POPULATION GENETICS, FOOD-PLANT SPECIALIZATION, AND LOCAL ADAPTATION OF INSECT HERBIVORES LIVING IN A FRAGMENTED LANDSCAPE

by

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"When nothing is sure, everything is possible."
Margaret Drabble

"Jos ymmärrät kaeken, oot varmasti käsittännä viärin!"
Savolainen sananlasku

Isälle.

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LIST OF ORIGINAL PAPERS

This thesis is based on the following publications and manuscripts, referred to in the text by their Roman numerals:

I   Laukkanen, L., Mutikainen, P., Muola, A. & Leimu, R.: Plant-species diversity correlates with genetic variation of an oligophagous seed predator. *Submitted manuscript.*

II   Laukkanen, L., Leimu, R., Muola, A., Lilley, M. & Mutikainen, P. 2013: Genetic factors affecting food-plant specialization of an oligophagous seed predator. – *Journal of Evolutionary Biology* 26(1):141–149. doi: 10.1111/jeb.12037

III   Laukkanen, L., Muola, A., Leimu, R., Kalske, A. & Mutikainen, P.: Selection for food-plant use of a seed predator: adaptation and trade-offs in fitness between different food plants. *Manuscript.*

IV   Muola, A., Laukkanen, L., Palin, N., Mutikainen, P. & Leimu, R.: Inbreeding and random drift modify food-plant specialization of a seed predator in a selection experiment. *Manuscript.*

V   Laukkanen, L., Leimu, R., Muola, A., Lilley, M., Salminen, J-P. & Mutikainen, P. 2012: Plant chemistry and local adaptation of a specialized folivore. – *PLoS One* 7(5): e38225. doi: 10.1371/journal.pone.0038225.

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1. INTRODUCTION

The evolution of terrestrial plants and herbivorous (phytophagous) insects are closely associated. Terrestrial insects have been feeding on green plants for about 400 million years (Labandeira 2007). During this period, insects have been the richest and the most important group of herbivores (Schoonhoven *et al.* 2005). Nearly 50 % of all existing insect species feed on living plants, and herbivory is common among multiple large insect taxa, for instance among bugs (Heteroptera), butterflies and moths (Lepidoptera), and flies (Diptera) (Schoonhoven *et al.* 2005). All terrestrial vascular plant species have some herbivores feeding on them (Schoonhoven *et al.* 2005). Therefore, it is obvious that the relationship between plants and insect herbivores is an essential part of the organisation of biotic communities and has fundamental importance in whole ecosystems.

Not only insect herbivores, but also the interactions between plants and herbivores are highly diverse. Some herbivore species have evolved to be dietary generalists and others specialists, and the object and degree of specialization may vary among populations or individuals within a population (e.g. Fox & Morrow 1981, Funk & Bernays 2001, Ueno *et al.* 2003, Singer 2008). In general, insect taxa that are specialized in their food utilization are often more diverse than taxa including more generalist feeders, as the rate of evolution is higher among specialists (Whitlock 1996). Of course, the high diversity of herbivores has not evolved independently, but is associated with simultaneous changes in plant populations and species. In fact, a growing number of studies have demonstrated that coevolution, i.e., the reciprocal evolutionary responses between interacting species, is a major force that modifies the diversity of insects and plants (Thompson 1994, 2005). Especially, when the coevolutionary processes between insects and their food plants are spatially divergent, they have the potential to contribute significantly to the diversity of insect herbivores (Thompson 1994).

Species interactions and their evolution are strongly influenced by the recent human induced environmental changes including loss and fragmentation of habitats, and as a consequence, biological diversity is rapidly declining in many ecosystems. The scale of the impact of this accelerating process ranges from populations to communities, as fragmentation may affect genetic variation at the population level, generate population differentiation at the species level, or reduce the species diversity at the community level (e.g. Saunders *et al.* 1991, Young *et al.* 1996, Fahrig 2003). Generally, the impacts of habitat fragmentation on a single species have been widely studied, and for example, migration rate, breeding system, and interactions with other species all influence the potential of the species to overcome the negative effects of habitat fragmentation (e.g. Dempster 1991). Moreover, the general loss of biodiversity in ecosystems due to habitat fragmentation is undeniable (e.g. Saunders *et al.* 1991, Collinge 2000), but the effects of fragmentation on specified species interactions that contribute to this large-scale process

are less well understood. Therefore, understanding the influences of fragmentation on plant-herbivore interactions may have extensive implications for conservation of biological diversity.

