional way, i.e. according to costs levied during each of these stages relative to the benefits of the entire dispersal strategy (Clobert et al. 2009). Typical costs associated with the transfer phase are energetic or risk costs directly affecting energy reserves or survival, but costs can be equally levied during settlement. Examples include the loss of advantages from being locally adapted, the loss of prior residence advantages like a familiar environment or losing group-living benefits (Bonte et al. 2012).

Typically, individuals from the same population vary in their morphological, physiological or behavioral state due to both genetic and environmental factors. The phenotype can be fixed when linked to the genotype, as for instance is the case for personalities, or plastic due to developmental contexts generating variation in body condition (Bonte and de la Peña 2009) or settling behavior (Bonte et al. 2011). In the Glanville fritillary butterfly, dispersing phenotypes are associated with a certain *pgi*-genotype and have a higher metabolic rate and a larger clutch size. However, these individuals experience trade-offs with longevity under stressful conditions, while more philopatric individuals live longer. As a result, the advantages of having a dispersal phenotype may

only hold under non-stressful conditions, for instance when population densities are low (Bonte and Saastamoinen 2012).

Independent of the mechanisms generating phenotypic variation, philopatric and dispersing phenotypes are expected to maximize fitness under the local environmental conditions that they experience (Clobert et al. 2009). Philopatric phenotypes do not invest in dispersal because their fitness benefits are expected to be marginal relative to the costs, while benefits are expected to be much higher in those individual phenotypes that leave their natal environment. This might be especially true for competitive subordinate (Bonte et al. 2011) or asocial individuals (Cote et al. 2010a, Cote et al. 2010b), rendering dispersal risk-taking an advantageous strategy. Because of dispersal costs and because individuals differ phenotypically, the fitness of dispersing individuals is not expected to be identical to those being philopatric, but always higher relative to the alternative strategy of staying home (Forero et al. 2002, Ronce 2007).

Earlier comparative studies demonstrated fitness correlates of dispersal, often interpreted as costs, but none have been able to demonstrate the principle of fitness maximization by dispersal (Bonte et al. 2012). While well established in dispersal theory (Ronce 2007), its empirical verification is extremely difficult. First, measures of fitness should be inclusive and also take into account offspring performance because changes in population structure may have a fitness impact over several generations (Delgado et al. 2011). Second, differences in lifetime reproductive success (LRS) between philopatric and dispersive individuals may be due to physiological constraints, rather than fitness costs, for instance when only individuals in the best condition are able to disperse long distances, or when those in subordinate condition are forced to leave (Bonte et al. 2012). As such, differences in LRS between philopatric and dispersive phenotypes do not necessarily represent dispersal costs, but may reflect phenotype dependent dispersal (Belichon et al. 1996). Third, dispersal is known to have a heritable component leading to individual consistency and parent–offspring resemblance, which raises statistical problems due to non-independence of data (Doligez and Part 2008); and finally, researchers may fail to detect (long distance) dispersing phenotypes in open populations (Belichon et al. 1996). Experimental approaches might overcome this problem by constraining emigration or translocating philopatric individuals (Johnson and Gaines 1987, Hahne et al. 2011), and should focus on understanding the state-dependence of dispersal to separate fitness differences due to body condition from dispersal costs.

Dispersing individuals may for instance perform equally as philopatric ones overall but nevertheless attain higher fitness than had they remained philopatric.

One promising avenue in evolutionary research is using mutants or transgenics to detect mechanisms driving fitness consequences of competing evolutionary strategies (Kawecki et al. 2012). When such mutants are easily detectable and do not differ in life history relative to the wild strain, they provide an excellent system to monitor the invasion success of different behavioral phenotypes in a novel context. We used a pesticide sensitive strain of the two-spotted spider mite *Tetranychus urticae* of which a mutant has been selected that exhibits strong maternally inherited pesticide resistance (Van Leeuwen et al. 2006). This strong resistant phenotype was shown to be caused by mutations in cytochrome *b*, a mitochondrially encoded protein in the respiratory pathway (Van Leeuwen et al. 2008). Despite the unusual nature of these mutations, these resistant mites do not differ in life history traits relative to those from the baseline strain from which they have been selected (Van Leeuwen et al. 2008). In *Tetranychus urticae*, density and relatedness act as proximate drivers of dispersal distance (Bitume et al. 2013), and dispersal distance heritability is strongly influenced by the prevailing densities (Bitume et al. 2011). In a range-expansion situation, the distribution of individuals after dispersal is typically right skewed with more individuals settling at smaller distances, and deviates significantly from a homogenous distribution, i.e. the ideal free distribution (Fretwell and Lucas 1970, Fretwell 1972, Krivan et al. 2008). This suggests already the presence of substantial dispersal costs (Bonte et al. 2012). It remains however challenging to understand which individuals eventually decide to disperse and to incur these costs. By means of a translocation experiment, we assessed the invasion success of dispersive and philopatric resistant phenotypes in terms of the number of offspring reaching maturity in both a philopatric and dispersive context by applying a pesticide treatment. This allowed us to directly assess genotypic success, so measuring fitness within the ecological context experienced by descendants. Because philopatric and dispersive phenotypes can be differently affected by dispersal costs (Belichon et al. 1996; Clobert et al. 2009), we simultaneously frustrated dispersers by translocating them to a philopatric context they left, and enforced philopatric phenotypes into a context they would have experienced had they dispersed. As such, we were able to keep the social context of the translocated individuals similar as under non-manipulated conditions.

# MATERIAL AND METHODS:

## STUDY SPECIES

The spider mite *Tetranychus urticae*, is a haplodiploid polyphagous herbivore feeding on plant cell fluids. Because of its high fecundity and short generation time, the species causes serious damage to crops and plants in general. The species is known to engage in aerial dispersal under suitable meteorological conditions when environmental conditions are strongly deteriorating (Li and Margolies 1994, Clotuche et al. 2011, Clotuche et al. 2013, De Roissart et al. 2013). The species does, however, predominantly disperse by ambulatory movements, i.e. by walking from leaf to leaf. In all cases, young inseminated female mites are the dominant dispersing life stage. Mites typically disperse when densities and relatedness increase (Bitume et al. 2013) and settle on uninfected leaves where densities are low. As such, the population spread follows a typical diffusive pattern leaving deteriorated plants behind the moving front. Dispersive mites can incur transfer costs because they do not feed during movement on stem tissues and because they leave the protective silk environment in the natal patch, thereby increasing vulnerability to predation and dehydration. Similar costs can be expected during settlement under low densities and may induce Allee effects (Le Goff et al. 2010). The simulated dynamics in our experiment represent this natural dispersal process, although potential mortality due to dehydration and predation is avoided since predators are absent and aerial humidity is always high in the experimental chambers.

Two strains of *T. urticae* with a common genetic background were used during the experiments. The original bifenazate susceptible strain (LS-VL) of *T. urticae* was collected in October 2000 from roses near Ghent, Belgium, where pesticides had not been used for at least 10 years. The BR-VL bifenazate resistant strain was selected from LS-VL by successively applying bifenazate at concentrations that killed 90% of individuals (Van Leeuwen et al. 2008). Both strains were kept in the laboratory on potted bean plants *Phaseolus vulgaris* L. cv 'Prelude' under controlled conditions (16:8 L:D photoperiod, 25 °C). Van Leeuwen et al. (2008) demonstrated that bifenazate resistance is maternally inherited and highly correlated with mutations in cytochrome b, a mitochondrially encoded protein in the respiratory pathway. Resistant mites show no statistical differences in the basic life history traits (fecundity, longevity, time to maturity and sex-ratio) and subsequent fitness costs in the absence of pesticide (Van Leeuwen et al. 2008).

## EXPERIMENTAL SETUP

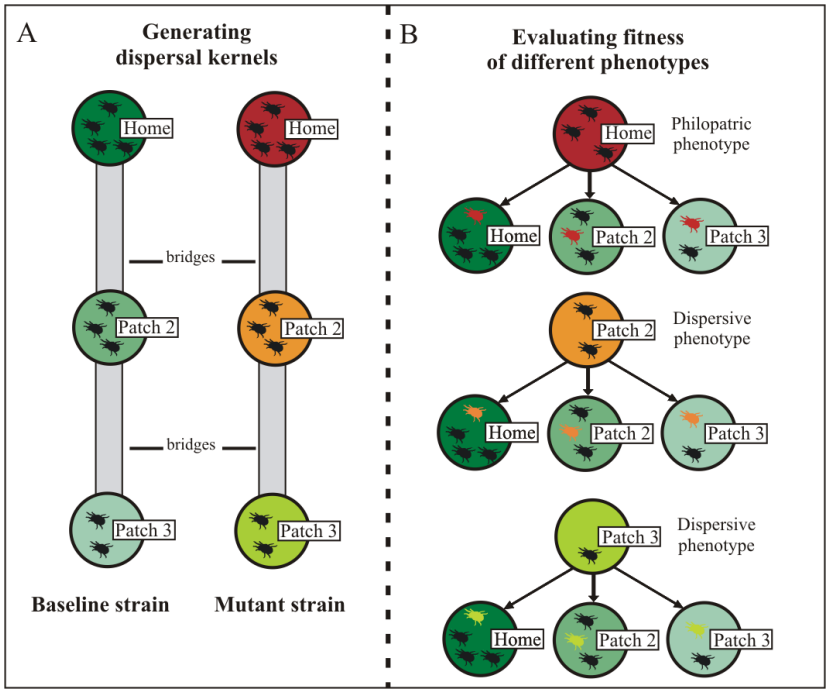
### MAIN EXPERIMENT

Fifty females of the wild susceptible type (LS-VL strain) and mutant resistant line (BR-VL strain) were collected and allowed to lay eggs for 48 hours on a fresh bean leaf (7 cm x 7 cm) to synchronize their offspring. After hatching and juvenile development, 50 one-to-two-day old mated females were chosen at random to start the dispersal procedure. These young *T. urticae* females are the dominant dispersers (Bitume et al. 2011). The dispersal procedure was initiated by translocating the females to a bean leaf square that subsequently served as the source population for dispersal (1 cm x 1 cm, in order to generate sufficiently high densities that induce emigration; see Bitume et al. (2013). This leaf fragment was connected linearly to two consecutive leaves by Parafilm bridges (8 cm x 2 cm), forming a line of 3 patches. The first patch is defined as the home patch, patches 2 and 3 are the patches reached after dispersal. Wet cotton beneath the leaves kept them fresh and prevented mites from escaping. Females were allowed to disperse for 48 hrs. Each dispersal trial consisted of one series of mites (1 x 50 mites) from the mutant (BR-VL) strain and 3 series of the wild type (LS-VL) strain (3 x 50 mites). We replicated this experiment, consisting of four series, 30 times. After removal of the bridges, we assessed local population sizes by counting the number of adult females on each patch (leaf).

Philopatric and dispersive mites from the resistant strain were then used as experimental subjects, assessing their reproductive performance when translocated to different patch types established by the susceptible strain (Fig. 3.1). For example, three resistant mites were randomly chosen from those that had dispersed to patch 3. One of these mites was then added to a population of susceptible mites in each of the three patch types, each time removing a susceptible mite so as to maintain the original population size and phenotypic context. Mites were allowed to reproduce for 14 days (until hatching of the next generation) at 25 °C and 16:8 L:D photoperiod. After 14 days all discs were sprayed with 0.8mL (1000 mg/ L bifenazate) of commercially formulated bifenazate (Floramite, 240SC) which killed all mites of the LS-VL strain. After 24 hrs, the number of surviving and dead mites in each patch was counted. In order to validate the treatment efficacy, we performed a genetic screening of more than 200 randomly selected surviving and dying mites after pesticide treatment. We followed the procedure of Van Leeuwen et al. (2008). In short, we sequenced amplified and purified MtDNA from individual mites using two internal primers: cytbWTF, 5'-

CGGAATAATTTTACAAATAACTCATGC; cytbWTR, 5'-TGGTACAGATCGTAGAATTGCG. Allsurviving mites bore the expected mutation and were descendants from the introduced resistant female, and dying mites were wild type and descendants from the susceptible baseline population.

***Fig. 3.1 – Schematic representation of the experimental setup of the invasion experiment.*** *(A) mites from the susceptible baseline strain and the acaracide resistant mutant strain disperse in a similar way and generate variation in density among the three connected patches. After 48 hours, the connecting bridges are removed and one susceptible mite from each of the three patches is replaced by a mutant resistant philopatric (from patch 1) or dispersive phenotype from patches 2 or 3 (B). By allowing reproduction, the mutant can invade the baseline population, and its invasion success as measured by the number of progeny in the next generation is assessed by applying an acaracide treatment. The experimental procedure was replicated such that 30 replicates per context-phenotype combination were obtained.*



## EXPERIMENTAL CONTROL

Since translocation experiments might affect the behavior of animals (Heidinger et al. 2009), we followed dispersal dynamics and subsequent population growth for the two strains under identical experimental conditions but without any translocation. The ratio of population size at 14 days to the number of founding females denotes the per capita reproductive output of a particular phenotype in a particular patch type. This average fitness measure was used as a control for the (density dependent) individual reproductive output of the resistant female introduced in a pesticide susceptible population, and allows an additional test of fitness differences between the two strains. By applying a pesticide treatment, we validated its effectiveness in relation to different population densities.

## DATA ANALYSIS

The probability distribution of spider mites among the three patch types was modeled by ordered multinomial logistic regression (see Bitume et al. 2013). The cumulative distribution of individuals across patches in the set up was modelled in relation to the strain, i.e. susceptible or resistant. We corrected for interdependence of the data within each experimental setup by including replicate as a random effect, and for potential interdependence among a set of simultaneously conducted experiments due to for instance common climatological conditions and similarity in host plant quality by adding day of the experimental assay as a second random component.

Generalized linear mixed models for Poisson distributed data with log-link and correction for potential overdispersion (Verbeke and Molenberghs 2000) were used to analyze the number of descendants within the different patches after 14 days (hereafter referred to as fitness). Independent factors were the patch occupied after possible dispersal (this then refers to the dispersal phenotype) and the patch or density to which the female was translocated (the dispersal context). We controlled in our analysis for non-independence generated by performing tests at the same time periods with the same source of mites by modelling random intercepts and slopes for each replicate to correct for respectively variation in average densities and distance-effects among the used replicates. Similar analyses were performed for the per capita reproductive output relative to the patch of settlement or density in the control experiments. All analyses were done in SAS 9.2 with the GLIMMIX procedure. This procedure fits generalized linear mixed models

by likelihood-based techniques conditional on normally distributed random effects. The GLIMMIX procedure allows data that can have any distribution in the exponential family and provides backtransformed least squares means estimates (not possible for multinomial regressions). Tukey Kramer posthoc tests were implemented to correct for multiple comparisons in the pair-wise contrasts.

## RESULTS

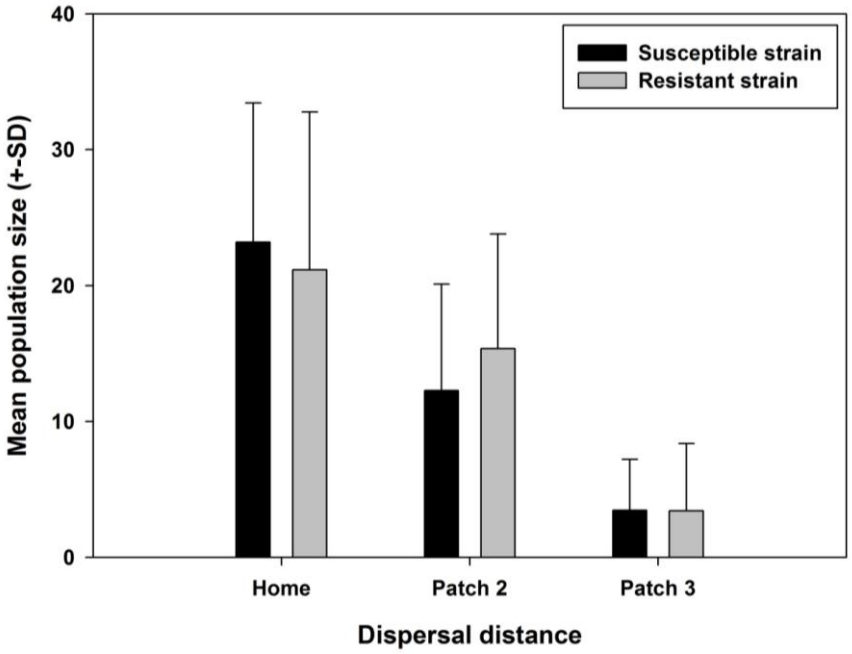
After 48 hours on average 21.7 individuals remained philopatric in the home patch (patch 1), 14.5 individuals dispersed to patch 2 and 3.5 individuals dispersed to patch 2 (Fig.2). The distribution of the mites did not differ between the strains ($F_{1,104}$=0.62; $P$=0.432), so evolved resistance did not influence the dispersal distance distribution.

When the fitness estimates were contrasted between the control experiments in which mites from the two strains were not translocated and the manipulated series in which a resistant individual has been introduced in its similar patch from baseline population, only patch-effects on fitness were found ($F_{2,57}$=11.27, $P$<0.0001). No effects of the used strain ($F_{2,57}$=1.02, $P$=0.3667) nor the interaction between the strain and the patch of dispersal were found ($F_{4,57}$=0.43, $P$=0.783). So, fitness differed only according to the distance moved and was not different between the resistant and baseline strain and neither affected by the experimental manipulation. Similar effects were found in relation to density (log(density) effect: $F_{1,59}$=30.20, P<0.0001; slope: -5.0595 ± 1.36 se; Strain effect: $F_{2,59}$=0.45, $P$=0.637; interaction: $F_{2,59}$=0.43, $P$=0.743). Density dependent recruitment was thus neither different among the two strain and the implemented translocations. The pesticide treatment was 100% effective in both the susceptible and resistant strains with respectively 100% mortality and survival.

Fitness after translocation did not differ according to the distance originally dispersed by the mites (original patch: F2,21=0.68; P=0.519). The main effect of patch of translocation was additionally significant (F2,20=4.61; P=0.020), but more importantly, fitness was affected by the interaction between the patch to which a mite originally dispersed and the patch of translocation (F4,27=6.92; P=0.0006). This significant interaction indicates that individual phenotypes dispersing different distances showed different fitness reaction norms according to the patch to which they were transferred. Indeed, while the number of descendants remained similar for philopatric phenotypes after translocation to any patch (all t-values <|1.2|, P>0.05 after multiple comparisons), prominent fitness differences were observed for individuals that dispersed to the second or third patch, especially for those that dispersed themselves to the furthest patch (Fig 3). The decline in fitness after translocation to a home patch was statistically significant for individuals that originally moved to the most distance patch (Patch 3 phenotypes: t=-3.86; adjusted P=0.01), and marginally significant for those that originally dispersed to the second patch (t=-3.20; adjusted

P=0.071). So, while philopatric phenotypes did not improve fitness when translocated into patches typically colonized by dispersive individuals (patches 2 and 3), dispersive phenotypes experience a statistically significant cost of 78.50% when remaining philopatric. Turned around, dispersive phenotypes increased their fitness by 458%.

***Fig. 3.2 – Density distribution of mites from the susceptible and resistant strain after allowing dispersal.*** *Densities declined significantly with increasing distance. Means and standard deviations are given.*



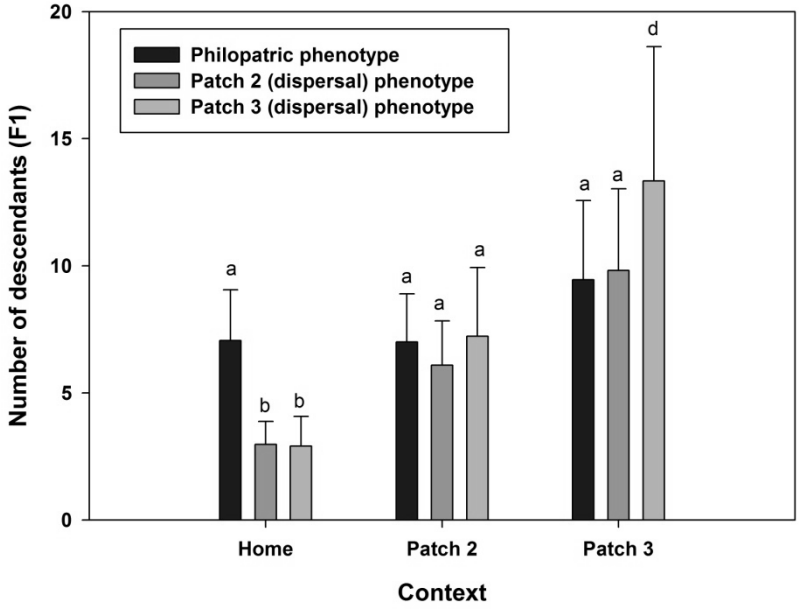



***Fig. 3.3 – Fitness measured as the number of descendants in the next generation (F1) of the translocated mites in relation to their dispersed distance (phenotype) and context of assisted immigration.*** *Fitness of the dispersive phenotypes was maximized when transferred to a low density context reached after dispersal. Fitness of philopatric phenotypes was independent of the density context. Means and standard deviations are given. Different letter symbols indicate statistically significant differences. Except for the difference of patch 2 phenotypes among the first and second patch (P=0.07), all P<0.05 after correction for multiple comparisons among all combinations.*

# DISCUSSION

Dispersal is a complex trait consisting of distinct behavioral phases related to departure, transfer and settlement, and will only be selected for when benefits of leaving the natal environment outweigh the costs (Clobert et al. 2009). We demonstrated that philopatric individuals reach equal fitness under different density conditions met when translocated different dispersal distances. Dispersive phenotypes, however, increased fitness by a factor of 4-5 by moving to new patches relative to the alternative strategy of remaining philopatric.

We thus demonstrate that the optimization of dispersal maximizes fitness to such an extent that substantial additional dispersal costs can be incurred. Individuals that leave the environment in which they were born do so in order to maximize their fitness in the novel environment relative to the natal environment.

While this principle of fitness maximization, being the first principle in evolutionary game theory (Nowak and Sigmund 2004), is well established in dispersal theory (Gyllenberg and Metz 2001, Poethke and Hovestadt 2002, Wild 2011), its empirical validation in nature is extremely difficult. Analyses of longitudinal data on vertebrates offer rare opportunities to measure fitness correlates of dispersal (Nevoux et al. 2013; Waser et al. 2013). Such correlative approaches, however, provide no causal evidence. Alternative experimental approaches by constraining emigration or transferring individuals may additionally fail to make inference of costs because such manipulations alter the population structure in both the natal and emigration environment and do not take into account multigenerational measures of fitness (Johnson and Gaines 1987, Hahne et al. 2011). Here, we demonstrated by a translocation experiment, using mutant mites that did not differ in life history and dispersal from their ancestor genotypes, that dispersing individuals are not a random subsample from the populations. Rather, dispersing phenotypes benefited most from released competition at distant patches and would have experienced tremendous fitness costs had they remained philopatric.

The translocation experiment was set up to quantify fitness as the invasion success of one mutant that replaced one native wild-type individual. By applying such an approach we kept the population (phenotypic) context of the environment similar for the focal individual as would be expected under natural conditions. However, while the mutant genotypes are selected from the susceptible base population, they can

be expected to show higher levels of relatedness due to the implemented artificial selection. Such differences in relatedness may affect social interactions and eventually impact dispersal (Bitume et al. 2013) or reproductive behavior (Roeder et al. 1996, Saito 2000). Our control experiment does not indicate any difference in basic reproductive success or dispersal between the two strains, and no impact of the experimental manipulations. We are consequently confident that the observed fitness differences among the dispersal phenotypes were not due to unintended effects of the experimental manipulation.

Because different dispersal phenotypes might experience diverse dispersal costs due to for instance, variation in morphology or physiology, care needs to be taken when interpreting the outcome of translocation experiments. Apparent absence of transfer costs might for instance be caused by adaptations and trade-offs in dispersive phenotypes to reduce costs of movement (Bonte et al. 2012). In our experiment, mites' fitness on average did not differ according to the travelled distance (no difference between individuals moving to the second or third patch). As a result, costs of transfer under natural conditions are unlikely to be related to energetic expenditure but rather to incurred risks. While we performed experiments in an artificial environment free of potential enemies, about 20% of the adult individuals died during the transfer phase by leaving the bridges and becoming drowned. These risk costs in the experiment were of the same magnitude as benefits of dispersal in the philopatric phenotypes. If these costs are of the same order under natural conditions, they likely explain the balanced costs-benefits for dispersive individuals. More-over, since dispersive phenotypes increased fitness in our experiments by more than 400%, additional costs in more natural systems can be incurred. Under natural conditions, where mites disperse from leaf to leaf, generating patterns of diffusive spread at the population level, additional costs of moving short distances might for instance be induced by systemic responses, where local herbivory induces chemical defenses at other plant locations (Schmidt et al. 2009, Sarmento et al. 2011) or attract predators (Dicke et al. 1993, Van Den Boom et al. 2004, Ament et al. 2010). However, the absence of transfer costs in our study might also be caused by the experimental sytem that consisted of merely three patches. A potential improvement of the experiment includes multiple patches, increasing the travel distance of dispersive mites. This might unravel the presence of currently hidden distance dependent costs.

While the translocation experiment allows inference of costs related to the integration into a novel context in both philopatric and dispersive phenotypes, it does not allow a full inference of the costs associated with transfer. Dispersive phenotypes cannot be forced to remain philopatric while philopatric phenotypes cannot be forced to move actively to a new location (dispersal behavior determines after all the phenotype). This problem is inevitable in translocation studies like this and mainly relevant when dispersal depends on different morphs or a size (which is not the case in our study system). However, in order to estimate the magnitude of transfer costs in the dispersive phenotypes, we used a three-patch system in which potential energetic costs of displacement can be quantified.

Fitness of dispersing phenotypes in a philopatric context was about half that of philopatric phenotypes while philopatric phenotypes performed equally well in all contexts. As a result, only dispersing phenotypes were negatively impacted by elevated levels of intraspecific competition met under natal conditions. Under high densities, especially subordinate individuals are expected to experience increased competitive interactions during foraging, either directly through behavioral interference or indirectly due to an increased rate of resource depletion, necessitating an increased investment into foraging movements. Such interactions lead on average to energy loss that cannot be invested in reproduction (Pyke et al. 1977) and to movement from high density to low density environments (Bitume et al. 2013). High densities are therefore expected to induce dispersal responses in those individuals that suffer most from increased intraspecific competition.

While this intraspecific competition likely drives the phenotype dependent dispersal in our experiment, it is not unlikely that the effect is strengthened by kin competition. A tight kin structure is expected under natural conditions where populations are founded by few inseminated females, and in our experiment where individuals were collected from a lab culture. In environments where dispersal costs are high and environments are spatiotemporally stable, kin competition is the major driver of dispersal (Bonte and de la Peña 2009). Subordinate competitors can then be the dispersing phenotype, depending on the competition dynamics and metapopulation structure (Gyllenberg et al. 2008, Bonte and de la Pena 2009, Gyllenberg et al. 2011, Kisdi et al. 2012). These individuals then leave the population thereby incurring dispersal costs in order to reduce competition. Interestingly, instead of reaching equal or lower levels of fitness relative to philopatric phenotypes transferred to a

low density context, dispersive individuals benefit even more from released competition. So, inferior competitors in natal environments can be superior ones in novel environments reached after dispersal. We here explicitly acknowledge that in our study, phenotypic variation is likely not related to genotypic polymorphisms but more to variation in body condition due to differentiation in resource acquisition during development. However, the retrieved pattern strongly resembles mechanisms of personality driven dispersal strategies with, in our case, subordinates having a lower reproductive capacity, being the more asocial phenotype (Cote et al. 2010a), thereby maximally benefiting from settlement in low density populations or groups.