In this thesis, the interaction between plants and herbivores will be mainly viewed from the herbivore perspective. I focus on the heritable variation within herbivore populations, differentiation among herbivore populations, food-plant specialization, and adaptation of herbivores to novel food plants and local plant populations. Gradual differentiation of populations and spatially divergent trajectories of adaptation and food-plant specialization are regarded as key processes behind the enormous diversity of herbivorous insects present today (Thompson 1994). Thus, studying these essential aspects of the interaction between plants and herbivores may help us to better understand the present interactions, estimate how they have evolved, and predict how they will be modified in the future.

1.1.   Genetic and phenotypic differentiation of populations

Usually species have spatially structured populations both at the genotypic and phenotypic level. The genetic differentiation of populations may be expressed as divergence in phenotypic traits and realised as differences in morphology, phenology, or physiology (Mayr 1947, Linhart & Grant 1996). Several abiotic and biotic factors influence the population genetics of herbivores, and population genetic structure may reveal the history of populations and species. In the first place, genetic differentiation among populations may be shaped by founder effects (Dlugosch & Parker 2008). In general, mutations, spatially varying selection pressures, random genetic drift, population bottlenecks, and inbreeding may all lead to genetic differentiation among populations (e.g. Lande 1976, Frankham 2005). For instance, the adaptive divergence of herbivore populations caused by spatially varying selection pressures may be revealed by spatially varying fitness and life-history traits, and preference for certain food-plant species (e.g. Sotka *et al.* 2003, Desurmont *et al.* 2012). Because differential natural selection, random drift, and accumulation of mutations have had more time to shape the genetic structure of populations that have persisted over longer times, these populations are expected to be genetically more differentiated than young populations (Lande 1976, Hartl & Clark 2007). Moreover, small populations may be more differentiated than large ones, because they are more vulnerable to random drift, population bottlenecks, and inbreeding (Frankham 2005). Even when mating is random, mating between close relatives is more common in small populations compared to larger populations (Frankham 2005).

On the other hand, gene flow reduces genetic differentiation among populations, and thus, isolated populations may be more differentiated than populations with several migrants from other populations importing novel genetic variation (Peterson & Denno 1998). As increased gene flow is thought to lead to decreased genetic and phenotypic

differentiation among populations, strong gene flow has traditionally been thought to reduce the possibility of adaptation of populations to local conditions (Gandon *et al.* 1996). Isolation by geographic distance is observed among many herbivore species (Peterson & Denno 1998, Rich *et al.* 2008, Gayathri Samarasekera *et al.* 2012). However, isolation may also increase due to the presence of physically and biologically unsuitable habitats for the species to survive or reproduce in (Lowe *et al.* 2004, Storfer *et al.* 2010). The genetic differentiation and divergence in plant secondary chemistry among food-plant populations can potentially cause differential natural selection among herbivore populations that may lead to the genetic differentiation of populations (Gols *et al.* 2008, Magalhaes *et al.* 2011). Furthermore, the genetic differentiation of herbivore populations and their differentiation in food-plant utilization are related to each other, and may finally lead to speciation (e.g. Waring *et al.* 1990, Stireman *et al.* 2005).