Our invasion-approach forces the integration of different phenotypes in either a similar or different context from which they originate. The dynamics as simulated in our experimental setup reflect patterns of diffusional spread as encountered during range expansions (Kubisch et al. 2014) or pest outbreaks (Kareiva 1983). It remains to be tested whether insights from this experiment can be generalized to organisms inhabiting more saturated environments where reinforced colonization is the rule. For instance in mammals and birds (Murray 1967, Matthysen 2005), dispersal benefits are less related to the release of intraspecific competition, but merely by avoidance of kin competition, inbreeding avoidance or risk spreading.

In conclusion, we demonstrate that dispersing phenotypes represent those individuals able to maximize their fitness in a novel, less populated environment reached after dispersal. In contrast to philopatric phenotypes, successful dispersive *Tetranychus urticae* performed better in a low density post-dispersal context, but worse in a high density philopatric context. They increased fitness with about 400% relative to the strategy of remaining philopatric. We thus provide the first empirical evidence that the optimization of phenotype-dependent dispersal maximizes individual fitness.

## ACKNOWLEDGEMENTS

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# 4. IMPACT OF METAPOPULATION STRUCTURE ON POPULATION DYNAMICS AND DENSITY DEPENDENT DEMOGRAPHY

*Adapted from: De Roissart A., Wang S. & Bonte D. (2014) Impact of metapopulation structure on population dynamics and density dependent demography. Major revision in Journal of Animal Ecology.

## ABSTRACT

The spatial and temporal variation in the availability of suitable habitat within metapopulations determines colonization-extinction events, regulates local population sizes and eventually affects local population and metapopulation stability. Insights into the impact of such a spatiotemporal variation on the local population and metapopulation dynamics are principally derived from classical metapopulation theory and have not been experimentally validated.

By manipulating spatial structure in artificial metapopulations of the spider mite *Tetranychus urticae*, we test to which degree spatial (island-mainland metapopulations) and spatiotemporal variation (classical metapopulations) in habitat availability affect the dynamics of the metapopulations relative to systems where habitat is constantly available in time and space (patchy metapopulations).

Our experiment demonstrates that (i) spatial variation in habitat availability decreases variance in metapopulation size, decreases density dependent dispersal at the metapopulation level, while (ii) spatiotemporal variation in habitat availability increases patch extinction rates, decreases local population and metapopulation sizes, and decreases density dependence in population growth rates. We found dispersal to be negatively density dependent and overall low in the spatial variable mainland-island metapopulation.

This demographic variation subsequently impacts local and regional population dynamics and determines patterns of metapopulation stability. Both local and metapopulation-level variability is minimised in mainland-island metapopulations relative to classical and patchy ones.

## INTRODUCTION

The development of the metapopulation concept by Levins (1969) resulted in an increasing awareness of the importance of spatial habitat configuration for colonization-extinction dynamics in fragmented landscapes. This subsequently triggered research on dispersal because of its central role in metapopulation dynamics (Hanski 2004) and simultaneously opened a new perspective in conservation biology through the consideration of landscape structure as a major driving force for the persistence of populations (e.g., Fahrig 2007). While the field has moved from a patch occupancy perspective to approaches that integrate population dynamics (Benton et al. 2001, 2004, Strevens & Bonsall 2011), most of our insights herein are derived from theory (e.g., Fronhofer et al. 2012). Establishing a thorough understanding of the impact of spatial and spatiotemporal variation in habitat availability in an empirical study-system is therefore a central challenge in ecology and conservation biology (Benton et al. 2002).

Populations in spatially structured habitats are expected to be regulated by external factors such as spatiotemporal variation in habitat/and or resource availability (Benton et al. 2001, Altwegg et al. 2014) but also by internal density dependent feedbacks (Turchin 1999, Benton et al. 2001). Density-dependent processes at the local level may impact population dynamics at the metapopulation-level by rescuing local populations at the brick of extinction or impacting the synchrony of the population fluctuation (Capucino 1995). In a constant environment, the population regulation by density dependent population growth can lead to a stable equilibrium in population size. However, because many populations are subject to continuous disturbance, this equilibrium is unlikely to be reached in natural populations (Friedenberg 2003, Cameron & Benton 2004).

Local densities are additionally regulated by emigration and immigration (Strevens & Bonsall 2011). Dispersal is typically positively density dependent when local exploitative and interference competition is strong (Bowler & Benton 2005). A negative density dependency in dispersal may, however, also evolve when densities are directly related to local habitat quality rather than to the level of competition, especially when dispersal costs are low (Rodrigues & Johnstone 2014). Because habitat fragmentation leads to substantial dispersal costs, dispersal will be especially a density-dependent process during emigration from the local patches (Dytham & Travis 2006; Poethke & Hovestadt 2002). This

local density dependence will be detectable at the metapopulation-level when organisms are able to move freely within the metapopulations, so when dispersal is global. When the population dynamics are strongly impacted by external forces, for instance by synchronised depletion of resources, local density dependency will not necessarily lead to density dependence at the metapopulation level (Bowler & Benton, 2005).

Density dependence of population growth and emigration principally affect local population dynamics. Dispersal also affect metapopulation stability through spatial coupling. The strength and direction of the stabilising impact depends directly on the strength and direction of the density dependent effects and the topology of the metapopulation (Tromeur et al. 2013). Not too low dispersal rates stabilise the size of local populations (Abbott 2011) but meanwhile enhance spatial synchrony among local populations (Liebhold et al. 2004), thereby potentially destabilising metapopulations persistence (Hanski 1998). Alternatively, recolonisation dynamics are an important prerequisite for the existence of metapopulation dynamics. Low dispersal rates will decrease the typical rescue events of local extinct patches (e.g., Heino et al. 1997; Hanski 1998; Holland & Hastings 2008) and thus decrease the viability of the metapopulation as a whole. Interestingly, dispersal is subject to fast evolutionary changes to changes in patch size asymmetry and patch extinction (Bowler & Benton 2005). Fast evolutionary dynamics can thus (re)enforce these metapopulation-level stabilising mechanisms (Heino & Hanski 2001).

Not all spatially structured populations in nature are, however, expected to be typical Levins' metapopulations, i.e., metapopulations consisting of equally sized patches, moderate levels of dispersal and some degree of stochastic extinctions (Baguette 2004, Fronhofer et al. 2012). Although we lack any empirical support, spatially structured populations in nature likely comply more to patchy or mainland-island metapopulations (Harrison & Taylor 1997). Mainland-island systems are characterized by high variation in patch size and occupancy as well as asymmetrical connectivity; patchy metapopulations are characterised by high levels of connectivity that prevent patch extinction (Fronhofer et al. 2012).

Substantial progress in understanding the relationship between the spatiotemporal metapopulation structure and population dynamics is hampered by the intrinsic difficulties to decouple patterns from processes in natural systems. We opted to follow an experimental approach using

microcosms to study population dynamics in three types of metapopulation that approach real systems as close as possible (Benton et al. 2007). The objective of this research was to understand to which degree spatial and spatiotemporal variation in habitat availability affects the demographic dynamics in experimental metapopulations of a spider mite. We specifically tested the hypotheses (Fig.4.1) that, relative to patchy metapopulations, (i) spatial variation in habitat availability (as in mainland-island metapopulations) would increase total metapopulation sizes and variance here-in, (ii) that spatiotemporal variable metapopulations (classical metapopulations) would be characterised by higher patch extinction rates and subsequent variation in local population size, thereby decreasing the overall metapopulation size. Our experiments were designed to induce aerial dispersal among local patches within the artificial metapopulations. Given the overall scarce availability of habitat within the metapopulations, we expect immigration costs to be high. Metapopulation stability is therefore anticipated to be primarily driven by external factors thereby destabilising the spatiotemporal variable metapopulations relative to the others due to a high level of local variability and a low level of spatial synchrony. Asymmetry in patch size, and thus decreased immigration probabilities for the majority of the individuals inhabiting large patches (Poethke et al. 2011), is additionally expected to select for lower levels of dispersal in mainland-island metapopulations, while no differences in the density dependent population growth are a priori expected.

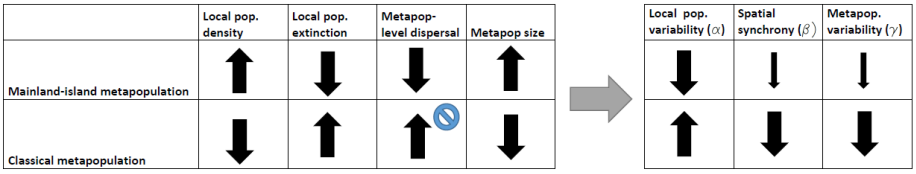



***Fig. 4.1 – Prediction on the expected changes in demography and population dynamics in the mainland-island (SPA) and classical (TEM) metapopulations relative to the patchy metapopulations (HOM).*** *Arrows indicate the expected direction and sizes of the effect. Only the expectation regarding dispersal increase in the TEM metapopulations was not validated.*

# MATERIALS AND METHODS

## EXPERIMENTAL SYSTEM OF ARTIFICIAL METAPOPULATIONS

Three types of artificial metapopulations were constructed with a varying spatial configuration of the patches but with an equal metapopulation carrying capacity. The "LS-VL" spider mite strain served as base population because earlier research demonstrated its high genetic variability (Bitume et al. 2013; Bonte et al. 2010; Van Leeuwen et al. 2008). *Tetranychus urticae* reaches adulthood from the egg stage in on average eight days. Freshly matured females constitute the dispersing phenotype in this species (Smitley & Kennedy 1985, Li & Margolis 1993, Fronhofer et al. 2014, Bonte et al. 2014, Van Petegem et al. 2015). Under standardised conditions where resources are not limiting, dispersal rates increase in relation to the density of young inseminated females (Bitume et al. 2013, De Roissart et al. 2013). Bean (*Phaseolus vulgaris L.*) leaves of standardized size were used to create patches and placed in closed boxes (71 x 44 x 31 cm). Patches were isolated from each other by a Tanglefoot matrix preventing mites from dispersing by walking. Metapopulation resources were renewed weekly by adding fresh bean leafs according to the treatment. No leaves were removed before complete deterioration preventing the enforcement of extinction. A wind current (2 m/s) facilitated aerial dispersal of the mites two times a week. The wind currents were tested in a preliminary experiment and ensured mite dispersal in all possible directions. Artificial metapopulations with the following spatial configurations (each three times replicated) were installed (Fig. 4.2):

i. a homogeneous configuration: metapopulations consisting of nine equally sized bean leaf patches of 20 cm²; patches were weekly refreshed (further referred to as HOM), thereby mimicking patchy metapopulations.
ii. a spatial heterogeneous configuration: metapopulations consisting of 3 patches of standard leaf size (20 cm²) and three double sized patches so corresponding to mainland-island metapopulations (further referred to as SPA). The position of the patches in the metapopulation was randomised among the three replicates but remained constant in time.
iii. a spatiotemporal heterogeneous configuration: metapopulations consisting of nine patches with temporally changing leaf sizes (further referred to as TEM). Here, standard leaves of 20cm² were every week randomly assigned to one of the nine patches. This resulted in a weekly changing availability

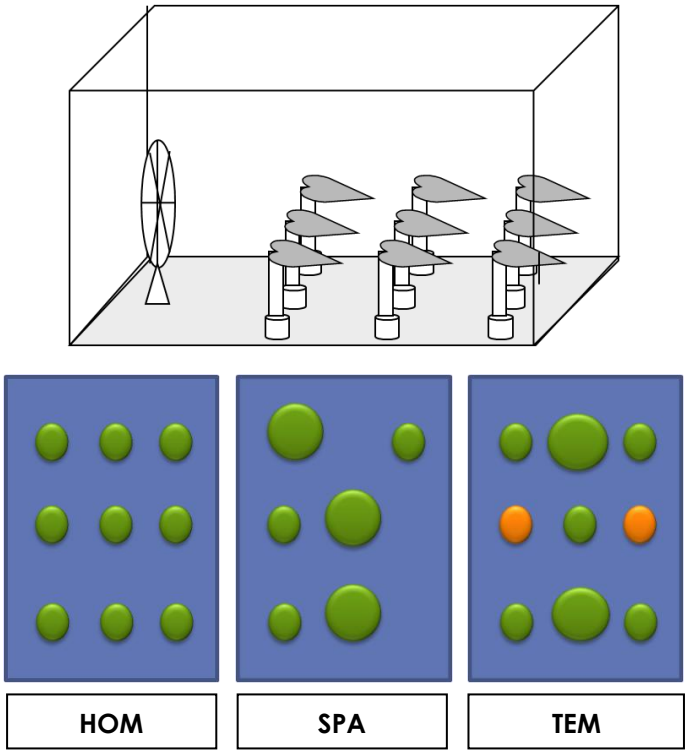
of resources over the nine patches. This treatment corresponds to classical metapopulations.

All metapopulations were thus weekly foreseen with 180 $cm^2$ leaf resources, but these were differently allocated among patches. The HOM metapopulations always consisted of nine patches with resources, the SPA metapopulation consisted of six patches, while for the TEM metapopulations, the total number of refreshed patches was variable, though always between six and nine patches. At the beginning of the experiment, 20 randomly collected female mites from the base population were assigned to each bean leaf patch and allowed to establish populations. All metapopulations were maintained under controlled conditions (23°C, 16:8 LD photoperiod). The experiment lasted 182 days but demographic data were only collected when metapopulations were fully established (after approximately 5 generations, 40 days).

## LOCAL DEMOGRAPHY

Average local population densities (individuals/cm2) of all the different life stages (eggs, juveniles, adult males and females) and local population extinctions were weekly quantified by counting the number of mites of all life stages (eggs, juveniles and adults) on three randomly chosen areas of 1cm2 of each bean leaf (thus, from each patch). Density-dependence of local populations was assessed as the slope of the relationship between population growth rate (ln(Nt+1/Nt)) and log population size (ln(Nt)). Population densities and extinctions were analysed using generalized linear mixed models (GLMM-GLIMMIX procedure SAS 9.3) (SAS Institute Inc 2006) with 'treatment' (HOM, SPA, TEM) as independent factor and each individual metapopulation as a random effect to control for dependence among replicates from each metapopulation treatment. Depending on the dependent variable, a Poisson ((meta)population density) or binomial error (extinction) structure was modelled with appropriate link functions. We corrected for potential overdispersion by modelling residual variation as an additional random factor. Non-significant contributions ($P>0.05$) were omitted by backwards procedure and validated by model selection. Effective degrees of freedom were estimated using Kenward-Rogers procedure (Kenward & Rogers 1997).

***Fig. 4.2 – Schematic representation of the experimental setup of the invasion experiment.*** *Top: metapopulation box containing local spider mite populations on bean leaves, bottom: metapopulation types: HOM – homogeneous configuration consisting of nine equally sized bean leaf patches; SPA- spatial heterogeneous configuration consisting of 3 standard leaf patches and 3 double-sized leaf patches; TEM – spatiotemporal heterogenous configuration consisting of nine patches with temporally changing levels of resources in local populations. Total metapopulation carrying capacity was kept constant and equal over all treatments.*



## DISPERSAL AND METAPOPULATION DYNAMICS

### DISPERSAL

Dispersal at the metapopulation level was estimated weekly by counting the number of mites on 12 rectangles (2cm x 3cm) of tanglefoot randomly placed in the metapopulation boxes. Adult females were, as foreseen, the only life stage present on the tanglefoot squares. Dispersal propensity was measured as the number of female adult mites on the total area of rectangles (12 x 6cm$^2$) relative to the current estimate of the number of females on a comparable surface of leaf in the metapopulation. It was statistically analysed by means of generalized linear mixed models (GLMM) for binomially distributed data with logit-link and correction for potential overdispersion. Independent factors were "average female mite density" (continuous factor) and the "treatment". In order to control for possible correlation due to the common date of testing, we included "date" as a random effect. Effective degrees of freedom were estimated using K-W procedure (Kenward & Rogers 1997).procedure. Post *hoc* Tukey tests were performed to correct pair wise differences among treatments. All analyses were performed with SAS 9.3 (Proc Glimmix; SAS Institute, 2001).

Cross-correlation analyses of time series of dispersal propensity and total metapopulation density were performed to determine the presence of serial dependence between the different time series. Our aim was to locate density-dependence and the lag between density and dispersal. Additionally we assessed whether lags and cross-correlation coefficients differed among treatments. Cross-correlation analyses of the time series of dispersal and metapopulation density were performed using the nlme package in R 3.1-97. The autocorrelation function (ACF) was used to demonstrate the presence of periodicity in the time series. Cross-correlation coefficients of the time-series were obtained by the cross-correlation function in R (CCF) and analysed using linear mixed models (MIXED procedure in SAS 9.3). 'Metapopulation replicate' was included as a random effect.

### METAPOPULATION SIZE/DENSITY

To estimate metapopulation size, we summed estimated local population sizes (see higher) for each replicated metapopulation for each time step they were recorded. Since the total amount of resources (total habitat availability) was equal in all metapopulations, metapopulation size is directly related to metapopulation density. Metapopulation sizes/densities were analysed using generalized linear

mixed models (GLMM-GLIMMIX procedure SAS 9.3) (SAS Institute Inc 2006) with 'treatment' (HOM, SPA, TEM) as independent factor and each individual metapopulation as a random effect to control for dependence among replicates from each metapopulation treatment. A Poisson error structure was modelled with appropriate link functions. We corrected for potential overdispersion by modelling residual variation as an additional random factor (Verbeke & Molenberghs 2000). Non-significant contributions (P>0.05) were omitted by backwards procedure and validated by model selection. Effective degrees of freedom were again estimated using Kenward-Rogers procedure. Density-dependence at the metapopulation level was estimated as the slope of the relation between population growth rate ($\ln(N_{t+1}/N_t)$) and log population size ($\ln(N_t)$). With the time series of metapopulation juvenile and adult density, a cross-correlation analysis was performed to determine the presence of serial dependence between the different time series. Our aim was to assess whether the ups and downs of the different time series are correlated and to locate possible lags between time series. Cross-correlation analyses of the time series of adult density and juvenile density were performed using the nlme package in R 3.1-97. Cross-correlation coefficients of the time-series were analysed using general linear models (MIXED procedure in SAS 9.3) with 'metapopulation replicate' included as a random effect. *Post-hoc* Tukey tests were performed to correct for pair wise differences among treatments.

## (META)POPULATION VARIABILITY AND SPATIAL SYNCHRONY

Temporal variability at both local population (α-variability) and metapopulation scales (γ-variability) were calculated following Wang & Loreau (2014). α-variability is calculated as the square of the weighted average of CV across local populations; γ-variability as the square of the CV of the metapopulations. Variability parameters were first separately analysed using general linear models (GLM procedure in SAS 9.3). Post-hoc Tukey tests were performed to correct pair wise differences among treatments. Spatial synchrony is defined as a metapopulation-wide measure of population synchrony and equals the metapopulation γ-variability divided by local α-variability (see Wang & Loreau 2014 for details).

# RESULTS

## LOCAL DEMOGRAPHY

Average local population size (adults, juveniles and eggs) differed among treatments ($F_{2,6.054}$=36.20; P=0.0004). Population sizes were on average larger in the spatially heterogeneous metapopulations (22.11 ± 2.32SE individuals) than in the homogeneous (15.43 ± 1.62SE individuals) ($t_{5.94}$=-6.96; P=0.0010) and spatiotemporal heterogeneous (14.80 ± 1.55SE individuals) ($t_{6.122}$=7.71; P=0.0006) metapopulations. The same effect (higher size in spatially heterogeneous metapopulations) was observed for the different life stages (adults, juveniles and eggs) separately (see supplementary tables S1 and S2). Average local population densities differed among treatments ($F_{2,6.083}$=25.26 P=0.0011) and were lower in spatiotemporally variable metapopulations (10.50 ± 1.17SE individuals) compared to homogeneous (15.20 ± 1.69SE individuals) and spatially heterogeneous metapopulations (14.41 ± 1.60SE individuals) (Fig. 4.3). The proportion of local populations going extinct was significantly affected by treatment ($F_{2,5.166}$=10.77; P=0.0144). The average proportion of extinctions was higher in spatiotemporal variable metapopulations (0.251 ± 0.091SE) than in homogeneous (0.01 ± 0.006SE) and spatial variable metapopulations (0.027 ± 0.015SE). All treatments exhibited negative density dependence of local population growth (HOM: slope= -0.662; SPA: slope= -0.7611; TEM: slope= -0.5219) and density dependence was lowest in the TEM metapopulations ($F_{2,1630}$=37.61; P<0.0001) (Fig. 4.4A).

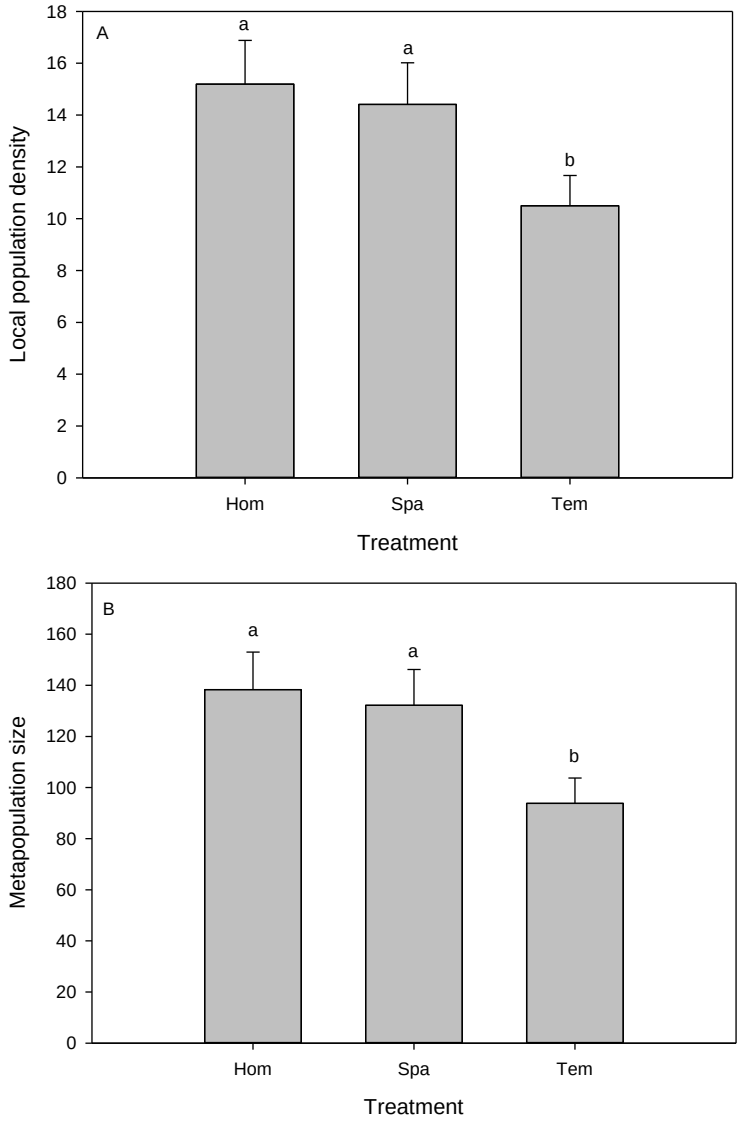



***Fig. 4.3 – Effects of variation in metapopulation structure on density (mean values ± 1 SE).*** *A: effect on local population density, B: metapopulation size. Densities comprise average number of females, males, juveniles and eggs/cm², metapopulation size estimates comprise the sum of the counted number of females, males, juveniles and eggs of all local populations. Equal notations indicate non-significant contrast for the respective measurements. Error bars represent standard errors.*

## DISPERSAL AND METAPOPULATION DYNAMICS

### DISPERSAL

Dispersal propensity was negatively affected by the average total (adults, juveniles and eggs) and female density in the metapopulation over treatments (Total: $F_{1,177}$=68.66; P<0.0001; Female: $F_{1,177}$=165.13; P<0.0001). The strength of density dependence of dispersal differed between treatments, with a steeper decline in the TEM metapopulations ($F_{2,177}$=20.91; P<0.0001) (Fig. 4.5). Overall, dispersal propensity differed among treatments ($F_{2,10.99}$=20.91; P=0.0002) and was lowest in spatial variable metapopulations 0.063 ± 0.01SE SE) compared to the other two treatments (HOM: 0.29 ± 0.004SE; TEM: 0.20 ± 0.003SE). Obviously, because the slopes of TEM differ from those from SPA en HOM, only the differences among the latter are meaningful. Cross-correlation analysis of the time series of dispersal propensity and metapopulation density revealed no differences of cross-correlation coefficients among treatments ($F_{2,54}$= 0.11; P=0.8945). Neither did we find differences among lags (lag-unit = 1 week) ($F_{9,54}$=1.28; P=0.2679) nor did the lags between both time series differ among all treatments ($F_{18,54}$=1.11; P=0.3694).

### METAPOPULATION SIZE/DENSITY

Metapopulation density differed among treatments ($F_{2,181}$=27.19; P<0.0001). Metapopulation density of spatio-temporal variable metapopulations (10.33 ± 1.07SE individuals) was on average lower than of homogenous (14.42 ± 1.46SE individuals) and spatially heterogeneous metapopulations (14.15± 1.44SE individuals) (Fig. 2B). Obviously, the same results hold for metapopulation size. All treatments exhibited negative density dependence of metapopulation growth (HOM: slope=-0.54; SPA: slope=-0.65; TEM: slope=-0.63) but density-dependence at this level of organisation did not differ among all treatments ($F_{2,181}$=0.42: P=0.6545) (Fig.4B). Cross-correlation coefficients of the time series of juvenile density and adult density did not differ statistically among treatments ($F_{2,54}$=0.66; P=0.5219). Significant differences among lags (lag-unit = 1 week) were detected ($F_{9,54}$=5.13 ; P<0.0001) and a significant interaction between lag and treatment was detected ($F_{18,54}$=2.76 ; P=0.0021). Cross-correlation coefficients were significantly different from 0 for lags 1, 7, 8, 9 and 10 (Supplementary table S3). Over all treatments cross correlation coefficients differed significantly between lag 1 and 2, 3, 4, 5, 6, 8 and between lag 3 and 10 (Supplementary table S4).

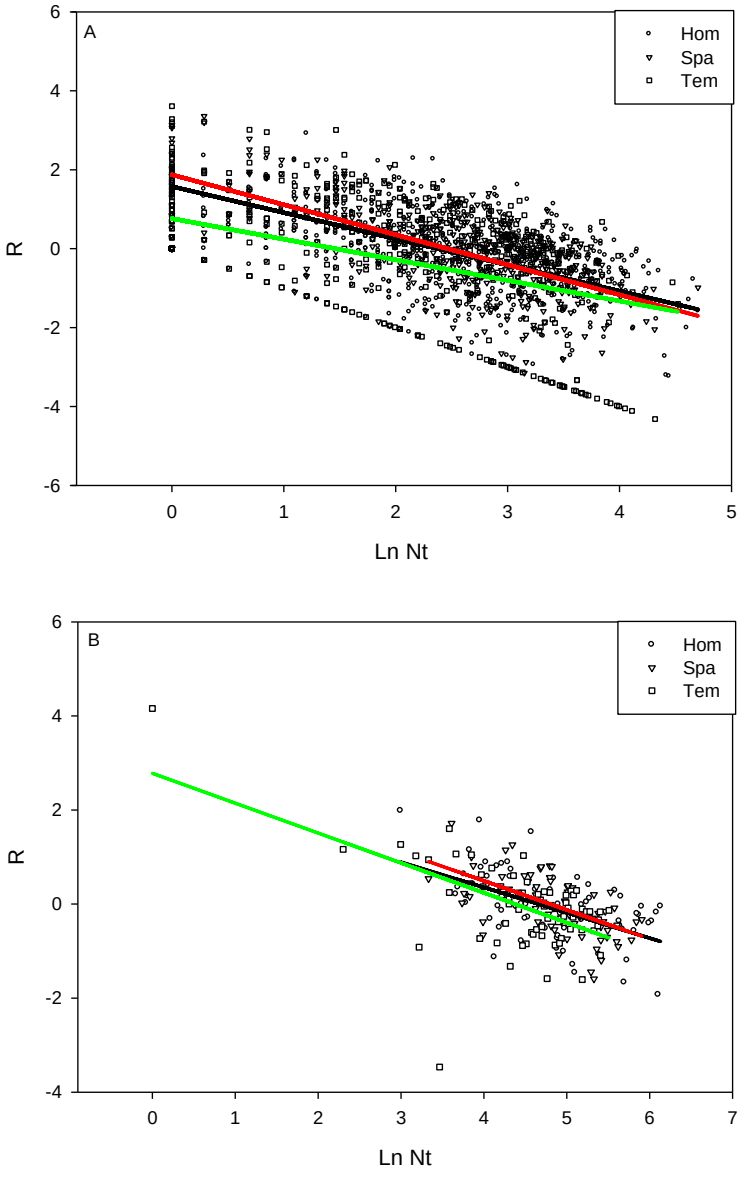



***Fig. 4.4 – Effects of variation in metapopulation structure on density dependence.*** *A: local growth rate in relation to local population density and metapopulation structure, B: metapopulation growth rate in relation to the metapopulation density and structure. Densities comprise average number of females, males, juveniles and eggs. The black line represents the modelled values of the HOM treatment, the red line of the SPA treatment and green line of the TEM treatment.*

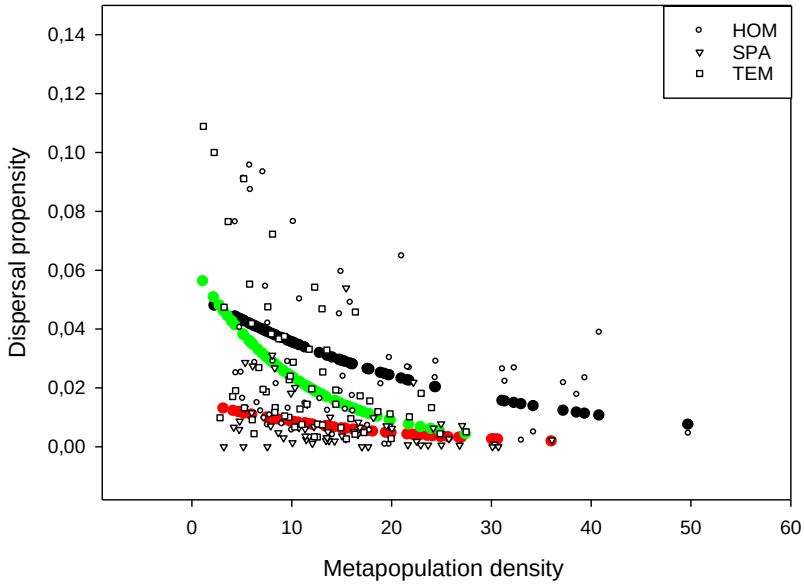


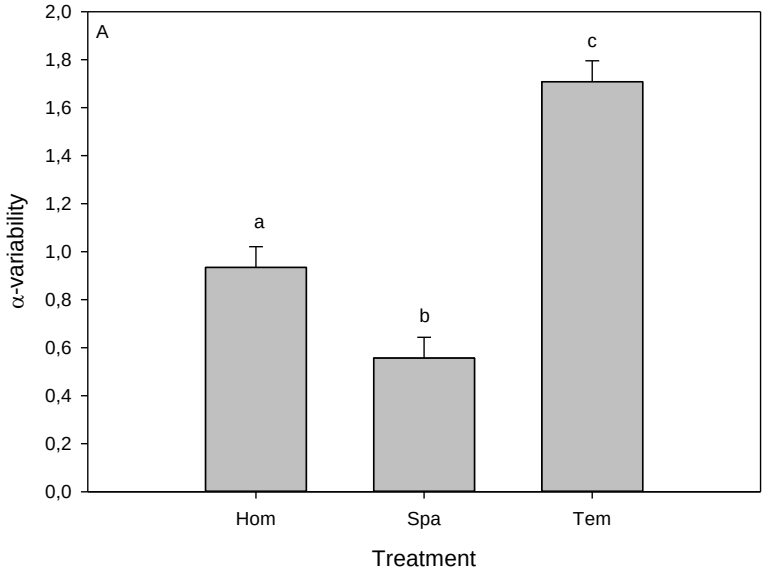

***Fig. 4.5 – Effects of metapopulation density and variation in metapopulation structure on dispersal.*** *Dispersal propensity (number of dispersive mites/metapopulation density) in relation to the experienced metapopulation density and the metapopulation structure. Densities comprise average number of females, males, juveniles and eggs. The dispersal propensities are plotted in the graph as dots. Lines indicate predictions derived from the Poisson mixed model. The black line represents the modelled values of the HOM treatment, the red line of the SPA treatment and green line of the TEM treatment.*

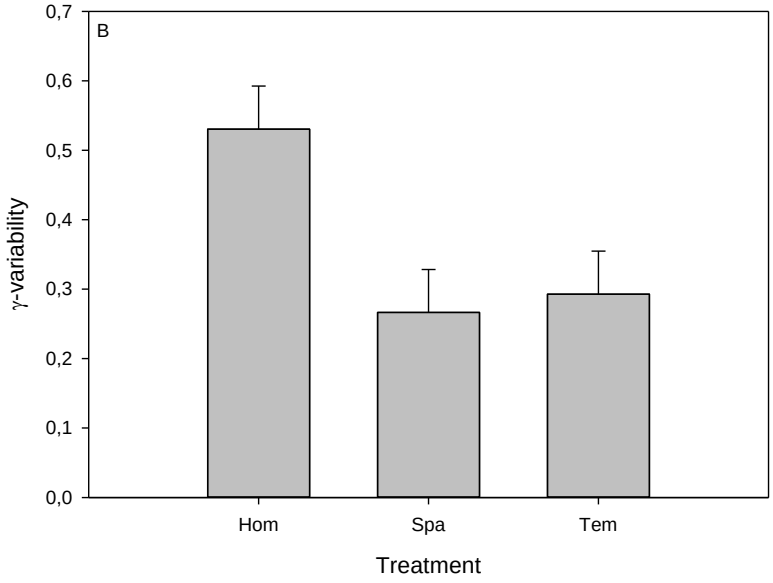
## (META)POPULATION VARIABILITY AND SPATIAL SYNCHRONY

Treatment affected local population variability ($\alpha$-variability) ($F_2$=46.14; P=0.0002). Local population variability was lowest in spatially variable metapopulations (0.56 ± 0.09SE), followed by homogeneous (0.93 ± 0.08SE) and spatiotemporal variable metapopulations (1.71± 0.08SE) (Fig. 6A). Metapopulation variability ($\gamma$-variability) differed significantly among treatments ($F_{2,7}$=5.50; P=0.044) (Fig.6B). Metapopulation variability was lower in spatially variable metapopulations (0.27 ± 0.07SE) and spatiotemporally variable metapopulations (0.29 ± 0.06SE) compared to

homogeneous metapopulations (0.53 ± 0.06SE) but only marginally significant (HOM-SPA $t_6$=3.01 P=0.054; $t_6$=2.71 HOM-TEM P=0.078). Over all treatments metapopulation variability was found to be lower than local population variability ($t_6$=33 P< 0.0001). Spatial synchrony differed significantly among treatments ($F_{2,7}$=22.47; P=0.0016) (Fig. 6C). Synchrony was lowest in metapopulations with spatiotemporal variation (0.17 ± 0.04SE) compared to homogeneous (0.57 ± 0.04SE) ($t_6$=6.43 P=0.0016) and spatially variable metapopulations (0.47 ± 0.04SE) ($t_6$=4.85 P=0.0068).

***Fig. 4.6 – Effects of variation in metapopulation structure on the variability (mean values ± 1 SE) of (meta)population size of mites.*** *A: local α-variability, B: metapopulation γ-variability, C: spatial synchrony. Equal notations indicate non-significant contrast for the respective measurements. Error bars represent one standard error.*

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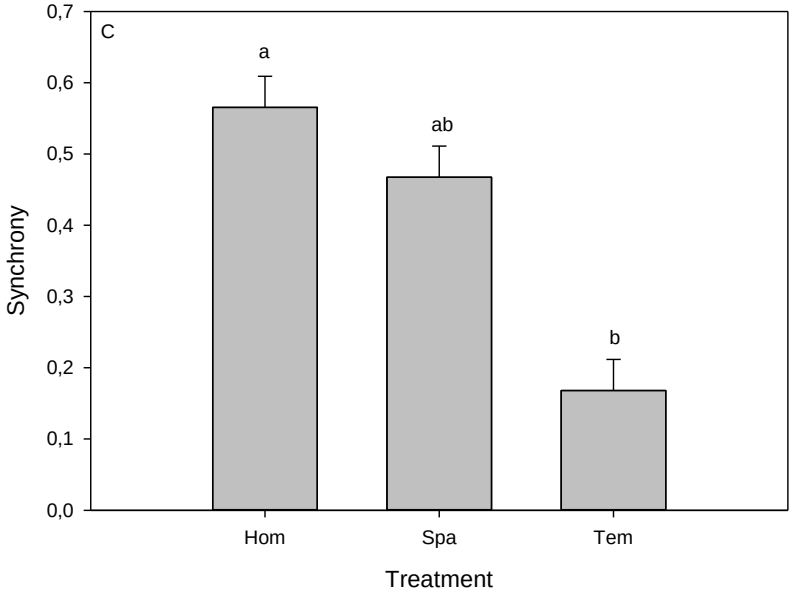
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# DISCUSSION

Using artificial metapopulations of the spider mite *Tetranychus urticae*, we demonstrate that, compared to spatially homogeneous metapopulations consisting of equally sized patches, spatial variation in habitat availability decreases variability in metapopulation size and density dependent dispersal at the metapopulation level. Spatiotemporal variation in habitat availability induced higher patch extinction rates, thereby lowering average local population and metapopulation sizes. Such spatiotemporal variation simultaneously increased variability in local population size, decreased the magnitude of density dependence in growth rates and the synchrony of the local population dynamics. Overall, metapopulation variability was found to be lower than local population variability and increased population variability in spatiotemporal variable metapopulations reduced variability at the scale of the metapopulation (Table 1).

A range of empirical as well as theoretical studies (Benton et al. 2002, Drake & Lodge 2004) already demonstrated higher population extinction rates in temporally fluctuating relative to constant environments. These are predominantly caused by bottom-up effects of increased stochasticity in the form of fluctuating resource levels (Bull et al. 2007). External forcing of temporal variation in patch size by the manipulation of resources significantly increased variance in local population sizes and patch extinction rates. Evidently, such catastrophic events where extinct patches are colonized by few emigrants at lagged time intervals decreased the average population sizes.

Local population sizes of all stages were on average 50% higher in spatially heterogeneous metapopulations than those in other treatments. This increase resulted obviously from the increased resource abundance in the double-sized patches (Fahrig 2007, Strevens & Bonsall 2011) and corresponds with previous studies on closed populations where the number of individuals is linked to the amount and distribution of available resources (Cameron & Benton 2004). Increases in local population sizes follow a linear response towards the availability of resources and are thus not associated with increased densities. Population growth under conditions where food resources are more abundant could be expected to lead to an extended period of time until density dependence kicks-in, thereby leading to higher local population densities and overshooting of the local carrying capacity. Absence of such a pattern indicates that population regulation occurs among all life stages, and that competition

among the life stages is less asymmetrical than anticipated by higher per capita consumption rates in adult females. Alternatively, individuals may have evolved slower individual growth rates under repetitive stress as expected under population sizes, and experience lower maximal per capita growth rate to improve environmental tolerance or the efficiency of resource use (Dey et al. 2008; Monro & Marshall 2014).

We observed overall a negative density dependence in population growth over the duration of the experiment, but a significant lower one in the spatiotemporal variable metapopulations. This accords with work of Strevens & Bonsall (2011), who indicated that the dynamics of homogenous and spatiotemporally variable systems were best described by density-dependent population dynamical models, while spatiotemporal variable systems were best described by density-independent models. They attributed this result to elevated levels of dispersal reducing the competition for resources at the local scale (Strevens & Bonsall, 2011). We did not observe overall higher levels of dispersal in the spatiotemporal variable metapopulations, especially not at high densities. We instead attribute absence of density dependence to the lagged colonization of empty patches. Indeed the lower density dependence became significant when extinct populations were excluded (HOM: slope= -0.89; SPA: slope= -0.95; TEM: slope= -1.09; $F_{2,1264}$=3.17 P=0.0425). Because local population sizes in the spatiotemporally variable metapopulations were on average lower than those in the other metapopulation types, decreased density dependence did not increase population growth rates and neither compensated for the externally induced variation in habitat availability.

Contrary to expectations and earlier work focusing at the local population-level (De Roissart et al. 2014), we witnessed negative dependence of dispersal propensity in the metapopulation for all treatments. Rodriguez & Johnstone (2014) demonstrated that a negative density dependence of dispersal can be selected for in temporally stable environments where local differences in resource quality persist over multiple generations. In their theoretical work, density is not scaled to $K$, and evolved dispersal strategies relate more to heterogeneity in habitat quality. Such a mechanism is not relevant in our experimental system since patches have resources of the same quality. In absence of any adaptive explanation, we therefore attribute this negative relationship towards changes in body condition. In the mite metapopulations where dispersal was only made possible within limited time windows, large population densities lead to increased competition, resource depletion

and subsequently an expected poorer body condition. Parallel work on more actively dispersing invertebrates and vertebrates has demonstrated that individuals in poor body condition can be constrained in dispersal due to lack of sufficient reserves to levy the costs and remain philopatric (Bonte et al. 2012, Debeffe et al. 2014, O' Sullivan et al. 2014). Generally, such a correlation is linked to energetic reserves to move between patches (energetic costs; Bonte et al. 20012). As for the spider mites and other passively dispersing organisms, larger amounts of reserves may also facilitate habitat selection (Bonte et al. 2011) and thus affect dispersal by changing cost-benefit balances during the immigration phase (Delagdo et al. 2014).

Our results revealed a lower level of spatial synchrony of the patches in spatiotemporally variable metapopulations relative to the other metapopulation types. Dispersal and regional stochasticity are the two prominent factors that influence spatial synchrony. It is generally difficult to establish whether spatial synchrony is due to dispersal or regional stochasticity but which one is the case makes an important difference to metapopulation dynamics (Hanski & Woiwod 1993, Bjornstad et al. 1999). If synchrony is due to high dispersal, high recolonisation rates will facilitate metapopulation persistence and may more than compensate for the negative effect of synchronous dynamics in generating correlated local extinctions. In contrary, if synchrony is due to regional stochasticity, metapopulation persistence time is necessarily decreased, in the same manner as the expected lifetime of a local population is decreased by increasing environmental stochasticity (Hanski & Woiwod 1993). Over all treatments, in our system, synchrony was observed to increase with dispersal (slope: 8.9; P=0.036). However for the different treatments separately, dispersal levels were lowest in homogeneous and spatial variable metapopulations and were coupled with higher synchrony relative to the spatiotemporal variable metapopulations, evidently caused by the spatially correlated resource renewals.

Metapopulation variability is significantly larger in homogeneous metapopulations than in spatially heterogeneous and spatiotemporal heterogeneous metapopulations. Metapopulation variability is determined by the product of local population variability and spatial synchrony among populations (Wang & Loreau 2014). Thus, the stabilizing effects can result from lower local variability, lower spatial synchrony, or both. In spatially heterogeneous metapopulations, patches with double resource abundance (i.e. carrying capacity) have larger population size and lower variability compared to smaller patches as also found in

Strevens & Bonsall (2011). This results in lower average local population variability. Resource supply in the HOM and SPA metapopulations are highly synchronous and causes high synchrony in population dynamics in both treatments. Therefore, SPA decreases metapopulation variability by reducing local population variability. In contrast, TEM decreases metapopulation variability through reducing spatial synchrony. The resource supply in TEM is highly variable both in time and in space. On the one hand, the large temporal variation results in high temporal variability of local population dynamics. On the other hand, the large spatial variation substantially reduces spatial synchrony. The latter effect is so strong that although TEM generates higher alpha variability and local extinction rates, the metapopulation variability is significantly lower than HOM. In consequence, an island-mainland metapopulation structure minimises both local and regional variability and thus extinction risk at these two scales. We suggest to take this important insights as a starting point to explain the potential dominance of such metapopulations relative to more classical ones in nature.

# SUPPLEMENTARY MATERIAL

## SUPPLEMENTARY TABLES

**Table S1:** Estimates of local population densities of the different stages.

| **Adults** | | | | | |
|---|---|---|---|---|---|
| **Treat** | **DF** | **t Value** | **Pr > \|t\|** | **Mean** | **Standard Error** |
| Hom | 26.79 | 6.26 | <.0001 | 1.9974 | 0.2209 |
| Spa | 28.55 | 5.59 | <.0001 | 1.8733 | 0.2105 |
| Tem | 29.62 | 3.30 | 0.0025 | 1.4534 | 0.1648 |
| **Juveniles** | | | | | |
| **Treat** | **DF** | **t Value** | **Pr > \|t\|** | **Mean** | **Standard Error** |
| Hom | 27.42 | 9.43 | <.0001 | 3.7193 | 0.5178 |
| Spa | 27.96 | 9.00 | <.0001 | 3.5252 | 0.4932 |
| Tem | 28.42 | 6.63 | <.0001 | 2.5382 | 0.3566 |
| **Eggs** | | | | | |
| **Treat** | **DF** | **t Value** | **Pr > \|t\|** | **Mean** | **Standard Error** |
| Hom | 27.36 | 17.49 | <.0001 | 8.9432 | 1.1201 |
| Spa | 27.64 | 17.05 | <.0001 | 8.5086 | 1.0685 |
| Tem | 27.88 | 14.50 | <.0001 | 6.2009 | 0.7803 |

**Table S2:** Estimates of differences between population sizes of the different treatments.

| **Adults** | | | | | | |
|---|---|---|---|---|---|---|
| **treat** | **_treat** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Adj P** |
| Hom | Spa | 0.06412 | 0.06201 | 6.033 | 1.03 | 0.5813 |
| Hom | Tem | 0.3179 | 0.06378 | 6.756 | 4.99 | 0.0042 |
| Spa | Tem | 0.2538 | 0.06681 | 8.134 | 3.80 | 0.0170 |
| **Juveniles** | | | | | | |
| **treat** | **_treat** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Adj P** |
| Hom | Spa | 0.05360 | 0.06479 | 5.62 | 0.83 | 0.7012 |
| Hom | Tem | 0.3821 | 0.06598 | 6.046 | 5.79 | 0.0027 |
| Spa | Tem | 0.3285 | 0.06742 | 6.588 | 4.87 | 0.0065 |
| **Eggs** | | | | | | |
| **treat** | **_treat** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Adj P** |
| Hom | Spa | 0.04981 | 0.05252 | 5.921 | 0.95 | 0.6318 |
| Hom | Tem | 0.3662 | 0.05314 | 6.204 | 6.89 | 0.0010 |
| Spa | Tem | 0.3164 | 0.05390 | 6.566 | 5.87 | 0.0023 |

**Table S3:** Estimates of cross-correlation coefficients between time series of juvenile and adult densities for the different lags.

| Lag | Estimate | Standard Error | DF | t Value | Pr > \|t\| |
|---|---|---|---|---|---|
| **1** | 0.1950 | 0.02752 | 54 | 7.09 | <.0001 |
| **2** | 0.05100 | 0.02752 | 54 | 1.85 | 0.0693 |
| **3** | -0.03644 | 0.02752 | 54 | -1.32 | 0.1909 |
| **4** | 0.04122 | 0.02752 | 54 | 1.50 | 0.1399 |
| **5** | -0.00444 | 0.02752 | 54 | -0.16 | 0.8723 |
| **6** | 0.05456 | 0.02752 | 54 | 1.98 | 0.0525 |
| **7** | 0.07111 | 0.02752 | 54 | 2.58 | 0.0125 |
| **8** | 0.05822 | 0.02752 | 54 | 2.12 | 0.0390 |
| **9** | 0.06944 | 0.02752 | 54 | 2.52 | 0.0146 |
| **10** | 0.1112 | 0.02752 | 54 | 4.04 | 0.0002 |

**Table S4:** Estimates of significant differences of cross-correlation coefficient between lags of the timeseries of juvenile and adult density (over all treatments). Non-displayed comparisons were not significant (P>0.05).

| Lag | _Lag | Estimate | Standard Error | DF | t Value | P |
|---|---|---|---|---|---|---|
| **1** | **2** | 0.1440 | 0.03891 | 54 | 3.70 | 0.0167 |
| **1** | **3** | 0.2314 | 0.03891 | 54 | 5.95 | <.0001 |
| **1** | **4** | 0.1538 | 0.03891 | 54 | 3.95 | 0.0079 |
| **1** | **5** | 0.1994 | 0.03891 | 54 | 5.13 | 0.0002 |
| **1** | **6** | 0.1404 | 0.03891 | 54 | 3.61 | 0.0216 |
| **1** | **8** | 0.1368 | 0.03891 | 54 | 3.51 | 0.0281 |
| **10** | **3** | 0.1477 | 0.03891 | 54 | 3.79 | 0.0127 |

# 5. EVOLUTION IN SPATIOTEMPORAL VARIABLE METAPOPULATIONS FACILITATES PERFORMANCE IN NOVEL CHALLENGING CONDITIONS.

* Adapted from: De Roissart A., Wybouw N., Renault D., Van Leeuwen T. & Bonte D. (2014) Evolution in spatiotemporal variable metapopulations facilitates performance in novel challenging conditions. First revision in Molecular Ecology.

## ABSTRACT

Colonization-extinction processes have a strong impact on metapopulation dynamics. While this fundament of metapopulation theory has been widely addressed in theoretical and empirical research, we lack a thorough understanding of how changes in habitat structure affect evolutionary processes. These deem especially important in face of rescue mechanisms when habitats become fragmented. We therefore investigated by means of experimental evolution how changes in metapopulation structure affect life history divergence and genome-wide gene expression in the phytophagous spider mite *Tetranychus urticae*. We experimentally manipulated metapopulation structure by controlling the spatial and temporal variation in patch size as determined by bean leaves as an optimal resource. We investigated evolutionary changes in life history, physiology as well as a potential shift in gene transcription underlying these traits. Spatiotemporal stable (homogeneous) metapopulations, spatially heterogeneous (mainland-island systems) and spatiotemporally variable metapopulation (spatiotemporal fluctuations in patch size) were the experimental treatments.

Evolution during approximately 30 generations induced a significant divergence in life history traits and gene expression in the spatially and spatiotemporally variable metapopulation relative to the homogeneous metapopulations. These evolutionary dynamics additionally increased the performance of mites on a novel, challenging host plant. The observed multivariate divergence points towards a general, adaptive stress response in disturbed metapopulations, thereby pre-adapting mites to novel environmental conditions. These evolutionary dynamics are suggested to be driven by metapopulation level variation in competition and patch extinction rates.

## INTRODUCTION

The introduction of the concept of metapopulations by Levins (1969), further developed by Hanski (2004), triggered the awareness that spatial structure affects demographic and population dynamical processes. Metapopulation dynamics are primarily influenced by patch colonization-extinction dynamics which in turn alter the synchrony of local population fluctuations and metapopulation stability (Hanski 1998). Equal patch sizes, moderate levels of dispersal and some stochastic patch extinction dynamics are assumed to be essential for the functioning of classical metapopulations. However, most spatially structured populations can be classified as patchy or mainland-island metapopulations (Harrison & Taylor 1997), and the omnipresence of classical metapopulations has been recently questioned (Fronhofer *et al.* 2012). Mainland-island systems are characterized by high variation in patch size and occupancy as well as asymmetrical connectivity, while patchy metapopulations are characterised by high levels of connectivity that prevent patch extinction (Fronhofer et al. 2012). Such a variation in spatial structure generates typical disequilibrium conditions characterised by population extinction and high levels of demographic fluctuations. The number of immigrants and their genetic composition, result in changes to local growth rates, density dependence and stochasticity (Olivieri et al. 1990).

In contrast to single, unconnected populations, local selection pressures are expected to act at more than one level of population structure in metapopulations (Olivieri et al. 1990). For instance, in classical metapopulations where local population extinctions occur regularly, increased dispersal is selected since long-term survival is only possible if genotypes are able to re-colonise patches from where they have become locally extinct (Dytham & Travis 2006). With increasing asymmetry in patch size, however, dispersal will evolve to lower rates because benefits of dispersal are only prevalent for a minority of the individuals (Poethke & Hovestadt 2002). Additional alternative adaptive strategies might also evolve through sex-ratio (Macke et al. 2011), age-at-maturation adjustments (Travis 2004) and density-dependent evolution of overall life history strategies (Bierbaum et al. 1989). While we lack a synthesis on the evolution of life histories in metapopulations, we can expect trait adaptations to affect demography and to be genetically associated with various metabolic pathways enhancing resistance to other stressors like starvation, toxicity and heat (Parsons 2005). In a heterogeneous world, metapopulation dynamics might stress organisms

(Margulis & Sagan 2000) and lead to the development of cross-tolerance to other stressors. There is indeed an increasing awareness that changes in spatial structure affect population dynamics, and that these ecological dynamics interact with evolutionary trajectories. However, with the exception of some theoretical work, there is no profound understanding on such eco-evolutionary dynamics in either natural or experimental metapopulations even though this is of exceptional relevance in light of evolutionary rescue. This designates the process where adaptive evolution that allows a population (Gomulkiewicz & Holt 1995) or metapopulation (Bell & Gonzales 2011; Travis et al. 2013) to recover from negative growth as initiated by environmental change that would have lead otherwise to extinction (Gomulkiewicz & Holt 1995). Evolutionary rescue is known to be strongly determined by demographic and genetic factors of local populations, but also entire metapopulations (Carlson et al. 2014).

The importance of eco-evolutionary dynamics is most obvious in metapopulations where dispersal determines the genetic composition of different populations, which in turn affects local growth and dispersal rates. Typical examples include the Glanville fritillary butterfly on the Åland archipelago (Hanski & Mononen 2011) or stick insect metapopulations (Farkas et al. 2013). The genetic architecture underpinning life history differentiation is not well understood, but currently facilitated by the development of several –omic approaches. Transcriptome analyses may uncover genes that significantly alter their transcript levels as a response to the implemented selection pressure and provide detailed insights on the pleiotropic effects underlying phenotypic divergence (Hodgins-David & Townsend 2009). For instance, in *Drosophila melanogaster,* many genes respond to stress that affect mobility and aggression (reviewed in Wheat 2012). In the phytophagous spider mite *Tetranychus urticae*, the transcriptomes of populations that developed pesticide resistance or that were exposed to challenging host plants revealed the presence of general adaptive pathways and identified key gene candidates for xenobiotic adaptation in this polyphagous mite (Dermauw et al. 2013, Wybouw et al. 2014).