1.2. Heritable variation within populations

Studying the level of genetic variation within populations may help us both to reveal the history and predict the future of populations and species. A sufficient level of genetic variation is required for the viability of populations and species (Lowe *et al.* 2004). A multitude of factors affect the level of genetic variation within populations. Initially, the size and origin of the founder population determines the level of genetic variation (Dlugosch & Parker 2008). In general, small and isolated populations are vulnerable to loss of within-population genetic variation. When the population size decreases for instance because of unfavourable abiotic conditions, i.e., there is a population bottleneck, genetic variation may be lost first due to chance and further due to increased inbreeding and random genetic drift (Frankham 1996, Lowe *et al.* 2004, Frankham 2005). When the level of heterozygosity is reduced, the deleterious recessive alleles normally present in a genome may be expressed potentially reducing the fitness of individuals (Frankham 2005). Inbreeding and random drift affect the whole genome, but in contrast, natural selection reduces genetic variation in fitness related traits, and thus, the neutral loci are not affected in the absence of genetic linkage (Willi *et al.* 2006). However, introduction of new genetic material via mutations and gene flow may counteract these forces reducing genetic variation and ensure further adaptive potential of small populations (Hartl & Clark 2007). If the mildly deleterious mutations are not purged due to ineffective selection in small populations, they may be fixed due to genetic drift and reduce reproductive fitness possibly leading to extinction of the population (Frankham 2005).

The low level of genetic variation in the use of resources may prevent the evolution of ecological specialization (Futuyma & Peterson 1985). For example, the lack of genetic variation may prevent the adaptation of herbivores to novel or changed environments and restrict their ability to feed on certain plant individuals, populations, or species (Futuyma & Peterson 1985, Lande & Shannon 1996). Thus, variation in herbivore performance on different food-plant species can be considered to indicate the ability of the herbivore

population to adapt further in food-plant use (e.g. Futuyma *et al*. 1995, Keese 1998, Mopper *et al.* 2000).

1.3.   Evolution of species interactions

Spatial genetic differentiation is a starting point both for the differentiation of populations at the phenotypic level and the spatially structured evolution of species interactions. The adaptive divergence caused by varying selection pressures occurring in different populations is a key in understanding how evolution eventually leads to speciation if the gene flow is reduced (Mayr 1947, Futuyma & Peterson 1985, Thompson 1994, 2005). For example, the degree of adaptation to local environmental conditions, and the level of specialization on the available resources may vary among populations (Thompson 1994, 2005).

A major part of evolution is coevolution (Ehrlich & Raven 1964, Janzen 1980, Thompson 1994, 2005). Among-population variation in species interactions is a prerequisite for variation in the coevolutionary process at larger spatial scales. According to the *Geographic mosaic theory of coevolution* (Thompson 1994, 2005), selection is reciprocal exclusively in coevolutionary hot spots, i.e., in those sites where the both interacting species impose selection on each other. Interacting species may not occur in every population, or the interaction between the species may not lead to evolutionary change in both counterparts. These sites are called coevolutionary cold spots.

It is often difficult to study coevolution in an evolutionary relevant time scale and show in practise that both counterparts in a species interaction are evolving as a result of reciprocal selection (Janzen 1980, Gomulkiewicz *et al*. 2007, Laine 2009). Commonly, the inadequate time scale of studies is compensated by studying coevolution at large spatial scales, i.e., by gathering snapshot data on traits that are closely linked with the fitness of the interacting species, and that may thus be important for the interaction (Gomulkiewicz *et al*. 2007, Laine 2009). Indeed, coevolution may occur at many levels, but it may be a challenge to link reciprocal evolutionary change at the population level to coevolution at a larger scale, i.e., the evolutionary change of species across the whole distribution range (Thompson 1994, 2005). Despite the difficulties that scientists studying reciprocal change in species interactions have to face, the body of evidence on coevolution is continuously growing (Gomulkiewicz *et al*. 2007).

1.4.   Plant-herbivore interactions

Plant-herbivore interactions usually show significant variation in space and time, and thus, green plants and herbivores have regularly been used as model systems in studying evolutionary change in species interactions (e.g. Ehrlich & Raven 1964, Berenbaum & Zangerl 1998, Pauw *et al*. 2009, Wise & Rausher 2013). Insect

herbivores in particular have been used in several evolutionary studies due to their high diversity, and the ease of rearing them due to their short generation time and small body size. In spite of spatiotemporally variable population sizes of herbivores due to biotic (e.g. spatially varying availability and quality of food, abundance of predators and parasitoids) and abiotic factors (e.g. weather conditions), insects feeding on plants have severe impacts on plant fitness in general (Schoonhoven *et al.* 2005). Herbivores may reduce plant survival, growth, and fecundity (Crawley 1989, Strauss 1991). Therefore, plants defend themselves against herbivores by a range of adaptations that may reduce the damage. The resistance mechanisms vary greatly among different plant species, and many plant secondary metabolites, structural defences, and phenological adaptations may be used to repel herbivores or reduce the digestibility of plant (Price *et al.* 1980, Bennett & Wallsgrove 1994, Núñez-Farfán *et al.* 2007). On the other hand, after herbivore damage has occurred plants often reduce the negative effects by tolerance mechanisms, i.e. improving their survival and reproduction (Núñez-Farfán *et al.* 2007, Fornoni 2011). Regardless of the versatile defence mechanisms used against herbivores, plants are usually consumed by multiple herbivore species each having different fitness effects on their shared food plant (Strauss 1991, Wise & Rausher 2013). Of course, not only do herbivores affect plants, but also *vice versa*. For example, variation in plant nutritional quality or secondary chemistry commonly affects herbivore populations through its effect on herbivore survival, reproductive output, and risk of predation (e.g. Price *et al.* 1980, Larsson *et al.* 2000, Lill *et al.* 2002).

1.4.1. *Effect of variation in plant-herbivore interactions on herbivore population genetics*

In addition to the size and age of populations and the degree of isolation, many biological characteristics of habitats also influence the population genetics of herbivores. In general, plant-species composition together with secondary chemistry and nutritive quality of food plants exert selection on herbivores and may affect their population genetics (e.g. Agrawal *et al.* 2006). It is important to take the diet breadth of herbivores into account, when evaluating the effects of plants on herbivores. In general, generalist and specialist herbivores may differ in their levels and spatial distribution of genetic variation (Nevo 1978, Peterson & Denno 1998, Kelley *et al.* 2000), and the effect of plant-species composition and plant quality on population genetics may also differ between generalist and specialist herbivores. Naturally, the occurrence and nutritive quality of a single food-plant species control the fitness of a specialist herbivore feeding on that food plant (e.g. Awmack & Leather 2002, Leimu & Syrjänen 2002, Colling & Matthies 2004). Correspondingly, the population size, population genetic structure, and secondary chemistry of this food-plant species have potential to influence the levels and spatial distribution of genetic variation of specialist herbivores (e.g. McCauley 1991). Moreover, high plant-species diversity may negatively affect herbivores using a single food-plant species due to the lower abundance of this food-

plant species in the environment or due to more abundant and more efficient predators (Tahvanainen & Root 1972, Bach 1980). In contrast, a generalist herbivore with an extensive diet may not be strongly affected by the occurrence and nutritive quality of single food-plant species. It seems logical to expect that within-population genetic variation and population differentiation of an oligophagous herbivore feeding on few food-plant species might be affected both by the occurrence, abundance, and quality of its primary food-plant species, as well as by the diversity of alternative food-plant species in the plant community. For instance, large variety of alternative food-plant species may prevent population bottlenecks caused by shortage of food, and thus, help to sustain a stable population size and further facilitate the maintenance of high levels of within-population genetic variation of oligophagous herbivores. In spite of the supposed significance of plant-species diversity on the population genetic structure of herbivores, studies verifying this are still needed.