Experimental evolution in artificial metapopulations provides a unique formal test to understand to which degree spatial variation in habitat availability affects life history divergence (Kawecki *et al.* 2012). Using this approach, we assessed divergence in life history traits, physiology and gene expression in three types of artificial metapopulations of the spider mite *T. urticae*. The mites evolved for 30 generations in metapopulations

differing in the spatial and temporal regime of habitat availability. This species functions as a model system in experimental evolution and is known to have a strong genetic component underlying dispersal (Li & Margolies 1993), sex ratio determination (Macke et al. 2011) and ecological specialisation (Magalhaes et al. 2007). The species' genome is fully sequenced and annotated (Grbic et al. 2011) and its small genome size renders genomic research straightforward and cost effective.

Based on the earlier outlined predictions from theory and our observed metapopulation dynamics (see supplementary material 1 for an overview of the main results), we expected relative to the stable metapopulation that served as a control (i) evolution towards higher dispersal rates in the spatiotemporally variable metapopulations due to increased extinction rates, accompanied by evolution towards r-selected traits to cope with the unstable, unpredictable resources (ii) evolution of reduced dispersal in spatially variable metapopulations and, in contrast to the spatiotemporally variable metapopulations, evolution towards moreK-selected life history traits caused bythe more stable and predictable resources. More specifically we expect the reproduction-survival balance to shift depending on the spatial structure caused by difference in stability of resource supply. Novel hosts and other environmental stressors typically invoke an orchestrated transcriptome response in *T. urticae* (Bryon et al. 2013; Dermauw et al. 2013). In both types of 'disturbed' metapopulations, we anticipated an overall increase of competition for resources and adaptation to cope with the resulting food stress. For mainland-island metapopulations, during development of invididuals, an increase in regulation of the density dependent population processes is expected, relative to the adult individuals. This is because of selection against dispersal and on average larger patch sizes. In metapopulations with externally driven patch extinctions, elevated density dependence is expected due to stochastic changes and absence of resources. In both metapopulation types, we anticipated an increased general stress response and assessed by means of transcriptomics to which degree these responses were differentially expressed in relation to metapopulation structure.

# MATERIALS AND METHODS

## EXPERIMENTAL SETUP OF THE ARTIFICIAL METAPOPULATIONS

Metapopulation dynamics of *Tetranychus urticae* were studied using experimental microcosms. We used as a base population the "LS-VL" *T. urticae* strain, because of its high genetic variability (Van Leeuwen et al. 2008; Fronhofer et al. 2014). Artificial metapopulations consisted of a transparent plastic box with 9 patches arranged in a 3 x 3 lattice. We constructed three types of artificial metapopulations with an equal metapopulation-level carrying capacity but varying spatial configuration of the patches. Patches were isolated bean (*Phaseolus vulgaris L.*) leaves placed on a Tanglefoot layer in closed boxes. This hostile matrix prevented mites from leaving the patches. Weekly, resources became depleted and bean leaves were renewed to avoid starvation of the mites. The size of the bean leaves introduced to each patch was dependent upon the treatment. Two times a week, for 8 hours a wind current (1.5m/s) was induced by a fan and allowed aerial dispersal (details of the magnitude of dispersal are provided as supplementary material S1) of the mites. Three metapopulation types were installed each of which was replicated three times:

i) a patchy metapopulation consisting of nine patches weekly refreshed with leaves of 20 $cm^2$ (spatially homogenous distribution of resources; further referred to as HOM)

ii) a mainland-island metapopulation consisting of three patches of standard leaf size (20 $cm^2$) and three of double size; another three patches of these metapopulations remained constantly empty (spatial heterogeneous distribution of resources; further referred to as SPA)

iii) a spatiotemporal heterogeneous metapopulation (further referred to as TEM) in which we assigned nine single-patch resources (standard leaf) randomly to one of the nine patches. Due to this algorithm, the distributions of the resources (and thus local carrying capacity or island size) changed weekly among the nine patches and varied between zero (no resource renewal and local extinction) and double or exceptionally triple island size. In consequence, patch sizes and thus local carrying capacities fluctuated over time and space, but we ensured again a

constant metapopulation carrying capacity (9 x 20 $cm^2$) over time.

At the beginning of the experiment, 20 randomly collected female mites, from the base population described above, were assigned to each patch within each metapopulation type and allowed to establish populations for 5 weeks. All metapopulations were kept under controlled conditions (23°C, 16:8 L:D photoperiod). Population dynamics were significantly different in the three types of metapopulations with high extinction rates in TEM, lower density dependent dispersal dynamics and initially higher population fluctuation in SPA. The main metapopulation dynamics are presented in supplementary material S1.

## QUANTIFICATION OF MITE LIFE-HISTORY

Spider mite life-history traits were measured at the initiation of the experiment and after 10 months, corresponding to approximately 30 generations. All traits were measured on F2 mites (raised for two generations in common garden) to minimise maternal effects caused for instance by local conditions of crowding (Magalhaes et al. 2007). Young inseminated females of each experimental metapopulation were individually allowed to oviposit on bean leaf discs. Leaf discs were placed with the abaxial part upwards on moistened filter paper to prevent mites from escaping and to maintain leaf turgor. Different life history parameters of the descendants were recorded daily: juvenile survival, developmental time (time from egg until the adult stage), fecundity (daily number of eggs), longevity and sex-ratio. Since spider mites deposit the majority of their eggs during the first seven days after maturity, we monitored fecundity only during that period. Dispersal propensity of the mites was assessed by transferring mated F2 females to test arenas for trials of aerial dispersal (after two whole generations under common garden to avoid confounding maternal effects). The same setup for aerial dispersal assessment as used by Li & Margolies 1993, was applied (details in supplementary material S2).

## MITE PERFORMANCE

Mite performance was followed by quantifying rate of intrinsic growth as a proxy of fitness (Cameron et al. 2013). To detect possible differences in individual performance between treatments, an integrated individual-level fitness measure, the rate of intrinsic growth ($r_m$), was calculated by combining the estimated parameter distributions of the different life history parameters according (see statistical analyses) to the equation Σ

$e^{-(rm)x}\, l_x\, f_x = 1$ (with $l_x$ survival till maturity x and $f_x$ the number of female offspring at age x) which represents the contribution of each female to the number of females in the subsequent generation. We performed 10000 simulations and reported the mean value and standard deviation while testing its significance in comparing whether 2.5% tails of the distribution overlap. We additionally measured a set of physiological endpoints (mass, glucose, trehalose and triglycerid levels) at the start of the experiment and after 30 generations of selection in a metapopulation context as indicators for mite performance. All physiological parameters were measured following (Laparie et al. 2012) on F2 mites (see suppl. material S2).

## DIFFERENTIAL GENE EXPRESSION AFTER EXPERIMENTAL EVOLUTION

To examine the effects of metapopulation structure on the mite transcriptome, Agilent dual colour gene expression micro-array analyses were performed on female F2 mites (raised for two generations in common garden) of every selection regime. The microarray data have been deposited in the Gene Expression Omnibus (GEO) (accession number: GSE55623). For the hierarchical clustering, data of previous *T. urticae* studies were incorporated (Bryon et al. 2013, Zhurov et al. 2014). Final processing and analysis was conducted in limma (Smyth 2005). GO-term annotation was performed using Blast2GO (Conesa *et al.* 2005). Within Blast2GO software, a Gene Set Enrichment Analysis (GSEA) was executed by Fisher's exact tests using the completely annotated *T. urticae* genome as the reference set. More details of the gene expression and GO-term analysis are provided in suppl. material S2.

## PERFORMANCE ON A CHALLENGING NEW HOST

Our LS-VL base population has been maintained on bean for more than 10 years. Our expectation was that changes in spatial structure affect population dynamics through increased population sizes in the SPA metapopulation and patch deterioration in the TEM metapopulation, which we assessed via performance measures in novel environments. This was done by quantifying isofemale growth rate on tomato (*Solanum lycopersicum;* variety Moneymaker) grown under controlled laboratory conditions (23°C, 16:8 L:D photoperiod). Experimental arenas were constructed with leaves from 4-week old tomato plants. Moist tissue paper was used to cover 10 cm² leaf edges that prevented mites from escaping. Twenty fertilized F2 females (raised for two generations in common garden to reduce maternal effects) from each artificial metapopulation were placed on a leaf-arena and allowed to establish a

population. All leaf-arenas were kept under controlled conditions (23°C, 16:8 L:D photoperiod). Population growth was assessed weekly for 3 weeks by counting the number of eggs, juveniles, adult males and females.

## STATISTICAL ANALYSIS

Because the measured traits follow different statistical distributions, we first tested for multivariate differences in the measured traits after experimental evolution by applying a Permutational Multivariate Analysis of Variance (PERMANOVA). Because our measurements were taken with different units on different scales, the correctly estimated replicate-level averages of the life history and physiological endpoints (see GLMM further) were scaled prior to PERMANOVA analysis based on Euclidean distances among replicates belonging to one of the three metapopulation treatments (PERMANOVA; with ADONIS function in R; Anderson 2001). To visualise metapopulation divergence based on life history, Nonmetric Multidimensional Scaling (NMDS) analyses were performed on the scaled distance matrix (all life history and physiological traits) using the METAMDS function (vegan library, R.2.15.1;). The significantly diverging traits were subsequently identified by a Multivariate Analysis of Variance (MANOVA) on the scaled averaged data per replicate (GLM procedure; SAS Institute Inc 2006).

We examined how metapopulation type affected the different life history traits and physiological endpoints using generalized linear mixed models (GLMM). The model included metapopulation type (HOM, SPA, TEM) as fixed factor and each individual metapopulation as a random effect to control for dependence among replicates from each metapopulation treatment. Depending on the dependent variable, a Gaussian (all physiological endpoints), Poisson (fecundity, developmental time, longevity and population size on the novel host) or binomial error (sex ratio, juvenile mortality) structure was modelled with appropriate link functions. We corrected for potential overdispersion by modelling residual variation as an additional random factor. Non-significant contributions ($P>0.05$) were removed by backwards procedure and validated by model selection. Effective degrees of freedom were estimated using Kenward-Rogers procedure. All analyses were conducted with SAS 9.3 (SAS Institute Inc 2006) by using the GLIMMIX procedure.

# RESULTS

## POPULATION-LEVEL DIVERGENCE IN LIFE HISTORY TRAITS

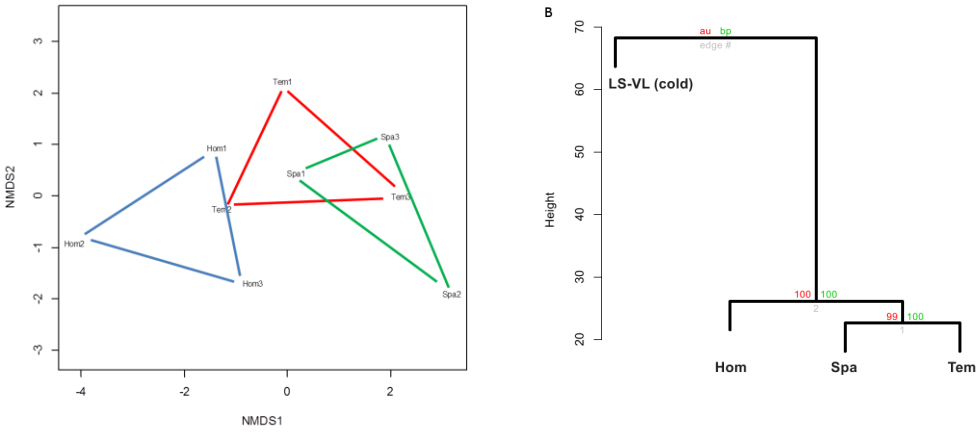
Experimental evolution caused significant divergence in life history traits of the variable treatments with the homogeneous treatment (PERMANOVA $F_2$= 2.75; p=0.03) (Fig. 5.1A). Sex-ratio (MANOVA $F_2$=7.77; p=0.02) and fecundity (MANOVA $F_2$=10.35; p=0.01) are the two main life history endpoints causing these differences using this conservative statistical procedure. An analysis of the trait variation at the start of the experiment is provided in supplementary material S3.

A detailed analysis on the individual trait distribution after experimental evolution confirmed divergence in fecundity and sex ratio, but also in longevity (Table 1). The average proportion of male offspring (Fig. 5.2A) was higher in clutches originating from the SPA metapopulations (0.34 ± 0.02SE) relative to the HOM (0.26 ± 0.02SE). Both daily fecundity (t=-3.79; p=0.01) and total fecundity (t=-3.53, p=0.0014) was lower in homogeneous metapopulations compared to mites from the SPA and TEM metapopulation (Fig. 5.2B).

Mites that evolved in the TEM metapopulations died earlier (after 9.65 ± 0.42SE days) than mites from homogeneous (HOM) metapopulations (after 11.24 ± 0.45SE days) and spatial variable (SPA) metapopulations (after 11.62 ± 0.47SE days) (Fig. 5.2C). Under the prevailing lab conditions, males developed in 7.99 days on average while the female reached maturity after 8.40 days (t=-3.28; p=0.0010). Mites from homogeneous metapopulations reached maturity earlier (7.97 ± 0.12) than mites from the spatially heterogeneous (SPA) metapopulation (8.43 ± 0.11) (t=-2.85; p=0.012) (Fig. 5.2D). The interaction between sex and treatment was not significant ($F_{2,2228}$=0.25; p=0.78). No significant differences in juvenile survival of mites among treatments were observed ($F_{2,7.201}$=0.25; p=0.79), and no differences were detected in aerial dispersal propensity ($F_{2,5.185}$=0.02; p=0.98).

The simulated growth rate at the start of the experiment was 3.56 (SD=0.19). After experimental evolution, growth rates were slightly lower in the homogeneous metapopulation treatment relative to the other two, but this difference was not significant based on the inferred 95% confidence intervals ($r_{Hom}$=3.38, SD=0.21; $r_{TEMP}$=3.52; SD=0.20; $r_{SPA}$=3.55, SD=0.19).

***Figure 5.1: Visualization of the life history data.*** *A: nonmetric multidimensional scaling of the metapopulations showing dissimilarities between metapopulations based on 'Euclidean distance', B: Hierarchical clustering of the transcriptomic profiles of the three metapopulation types and the ancestral mite population living in cold conditions (17°C), relative to a reference strain (London).*




## DIVERGENCE IN PHYSIOLOGICAL ENDPOINTS

Although not significant ($F_{2,32}$= 3.08; p=0.06), a trend towards a lower mass per 50 mites was observed for mites from homogeneous metapopulations (424 ± 25SE µg) compared to mites from metapopulations with spatial (510 ± 25SE µg) or spatiotemporal variation (441 ± 31SE µg). Glucose levels were significantly different among the metapopulation treatments ($F_{2,67}$=3.52; p=0.03; Fig. 5.3), with the lowest levels for HOM (1.39 ± 0.25SE) relative to those from SPA (2.33 ± 0.25SE) (t=-2.64; p=0.027). No significant differences in trehalose ($F_{2,60}$=0.43; p=0.51) or triglyceride level were observed among treatments ($F_{2,56}$=2.07; p=0.14).

***Figure 5.2: Effects of variation in metapopulation structure on life history parameters (mean values ± SE) of mites.*** *A: longevity, B: total fecundity, C: sex ratio (males/total clutch size), D: developmental time. Dotted lines represent parameter values before 30 generations of selection. Equal notations indicate non-significant contrast for the respective measurements. Error bars represent standard errors.*

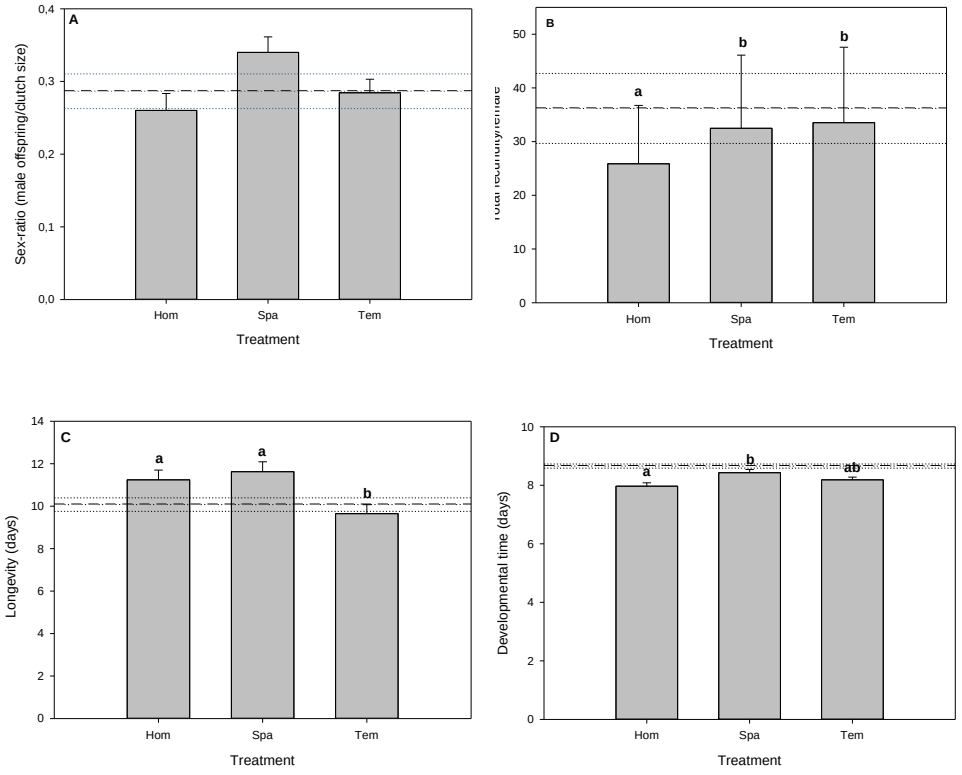
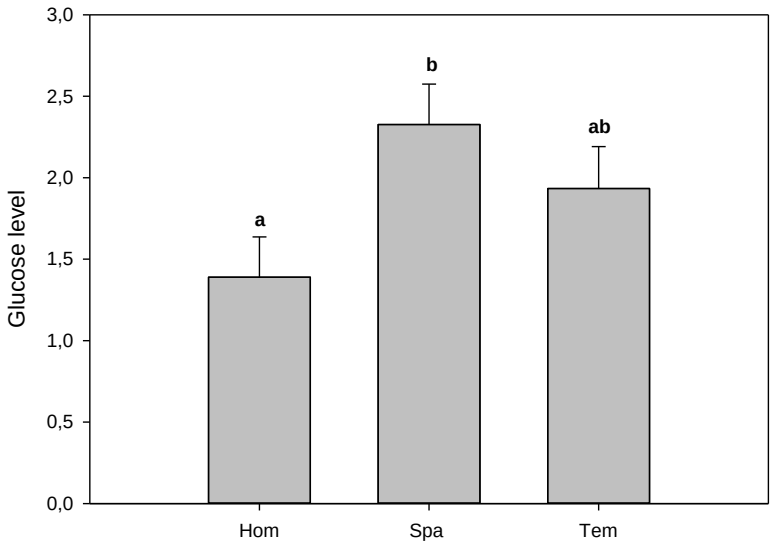


***Figure 5.3: Effects of variation in metapopulation structure on glucose level (nmol) per 50 mites (mean values ± SE).****Equal notations indicate non-significant contrast for the respective measurements. Error bars represent standard errors.*

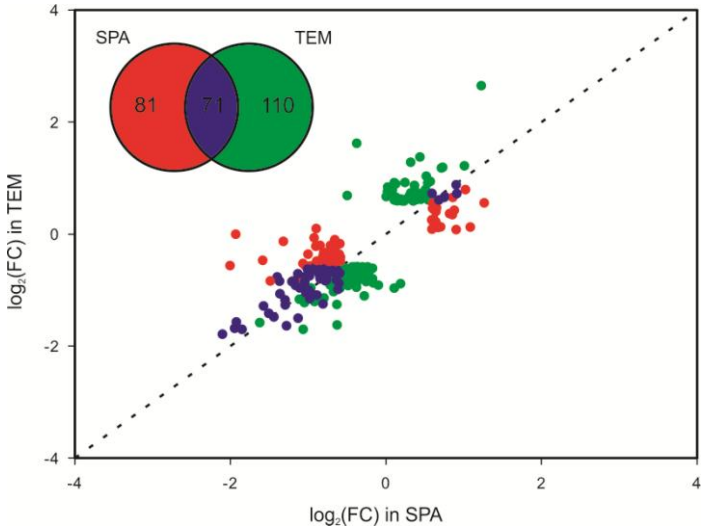



## DIVERGENCE IN GENE EXPRESSION

Based on adult female mite transcriptomes, SPA and TEM treatments diverged from the control HOM treatment, but SPA and TEM converged mutually (Fig. 5.1B). Using the HOM lines as a reference, we found 152 and 181 differentially expressed genes in SPA and TEM lines, respectively (Fig. 5.4). Fig. S2 depicts the expression patterns of the three replicated lines within the treatments separately. These transcriptional differences were not the result of environmental or maternal factors since prior to expression analysis, all mites were reared in identical conditions for two generations. Of these differentially expressed genes, 81.6% and 70.7% exhibited down-regulation in SPA and TEM, relative to HOM respectively (Fig. 5.4, Fig. S2). Pearson correlation indicated that the differential transcript levels in SPA and TEM were significantly correlated ($\rho$=0.80, df=260, $p<22e^{-16}$). A statistical analysis did not find significantly different transcript levels when SPA and TEM were contrasted directly.

Using Blast2GO, a total of 164 GO-categories linked to biological processes were assigned to the heritable transcriptional changes of TEM and SPA. Approximately half of these GO-terms (84) were present in both selection regimes. The most abundant GO-terms in the up- and down-regulation are shown in Fig. 5.5. In addition, GSEA revealed significant enrichment of GO-terms in the transcriptional response of the SPA and TEM treatments. Table S1 lists the significantly enriched GO-terms in the down-regulation of both regimes. Of interest among the genes connected to the significantly over-represented GO-terms were genes encoding for enzymes of the carbohydrate and citric acid metabolism.

***Figure 5.4: Scatterplot showing the $log_2$(FC) of the differentially expressed genes in SPA and TEM.*** *A venn-diagram depicting the number of differentially expressed genes in the TEM and SPA lines, relative to HOM.*

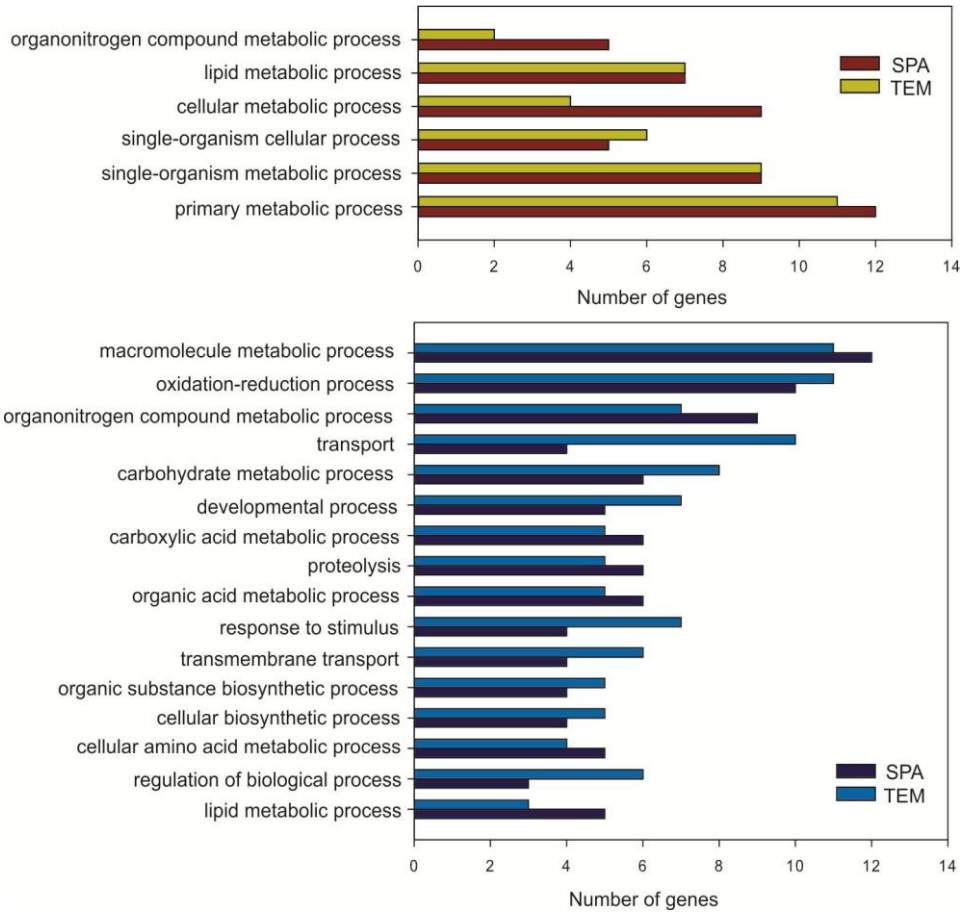



## POPULATION PERFORMANCE ON NOVEL HOSTS

After one week of challenging the novel host, the number of alive mites originating from the different metapopulation treatments did not differ significantly among metapopulation types (HOM: 6.33 ± 0.38SE alive females, SPA: 7.49 ± 0.43SE alive females, TEM: 6.66 ± 0.40 alive females) ($F_{2,122}$=2.19; p=0.12). However, significant differences in fecundity were observed ($F_{2,122}$=66.81; p<0.0001), with a lower number of deposited eggs

in mites that evolved in the homogeneous populations (49.33 ± 1.07SE eggs) relative to SPA (68.29 ± 1.29SE eggs) and TEM (55.83 ± 1.17 eggs). All pairwise differences were significant (Fig5..6A). After three weeks, the first cohort of offspring matured which differed in population size among treatments ($F_{2,5.635}$=5.83; p=0.04; Fig. 5.6B). Again, population sizes were lowest in mites originating from HOM (5± 0.90SE) relative to SPA (10.26 ± 1.71SE) and TEM (10.62 ± 1.77SE).