1.4.2. Food-plant specialization

Less than 10 % of herbivore species utilize plants from more than three plant families, and the majority of herbivores specialize on few plant species belonging to a single plant family (Schoonhoven *et al.* 2005). In general, food-plant specialization is a spatiotemporally varying, dynamic process that may ultimately lead to speciation (Thompson 1994). A change in the availability of a previous food-plant species in an environment may lead to a shift in food-plant use. The degree of specialization may vary among herbivore populations, and even individuals within the same population may differ in their performance on different food plants and in food-plant preference (e.g. Fox & Morrow 1981, Ueno *et al.* 2003, Schoonhoven *et al.* 2005, Singer 2008). Furthermore, introduction of a novel food-plant species may lead to strong selection for herbivore adaptation to this plant species. Adaptation to a novel food plays a key role, for example, in the establishment of introduced species to new environments. Adaptation of herbivores to novel food plants has been observed in a wide range of insect herbivores, and in some cases the evolutionary response of a herbivore to a novel food plant has been very rapid (Carroll *et al.* 1997, Agrawal 2000). For instance, soapberry bugs (*Jadera haematoloma*) adapted morphologically to the goldenrain tree (*Koelreuteria elegans*) in just few decades (Carroll *et al.* 1997). Rapid evolutionary change has been documented also in herbivore preference for food plants (Wasserman & Futuyma 1981) and in performance on different food plants (Gould 1979, Fry 1989). Despite the importance of specialization and adaptation in ecology and evolution, the rate of evolutionary change and the processes driving variation in food-plant specialization are still largely unknown.

Multiple ecological and genetic factors affect food-plant specialization of herbivorous insects (Fox & Morrow 1981, Bernays & Graham 1988, Futuyma *et al.* 1995, Forister *et al.* 2007). According to the traditional view, being a specialist is beneficial in stable conditions, where plant resources are abundant over space and time enabling herbivore

adaptation to plant nutritional quality and secondary chemistry. Naturally, spatiotemporal variation in food abundance may prevent specialization of a herbivore to a single food-plant species and favour generalist herbivores (e.g. Futuyma 1976, Fox & Morrow 1981, Fox & Caldwell 1994). Moreover, among-species variation in plant chemical compounds may determine the preference, performance, and specialization of the herbivores to different food-plant species (e.g. Rank 1992, Becerra 1997, Rasmann & Agrawal 2011). In addition to the direct effects of plant chemicals on food-plant specialization, they often have an effect via a third trophic level, as herbivores may use chemicals as defence against their predators and parasitoids (Price *et al*. 1980). Insects might even prefer nutritionally suboptimal food plants that provide them 'enemy-free-space' (Singer *et al*. 2004).

In addition to factors related to biotic interactions, genetic factors may also influence the evolution of specialization in herbivores. Firstly, adaptation and specialization of a herbivore to current and novel food-plant species may be constrained by insufficient level of genetic variation for selection to act on (e.g. Futuyma *et al*. 1995, Forister *et al*. 2007). Secondly, trade-offs, i.e., negative genetic correlations in performance of the herbivores between different food-plant species favour the evolution of specialization (Fry 2003, Scheirs *et al*. 2005). A trade-off exists if a genotype performing well on one food-plant species has relatively poor performance on other. Trade-offs may result from antagonistic pleiotropy or linkage equilibrium of genes (Agosta & Klemens 2009). Most studies have found no evidence for trade-offs in herbivore performance between different food plants (e.g. Fry 2003, Scheirs *et al*. 2005, Agosta & Klemens 2009). Asexual reproduction may promote the possibility of trade-offs in food-plant use, as the majority of studies that have found evidence for them used asexually reproducing herbivores, such as mites and aphids, as study organisms (e.g. Gould 1979, Fry 1990, Via 1991, Mackenzie 1996, Via & Hawthorne 2002). In addition, only a very limited variety of specialized herbivores have been used in studies testing the existence of trade-offs, and the results are contradictory (Via 1991, Thompson 1996, Keese 1998, Via & Hawthorne 2002, Forister *et al*. 2007, García-Robledo & Horvitz 2011).

1.4.3. *Local adaptation*

The abiotic and biotic environment experienced by organisms varies in space and time (Thompson 2005), and natural selection produces adaptations to these different environments, i.e., spatial variation in fitness-related traits. This often results in local adaptation of populations (Kawecki & Ebert 2004, Hoeksema & Forde 2008). Indeed, there is evidence of herbivore local adaptation both to regional climates and local food-plant populations (e.g. Ayres & Scriber 1994, Mopper *et al*. 1995, Abdala-Roberts & Marquis 2007). Herbivores are locally adapted to their food-plant populations if their mean fitness is relatively higher on plants from their sympatric

(i.e. home) population compared to those from allopatric (i.e. away) populations (Kawecki & Ebert 2004).

Generation times and migration rates of interacting species are likely to influence their local adaptation (Gandon *et al.* 1996, Gandon & Michalakis 2002, Greischar & Koskella 2007, Hoeksema & Forde 2008). In an interaction with a long-lived plant and its herbivore, adaptation of the herbivore to sympatric food-plant populations is likely, as the herbivore has a much shorter generation time and thus an adaptive advantage compared to the plant (Hanks & Denno 1994, Hoeksema & Forde 2008, Garrido *et al.* 2011). Furthermore, a herbivore is predicted to be locally adapted to its local food-plant populations when it has strong negative effects on plant fitness (Gandon *et al.* 1996, Lively 1999). Herbivore local adaptation is also predicted if the migration rate of the herbivore is higher than that of the food plant (Gandon *et al.* 1996, Greischar & Koskella 2007, Hoeksema & Forde 2008, Garrido *et al.* 2011). This is because gene flow provides genetic variation for selection to act on. However, high levels of gene flow may also homogenize populations and thus prevent local adaptation (Gandon *et al.* 1996). In general, local adaptation is predicted to be more probable with growing divergence among the populations (Becker *et al.* 2006, Hereford & Winn 2008, Hereford 2009). Consequently, the difference in the degree of local adaptation among populations should correlate positively with the geographic distance, genetic differentiation, and phenotypic divergence among the populations (Hereford 2009). However, local adaptation may occur even between connected populations or within continuous populations if the forces of selection are strong enough to counteract gene flow among patches or populations (Kawecki & Ebert 2004, Tack & Roslin 2010). Moreover, the occurrence and degree of local adaptation in antagonistic interactions is predicted to vary both in time and space due to the dynamic nature of the evolutionary process (Thompson 2005, 2009). At a given point in time, populations of herbivores might show different degrees of local adaptation, or even lack of local adaptation depending on the strength of the selection imposed by the interacting food-plant species (Thompson *et al.* 2002, Ruhnke *et al.* 2006, Hoeksema & Forde 2008, Garrido *et al.* 2011). Lack of local adaptation may result from no difference in fitness between sympatric and allopatric food plants, or maladaptation, i.e., lower fitness in sympatry compared with allopatry (Ruhnke *et al.* 2006, Garrido *et al.* 2011). Maladaptation might either reflect the dynamic nature of evolution of species interactions (Lively 1999, Thompson 2005, 2009, Laine 2009), or indicate that there might be gene flow from populations adapted to different conditions (Thompson *et al.* 2002). In accordance with these predictions, some studies demonstrate local adaptation of herbivores in at least some of the populations (e.g. Hanks & Denno 1994, Mopper *et al.* 1995, Van Zandt & Mopper 1998, Ortegón-Campos *et al.* 2009, Garrido *et al.* 2011) while other studies provide no evidence on local adaptation (e.g. Strauss 1997, Spitzer 2006).

Variation in local adaptation is predicted to be driven by differences in the traits that are central for the interaction (Hoeksema & Forde 2008). In many plant species, there

is considerable geographic variation in resistance against herbivores, and populations of many herbivore species are known to be differentiated in their food-plant use (e.g. Roininen *et al*. 1993, Sork *et al*. 1993, Carroll *et al*. 1997, Sotka *et al*. 2003). Plant resistance exerts a strong selection pressure on insect herbivores, as plant chemicals commonly affect the behaviour, growth, or mortality of the herbivores (Schoonhoven *et al*. 2005). It has been demonstrated that between-species variation in plant defence chemicals may affect adaptation of herbivores to different food-plant species (Del Campo *et al*. 2003), and that plant resistance and herbivore adaptation to local plant populations are associated (Garrido *et al*. 2011). However, the studies combining variation in plant secondary chemistry and local adaptation of herbivores are needed to understand the effects of specific secondary chemicals on the process of herbivore local adaptation.