***Figure 5.5: Overview of the most abundant GO-terms connected to the proteins encoded by differentially expressed genes*** *(red: up-regulation, blue: down-regulation). Only the GO-terms with at least 5 members in either the transcriptional response to SPA or TEM selection are shown.*



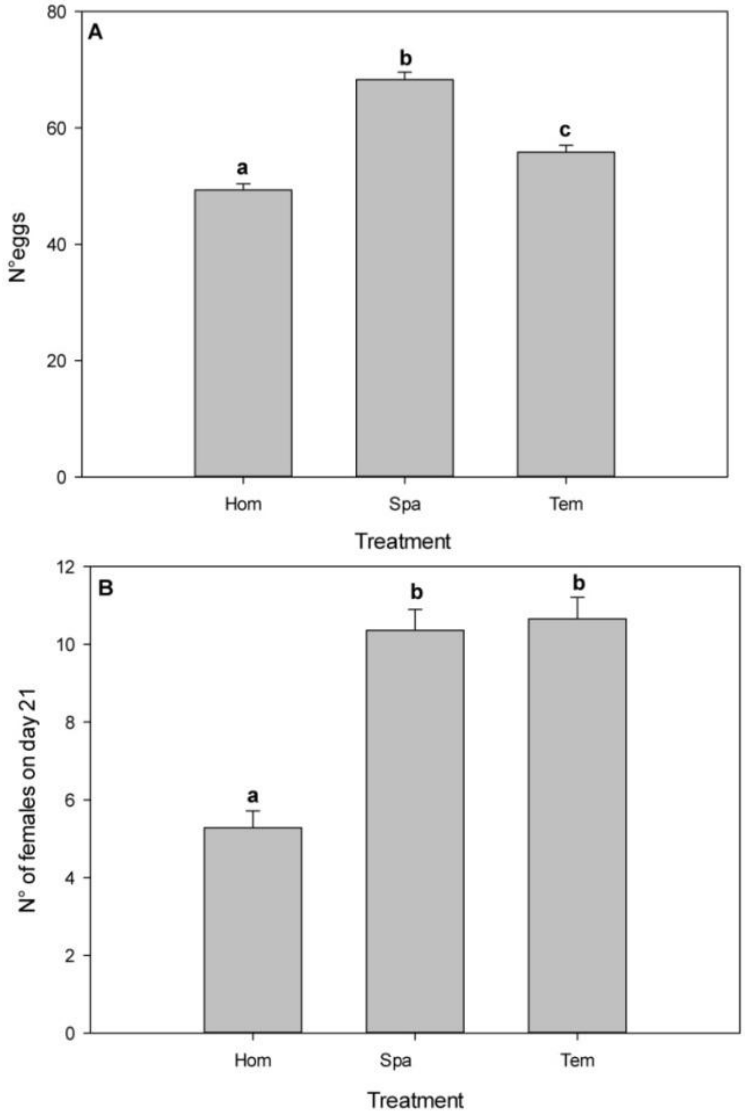



***Figure 5.6: Effects of long-term evolution in the different metapopulation contexts on population growth on a novel host (mean values ± SE).*** *A: number of eggs after one week, B: number of female offspring reaching adulthood after 21 days. Equal notations indicate non-significant contrast for the respective measurements. Error bars represent standard errors.*

## DISCUSSION

While there is an increasing awareness that changes in spatial structure affect population dynamics, and that these ecological dynamics interact with evolutionary trajectories, there is no profound understanding of how these eco-evolutionary dynamics are governed by metapopulation-level selection pressures. While the few available long-term field studies point at the interplay between local adaptation and gene flow among patches (Farkas et al. 2013), or at coupled eco-evolutionary dynamics by non-random dispersal (Hanski & Mononen 2011), the vast majority of theory has focused so far on the evolution of single life history traits like dispersal (Hanski & Mononen 2011) or aging (Ronce & Olivieri 1997) in response to changes in metapopulation structure. Experimental manipulations of demographic properties suggest the possibility of life history evolution, for instance after harvesting (Cameron et al. 2013), adjusting local mate competition (Macke et al. 2011) or dispersal (Friedenberg 2003), but to date, no metapopulation-level experimental evolutionary studies have been contrasted with the available theory.

By explicitly manipulating the spatial structure in a metapopulation, we demonstrate that changes in spatial structure alone induce evolutionary divergence in life history traits as well as a coordinated divergence in gene expression potentially underlying these traits. More precisely, compared to homogeneous metapopulations mites evolved an increased fecundity in the spatiotemporal (TEM) and spatial (SPA) variable metapopulations, in addition to a shortened lifespan in spatiotemporal variable metapopulations (TEM) and a decreased developmental rate in spatial variable metapopulations (SPA). The observed high patch extinction rates due to temporally unpredictable resource availability in the TEM metapopulations (Supplementary material S1) thus likely altered the pattern of resource allocation between survival and reproduction in mites (Magalhaes *et al.* 2007), leading to the evolution of *r*-strategic traits (Ronce & Olivieri 1997). In contrast, SPA metapopulations experienced more k-like selection resulting in a higher longevity, more male-biased sex ratio's, slower development but not reduced fecundity (Reznick et al. 2002).

In classical metapopulations characterised by high patch turnover, selection for increased dispersal or longer dispersal distances was expected (Ronce et al. 2000). Surprisingly, such patterns were not detected by our behavioural assays. For practical reasons, we were only able to test dispersal propensity under single standard conditions,

thereby potentially missing the proper environmental conditions (e.g., density, relatedness, sex ratio; see Bitume et al. 2013) under which differentiation is detectable. Neither were elevated dispersal rates in TEM detected at the metapopulation level (see supplementary material S1). It is however important to recognize that multiple factors (e.g. kin competition, extinction risk, dispersal costs) are known to impose selection on dispersal, sometimes acting in opposite directions (Bonte et al. 2012).

The behavioural trials neither demonstrated reduced dispersal behaviour in the mainland-island metapopulation (SPA), although more detailed analyses of the mite population dynamics indicated overall lower dispersal rates in the SPA metapopulation (see supplementary material S1). Individual mites evolved in the SPA metapopulation less female biased sex-ratios relative to the two other types of metapopulations. Female spider mites can control sex-ratio by altering egg size and subsequent fertilization success, with more unfertilized eggs leading to a more male-biased sex ratio. As such sex ratio changes are subject to evolutionary changes (Macke et al. 2011) and are known to evolve in response to local mate competition theory (Macke et al. 2011). In the SPA metapopulations, patch specific emigration/immigration ratios are larger relative to those in classical metapopulations, in combination with potentially evolved dispersal rates, leading to higher levels of relatedness within patches. These conditions are expected to select for more female biased sex-ratios, so evolution into the opposite direction than demonstrated in our experiment. Such a strategy combined with reduced developmental rates does not optimize lifetime reproductive success. Combined with the increased longevity, this indicates a shift in the trade-off between survival and reproduction towards survival (Magalhaes et al. 2007).

In contrast, local mate competition theory can be expected to have induced the evolution of more female-biased sex rations in the homogeneous metapopulation. In these metapopulations, patch-extinction rates were lowest. This stability together with overall low immigration rate can here have increased relatedness substantially. We unfortunately have no data on relatedness available. The induced spatial and spatiotemporal variation consequently induced a divergence of life history traits relative to the more stable patchy metapopulation used as a reference. We attribute this evolved divergence to changes in demographic conditions. Clearly, these multivariate evolutionary dynamics have not optimised population

growth rates since they remained similar in all three types of metapopulation. The evolved overall higher fecundity rates in the TEM and SPA metapopulation should, however, increase resource competition during development (Persson & De Roos 2013) since, in contrast to adults, juvenile males and females equally compete for food, and are clumped distributed on the leaves. Indeed, evolved physiological responses combined with transcriptomic analyses suggest that life histories evolution in the SPA and TEM metapopulations prompt an important general stressor related to starvation. Indeed, elevated glucose levels from the SPA and TEM metapopulations is associated with responses to cope with increased starvation and likely related to food stress resistance (Laparie et al. 2012). While under food-unlimited conditions, a positive relation between metabolic rate and the levels of circulating sugars has been detected (Bozic & Woodring 1997), a restricted access to sugars likely results in an opposite relation (Packard & Boardman 1999). The decreased developmental rates, the transcriptional response of sugar metabolism genes and the higher glucose levels of stressed mites in this study are in concordance with this assumed negative relationship.

In arthropods and other animals, genes that transcriptionally respond to environmental stressors repeatedly encode for basal metabolic enzymes, with enzymes of the gluconeogenesis/glycolysis and citric acid pathways as one of the prime targets (Marden 2013a). These core genes have multifaceted roles; in addition to potentially altering metabolic flux rates (rate of turnover of molecules through a metabolic pathway) by the heritable transcriptional changes, its metabolites can also affect signalling pathways. Because of these multifaceted roles, variations in these core genes can greatly influence fitness by traits associations. For instance, in the Glanville fritillary butterfly, of which the genome is recently uncovered (Ahola et al. 2014), differential allelic composition with a correlated differential expression of succinate dehydrogenase (SDH) regulates the activation of the HIF-1a transcription factor. By this regulation, SDH expression influences flight muscle morphology and flight performance, making the SDH gene a favoured target for selection (Marden et al. 2013b). However, the most studied and important gene encodes for PGI, a phosphoglucose isomerase, which has been associated with numerous life history traits in various arthropod species (Wheat 2012). For instance, dispersal behaviour, oviposition, development, lifespan and basal metabolic rate are all influenced by *pgi* (Hanski & Mononen 2011, Wheat 2012).

We argue that the life history changes observed in this study are at least (partially) determined by the heritable changes in gene expression in the different spider mite metapopulations. *T. urticae* genes involved in the citric acid cycle, gluconeogenesis and glycolysis were part of the heritable transcriptional response to the induced TEM metapopulation selection with corresponding GO-terms (GO:0005975 and GO:0004108) being significantly enriched in TEM (Table S1). In Fig S3 an overview is presented that focuses on these core pathways. Surprisingly, one of the *T. urticae* genes with altered transcript levels after selection in the TEM and SPA metapopulations, glucose 6-phosphatase (G6Pase) (*tetur33g00210*), shares a substrate (glucose 6-phosphate) with the protein encoded by the *pgi* gene. In *Drosophila,* studies suggest that G6Pase could control the metabolic flux by showing adaptive evolution of G6Pase (Flowers et al. 2007). The observed transcript variation could thus influence the metabolic flux in *T. urticae*. The G6Pase variation could even affect the overall carbohydrate metabolism in *T. urticae* as the concentrations of its metabolites impact signalling pathways controlling this (Diaz-Moralli et al. 2012).

*T. urticae* is known to alter the transcription of horizontally transferred genes in heterogeneous, challenging conditions (Dermauw et al. 2013, Wybouw et al. 2014). The hypothesis that such laterally acquired genes are employed for surviving environmental stress is supported in this study as these genes constituted a part of the transcriptional evolution. Among the differentially expressed genes connected to the significantly enriched GO-term of carbohydrate metabolism (GO:0005975) (Table S1), were two genes encoding for glycoside hydrolases (*tetur29g01280* and *tetur29g01230*) that were laterally transferred from bacteria to the mite genome (Grbic et al. 2011) (Figure S3). These hydrolases are crucial for many arthropod herbivores to survive suboptimal nutrition (Kirsch et al. 2014). Moreover a horizontally transferred methionine synthase gene (*tetur16g00990*) and genes encoding for enzymes catalysing reactions upstream in the methionine synthesis pathway showed correlated down-regulation (Figure S4).

Most likely, an elevated competitive stress during juvenile development due to (i) higher evolved daily fecundity in TEM and SPA metapopulations and (ii) higher patch extinction due to leaf deterioration in TEM caused the observed altered metabolic responses in both types of metapopulations. These metabolic changes are a common stress response (Parsons 2005). Hence, adaptive responses to one stressor consequently lead to the development of cross-tolerance in

organisms, enabling them to cope with new stressors that have never been experienced. Also, population expansion or metapopulation dynamics in a heterogeneous world inevitably stresses organisms since environmental conditions continuously change from an individual or lineage-level perspective (Parsons 2005). In our study, stressors resulting from such changes of the spatial structure, pre-adapt mites to cope with a challenging novel host. Such eco-evolutionary dynamics are then likely to have a strong impact on community- and food web dynamics under natural conditions (Farkas et al. 2013).

To date, few studies have investigated the eco-evolutionary feedback under controlled environmental change. Here, we demonstrate that spatial variation in habitat configuration creates divergence in life history and transcriptome evolution. This selection is suggested to be induced by metapopulation-level variation in competition and patch extinction rates. Besides life history evolution, we observed variation in physiological endpoints, but also divergent patterns in gene expression. Both spatial and spatiotemporal heterogeneous metapopulations induce spatial stress that pre-adapts the mites towards a better performance on novel challenging hosts. In conclusion, we demonstrated the impact of spatial configuration on evolutionary dynamics in metapopulations that move beyond dispersal evolution. But more importantly, we witnessed the eco-evolutionary feedbacks towards tolerance of a novel challenging host. These adaptive dynamics might lead to evolutionary rescue (Carlos et al. 2014) when habitat fragmentation is accompanied by sudden changes in habitat quality.

## ACKNOWLEDGEMENTS

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# SUPPLEMENTARY MATERIAL

## SUPPLEMENTARY FIGURE LEGENDS

**Figure S1.** Effects of variation in metapopulation structure on dispersal propensity (mean values ± SE) of mites. Equal notations indicate non-significant contrast for the respective measurements. Error bars represent standard errors.

**Figure S2.** Expression heatmap depicting the expression levels of the three biological lines within the TEM and SPA treatments, relative to the HOM reference. Fold changes were $\log_2$ transformed. Genes were clustering using Euclidean distance (Ward-method).

**Figure S3**. Overview of the differentially expressed genes in the TEM (red) and SPA (orange) lines, relative to HOM, encoding for proteins of the citric acid cycle, glycolysis and gluconeogenesis**.** The reaction catalysed by the phosphoglucose isomerase enzyme is indicated by PGI. Mitochondrial localization is depicted by a grey background. The citric acid pathway is indicated by a dotted black line.

**Figure S4.** Overview of the differentially expressed genes encoding for proteins of the methionine synthesis pathway through one carbon metabolism in the TEM (red) and SPA (orange) lines, relative to HOM. *Tetur16g00990* is a laterally acquired gene from bacteria in the spider mite genome (Grbic et al. 2011).

## SUPPLEMENTARY TABLES

**Table S1.** Significantly enriched GO-terms in the combined down-regulation of TEM and SPA, relative to HOM as identified by the GSEA.

| GO-ID | Term | FDR | P-Value | presented |
|---|---|---|---|---|
| **GO:0042302** | structural constituent of cuticle | 4,90E-04 | 1,60E-07 | over |
| **GO:0005515** | protein binding | 4,90E-04 | 1,80E-07 | under |
| **GO:0044260** | cellular macromolecule metabolic process | 8,02E-04 | 3,69E-07 | under |
| **GO:0031409** | pigment binding | 0,001365 | 1,26E-06 | over |
| **GO:0005634** | Nucleus | 0,011438 | 1,37E-05 | under |
| **GO:0050794** | regulation of cellular process | 0,020216 | 2,60E-05 | under |
| **GO:0055114** | oxidation-reduction process | 0,03712 | 5,12E-05 | over |
| **GO:0005975** | carbohydrate metabolic process | 0,037261 | 5,48E-05 | over |
| **GO:0044763** | single-organism cellular process | 0,041129 | 6,63E-05 | under |
| **GO:0006139** | nucleobase-containing compound metabolic process | 0,041129 | 6,81E-05 | under |
| **GO:0004108** | citrate (Si)-synthase activity | 0,048703 | 8,96E-05 | over |
| **GO:0004099** | chitin deacetylase activity | 0,048703 | 8,96E-05 | over |

## SUPPLEMENTARY MATERIAL S1: POPULATION DYNAMICS

### METHODS

Local population extinction was assessed weekly by counting the number of extinct patches. Density-dependence of local populations was estimated as the slope of the relation between population growth rate ($\ln(N_{t+1}/N_t)$) and log population size ($\ln(N_t)$).

Dispersal at the metapopulation level was estimated weekly by counting the number of mites on 12 rectangles (2 x 3 cm²) of tanglefoot randomly placed in the metapopulation boxes. Dispersal propensity was defined as the number of mites on the rectangles relative to the estimate of population density.

Population densities of the different life stages (eggs, juveniles and adults) were estimated weekly by counting the number of mites of all life stages (eggs, juveniles and adults) on 3 randomly chosen $cm^2$ of each local population. Variation in spider mite local population size was compared using the coefficient of variation (CV).

A detailed analysis and report of the local and metapopulation dynamics is in preparation.

### RESULTS

Population dynamics were significantly different in the three types of metapopulations. The proportion of local populations going extinct was significantly affected by metapopulation structure ($F_{2,5.166}=10.77$; $P=0.01$). The average proportion of extinctions was higher in spatio-temporal variable metapopulations (TEM) (0.25 ± 0.096SE) than in homogeneous (0.011 ± 0.0067SE) and spatial variable metapopulations (0.027 ± 0.015SE).

Dispersal rates also differed between metapopulation types ($F_{2,11.74}=29.97$; $P<0.0001$) with the lowest dispersal rate (0.0065 ± 0.0011SE) in spatial variable metapopulations compared to spatiotemporal (0.016 ± 0.0025SE) and homogeneous metapopulations (0.03 ± 0.0046SE) (all pairwise differences were significant) (Figure S1). The coefficient of variation of the average local population size across time differed among treatments ($F_{2,63}=14.91$; $P<0.0001$). Variation in local population size was higher in spatio-temporal variable metapopulations (1.26 ± 0.055SE) than in homogenous (0.99 ± 0.055SE) and spatially heterogeneous metapopulations (0.80 ± 0.067SE).

# SUPPLEMENTARY MATERIAL S2

## METHODOLOGIES

### AERIAL DISPERSAL ASSAY

To evaluate the effect of metapopulation context on the rearing dispersal behaviour, mated females were transferred to test arenas for trials of aerial dispersal after one whole generation on bean to minimise maternal effects. The same setup as used by (Li & Margolies 1993; Li & Margolies 1994) was applied. The test arenas consisted of 1cm² black painted plastic discs located on soaked cotton dishes in order to avoid escape of the mites. Mites of the three treatments were placed on one cm² disc and test arenas were provided with an upward (30°) wind current of 1.5 m/s (produced by a fan) and a strong light source (990 lumen) at the source of the air current. As mites perform rearing behavior before entering the air column, we were able to count and select the number of mites performing this behaviour (for at least 5 seconds) in the subsequent 3 hours. Mites that showed this behaviour were immediately removed in order to avoid double records. We simultaneously tested mites originating from metapopulations with the three treatments.

### PHYSIOLOGICAL TRAIT ASSAYS

Due to the extremely low mass of a single mite, 50 one-day-old females were pooled together for each sample, and all samples were snap-frozen at -80°C immediately after mite collection. Fresh mass of the pools was measured using a microbalance accurate to 0.01mg. The concentrations of triglycerids (TGs), glucose and trehalose were measured following the protocol of Laparie *et al.* (2012). Samples were homogenised in 300 µl of methanol-chloroform solution (2:1, v:v for sugars; 1:2, v:v for TGs) with a bead-beating apparatus (Retsch MM301, Retsch GmbH, Germany) for 60 s at 30/s frequency. For sugars, 200 µL of ultrapure water was added to each sample, further vortexed and centrifuged at 8000g for 10 min at 4°C. 300 µL of the upper aqueous phase containing the sugars were transferred to microtubes, and dissolved in 200 µL of ultrapure water before analysis. For TGs, samples were stored at -20°C for 12 h after the homogenization with the bead-beating. Then, 60 µL of KCl (2g/L) were added, and the samples were incubated for 5 min at 40 °C. One hundred and 50 µL of the lower phase (containing lipids in chloroform) were transferred to microtubes and dried at 30°C under a nitrogen stream. Eventually the residual lipids were dissolved in 100 µL of Triton X 100 solution (0.2%) and delipidated BSA (3%). Finally samples were vortexed and incubated for 10 min at 60°C

before metabolic assays. Spectrophotometric assay kits (K-TREH, Megazyme International Ireland Ltd. and Triglyceride assay kit, Cayman Chemical Company, Ann Arbor, MI, USA) were used to measure glucose, trehalose, and triglycerides. Manufacturer's protocol was followed.

### MICRO-ARRAY AND BLAST2GO ANALYSIS

Mites of all three treatments underwent two whole generations under identical conditions (common garden) before sampling for transcriptome analysis. Of every independent biological replication, a sample was collected. RNA samples were extracted from 50 pooled one-to-two day old female mites using the RNeasy mini kit (Qiagen). The quality and quantity of the RNA were assessed by a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies) and by running an aliquot on a 1% agarose gel. RNA was labelled as previously described (Dermauw *et al.* 2013). RNA samples of SPA and TEM were labelled with cy5, while the three HOM samples with cy3. Hybridization of cRNA samples was performed as previously described (Dermauw *et al.* 2013). On every array, a cy3-labelled HOM sample was mixed with either a cy5-labelled SPA or a TEM sample. Slides were scanned with an Agilent Microarray High-Resolution Scanner and extracted with Agilent Feature Extraction software using the GE2_107_Sep09 protocol. The microarray data have been deposited in the Gene Expression Omnibus (GEO) (accession number: GSE55623). Data was then processed and analysed in limma (Smyth 2005). Background intensities were corrected by the "normexp"-method using an offset of 50 (Ritchie *et al.* 2007). A within- and between-array normalisation ("loess"- and "Aquantile"-method, respectively) was subsequently performed. In the linear modelling of the data, intra-spot correlations were incorporated (Smyth & Altman, 2013). Significant differentially expressed genes were identified by an empirical Bayes approach with cut-offs of the Benjamin-Hochberg corrected p-values and $\log_2$FC at 0.05 and 0.585, respectively. For the hierarchical clustering analysis, data of Bryon *et al.* (2013) and Zhurov *et al.* (2014) were incorporated. Here, the design on which the linear model was fitted compared our data and the same LS-VL strain in cold conditions (17°C, 60%RH) to a reference *T. urticae* strain (London) on bean at standard laboratory conditions (25°C, 60% RH). The obtained data was subsequently hierarchically clustered (Euclidean, ward) using the pvclust package (Suzuki & Shimodaira 2006).

Assignment of GO-terms to the differentially expressed genes and the full genome of *T. urticae* was excecuted using Blast2GO software v.2.6.6 (Conesa *et al.* 2005). An E-value cut off of $1e^{-15}$ was used for BLAST

analysis. Annotation was performed with a cut off of $1e^{-15}$ and updated using InterPro. A two-tailed Fisher's exact test was performed to identify enriched GO-terms in the differentially expressed genes using the full *T. urticae* genome as the reference. Only GO-terms with a Benjamini-Hochberg corrected p-value of lower than 0.05 were considered significantly enriched. The results were reduced to the most specific GO-terms.

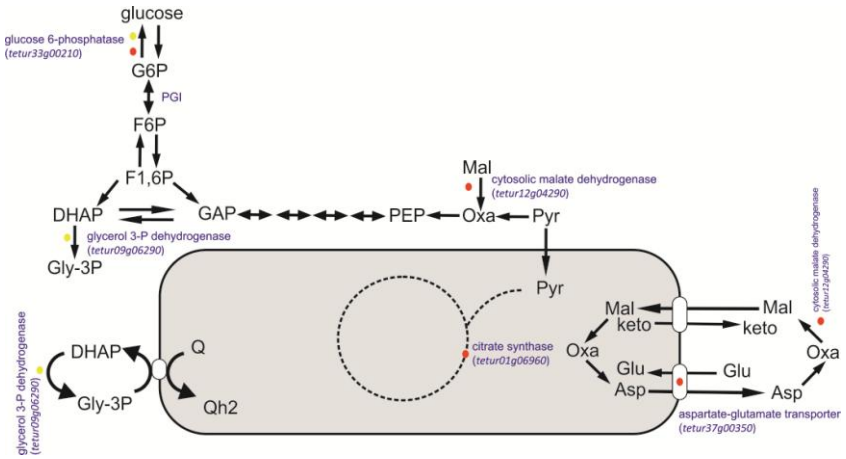
## SUPPLEMENTARY MATERIAL S3

### TRAIT VARIATION AT THE START OF THE EXPERIMENT

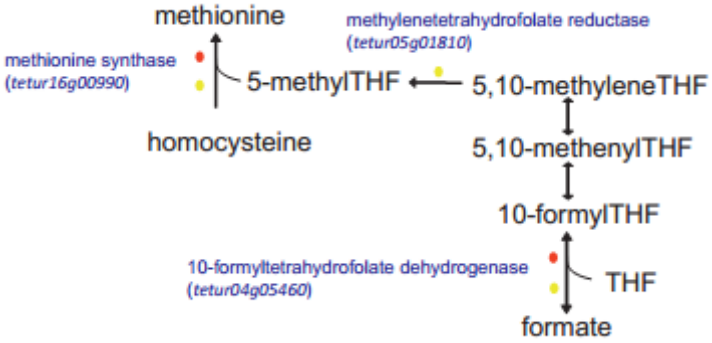
Mite from the base population lay on average 6.9368 ± 0.3132SE eggs per day. After 7 days their total fecundity was 36.2640 ± 3.3226SE eggs. A clutch contained on average 0.3225 ± 0.0806SE % males. 85.4629 ± 2.7823SE % of juveniles survived until adulthood. Mites developed in 8.6753 ± 0.0358SE days and had longevity of 10.1 ± 0.1599SE days.

## SUPPLEMENTARY FIGURES

### FIGURE S1




### FIGURE S2



# 6. GENERAL DISCUSSION

Annelies De Roissart

In this thesis we aimed to gain insight in the eco-evolutionary feedback between spatial structure, population dynamics and the evolution of life history traits including dispersal. A thorough understanding of these links allows making predictions about the distribution and resilience of species in a continuously changing world. In this last chapter, the obtained results will be evaluated and discussed. Additionally, pathways for further research will be recommended.

## OVERVIEW OF THE MAIN RESULTS

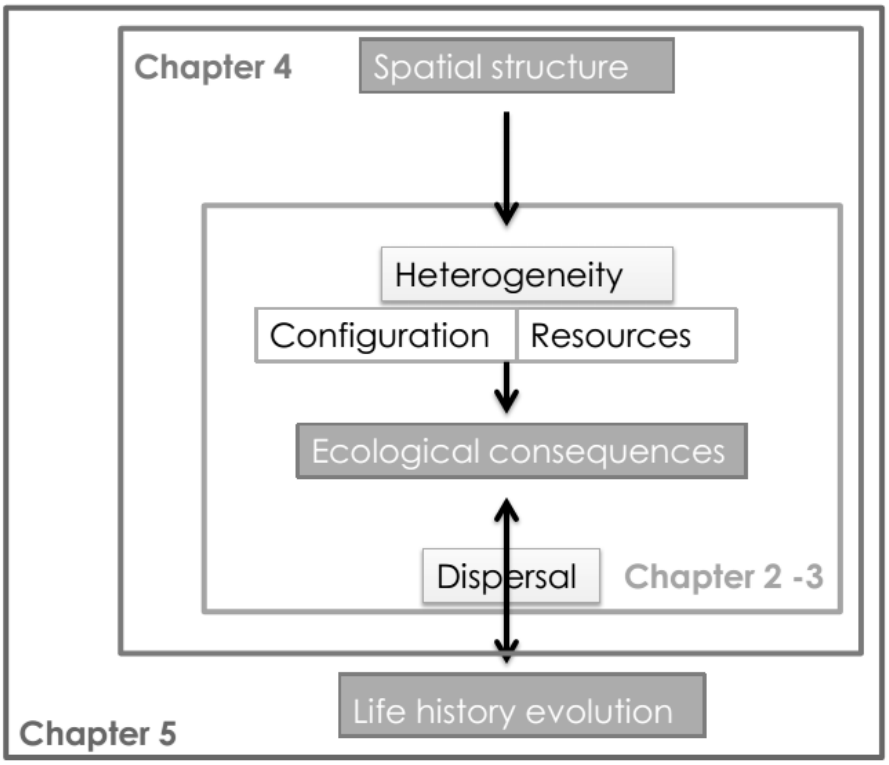
In **chapter 2,** the influence of heterogeneity caused by soil biota (the presence of root-feeding nematodes or arbuscular mycorrhizal fungi) on the aerial dispersal strategies of a phytophagous mite *(Tetranychus urticae)* was assessed using wind tunnel experiments. Spider mite pre-dispersal behavior significantly increased with the experienced mite density on the host during development. Additionally, belowground herbivory resulted in decreased water content and an increased spider mite aerial dispersal behavior. Since no change in the density threshold has been observed, increased dispersal rates appear to be caused by their development on plants with belowground nematode herbivory. Although, in our experiment, no declines in nutritional plant tissue quality and cyanogenic potential of nematode treated plants were detected (Ballhorn et al. 2007, Bonte et al. 2010), this does not rule out the prevalence of unidentified nitrogen-containing defensive metabolites (e.g. alkaloids or tannins) which may have caused the increased dispersal response.