1.5. Aims of the study

The aim of this thesis was to address several fundamental questions on the evolution of plant-herbivore interactions focusing on two herbivores that share a food-plant species. Specifically, I aimed to study heritable variation within and differentiation among herbivore populations, adaptation of herbivores to local food-plant populations and to novel food-plant species, and the roles of selection, inbreeding, and random processes in herbivore food-plant specialization (Table 1). These specific processes determine the ecological and evolutionary outcomes of plant-herbivore interactions, not separately but in combination with each other: for instance, heritable variation is a prerequisite for herbivore's ability to adapt to local food-plant populations and to novel food-plant species, and the genetically differentiated herbivore populations are more likely to show divergence in food-plant utilization compared to the populations with no genetic differentiation. As the study system I used the folivore *Abrostola asclepiadis* (Lepidoptera), seed predator *Lygaeus equestris* (Heteroptera), and their shared food plant *Vincetoxicum hirundinaria* (Apocynaceae). I also used alternative or novel food-plant species to examine food-plant adaptation and specialization of *L. equestris*. The results of my thesis provide novel insights into the importance of the scale and level of spatial variation for the evolution of plant-herbivore interactions. Furthermore, my results add to our understanding of the mechanism and relevant timescale of adaptation and specialization occurring in specialized plant-herbivore interactions in a spatial framework.

Table 1. Research questions, study species, and methods used in this study.

Research questions:	Chapter
Heritable variation	
in neutral markers	I
in fitness-related traits	II, III
Population differentiation	
in neutral markers	I
in fitness-related traits	III, IV
Food-plant specialization	II-IV
Adaptation to novel food plant	III
Random processes	IV
Inbreeding	IV
Local adaptation	V
Study species:	
Herbivores	
Heteropteran seed predator *Lygaeus equestris*	I-IV
Folivorous moth *Abrostola asclepiadis*	V
Food-plant species	
Centaurea phrygia	III
Crepis tectorum	II
Helianthus annuus	III, IV
Tanacetum vulgare	II
Verbascum thapsus	II, III
Vincetoxicum hirundinaria	I-V
Methods:	
Data from the natural populations	I
Laboratory experiments	
Crossing experiments	IV
Multi-generation selection experiment	III, IV
Split-brood feeding experiment	II, III, V
Genetic analysis (AFLP)	I
Analysis of plant secondary chemicals (HPLC)	V
Statistical analysis	I-V

2. MATERIALS AND METHODS

2.1. Study species

Vincetoxicum hirundinaria Med. (= *Cynanchum vincetoxicum* (L.) Pers.) (Apocynaceae, former: Asclepiadaceae) (Figure 1) is a long-lived, perennial herb that prefers calcareous substrates and grows typically on sunny exposed cliffs and slopes. *Vincetoxicum hirundinaria* has a wide continental Eurasian distribution. The north-western limit of the distribution is in Scandinavia, where *V. hirundinaria* inhabits the islands and coastal areas of the Baltic Sea. In Scandinavia, *V. hirundinaria* flowers from the middle of June until the beginning of August, and the flowers are pollinated mainly by large flies, moths, and bees (Timonin & Savitsky 1997, L. Laukkanen & A. Muola pers. obs.). The pods, containing approximately 20 wind-dispersed seeds, normally ripen at the end of August and in September (Hämet-Ahti *et al.* 1998, Leimu 2004).