Within **chapter 3,** the fitness-effects of dispersal were empirically assessed in a game-theoretical context. By a translocation experiment with mutant mites, we demonstrated that dispersing individuals are not a random subsample of the population. Our results indicate that philopatric individuals reach equal fitness under different density conditions when translocated to different dispersal distances. In contrary, dispersing individuals were able to maximize their fitness when reaching a novel less populated environment after dispersal. We thus provide the first empirical evidence that the optimization of phenotype-dependent dispersal maximizes individual fitness to such an extent that substantial additional dispersal costs can be levied.

The impact of spatial structure on metapopulation dynamics was investigated within **chapter 4** using artificial metapopulations. Our results demonstrate that spatial and temporal variation in habitat availability

impacts on local as well as regional density-dependent population dynamical properties and alters the density dependence of dispersal. The observed changes are probably caused by differing levels of resource abundance and competitive interactions. Furthermore, our results indicated that metapopulation stability was impacted by spatial structure through changes in synchrony and variability.

In **chapter 5,** we focused on the impact of spatial structure on the evolutionary dynamics in metapopulations. The spatial structure of artificial metapopulations was manipulated in order to assess the effect on life history evolution, stoichiometry and gene expression. We demonstrated that changes in spatial structure induce evolutionary divergence in life history. Besides life history evolution, we retrieved variation in physiological endpoints, but also divergent patterns in gene expression. Both spatial and spatiotemporal variability induce spatial stress that pre-adapted the mites towards a better performance on novel challenging hosts. The multivariate response points towards general adaptations in stress resistance pathways and is suggested to be driven by metapopulation-level variation in competition and patch extinction rates.




***Overview of the research chapters.*** *Spatial structure has an impact on the level of heterogeneity that an individual experiences. Heterogeneity can occur through differences in landscape configuration or through differences in the amount of resources (habitat quality). The level of heterogeneity of the habitat has consequences for the ecology of organisms which than impacts on the evolution of life history traits including dispersal.*

## THE CONSEQUENCES OF HETEROGENEITY

Both natural and anthropogenic processes often lead to the fragmentation or reduction of quality of habitat and forces many organisms to live in a heterogeneous landscape (Hanski 2011). Spatial as well as temporal heterogeneity in habitat structure and/or resources affect both within- and among patch dynamics resulting in a range of ecological consequences. Understanding how the temporal and spatial variability of habitat affects ecological processes like **extinction-colonisation dynamics**, **population regulation**, and **synchrony** is elementary in contemporary ecology.

The impact of spatial and temporal heterogeneity on the **regulation** of population size was demonstrated in **chapter 4.** As predicted by theory (Turchin 1999), our results indicate that internal density-dependent processes regulate population dynamics in spatially structured habitats. In our study population growth rate seemed to be negatively dependent of density which implies that differences in resource availability and/or inter- and/or intraspecific competition (Royama 1992, Dooley 2013) probably have negative consequences for the fecundity of the next generation (Turchin 1999). More specifically we found that spatial and temporal variation in habitat availability significantly affected the strength of density-dependence of population growth. In spatiotemporally variable metapopulations, the lagged colonization of empty patches caused a decrease in the strength of density-dependendence of growth.

Next to the effect of spatial and/or temporal variation in habitat structure on density dependence, the prevalence of for instance changes in **colonization-extinction dynamics** as an ecological consequence of heterogeneity is well documented. In **chapter 4** an increased level of local population extinctions was found in spatiotemporal variable metapopulations. A range of empirical as well as theoretical studies (Benton et al. 2002, Drake & Lodge 2004) demonstrated higher population extinction rates in temporally fluctuating relative to constant environments. These are predominantly caused by the increased stochasticity in the form of fluctuating resource levels (Bull et al. 2007). The external forcing of temporal variation in patch size by the manipulation of resources significantly increased variance in local population sizes and patch extinction rates. Evidently, frequent population extinction followed by recolonization by few emigrants decreased the average population sizes and simultaneously increased (meta)population variability and **synchrony** in the spatiotemporally

variable metapopulations. This supports the theory that local density-dependent processes may as such impact population dynamics at the metapopulation level by their influence on the colonization-extinction balance and stability-parameters (Capucino 1995).

Since our results indicate that local as well as regional dynamics might be affected by heterogeneity, the relevance of an integrated knowledge of the ecological processes and mechanisms underlying metapopulation dynamics is accentuated.

## DISPERSAL IN RESPONSE TO HETEROGENEITY

Completely blended populations in homogeneous environments hardly ever occur. Instead, most populations exhibit some kind of spatial structure and are connected by the movement of individuals between patches. The study of dispersal is crucial for analyzing the way population dynamics respond to heterogeneity of the environment, whether caused by **biotic** (such as the presence of competitors or conspecifics) or **abiotic** (such as habitat degradation, loss or fragmentation or climate change)changes .

The impact of heterogeneity caused by differences in resource availability due to **interspecific** (the presence of nematodes or AMF) and **intraspecific** (density) biotic interactions on dispersal, was validated in **chapter 2.** The results of this chapter confirm that **interspecific interactions** with belowground nematodes can affect the dispersal strategies of aboveground herbivores and their eventual spatial population dynamics through changes in host plant quality (Sacket et al. 2010). Mechanisms behind these multitrophic-interactions are always plant-mediated and act through changes in host plant quality and/or the regulation of plant defense strategies (Masters & Brown 1997; Van der Putten et al. 2001). Although no effect on cyanogenic potential was found, belowground herbivory most probably induced a systemic response in the plants leading to the production of nonidentified secondary metabolites. As opposed to what we expected, no effects of AMF neither on dispersal propensity nor plant quality were observed. Interactions between plant mutualists, such as arbuscular mycorrhizal fungi (AMF) result in a positive plant response in most cases (Hoffmann et al., 2009) but a neutral or negative effect on the plant has also been documented (Graham and Abbott 2000; Reynolds et al. 2006, Sudova and Vosatka 2008, Hartley and Gange 2009, Koricheva et al. 2009). In our study, the neutral effect of AMF on the plant quality and dispersal propensity is most probably

related to our experimental design. During our experiments we used young 2-week old bean plants (Johnson et al. 1997). In these first weeks plants obtain their necessary resources from seed reserves and the loss of carbon to the fungus can decrease the allocation to photosynthesis or defense causing AMF to become parasitic (Johnson et al. 1997).

Heterogeneity of the environment caused by **intraspecific interactions** more specifically, the presence of conspecifics, can also impact on dispersal propensity. Population density is most probably one of the most well studied environmental variables affecting dispersal. A broad spectrum of empirical and theoretical studies analyzes the effects of density on the different aspects of the dispersal process (e.g. emigration, invasion, dispersal distance). Most studies indicate an increase of dispersal propensity with density (positive density dependence) in order to avoid a reduced fitness due to competitive interactions and local overpopulation (Li & Margolies 1993, Demeester & Bonte 2010). Bitume et al. (2011, 2013) demonstrated in their study on dispersal distance by walking in *T. urticae* that high densities can represent a stressfull environment to the mites and act as a cue for dispersal. This is confirmed in **chapter 2 and 3.**

Opposed to the positive density dependence of dispersal found in chapter 2 and 3, a negative relationship between dispersal propensity and density was observed at the metapopulation level (**chapter 4** – see further).

The impact of **heterogeneity caused by abiotic changes** in spatial structure also clearly affects dispersal dynamics. Spatial and/or temporal variable environmental conditions are predicted to impact on dispersal and have been examined in a range of theoretical studies (McPeek & Holt 1992, Olivieri et al.1995, Travis & Dytham 1999). Few examples of empirical research on this subject exist. Friedenberg (2003) demonstrated that dispersal of *Caenorhabiditis elegans* increased in populations that had undergone multiple extinctions. In the study of Strevens & Bonsall (2011) the impact of spatial and temporal heterogeneity was assessed in metapopulations of the bruchid beetle (*Callosobruchus maculates*). They illustrated in their study that different types of landscape heterogeneity had dissimilar effects on dispersal in metapopulations.

In **chapter 4** we empirically analyzed the impact of spatial and temporal variability of habitat on the dispersal propensity of spider mites in a metapopulation structure. Contrary to expectations and earlier work on dispersal at the local population level, a negative density-dependence

of dispersal at the metapopulation level was observed. We attribute the negative density dependence of dispersal to the interaction with body condition. Individuals in poor body condition can be constrained in dispersal and remain philopatric due to lack of sufficient reserves to levy the costs (Bonte et al. 2012). In the context of range-expansion Van Petegem et al. observed similar trends. In their experiment, over all latitudes, spider mites seemed to disperse more in a low-density compared to a high-density context. These results were validated by genomic analysis revealing a higher body-condition of dispersing compared to philopatric mites (Van Petegem et al. in prep.). The complex interaction between external conditions and the dispersal phenotype can reveal a completely different view on the emerging spatial dynamics. More generally, the deviating pattern from theoretical expectations emphasises the need to carefully evaluate model assumptions, as well as to remain aware of the often large simplifications relative to more natural situations.

In general dispersal is a complex process that will only be selected for when the benefits of leaving the natal habitat, outweigh the costs of staying (Clobert et al. 2009). While this principle of fitness maximization is well established in dispersal theory (Gyllenberg and Metz 2001, Poethke and Hovestadt 2002, Wild 2011), its empirical validation in nature is extremely difficult and studies that provide causal evidence are mostly lacking (Nevoux et al. 2013; Waser et al. 2013). Experimental approaches that try to validate fitness maximization theory by constraining emigration or transferring individuals may additionally fail to make inference of costs because such manipulations alter the population structure in both the natal and emigration environment and do not take into account multigenerational measures of fitness (Johnson and Gaines 1987, Hahne et al. 2011).

Fitness maximization through dispersal by walking was assessed in **chapter 3** where we demonstrated that philopatric individuals, when translocated over different distances, reach equal fitness under variable density conditions. Addtitionally we found that dispersive phenotypes increased fitness, by a factor of 4-5, when moving to new patches relative to the alternative strategy of remaining philopatric. We thus demonstrate that the optimization of dispersal maximizes fitness to such an extent that substantial additional dispersal costs can be incurred. Individuals that leave the environment in which they were born do so in order to maximize their fitness in the novel environment relative to the natal environment.

The results of **chapter 3** demonstrate that dispersing individuals are no random subset of the population, but differ from philopatric individuals in phenotype. Dispersing phenotypes benefited most from released competition at distant patches and would have experienced tremendous fitness-costs had they remained philopatric. The phenotypic differences between dispersing and philopatric individuals are the focus of many recent studies (Cote et al. 2010) and may or may not involve consistent suites of traits (see evolution in metapopulations) (Cote et al. 2010). Bitume et al. (2011, 2013) found similar results in their study on the link between relatedness, density and dispersal distance. In their dispersal trials they applied a comparable approach to the approach we used in **chapter 3.** A linear set-up of patches was constructed along which spider mites dispersed by walking. In accordance with our results they found indications for the presence of 'resident' and 'disperser' phenotypes.

In general, our results stress the need to consider competition with con- and heterospecifics as proximate drivers of individual dispersal (Clobert et al. 2009) and demonstrate that dispersal dynamics should not be studied as an isolated phenomenon but should rather be considered as an emergent property of a complex system comprising many interacting individuals and species. Due to the complexity of the system and the feedbacks within it, we should study the whole system in which the dispersal dynamics emerge.

Since some of our observed results deviate from theory, the need to carefully evaluate model assumptions is emphasized, as well as to remain aware of the often large simplifications relative to more natural situations.

## INCORPORATING EVOLUTION IN ECOLOGICAL RESEARCH

While ecological research focuses on the maintenance and implications of biological diversity, evolutionary research largely focuses on the processes that generate diversity (Post & Palkovacs 2009). Eco-evolutionary research encompasses the interaction between both (Hairston et al. 2005, Kinnison & Hairston 2007). Three types of interactions between ecological and evolutionary dynamics exist and were explored in this thesis: ecological change that influences evolutionary change, evolutionary change that influences ecological change and reciprocal influences between ecological and evolutionary changes called eco-evolutionary feedbacks (Hanski 2012).

The **impact of ecological change on evolutionary change** was assessed in a metaopopulation context (**chapter 5**). Metapopulation theory

suggests that the non-equilibrium conditions linked to spatial and genetic structure in metapopulations affect the evolutionary dynamics (de Jong et al. 2000). However, empirical work on the evolution of life history traits in a metapopulation context is relatively rare. Most of the research done has a theoretical background and does not include extinction-recolonisation dynamics typical of metapopulations but rather focuses on the evolution of dispersal propensity and aging (Friedenberg 2003, Fjerdingstad et al. 2007, Ronce & Olivieri 1997, Ronce et al. 2000). In **chapter 5** we found a clear effect of spatial structure on the evolution of variable life history traits other than dispersal in a metapopulation context. Additionally we illustrated a divergence in physiology and transcriptome evolution.

The **impact of evolutionary change on ecology** was investigated in **chapter 5**. The notion that evolutionary dynamics influence demographic population dynamics (ecology) is dual. Since the reproduction and mortality of individuals are partly determined by the genes, it is obvious that the genetic composition of a population should influence its demography. But according to another common knowledge, evolutionary changes occur so slowly that the demographic and evolutionary dynamics become effectively decoupled from each other (Hanski 2011). Nonetheless, population biologists are increasingly concluding that microevolutionary changes (changes in gene frequencies within populations) can be fast enough in natural populations (Thompson1998; Hendry & Kinnison 1999; Saccheri & Hanski 2006) to influence ecological population dynamics (Hanski & Mononen 2011). Our results indicated that the evolutionary changes caused by spatial and temporal heterogeneity, pre-adapted the mites towards better performance in novel ecological conditions. More specifically, mites confronted with spatial stress from spatial and spatiotemporal heterogeneous metapopulations performed better on novel challenging hosts.

**Eco-evolutionary feedbacks** are defined as the reciprocal interaction beween ecology and evolution such that changes in ecological interactions drive evolutionary change in organismal traits that in turn alter the form of ecological interactions and so on (Post & Palkovacs 2009). In **chapter 4** we found a clear effect of habitat heterogeneity on the ecological dynamics (population dynamics). Variation in these dynamics impacted on the evolution of diverse life history traits, physiology and transcriptome (**chapter 5**). Since these evolutionary changes pre-adapted the mites towards a better performance on novel

challenging hosts, this confirms the presence of an eco-evolutionary feedback. Although few studies have investigated the eco-evolutionary feedback under controlled environmental change, such eco-evolutionary dynamics are likely to have a strong impact on community- and food web dynamics under natural conditions (Farkas *et al.* 2013). Our results prove that eco-evolutionary feedbacks may strongly affect community processes by altering the ecological role of differentiated populations (Bailey et al. 2006, Whitham et al. 2006, Post et al. 2008). Moreover these adaptive dynamics might lead to evolutionary rescue (Carlson et al. 2014) when habitat fragmentation is accompanied by sudden changes in habitat quality.

## THE USE OF GENOMICS IN ECO-EVOLUTIONARY RESEARCH

Recently, advances have been made in large-scale gene sequencing technologies and the possibilities to acquire genetic data have exploded through the use of techniques like micro-array analysis, next generation sequencing technologies and the analysis of noncoding small RNA-fragments (Aubin-Horth & Renn 2009, Ellegren 2014).

The field of ecological and evolutionary genomics copes with the understanding of how the genome of an organism evolves under diverse environmental circumstances (Pavey et al. 2012). This leads to the field of 'integrative biology' in which ecology, evolution and genomics are united (Aubin-Horth & Renn 2009). The association of molecular and cellular mechanisms with ecological or evolutionary traits of interest in 'integrative biology' is two-sided. The use of molecular techniques on the one hand enhances our knowledge on gene functions and molecular mechanisms underlying ecologically important traits. While ecological studies on the other hand remain crucial for a comprehensive understanding of functional genomics (Aubin-Horth & Renn 2009).

An example of the applicability of genomics for eco-evolutionary studies lies in tracing the origin of adaptive traits by sequencing multiple genomes from related species. Substitutions can be mapped onto a phylogeny of species to identify in which node adaptive evolution has taken place (Pavey et al. 2012). This is critical for the comprehension of the link between evolution at the genetic and phenotypic level (Pavey et al. 2012).

In a recent study by Zhan et al. (2014), genomics were applied to unravel the evolutionary history of the monarch butterfly (*Danaus plexippus*) migration behaviour and its recent global dispersal. The monarch

butterfly is well known for its annual migration, recent global dispersal and characteristic orange warning colouration. Despite the multitude of studies, knowledge on the genetic basis of these traits is scarce. By analyzing the sequences of *Danaus*-genomes from around the world, the results of the study of Zhan et al. (2014) indicate that *D. plexippus* was ancestrally migratory and dispersed globally to occupy its broad distribution.

Another benefit of incorporating genomics of non-model organisms in eco-evolutionary research lies in the molecular study of plasticity. Novel genome-level molecular approaches help to address many questions related to the processes and mechanisms of phenotypic plasticity like the genomic make-up of plastic traits, the understanding of higher level biological processes involved and determination of the molecular machinery that interfaces the genotype and the environment (Aubin-Horth & Renn 2009).

However, simply correlating phenotypes with genotypes is often not enought to distinguish causation from consequence since gene expression itself can be plastic in response to environmental change (Côté et al. 2007). It might therefore be necessary to represent the different expression levels of a gene in different environments as a reaction norm.

Although it is now possible to study the genome of many different taxa, the possibility to thoroughly interpret this abundance of data is lagging behind and often limited by the need to refer to the traditional model organisms (Pavey et al. 2012). Even if the entire genome of the species is sequenced, the annotation of the genes will remain the limiting factor if the results are interpreted mechanistically (Pavey et al. 2012).

## APPLIED RELEVANCE OF THE RESULTS

### GENERALITY OF THE RESULTS

Through the results of this thesis we gained insight in the eco-and evolutionary effects of spatial and/or temporal heterogeneity. A thorough understanding of these dynamics would allow making predictions about the distribution and resilience of species in a continuously changing world. However, we must be aware of a number of pitfalls that prevent the extrapolation of our lab-results to more natural situations.

### COMMUNITY-PERSPECTIVE

Because of its rapid population growth, evolutionary potential and easy laboratory maintenance *T. urticae* was used as a model species during all of our experiments. Although tempting, our lab-results should not be applied in more general, natural situations. In nature *T.urticae* is part of a multitrophic community containing other herbivores, parasites, predators and different plant species that can not be ignored. The extension to two-species systems or more complex multiple species systems, will certainly have implications for the dynamics of *T. urticae* since the presence of other species will impact on the distribution and amount of resources available (Amarasekare 2008). In these metacommunities, evolutionary and ecological dynamics of the focal species will become a function of both habitat characteristics and the presence of con- and heterospecifics (Holt & Gilpin 1997), adding an important level of depth and realism.

### 'METAPOPULATION TYPES' AND SCALE

In **chapter 4 and 5** the impact of spatial and temporal variability in metapopulation structure on eco-evolutionary dynamics was assessed by the use of 3 types of metapopulation configurations. Although it is tempting to classify different spatially structured populations in to different metapopulation types for the ease of use (Harrisson et al. 1997), the existence of an enormous diversity of landscapes in nature most probably leads to a huge diversity of "metapopulation structures" (Hanski & Gaggiotti 2004). Although the use of metapopulation types with delineated patches is considered a simplification it remains conceptually useful and makes it able to link theoretical predictions with empirical data.

Another aspect to be aware of, is the scale at which our experiments took place. All experiments were performed in a laboratory-environment. Therefore, artificial metapopulations were downscaled compared to natural spatially structured populations of spider mites. Moreover, our metapopulations consisted of configurations of cut bean leaf patches that were relatively close to each other. Since leaves were cut from the plant they most probably induced a general defensive response which might have intervened with the dynamics. Additionally a more natural situation, mites can disperse eather aerially or by walking, over much higher dispersal distances. Metapopulation experiments could be improved by increasing the scale (e.g. by using whole plants in stead of leaves) enabling aerial as well as short distance dispersal.

### ECO-EVOLUTIONARY FEEDBACKS

In our results we found evidence for the presence of eco-evolutionary feedbacks in *Tetranychus urticae*. Whether these eco-evolutionary feedbacks occur in other spatially structured populations of organisms will depend on the rate of evolution to the rate of ecological change. The presence of eco-evolutionary feedbacks requires rapid evolution which is coupled with strong selection. If selection is weak the ecological and evolutionary dynamics will most probably not be coupled at all (Hanski 2011). Episodes of rapid evolution are often linked to a continuously changing environment and are especially prevalent in metapopulation dynamics of heterogeneous environments (Hanski 2011).

## RELEVANCE FOR CONSERVATION BIOLOGY

In the past years, human impact on the earth in the form of land-use changes, climate change and the spread of invasive species, has increased. Human-induced changes of the landscape often lead to the fragmentation or reduction of quality of habitat for a diverse range of organisms. Many organisms are consequently forced to live in suboptimal habitat and experience a high level of landscape heterogeneity and stochasticity (Kinnison & Hairston 2007).

The ecological response to this stochasticity results in the decline and at the extreme end, the extinction of populations. At the same time these changes altered the course or strength of natural selection leading to micro-evolutionary changes in populations. In this study we found a link between the evolutionary as well as ecological consequences of heterogeneity in the spider mite populations. It seems that the spider mite populations in our experiments were able to track the environmental changes and adapt to them on a relatively short timescale. Our results prove that small populations of spider mites as many other pest species, diseases, introductions and invasions may become too well adapted to their new environments and grow to costly abundance (Kinnison & Hairston 2007, Hanski 2011).

In general our observations highlight that the potential evolutionary consequences of habitat mananagement should not be neglected in conservation actions to prevent extinction and promote conservation of fragmented populations. However, while rapid evolutionary changes and eco-evolutionary feedbacks can occur in natural populations, we cannot assume that evolution wil generally rescue populations that are in

decline due to anthropogenic deterioration of their environment (Kinnison & Hairston 2007). As opposed to our spider mites, many populations have difficulty with keeping evolutionary pace with severe anthropogeic challenges (Kinnison & Hairston 2007, Hanski 2011).

## CHALLENGES FOR THE FUTURE

### COMBINING ECOLOGY AND EVOLUTION

While there is an increasing awareness that heterogeneity impacts on population dynamics, and that these ecological dynamics interact with evolutionary trajectories, there is no profound understanding of these eco-evolutionary feedbacks. The few available long-term field studies point at the interplay between local adaptation and gene flow among patches (Farkas *et al.* 2013), or at coupled eco-evolutionary dynamics by non-random dispersal (Hanski & Mononen 2011). Most studies, however, have focused on the evolution of single life history traits like dispersal (Hanski & Mononen 2011) or aging (Ronce & Olivieri 1997) in response to heterogeneity.

There is a crucial need for empirical research that explores the interaction between ecological and evolutionary consequences of heterogeneity in order to validate existing eco-evolutionary models. Future research should therefore focus on eco-evolutionary dynamics in natural populations living under various environmental settings.

Additionally the exploration of the significance of eco-evolutionary dynamics in interspecific interactions and muti-species communities is an especially important challenge. In these metacommunities, evolutionary and ecological dynamics of the focal species will become a function of both habitat characteristics and the presence of con- and heterospecifics (Holt & Gilpin 1997), adding an important level of depth and realism.

### COMBINING EMPIRICAL AND THEORETICAL RESEARCH

As Einstein once mentioned: "In theory, theory and practice are the same, in practice they're not", this surely counts for (meta)population dynamical research. Although the significance of heterogeneity for insights in population and conservation biology is becoming theoretically acknowledged, solid empirical insights that validate theory and that can

be confronted with correlative insights from natural systems are still missing.

More data are needed to assess the validity of model assumptions. To our opinion, the patterns observed in this work, emphasize the need to critically evaluate theoretical predictions by empirical work since theoretical approaches often encompass large simplification of natural, real systems.

## GENERAL CONCLUSION

From this thesis we conclude that heterogeneity impacts on the ecological as well as evolutionary dynamics of spider mite populations. Moreover, ecology and evolution might interact and the presence of an eco-evolutionary feedback in the mite population was confirmed. Therefore, we conclude that ecological dynamics and evolutionary dynamics should not be studied as isolated traits but should rather be considered as emergent properties of a complex system comprising many individuals that interact with their biotic as well as abiotic environment. Due to the complexity of this system and the feedbacks within it, we should therefore study the whole system in which these dynamics emerge. The integration of ecology, genetics and evolution in theoretical as well as empirical studies should therefore be continued in the near future.

# SUMMARY

Human-induced changes of the landscape often lead to the fragmentation or reduction of quality of habitat and forces many organisms to live in a heterogeneous landscape. Heterogeneity of the landscape can arise through variable biotic (e.g. interactions with inter- and intraspecifics) and abiotic (e.g. landscape configuration) characteristics within patches as well as among habitat patches. Understanding dynamics in heterogeneous systems is currently considered as one of the major challenges within the field of evolutionary biology, epidemiology and conservation biology. In this thesis we aimed to gain insight in the eco-evolutionary dynamics in heterogeneous systems and to analyze the links between spatial structure, population dynamics and the evolution of life history traits including dispersal. A thorough understanding of these links allows making predictions about the distribution and resilience of species in a continuously changing world.

We studied the impact of heterogeneity caused by soil biota on the dispersal strategies of a phytophagous mite *(Tetranychus urticae)* using wind tunnel experiments **(Chapter 2)**. The results indicated that spider mite pre-dispersal behavior significantly increased with the experienced mite density on the host during development. Additionally, belowground herbivory resulted in decreased water content and an increased spider mite aerial dispersal behavior. Since no change in the density threshold has been observed, increased dispersal rates appear to be caused by their development on plants with belowground nematode herbivory. Although, in our experiment, no declines in nutritional plant tissue quality were detected nor detectable levels of cyanogenic potential in nematode treated plants, this does not rule out the prevalence of unidentified nitrogen-containing defensive metabolites (e.g. alkaloids or tannins) which may have caused the increased dispersal response.

Additionally, the fitness-effects of dispersal were empirically assessed in a game-theoretical context by a translocation experiment with mutant mites **(Chapter 3)**. We demonstrate that dispersing individuals are not a random subsample of the population. Our results indicate that philopatric individuals reach equal fitness under different density conditions when translocated to different dispersal distances. In contrary, dispersing individuals were able to maximize their fitness when reaching a novel less populated environment after dispersal. We thus provide the first

empirical evidence that the optimization of phenotype-dependent dispersal maximizes individual fitness to such an extent that substantial additional dispersal costs can be levied.

The impact of heterogeneity caused by spatial structure on ecological **(Chapter 4)** and evolutionary **(Chapter 5)** dynamics was investigated using artificial metapopulations. Our results demonstrate that spatial and temporal variation in habitat availability impacts on local as well as regional density-dependent population dynamical properties and alters the density dependence of dispersal. The observed changes are probably caused by differing levels of resource abundance and competitive interactions. Furthermore, metapopulation stability was impacted by spatial structure through changes in synchrony and the coefficient of variation.

Next to these ecological effects, we found spatial structure to induce evolutionary divergence in life history traits, variation in physiological endpoints and divergent patterns in gene expression. Both spatial and spatiotemporal variability induce spatial stress that pre-adapted the mites towards a better performance on novel challenging hosts. The multivariate responses points towards general adaptations in stress resistance pathways and is suggested to be driven by metapopulation-level variation in competition and patch extinction rates.

In general this thesis accentuates the relevance of an integrated knowledge of the eco-evolutionary processes and mechanisms underlying spatially structured populations. Dispersal dynamics, population dynamics and evolutionary dynamics should not be studied as isolated traits but should rather be considered as emergent properties of a complex system comprising many individuals that interact with their biotic as well as abiotic environment. Due to the complexity of this system and the feedbacks within it, we should therefore study the whole system in which these dynamics emerge. The integration of ecology, genetics and evolution in theoretical as well as empirical studies should therefore be continued in the near future.

# SAMENVATTING

Wijzigingen in het landschap, al dan niet door menselijke impact, leiden vaak tot fragmentatie en/of kwaliteitsdaling van het habitat waarin organismen leven. Dit dwingt veel organismen tot een leven in een heterogeen landschap. Deze heterogeniteit kan veroorzaakt worden door variatie in abiotische (vb. landschapsconfiguratie) en/of biotische (vb. interacties met soortgenoten of individuen van een andere soort) kenmerken, zowel binnen als tussen habitat vlekken.

Kennis van de dynamieken die binnen heterogene systemen heersen wordt momenteel als een van de grootste uitdagingen beschouwd binnen de evolutionaire biologie, epidemiologie en conservatie-biologie. In dit werk trachtten we inzicht te verwerven in de eco-evolutionaire dynamieken van heterogene systemen en analyzeerden we de connecties tussen ruimtelijke structuur, populatiedynamiek en de evolutie van levensgeschiedeniskenmerken waar ook dispersie toe behoort.

In een eerste deel werd de impact van heterogeniteit veroorzaakt door bodembiota op de dispersiestrategie van *Tetranychus urticae* bestudeerd **(Hoofdstuk 2).** De resultaten toonden aan dat het dispersiegedrag van deze mijten significant steeg bij een verhoging van de densiteit op hun gastheer. Ondergrondse herbivorie door nematoden resulteerde tijdens dit experiment in een verminderde waterinhoud van de planten gekoppeld met een verhoging van het dispersiegedrag van de mijten. Gezien er geen verandering in de dispersie-drempelwaarde voor densiteit geobserveerd werd, kan verondersteld worden dat de verhoging in het dispersiegedrag veroorzaakt wordt door de ontwikkeling van de mijten op planten met ondergrondse herbivorie. Hoewel in de planten met herbivorie door nematoden, geen verminderde nutritionele kwaliteit van het plantweefsel noch de aanwezigheid van cyanogeen potentieel konden detecteren, kan de aanwezigheid van ongeïdentificeerde stikstofbevattende verdedigings metabolieten zoals alkaloiden of tannines de geobserveerde respons nog steeds verklaren .

In een tweede deel van deze thesis werden de fitness-effecten van dispersie geanaliseerd a.d.h.v. een empirisch translocatie experiment met mutante mijten **(Hoofdstuk 3)**. In dit experiment toonden we aan dat disperserende mijten geen random steekproef uit de populatie zijn. Philopatrische individuen bereikten een identieke fitness bij verschillende densiteiten en bij verplaatsing naar verschillende dispersie-afstanden.

Disperserende individuen daarentegen, konden hun fitness maximaliseren bij het bereiken van een nieuwe omgeving die een minder dense mijtenpopulatie bevatte. De resultaten van dit experiment leveren het eerste empirisch bewijs dat de optimalisatie van fenotype-afhankelijke dispersie de individuele fitness maximaliseert tot een niveau waarbij bijkomende dispersiekosten opgeheven worden.

De impact van heterogeniteit door ruimtelijke structuur op ecologische **(Chapter 4)** en evolutionaire **(Chapter 5)** dynamieken werd onderzocht door gebruik te maken van artificiële metapopulaties. De resultaten toonden aan dat ruimtelijke en temporele fluctuaties in habitatbeschikbaarheid een invloed hebben op lokale en regionale densiteitsafhankelijke populatiedynamiek. Bovendien wordt aangetoond dat deze fluctuaties, de densiteitsafhankelijkheid van dispersie kunnen wijzigen. Deze geobserveerde trends worden hoogstwaarschijnlijk veroorzaakt door de wisselende niveaus van bronbeschikbaarheid en competitieve interacties. Ook de synchronie en coefficient van variatie werden beinvloed door de ruimtelijke structuur.

Bovenop deze ecologische effecten, heeft ruimtelijke structuur een gevolg voor de evolutionaire dynamiek. We vonden een evolutionaire divergentie in levensgeschiedeniskenmerken, variatie in fysiologie en divergente patronen in genexpressie ten gevolge van een wisselende ruimtelijke structuur. Zowel ruimtelijk als temporele variabiliteit induceren ruimtelijke stress die ervoor zorgde dat mijten beter aangepast waren aan een leven op een nieuwe uitdagende gastheerplant. Deze multivariate respons wijst in de richting van algemene adaptaties in stress-gerelateerde pathways en lijkt te worden gedreven door variatie in competitie en patch-extincties op metapopulatieniveau.

In conclusie kunnen we stellen dat deze thesis het belang benadrukt van een geïntegreerde kennis van de eco-evolutionaire processen en mechanismen die ruimtelijk gestructureerde populaties sturen. Dispersie, populatie –en evolutionaire dynamieken moeten beschouw worden als intrinsieke onderdelen van een complex systeem bestaande uit interagerende individuen en dus niet in isolatie van elkaar bestudeerd worden. Gezien de complexiteit van dit systeem en de feedbacks dat het bevat, is het aan te raden om de integratie van ecologie, genetica en evolutie in zowel theoretische als empirische studies in de nabije toekomst verder uit te diepen.

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# REFERENCES


Abbott K.C. (2011). A dispersal-induced paradox: synchrony and stability in stochastic metapopulations. *Ecol Lett*, **14:** 1158-1169.

Ahola V., Lehtonen R., Somervuo P., Salmela L., Koskinen P., Rastas P. *et al.* (2014). The Glanville fritillary genome retains an ancient karyotype and reveals selective chromosomal fusions in Lepidoptera. *Nat Commun*, **5,** doi:10.1038/ncomms5737.

Altwegg R., Doutrelant C., Anderson M., Spottiswoode C. & Covas R. (2014). Climate, social factors and research disturbance influence population dynamics in a declining sociable weaver metapopulation. *Oecologia*, **174,** 413-425.

Amarasekare P. (2008). Spatial dynamics of keystone predation. *J Anim Ecol*, **77,** 1306-1315.

Ament K., Krasikov V., Allmann S., Rep M., Takken F.L.W. & Schuurink R.C. (2010). Methyl salicylate production in tomato affects biotic interactions. *Plant Journal,* **62**,124-134.

Anderson M.J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology,* **26,** 32-46.

Anderson P., Sadek M. M. & Wackers F. L. (2011). Root herbivory affects oviposition and feeding behavior of a foliar herbivore. *Behavioral Ecology,* **22**, 1272-1277.

Andreassen H.P. & Ims R.A. (2001). Dispersal in patchy vole populations: role of patch configuration, density dependence, and demography. *Ecology*, **82,** 2911-2926.

Aubin-Horth N. & Renn S.C.P. (2009). Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity. *Mol Ecol*, **18,** 3763-3780.

Awmack C. S. & Leather S. R. (2002). Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology,* **47,** 817-844.

Baguette M. (2004). The classical metapopulation theory and the real, natural world: a critical appraisal. *Basic Appl Ecol*, **5,** 213-224.

Baguette M., Petit S. & Quéva F.o. (2000). Population spatial structure and migration of three butterfly species within the same habitat network: consequences for conservation. *Journal of Applied Ecology*, **37,** 100-108.

Baldwin I. T. (1991). Damage-induced alkaloids in wild tobacco. In M. J. Raupp & D. W. Tallamy (Eds.)., Phytochemical Induction by Herbivores (pp. 47-69)..

Ballhorn D. J., Godschalx A. L. & Kautz S. (2013a). Co-Variation of chemical and mechanical defenses in lima bean (*Phaseolus lunatus* L.).. *Journal of Chemical Ecology*, **39**, 413-417.

Ballhorn D. J., Kautz S., Jensen M., Schmitt I., Heil M. & Hegeman A. D. (2011). Genetic and environmental interactions determine plant defences against herbivores. *Journal of Ecology*, 99, 313-326.

Ballhorn D.J., Pietrowski A., Heil M. & Lieberei R. (2007). Quantitative effects of cyanogenesis on an adapted herbivore. *Journal of Chemical Ecology*, **33**, 2195-2208.

Belichon S., Clobert J. & Massot M. (1996). Are there differences in fitness components between philopatric and dispersing individuals? *Acta Oecologica,* **17,** 503-517.

Bell G. & Gonzalez A. (2011). Adaptation and evolutionary rescue in metapopulations experiencing environmental deterioration. *Science*, **332,** 1327-1330.

Benard M. F. & McCauley S.J. (2008). Integrating across life-history stages: Consequences of natal habitat effects on dispersal. *American Naturalist,* **171,** 553-567.

Benton T.G., Cameron T.C. & Grant A. (2004). Population responses to perturbations: predictions and responses from laboratory mite populations. *J Anim Ecol*, **73,** 983-995.

Benton T.G., Lapsley C.T. & Beckerman A.P. (2001). Population synchrony and environmental variation: an experimental demonstration. *Ecol Lett*, **4,** 236-243.

Benton T.G., Solan M., Travis J.M.J. & Sait S.M. (2007). Microcosm experiments can inform global ecological problems. *Trends Ecol Evol*, **22,** 516-521.

Berg M.P., Kiers E.T., Driessen G., van der Heijden M., Kooi B.W., Kuenen F., Liefting M., Verhoef H.A. & Ellers J. (2010). Adapt or disperse: understanding species persistence in a changing world. Global Change Biology **16,** 587-598.

Bernstein C. (1984). Prey and predator emigration responses in the acarine system Tetranychus urticae-Phytoseiulus persimilis. *Oecologia*, **61,** 134-142.

Bezemer T. M. & van Dam N. M. (2005). Linking aboveground and belowground interactions via induced plant defenses. *Trends in Ecology & Evolution,* **20,** 617-624.

Bierbaum T.J., Mueller L.D., Ayala F.J. (1989). Density-dependent evolution of life-history traits in *Drosophila melanogaster*. *Evolution,* **43,** 382–392.

Bitume E.V., Bonte D., Magalhaes S., Martin G.S., Van Dongen S., Bach F., Anderson J.M., Olivieri I. & Nieberding C.M. (2011). Heritability and Artificial Selection on Ambulatory Dispersal Distance in Tetranychus urticae: Effects of Density and Maternal Effects. *Plos One,* **6**.

Bitume E.V., Bonte D., Ronce O., Bach F., Flaven E., Olivieri I. & Nieberding C.M. (2013). Density and genetic relatedness increase dispersal distance in a subsocial organism. *Ecol Lett*, **16,** 430-437.

Bjornstad O.N., Ims R.A. & Lambin X. (1999). Spatial population dynamics: analyzing patterns and processes of population synchrony. *Trends in Ecology and Evolution,* **14,** 427-432.

Blossey B. & Hunt-Joshi T. R. (2003). Belowground herbivory by insects: Influence on plants and aboveground herbivores. *Annual Review of Entomology,* **48,** 521-547.

Boggs C.L. (2009). Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology*, **23,** 27-37.

Bonenfant C., Gaillard J.M., Coulson T., Festaâ€Bianchet M., Loison A., Garel M., Loe L.E., Blanchard P., Pettorelli N. & Owenâ€Smith N. (2009). Empirical evidence of densityâdependence in populations of large Herbivores. *Advances in ecological research*, **41,** 313-357.

Bonte D. & de la Peña E. (2009). Evolution of body condition-dependent dispersal in metapopulations. *J Evolution Biol*, **22,** 1242-1251.

Bonte D. and Saastamoinen M. (2012). Dispersal syndromes in spiders and butterflies. Pages 161-170 *in* J. Clobert, M. Baguette, T. Benton, and J. Bullock, editors. Dispersal and Spatial Evolutionary Ecology. OUP, Oxford.

Bonte D., De Meester N. & Matthysen E. (2011). Selective integration advantages when transience is costly: immigration behaviour in an agrobiont spider. Animal Behaviour **81**:837-841.

Bonte D., De Roissart A., Vandegehuchte M.L., Ballhorn D.J., Van Leeuwen T. & de la Pena E. (2010). Local adaptation of aboveground herbivores towards plant phenotypes induced by soil biota. *Plos One*, **5,** e11174

Bonte D., De Roissart A., Wybouw N. & Van Leeuwen T. (2014). Fitness maximization by dispersal: evidence from an invasion experiment. *Ecology*, **95,** 3104-3111.

Bonte D., Van Dyck H., Bullock J. M., Coulon A., Delgado M., Gibbs M., Lehouck V., Matthysen E., Mustin K., Saastamoinen M., Schtickzelle N., Stevens V. M., Vandewoestijne S., Baguette M., Barton K., Benton T. G., Chaput-Bardy A., Clobert J., Dytham C., Hovestadt T., Meier C. M., Palmer S. C. F., Turlure C. & Travis J. M. J. (2012). Costs of dispersal. *Biological Reviews*, **87,** 290-312.

Bozic J. & Woodring J. (1997). Effect of activity on the haemolymph sugar titres in honey bees. *Journal of Apicultural Research,* **36,** 33-39.

Brook B.W. & Bradshaw C.J.A. (2006). Strength of evidence for density dependence in abundance time series of 1198 species. *Ecology*, **87,** 1445-1451.

Bryon A., Wybouw N., Dermauw W., Tirry L. & Van Leeuwen T. (2013). Genome wide gene-expression analysis of facultative reproductive diapause in the two-spotted spider mite *Tetranychus urticae*. *BMC genomics,* **14,** 815.

Bull J.C., Pickup N.J., Pickett B., Hassell M.P. & Bonsall M.B. (2007). Metapopulation extinction risk is increased by environmental stochasticity and assemblage complexity. *P Roy Soc B-Biol Sci*, **274,** 87-96.

Cameron T.C. & Benton T.G. (2004). Stage-structured harvesting and its effects: an empirical investigation using soil mites. *J Anim Ecol*, **73,** 996-1006.

Cameron T.C., O'Sullivan D., Reynolds A., Piertney S.B. & Benton T.G. (2013). Eco-evolutionary dynamics in response to selection on life-history. *Ecology Letters,* 16, 754–763.

Cappuccino (1995). Novel approaches to the study of population dynamics. *Population Dynamics: New Approaches and Synthesis. Academic press,* 3-16.

Carlson S.M., Cunningham C.J. & Westley P.A.H. (2014). Evolutionary rescue in a changing world. *Trends Ecol Evol*, **29,** 521-530.

Clarke A.L., Saether B.-E. & Roskaft E. (1997). Sex biases in avian dispersal: a reappraisal. *Oikos*, 429-438.

Clobert J., Ims R. A., Rousset F. o. & Gaggiotti I. H. E. (2004) 13 - Causes, Mechanisms and Consequences of Dispersal. *Ecology, Genetics and Evolution of Metapopulations*, pp. 307-335. Academic Press, Burlington.

Clobert J., Le Galliard J. F., Cote J., Meylan S. & Massot M. (2009). Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. *Ecology Letters,* **12,** 197-209.

Clobert, J., Nichols, J.D., Danchin, E. & Dhondt, A. (eds) (2001). Dispersal. Oxford University Press, Oxford, UK.

Conesa A., Götz S., García-Gómez J.M., Terol J., Talón M. & Robles M. (2005). Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics*, **21,** 3674-3676.

Côté G., Perry G., Blier P. & Bernatchez L. (2007). The influence of gene-environment interactions on GHR and IGF-1 expression and their association with growth in brook charr, Salvelinus fontinalis (Mitchill). *BMC genetics*, **8,** 87.

Cote J., Clobert J., Brodin T., Fogarty S. & Sih A. (2010). Personality-dependent dispersal: characterization, ontogeny and consequences for spatially structured populations. Philosophical Transactions of the Royal Society B-Biological Sciences **365,** 4065-4076.

Cote J., Fogarty S., Weinersmith K., Brodin T. & Sih A. (2010). Personality traits and dispersal tendency in the invasive mosquitofish (Gambusia affinis). Proceedings of the Royal Society B-Biological Sciences **277**, 1571-1579.

Courchamp F., Berec L. & Gascoigne J. (2008). Allee effects in ecology and conservation. *Environ. Conserv*, **36,** 80-85.

Courchamp F., Clutton-Brock T. & Grenfell B. (1999). Inverse density dependence and the Allee effect. *Trends Ecol Evol*, 14, 405-410.

De Deyn G. B. & Van der Putten W. H. (2005). Linking aboveground and belowground diversity. *Trends in Ecology & Evolution,* **20,** 625-633.

De Deyn G. B., van Ruijven J., Raaijmakers C. E., de Ruiter P. C. & van der Putten W. H. (2007). Above- and belowground insect herbivores differentially affect soil nematode communities in species-rich plant communities. Oikos, **116,** 923-930.

de Jong T.J., Klinkhamer P.G.L. & de Heiden J.L.H. (2000). The evolution of generation time in metapopulations of monocarpic perennial plants: some theoretical considerations and the example of the rare thistle Carlina vulgaris. *Evol Ecol*, **14,** 213-231.

De Meester N. & Bonte D. (2010). Information use and density-dependent emigration in an agrobiont spider. *Behavioral Ecology,* **21,** 992-998.

De Roissart, A., de la Pena, E., Van Oyen, L., Van Leeuwen, T., Ballhorn, D. & Bonte, D. (2013). The presence of root-feeding nematodes, but not AMF affects an herbivore dispersal strategy. *Acta Oecologica*, **52,** 38-44.

Debeffe L., Morellet N., Verheyden-Tixier H., Host H., Gaillard J.M., Cargnelutti B., Picot D., Sevila J. & Hewison A.J.M.. (2014). Parasite load contributes to condition-dependent dispersal in a wild population of large herbivores. *Oikos*, **123,** 1121–1125.

Delgado M., Barton K.A., Bonte D. & Travis J. (2014). Prospecting and dispersal: their eco-evolutionary dynamics and implications for population patterns. *Proceedings of the Royal Society B-Biological Sciences* **281**.

Delgado M., Penteriani V., Revilla E. & Nams V.O. (2010) The effect of phenotypic traits and external cues on natal dispersal movements. *J Anim Ecol*, **79,** 620-632.

Delgado M.D., Ratikainen I.I. & Kokko H. (2011). Inertia: the discrepancy between individual and common good in dispersal and prospecting behaviour. *Biol Rev*, **86,** 717-732.

Dermauw W., Van Leeuwen T., Vanholme B. & Tirry L. (2009). The complete mitochondrial genome of the house dust mite *Dermatophagoides pteronyssinus* (Trouessart): a novel gene arrangement among arthropods. *BMC Genomics,* **10,** 107.

Dermauw W., Wybouw N., Rombauts S., Menten B., Vontas J., Grbic M. *et al.* (2013). A link between host plant adaptation and pesticide resistance in the polyphagous spider mite *Tetranychus urticae*. *Proceedings of the National Academy of Sciences of the United States of America,* **110,** E113-E122.

Dey S., Prasad N.G., Shakarad M. & Joshi A. (2008). Laboratory evolution of population stability in Drosophila: constancy and persistence do not necessarily coevolve. *J Anim Ecol*, **77,** 670-677.

Diaz-Moralli S., Ramos-Montoya A., Marin S., Fernandez-Alvarez A., Casado M. & Cascante M. (2012). Target metabolomics revealed complementary roles of hexose- and pentose-phosphates in the regulation of carbohydrate-dependent gene expression. *American Journal of Physiology - Endocrinology and Metabolism*, **303,** E234-E242.

Dicke M. (2000). Chemical ecology of host-plant selection by herbivorous arthropods: a multitrophic perspective. *Biochemical Systematics and Ecology,* **28,** 601-617.

Dicke M., Vanbaarlen P., Wessels R. & Dijkman H. (1993). Herbivory induces systemic production of plant volatiles that attract predators of the herbivore - extraction of endogenous elicitor. Journal of Chemical Ecology **19**, 581-599.

Doligez, B. and T. Part. (2008). Estimating fitness consequences of dispersal: a road to 'know-where'? Non-random dispersal and the underestimation of dispersers' fitness. Journal of Animal Ecology **77,** 1199-1211.

Dooley C.A., Bonsall M.B., Brereton T. & Oliver T. (2013). Spatial variation in the magnitude and functional form of density-dependent processes on the large skipper butterfly Ochlodes sylvanus. *Ecol Entomol*, **38,** 608-616.

Drake J.M. & Griffen B.D. (2010). Experimental demonstration of accelerated extinction in source-sink metapopulations. *Ecol. Evol.*, **3,** 3369-3378.

Drake J.M. & Lodge D.M. (2004). Effects of environmental variation on extinction and establishment. *Ecol Lett*, **7,** 26-30.

Dytham C. & Travis J.M.J. (2006). Evolving dispersal and age at death. *Oikos,* **113,** 530-538.

Ebenhard T.r. (1990). A colonization strategy in field voles (Microtus agrestis): reproductive traits and body size. *Ecology*, 1833-1848.

Edelsparre A.H., Vesterberg A., Lim J.H., Anwari M. & Fitzpatrick M.J. (2014). Alleles underlying larval foraging behaviour influence adult dispersal in nature. *Ecol Lett*, **17,** 333-339.

Erb M., Flors V., Karlen D., de Lange E., Planchamp C., D'Alessandro M., Turling, T. C. J. & Ton J. (2009). Signal signature of aboveground-induced resistance upon belowground herbivory in maize. *Plant Journal,* **59,** 292-302.

Erb M., Kollner T. G., Degenhardt J., Zwahlen C., Hibbard B. E. & Turlings T. C. J. (2011). The role of abscisic acid and water stress in root herbivore-induced leaf resistance. *New Phytologist,* **189,** 308-320.

Ezard T.H.G., Côte S.D. & Pelletier F. (2009). Eco-evolutionary dynamics: disentangling phenotypic, environmental and population fluctuations. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **364,** 1491-1498.

Fahrig L. (2007). Landscape heterogeneity and metapopulation dynamics. *Key topics and perspectives in landscape ecology. Cambridge University Press, Cambridge*, 78-89.

Farkas T.E., Mononen T., Comeault A.A., Hanksi I. & Nosil P (2013). Evolution of camouflage drives rapid ecological change within an insect community. *Current Biology,* **23,** 1835-1843.

Ferriere R., Belthoff J.R., Olivieri I. & Krackow S. (2000). Evolving dispersal: where to go next? *Trends Ecol Evol*, **15,** 5-7.

Fitzpatrick J.W., Lammertink M., Luneau M.D., Gallagher T.W., Harrison B.R., Sparling G.M., Rosenberg K.V., Rohrbaugh R.W., Swarthout E.C.H., Wrege P.H., Swarthout S.B., Dantzker M.S., Charif R.A., Barksdale T.R., Remsen J.V., Simon S.D. & Zollner D. (2005). Ivory-billed Woodpecker (Campephilus principalis) Persists in Continental North America. *Science*, 308, 1460-1462.

Fjerdingstad E.J., Schtickzelle N., Manhes P., Gutierrez A. & Clobert J. (2007). Evolution of dispersal and life history strategies - Tetrahymena ciliates. *Bmc Evol Biol*, **7**.

Flowers J.M., Sezgin E., Kumagai S., Duvernell D.D., Matzkin L.M., Schmidt P.S. *et al.* (2007). Adaptive evolution of metabolic pathways in *Drosophila*. *Molecular Biology and Evolution,* **24,** 1347-1354.

Forero M.G., Donazar J.A. & Hiraldo F. (2002). Causes and fitness consequences of natal dispersal in a population of black kites. Ecology **83**, 858-872.

Fretwell S.D. & Lucas H.L. (1970). On territorial behavior and other factors influencing habitat distribution in birds. I. Theoretical Development. Acta Biotheoretica **19**, 16–36.

Fretwell S.D. (1972). Populations in a Seasonal Environment. Princeton University Press., Princeton.

Friedenberg N.A. (2003). Experimental evolution of dispersal in spatiotemporally variable microcosms. *Ecology Letters,* **6,** 953-959.

Fronhofer E.A., Kubisch A., Hilker F.M., Hovestadt T. & Poethke H.J. (2012). Why are metapopulations so rare? *Ecology,* **93,** 1967-1978.

Fronhofer E.A., Stelz J.M., Lutz E., Poethke H.J. & Bonte D. (2014). Spatially correlated extinctions select for less emigration but larger dispersal distances in the spider mite *Tetranychus* urticae. *Evolution*, **68,** 1838-1844.

Gandon S. & Michalakis Y. (2002). Local adaptation, evolutionary potential and host-parasite coevolution: interactions between migration, mutation, population size and generation time. *J Evolution Biol*, **15,** 451-462.
Gandon S. (1999). Local adaptation and host-parasite interactions. *Trends Ecol Evol*, **13,** 214-216.

Giovannetti M. & Mosse B. (1980). Evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist,* **84,** 489-500.

Gomulkiewicz R. & Holt R.D. (1995). When does evolution by natural selection prevent extinction? *Evolution*, **49,** 201-207.

Graham J.H. & Abbott L.K. (2000) Wheat responses to aggressive and non- aggressive arbuscular mycorrhizal fungi. *Plant and Soil,* **220,** 207-218.

Grbic M., Khila A., Lee K.Z., Bjelica A., Grbic V., Whistlecraft J., Verdon L., Navajas M. & Nagy L. (2007). Mity model: Tetranychus urticae, a candidate for chelicerate model organism. *Bioessays*, **29,** 489-496.

Grbic M., Van Leeuwen T., Clark R.M., Rombauts S., Rouze P., Grbic V. *et al.* (2011). The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. *Nature,* **479,** 487-492.

Gros A., Hovestadt T. & Poethke H.J. (2008). Evolution of sex-biased dispersal: The role of sex-specific dispersal costs, demographic stochasticity, and inbreeding. *Ecol Model*, **219,** 226-233.
Gros A., Poethke H.J. & Hovestadt T. (2006). Evolution of local adaptations in dispersal strategies. *Oikos*, **114,** 544-552.

Gyllenberg M. & Metz J.A.J. (2001). On fitness in structured metapopulations. *Journal of Mathematical Biology,* **43,** 545-560.

Gyllenberg M., Kisdi E. & Utz M. (2008). Evolution of condition-dependent dispersal under kin competition. *Journal of Mathematical Biology,* **57,** 285-307.

Gyllenberg M., Kisdi E. & Utz M. (2011). Body condition dependent dispersal in a heterogeneous environment. *Theoretical Population Biology,* **79,** 139-154.

Hahne J., Jenkins T., Halle S. & Heckel F. (2011). Establishment success and resulting fitness consequences for vole dispersers. *Oikos*, **120**, 95-105.

Hairston N.G., Ellner S.P., Geber M.A., Yoshida T. & Fox J.A. (2005). Rapid evolution and the convergence of ecological and evolutionary time. *Ecol Lett*, **8,** 1114-1127.

Hamback P.A. & Englund G. (2005). Patch area, population density and the scaling of migration rates: the resource concentration hypothesis revisited. *Ecol Lett*, **8,** 1057-1065.

Hamilton W. D. (1964). The genetical evolution of social behaviour. II. *Journal of Theoretical Biology,* **7,** 17-52.

Hamilton W.D. & May R.M. (1977). Dispersal in stable habitats. *Nature*, **269**, 578-581.

Hance T. & Van Impe G. (1999). The influence of initial age structure on predator-prey interaction. *Ecological Modelling,* **114,** 195-211.

Hanski I. & Gilpin I.H.E. (1997). 4 - Metapopulation Dynamics: From Concepts and Observations to Predictive Models. In: *Metapopulation Biology*. Academic Press San Diego, pp. 69-91.

Hanski I. & Mononen T. (2011). Eco-evolutionary dynamics of dispersal in spatially heterogeneous environments. *Ecology Letters,* **14,** 1025-1034.

Hanski I. & Woiwod I.P. (1993). Spatial synchrony in the dynamics of moth and aphid populations. *J Anim Ecol*, **62,** 656-668.

Hanski I. (1994). A practical model of metapopulation dynamics. *J Anim Ecol*, 151-162.

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

Hanski I. (1999). Habitat connectivity, habitat continuity, and metapopulations in dynamic landscapes. *Oikos*, 209-219.

Hanski I. (2004). Metapopulation theory, its use and misuse. *Basic and Applied Ecology,* **5,** 225-229.

Hanski I., Breuker C.J., Schöps K., Setchfield R. & Nieminen M. (2002). Population history and life history influence the migration rate of female Glanville fritillary butterflies. *Oikos*, **98,** 87-97.

Hanski I., Gaggiotti O. & Gaggiotti I.H.E. (2004). 1 - Metapopulation Biology: Past, Present, and Future. In: *Ecology, Genetics and Evolution of Metapopulations*. Academic Press Burlington, pp. 3-22.

Hanski I.A. (2011). Eco-evolutionary spatial dynamics in the Glanville fritillary butterfly. *Proceedings of the National Academy of Sciences*, 108, 14397-14404.

Hanski I. (2012). Eco-evolutionary dynamics in a changing world. *Annals of the New York Academy of Sciences,* **1249,** 1-17.

Harrison S. & Taylor A.D. (1997). Empirical evidence for metapopulation dynamics. pp. 27–42. In I. A. Hanski and M. E. Gilpin (eds.), Metapopulation Biology.

Hartley S.E. & Gange A.C. (2009). Impacts of plant symbiotic fungi on insect herbivores: Mutualism in a multitrophic context. *Annual Review of Entomology,* **54,** 323-342.

Hastings A. (1983). Can spatial variation alone lead to selection for dispersal? *Theoretical Population Biology,* **24,** 244–251.

Haynes K.J., Dillemuth F.P., Anderson B.J., Hakes A.S., Jackson H.B., Jackson S.E. & Cronin J.T. (2007). Landscape context outweighs local habitat quality in its effects on herbivore dispersal and distribution. *Oecologia*, **151,** 431-441.

Heino M. & Hanski I. (2001). Evolution of migration rate in a spatially realistic metapopulation model. *The American Naturalist*, **157,** 495-511.

Heino M., Kaitala V., Ranta E. & Lindstrom J. (1997). Synchronous dynamics and rates of extinction in spatially structured populations. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **264,** 481-486.

Helle W., Sabelis M.W., editors. (1985). *Spider Mites*: Their biology, natural enemies, and control. Amsterdam: Elsevier. 458p.

Hendry A.P. & Kinnison M.T. (1999). Perspective: the pace of modern life: measuring rates of contemporary microevolution. *Evolution*, 1637-1653.

Hodgins-Davis A. & Townsend P.J. (2009). Evolving gene expression: from G to E to G x E. *Trends in Ecology and Evolution,* **24,** 649-658.

Hoffmann D. & Schausberger P. (2012). Plant-mediated aboveground-belowground interactions: the spider mite perspective. *Acarologia,* **52,** 17-27.

Hoffmann D., Vierheilig H., Riegler P. & Schausberger P. (2009). Arbuscular mycorrhizal symbiosis increases host plant acceptance and population growth rates of the two-spotted spider mite *Tetranychus urticae*. *Oecologia,* **158,** 663-671.

Holland M.D. & Hastings A. (2008). Strong effect of dispersal network structure on ecological dynamics. *Nature,* **456,** 792-795.

Holt R.D. & Gilpin I.H.E. (1997). 7 - From Metapopulation Dynamics to Community Structure: Some Consequences of Spatial Heterogeneity. In: *Metapopulation Biology*. Academic Press San Diego, pp. 149-164.

Hovestadt T. & Poethke H.J. (2006). The control of emigration and its consequences for the survival of populations. *Ecol Model*, 190, 443-453.

Hovestadt T., Messne, S. & Poethke H. J. (2001). Evolution of reduced dispersal mortality and 'fat-tailed' dispersal kernels in autocorrelated landscapes. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **268,** 385-391.

Hovestadt T., Kubisch A. & Poethke H.-J. (2010) Information processing in models for density-dependent emigration: a comparison. *Ecological Modelling,* **221,** 405-410.

Hudson P.J. & Cattadori I.M. (1999). The Moran effect: a cause of population synchrony. *Trends Ecol Evol*, **14,** 1-2.

Ims R.A. & Andreassen H.P. (2005). Density-dependent dispersal and spatial population dynamics. *Proceedings of the Royal Society B: Biological Sciences*, **272,** 913-918.

Janssen A. & Sabelis M.W. (1992). Phytoseiid life-histories, local predator-prey dynamics, and strategies for control of tetranychid mites. *Experimental & applied acarology*, **14,** 233-250.

Jia F., Margolies D. C., Boyer J. E. & Charlton R. E. (2002). Genetic variation in foraging traits among inbred lines of a predatory mite. *Heredity,* **89,** 371-379.

Johnson N. C., Graham J. H. & Smith F. A. (1997). Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist*, **135,** 575-586.

Johnson, M. L. and M. S. Gaines. 1987. The selective basis for dispersal of the prairie vole, microtus-ochrogaster. Ecology **68**:684-694.

Kaplan I., Halitschke R., Kessler A., Sardanelli S. & Denno R. F. (2008). Constitutive and induced defenses to herbivory in above- and belowground plant tissues. *Ecology,* **89,** 392-406.

Kawecki T.J., Lenski R.E., Ebert D., Hollis B., Olivieri I. & Whitlock M.C. (2012). Experimental evolution. *Trends Ecol Evol*, **27,** 547-560.

Kenward M. G. & Roger J. H. (1997). "Small Sample Inference for Fixed Effects from Restricted Maximum Likelihood," *Biometrics*, **53,** 983–997.

Kim S.-Y., Torres R. & Drummond H. (2009). Simultaneous positive and negative density-dependent dispersal in a colonial bird species. *Ecology,* **90,** 230-239.

Kindvall O. & Peterson A. (2000). Consequences of modelling interpatch migration as a function of patch geometry when predicting metapopulation extinction risk. *Ecological Modelling* **129,** 101-109.

Kinnison M. T. & Hairston N. G. (2007). Eco-evolutionary conservation biology: contemporary evolution and the dynamics of persistence. *Functional Ecology,* **21,** 444-454.

Kirsch R., Gramzow L., Theiβen G., Siegfried B.D., French-Constant R.H., Heckel D.G. & Pauchet Y. (2014). Horizontal gene transfer and functional diversification of plant cell wall degrading polygalacturonases: Key events in the evolution of herbivory in beetles. *Insect Biochemistry and Molecular Biology*, **52,** 33-50.

Kisdi É., Utz M. & Gyllenberg M. (2012). Evolution of condition-dependent dispersal. Pages 139-151. *in* M. B. J. Clobert, T. Benton & J. Bullock, editor. Dispersal Ecology and Evolution. Oxford University Press, Oxford.

Klomp H. (1964). Intraspecific competition and the regulation of insect numbers. *Annual Review of Entomology*. , **9,** 17- 40.

Koenig W.D. (1999). Spatial autocorrelation of ecological phenomena. *Trends Ecol Evol*, **14,** 22-26.

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

Kokko H. & Lopez-Sepulcre A. (2007). The ecogenetic link between demography and evolution: can we bridge the gap between theory and data? *Ecol Lett*, **10,** 773-782.

Kokko H. (2007). *Modelling for field biologists and other interesting people*. Cambridge University Press.

Koricheva J., Gange A. C. & Jones T. (2009). Effects of mycorrhizal fungi on insect herbivores: a meta-analysis. *Ecology*, **90,** 2088-2097.

Krebs C.J., Boutin S., Boonstra R., Sinclair A.R.E., Smith J.N.M., Dale M.R.T., Martin K. & Turkington R. (1995). Impact of food and predation on the snowshoe hare cycle. *Science*, **269,** 1112-1115.

Krips O.E., Witul A., Willems P.E.L. & Dicke M. (1998). Intrinsic rate of population increase of the spider mite *Tetranychus urticae* on the ornamental crop gerbera: Intraspecific variation in host plant and herbivore. *Entomol Exp Appl*, **89,** 159-168.

Kun A. & Scheuring I. (2006). The evolution of density-dependent dispersal in a noisy spatial population model. *Oikos,* **115,** 308-320.

Laparie M., Larvor V., Frenot Y. & Renault D. (2012). Starvation resistance and effects of diet on energy reserves in a predatory ground beetle (*Merizodus soledadinus*; Carabidae) invading the Kerguelen Islands. *Comparative Biochemistry and Physiology A,* **161,** 122-129.

Lawson Handley L.J. & Perrin N. (2007). Advances in our understanding of mammalian sex-biased dispersal. *Molecular Ecology,* **16,** 1559-1578.

Le Goff G.J., Mailleux A.C., Detrain C., Deneubourg J. L., Clotuche G. & Hance T. (2010). Group effect on fertility, survival and silk production in the web spinner *Tetranychus urticae* (Acari: Tetranychidae) during colony foundation. *Behaviour* **147,**1169-1184.

Léna J.P., Clobert J., De Fraipont M., Lecomte J. & Guyot G. (1998). The relative influence of density and kinship on dispersal in the common lizard. *Behav Ecol*, **9,** 500-507.

Levins R. (1969). Effect of random variations of different types on population growth. *P Natl Acad Sci USA*, **62,** 1061-1065.

Li J. B. & Margolies D. C. (1994). Responses to direct and indirect selection on aerial dispersal behavior in *Tetranychus urticae*. *Heredity,* **72,** 10-22.

Li J.B. & Margolies D.C. (1993a). Quantitative genetics of aerial dispersal behavior and life-history traits in *Tetranychus urticae*. *Heredity,* **70,** 544-552.

Li, J.B. & Margolies D.C. (1993b). Effects of mite age, mite density, and host quality on aerial dispersal behavior in the 2-spotted spider-mite. *Entomologia Experimentalis et Applicata,* **68,** 79-86.

Liebhold A., Koenig W.D. & Bjornstad O.N. (2004). Spatial synchrony in population dynamics. *Annu Rev Ecol Evol S*, **35,** 467-490.

Lin Y.K. & Batzli G.O. (2004). Emigration to new habitats by voles: the cost of dispersal paradox. *Anim Behav*, **68,** 367-372.

Macke E., Magalhaes S., Bach F. & Olivieri I. (2011). Experimental evolution of reduced sex ratio adjustment under local mate competition. *Science,* **334,** 1127-1129.

Magalhaes S., Fayard J., Janssen A., Carbonell D. & Olivieri I. (2007). Adaptation in a spider mite population after long-term evolution on a single host plant. *Journal of Evolutionary Biology,* **20,** 2016-2027.

Marden J.H. (2013a). Nature's inordinate fondness for metabolic enzymes: why metabolic enzyme loci are so frequently targets of selection. *Molecular Ecology,* **22,** 5743-5764.

Marden J.H., Fescemyer H.W., Schilder R.J., Doerfler W.R., Vera J.C. & Wheat C.W. (2013b). Genetic variation in HIF signaling underlies quantitative variation in physiological and life-history traits within lowland butterfly. *Evolution,* **67,** 1105-1115.

Margulis L. & Sagan D. (2000). What is life? University of California Press

Masters G. J. (1995). The Impact of root herbivory on aphid performance - field and laboratory evidence. *Acta Oecologica - International Journal of Ecology*, **16,** 135-142.

Masters G.J. & Brown V.K. (1997). Host-plant mediated interactions between spatially separated herbivores: Effects on community structure. *Multitrophic Interactions in Terrestrial Systems*, pp. 217-237.

Matthysen E. (2005). Density-dependent dispersal in birds and mammals. *Ecography,* **28,** 403-416.

Matthysen E. (2012) Multicausality of dispersal: a review. *Dispersal ecology and evolution*, 462.

Mcpeek M.A. & Holt R.D. (1992). The Evolution of Dispersal in Spatially and Temporally Varying Environments. *Am Nat*, **140,** 1010-1027.

Monro K. & Marshall D.J. (2014). Faster isn't always better: selection on growth rate fluctuates across the life history and environments. *The American Naturalist,* **183,** 798–809.

Moran P.A.P. (1953). The statistical analysis of the Canadian lynx cycle. II. Synchronization and meteorology. *Aust J Zool,* **1,** 291-298.

Murdoch W.W. (1994). Population regulation in theory and practice. *Ecology*, **75,** 271-287.

Nevoux M., Arlt D., Nicoll M., Jones C. & Norris K. (2013). The short- and long-term fitness consequences of natal dispersal in a wild bird population. *Ecology Letters,* **16**, 438-445.

Niitepõld K., Smith A.D., Osborne J.L., Reynolds D.R., Carreck N.L., Martin A.P. *et al.* (2009). Flight metabolic rate and Pgi genotype influence butterfly dispersal rate in the field. *Ecology,* **90,** 2223-2232.

Nowak M.A. & Sigmund K. (2004). Evolutionary dynamics of biological games. Science **303**, 793-799.

O'Sullivan D., Benton T.G. & Cameron T.C. Inter-patch movement in an experimental system: the effects of life history and the environment. *Oikos*, **123,** 623-629.

Ohgushi T. (2005). Indirect interaction webs: Herbivore-induced effects through trait change in plants. *Annual Review of Ecology Evolution and Systematics,* **36,** 81-105.

Olivieri I., Couvet D., Gouyon P. (1990). The genetics of transient populations: Research at the metapopulation level. *Trends in Ecology & Evolution,* **5,** 207-210.

Olivieri I., Michalakis Y. & Gouyon P.-H. (1995). Metapopulation genetics and the evolution of dispersal. *Am Nat*, 202-228.

Orsini L., Wheat C.W., Haag C.R., Kvist J., Frilander M.J. and Hanski I. (2009). Fitness differences associated with PgiSNP genotypes in the Glanville fritillary butterfly (*Melitaea cinxia*). *Journal of Evolutionary Biology,* **22,** 367-375.

Osakabe M., Isobe H., Kasai A., Masuda R., Kubota S. & Umeda M. (2008). Aerodynamic advantages of upside down take-off for aerial dispersal in *Tetranychus* spider mites. *Experimental and Applied Acarology,* **44,** 165-183.

Osborne K.A., Robichon A., Burgess E., Butland S., Shaw R.A., Coulthard A., Pereira H.S., Greenspan R.J. & Sokolowski M.B. (1997). Natural behavior polymorphism due to a cGMP-dependent protein kinase of Drosophila. *Science*, **277,** 834-836.

Packard G.C. & Boardman T.J. (1999). The use of percentages and size-specific indices to normalize physiological data for variation in body size: wasted time, wasted effort? *Comp. Biochem. Physiol. A,* **122,** 37-44.

Parsons P.A. (2005). Environments and evolution: interactions between stress, resource inadequacy and energetic efficiency. *Biological Reviews,* **80,** 589–610.

Pavey S.A., Bernatchez L., Aubin-Horth N. & Landry C.R. (2012). What is needed for next-generation ecological and evolutionary genomics? *Trends Ecol Evol*, **27,** 673-678.

Persson L. & de Roos A.M. (2013). Symmetry breaking in ecological systems through different energy efficiencies of juveniles and adults. *Ecology*, **94,** 1487-1498.

Poethke H.J. & Hovestadt T. (2002). Evolution of density-and patch-size-dependent dispersal rates. *Proceedings of the Royal Society B-Biological Sciences,* **269,** 637-645.

Poethke H.J., Dytham C. & Hovestadt T.A. (2011). Metapopulation paradox: partial improvement of habitat may reduce metapopulation persistence. *American Naturalist,* **177,** 792-799.

Post D.M. & Palkovacs E.P. (2009). Eco-evolutionary feedbacks in community and ecosystem ecology: interactions between the ecological theatre and the evolutionary play. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **364,** 1629-1640.

Prugnolle F. & De Meeus T. (2002). Inferring sex-biased dispersal from population genetic tools: a review. *Heredity,* **88,** 161-165.

Reynolds H.L., Vogelsang K.M., Hartley A.E., Bever J.D. & Schultz, P.A. (2006). Variable responses of old-field perennials to arbuscular mycorrhizal fungi and phosphorus source. *Oecologia*, **147,** 348-358.

Reznick D., Bryant M.J. & Bashey F. (2002). r- and K-selection revisited: the role of population regulation in life-history evolution. *Ecology*, **83,** 1509-1520.

Rodrigues A.M.M. & Johnstone R.A. (2014). Evolution of positive and negative density-dependent dispersal. *Proceedings of the Royal Society B*, in press.

Roeder C., Harmsen R. & Mouldey S. (1996). The effects of relatedness on progeny sex ratio in spider mites. Journal of Evolutionary Biology **9**,143-151.

Roff D.A. & Fairbairn D.J. (2001). The genetic basis of dispersal and migration, and its consequences for the evolution of correlated traits. *Dispersal. Oxford University Press, Oxford*, 191-202.

Ronce O. & Olivieri I. (1997). Evolution of reproductive effort in a metapopulation with local extinctions and ecological succession. *American Naturalist,* **150,** 220-249.

Ronce O. (2007). How does it feel to be like a rolling stone? Ten questions about dispersal evolution. *Annual Review of Ecology Evolution and Systematics,* **38,** 231-253.

Ronce O., Perret F. & Olivieri I. (2000). Landscape dynamics and evolution of colonizer syndromes: interactions between reproductive effortand dispersal in a metapopulation. *Evolutionary Ecology,* **14,** 233-260.

Ronce O.I., Clobert J. & Massot M. (1998). Natal dispersal and senescence. *Proceedings of the National Academy of Sciences*, **95,** 600-605.

Rothery P., Newton I., Dale L. & Wesolowski T. (1997). Testing for density dependence allowing for weather effects. *Oecologia,* **112,** 518-523.

Royama T. (1992). *Analytical population dynamics*. Springer.

Saccheri I. & Hanski I. (2006). Natural selection and population dynamics. *Trends Ecol Evol*, **21,** 341-347.

Sackett T. E., Classen A. T. & Sanders N. J. (2010). Linking soil food web structure to above- and belowground ecosystem processes: a meta-analysis. *Oikos*, **119:** 1984–1992.

Saito Y. (2000). Do kin selection and intra-sexual selection operate in spider mites ? Experimental and Applied Acarology **24,** 351-363.

Sarmento R. A., Lemos F., Bleeker P. M., Schuurink R. C., Pallini A., Almeida Oliveira M. G., Lima E. R., Kant M., Sabelis M. W. & Janssen A. (2011). A herbivore that manipulates plant defence. *Ecology Letters,* **14,** 229-236.

Schmidt L., Schurr U. & Roese U. S. R. (2009). Local and systemic effects of two herbivores with different feeding mechanisms on primary metabolism of cotton leaves. *Plant Cell and Environment,* **32,** 893-903.

Sibly R.M., Barker D., Denham M.C., Hone J. & Pagel M. (2005). On the regulation of populations of mammals, birds, fish, and insects. *Science*, **309,** 607-610.

Smitley D.R. & Kennedy G.G. (1985). Photo-oriented aerial-dispersal behavior of *Tetranychus urticae* (Acari: Tetranychidae). enhances escape from the leaf surface. *Annals of the Entomological Society of America*, **78,** 609-614.

Smyth G.K. (2005). limma: Linear models for microarray data. *Bioinformatics and computational biology solutions using R and BioconductorStatistics for biology and health*, Springer New York, pp 397-420.

Sokolowski M.B. (2001). Drosophila: genetics meets behaviour. *Nature Reviews Genetics*, **2,** 879-890.

Southwood T.R.E. (1977). Habitat, Templet for Ecological Strategies - Presidential-Address to British-Ecological-Society, 5 January 1977. *J Anim Ecol*, **46,** 337-365.

Starrfelt J. & Kokko H. (2012). Bet-hedging -a triple trade-off between means, variances and correlations. *Biol Rev*, **87,** 742-755.
Stevens V.M., Trochet A., Van Dyck H., Clobert J. & Baguette M. (2012). How is dispersal integrated in life histories: a quantitative analysis using butterflies. *Ecol Lett*, **15,** 74-86.

Strevens C.M.J. & Bonsall M.B. (2011). Density-dependent population dynamics and dispersal in heterogeneous metapopulations. *J Anim Ecol*, **80,** 282-293.

Sudova R. & Vosatka M. (2008). Effects of inoculation with native arbuscular mycorrhizal fungi on clonal growth of *Potentilla reptans* and *Fragaria moschata* (Rosaceae).. *Plant and Soil*, **308**, 55-67.

Thamer S., Schädler M., Bonte D. & Ballhorn D. J. (2011). Dual benefit from a belowground symbiosis: nitrogen fixing rhizobia promote growth and defense against a specialist herbivore in a cyanogenic plant. *Plant and Soil,* **341,** 209-219.

Thomas C.D. (2011). Anthropocene Park? No alternative. *Trends in Ecology & Evolution,* **26,** 497-498.

Thomas C.D. (2011). Translocation of species, climate change, and the end of trying to recreate past ecological communities. Trends in Ecology & Evolution **26,** 216-221.

Tindall K. V. & Stout M. J. (2001). Plant-mediated interactions between the rice water weevil and fall armyworm in rice. *Entomologia Experimentalis et Applicata*, **101,** 9-17.

Travis J. M. J., Delgado M., Bocedi G., Baguette M., Bartoń K., Bonte D., Boulangeat I., Hodgson J. A., Kubisch A., Penteriani V., Saastamoinen M., Stevens V.M. & Bullock J.M. (2013). Dispersal and species responses to climate change. *Oikos*, **11,**1532-1540.

Travis J.M.J. & Dytham C. (1999). Habitat persistence, habitat availability and the evolution of dispersal. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* **266,** 723-728.

Travis J.M.J. (2004). The evolution of programmed death in a spatially structured population. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences,* **59,** 301-305.

Travis J.M.J., Murrell D.J. & Dytham C. (1999). The evolution of density-dependent dispersal. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **266,** 1837-1842.

Tromeur E., Rudolf L. & Gross T. Impact of dispersal on the stability of metapopulations. *arXiv preprint arXiv:1305.2769*.

Turchin P. (1999). Population regulation: a synthetic view. *Oikos*, **84,** 153-159.

van Dam N. M. & Heil M. (2011). Multitrophic interactions below and above ground: en route to the next level. *Journal of Ecology,* **99,** 77-88.

van Dam N. M., Harvey J. A., Wackers F. L., Bezemer T. M., van der Putten W. H. & Vet L. E. M. (2003). Interactions between aboveground and belowground induced responses against phytophages. *Basic and Applied Ecology,* **4,** 63-77.

van Dam N. M., Raaijmakers C. E. & van der Putten W. H. (2005). Root herbivory reduces growth and survival of the shoot feeding specialist

Pieris rapae on Brassica nigra. *Entomologia Experimentalis Et Applicata,* **115,** 161-170.

Van Den Boom C. E. M., Van Beek T. A., Posthumus M. A., De Groot A. & Dicke M.. (2004). Qualitative and quantitative variation among volatile profiles induced by Tetranychus urticae feeding on plants from various families. *Journal of Chemical Ecology,* **30,** 69-89.

Van der Putten W.H., Vet L.E.M., Harvey J.A. & Wackers F.L. (2001). Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends in Ecology & Evolution,* **16,** 547-554.

Van Leeuwen T., Tirry L. & Nauen R. (2006). Complete maternal inheritance of bifenazate resistance in *Tetranychus urticae* Koch (Acari: Tetranychidae) and its implications in mode of action considerations. Insect Biochemistry and Molecular Biology **36**, 869-877.

Van Leeuwen T., Vanholme B., Van Pottelberge S., Van Nieuwenhuyse P., Nauen R., Tirry L. & Denholm I. (2008). Mitochondrial heteroplasmy and the evolution of insecticide resistance: Non-Mendelian inheritance in action. *Proceedings of the National Academy of Sciences of the United States of America,* **105,** 5980-5985.

Van Leeuwen T., Vontas J., Tsagkarakou A., Dermauw W. & Tirry L. (2010). Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: A review. *Insect Biochemistry and Molecular Biology,* **40,** 563-572.

Van Petegem H.P.K., Pétillon J., Renault D., Wybouw N., Van Leeuwen T., Stoks R. & Bonte, D. (2015). Empirically simulated spatial sorting points at fast epigenetic changes in dispersal behaviour. Evol Ecol **29,** 299-310.

Vandegehuchte M. L., de la Pena E. & Bonte D. (2010). Relative importance of biotic and abiotic soil components to plant growth and insect herbivore population dynamics. *PLoS ONE,* **5,** e12937.

Verbeke G. & Molenberghs G. (2000). *Linear mixed models for longitudinal data.* Springer-Verlag, New-York. 568p.

Vierheilig H., Coughlan A. P., Wyss U. & Piche Y. (1998). Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology,* **64,** 5004-5007.

Wang S. & Loreau M. (2014). Ecosystem stability in space: α, β and γ variability. *Ecol Lett*, **17,** 891-901.

Wardle D. A., Bardgett R. D., Klironomos J. N., Setala H., van der Putten W. H. & Wall D. H. (2004). Ecological linkages between aboveground and belowground biota. *Science,* **304,** 1629-1633.

Waser P.M., Nichols K.M. & Hadfield J.D. (2013). Fitness consequences of dispersal: Is leaving home the best of a bad lot? *Ecology* ,**94**, 1287-1295.

Wheat C.W. (2012). Dispersal genetics: emerging insights from fruit flies and butterfies in J. Clobert, M. Baguette, T. Benton, and J. Bullock, eds. *Informed dispersal and spatial evolutionary ecology*. Oxford University Press.

Wilbur H.M. & Rudolf V.H.W. (2006). Life-history evolution in uncertain environments: Bet hedging in time. *Am Nat*, **168,** 398-411.

Wild G. (2011). Direct fitness for dynamic kin selection. *Journal of Evolutionary Biology,* **24,**1598-1610.

Williams G.C. (1966). Natural selection, the costs of reproduction, and a refinement of Lack's principle. *Am Nat*, 687-690.

Wybouw N., Dermauw W., Tirry L., Stevens C., Grbiç M., Feyereisen R. *et al.* (2014). A gene horizontally transferred from bacteria protects arthropods from host plant cyanide poisoning. *eLife,* **3,** doi:10.7554/eLife.02365.

Yano S. & Takafuji A. (2002). Variation in the life history pattern of *Tetranychus urticae* (Acari: Tetranychidae) after selection for dispersal. *Experimental and Applied Acarology,* **27,** 1-10.

Yano S., Wakabayashi M., Takabayashi J. & Takafuji A. (1998). Factors determining the host plant range of the phytophagous mite, *Tetranychus urticae* (Acari: Tetranychidae): a method for quantifying host plant acceptance. *Experimental & applied acarology,* **22,** 595-601.

Zhurov V., Navarro M., Bruinsma K.A., Arbona V., Santamaria M.E., Cazaux M. *et al.* (2014). Reciprocal responses in the interaction between *Arabidopsis* and the cell-content-feeding chelicerate herbivore spider mite. *Plant Physiology,* **164,** 384-399.

Zera A. J. & Denno R. F. (1997). Physiology and ecology of dispersal polymorphism in insects. *Annual Review of Entomology,* **42,** 207-230.