Vincetoxicum hirundinaria

Lygaeus equestris   *Abrostola asclepiadis*
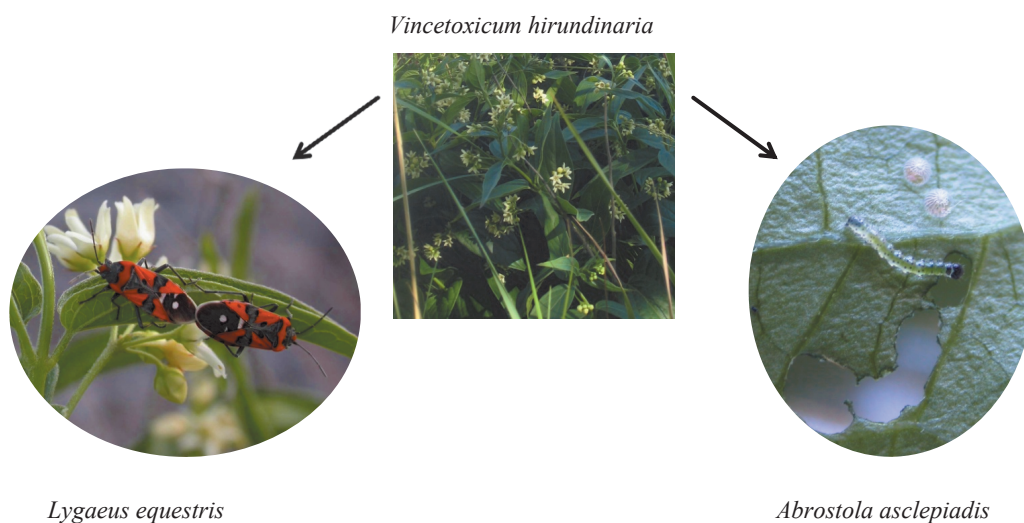
Photographs: *L. equestris* / Kalle Rainio, *V. hirundinaria* / Anne Muola, *A. asclepiadis* / Liisa Laukkanen

Figure 1. Study species. The perennial plant *Vincetoxicum hirundinaria* and herbivores feeding on it. Both adults and larvae of the true bug *Lygaeus equestris* feed mainly on seeds of *V. hirundinaria*. Larvae of the moth *Abrostola asclepiadis* is feeding on the leaves of *V. hirundinaria*.

Vincetoxicum hirundinaria is highly poisonous and contains several types of secondary compounds, such as antofine and phenolic compounds (Staerk *et al*. 2000, Muola *et al*. 2010b), which might explain the low number of herbivores feeding on it. Indeed, mammals and many generalist insects avoid *V. hirundinaria*. Despite its toxicity, in my study area three specialized herbivores, *Lygaeus equestris* L. (Heteroptera: Lygaeidae), *Abrostola asclepiadis* Schiff. (Lepidoptera: Noctuidae), and *Euphranta connexa* (Fabr.) (Diptera) feed on *V. hirundinaria*.

The aposematic seed predator *L. equestris* (Figure 1) is specialized to feed on the green ovulae, developing seeds, and mature seeds of *V. hirundinaria*, but it can occasionally also suck other parts, such as stems and leaves, of *V. hirundinaria*, and other plant species (Solbreck & Kugelberg 1972, Kugelberg 1973ab, 1974). Especially in spring, when the seeds of *V. hirundinaria* from previous year may be difficult to find, and during and after the severe summer droughts, alternative food plants might be essential for the survival of *L. equestris* (Solbreck & Kugelberg 1972). However, feeding on alternative food plants is known to affect several life-history traits negatively such as mortality, adult biomass, and the number of eggs laid (Kugelberg 1973ab), and thus, it may have both ecological and evolutionary consequences. Moreover, in Finland and Sweden *L. equestris* is found just in *V. hirundinaria* populations, and therefore, I consider *L. equestris* as an oligophagous herbivore. *Lygaeus equestris* is relatively common in my study area (Leimu & Syrjänen 2002, Rintala & Rinne 2010), although its population sizes vary considerably among years and populations (Solbreck & Sillén-Tullberg 1990ab). The usually univoltine *L. equestris* overwinters as an adult. The female *L. equestris* lay eggs on the ground-layer vegetation in June and July. Adults of the new generation generally appear from late-July onwards (Solbreck & Kugelberg 1972).

The folivorous noctuid moth *A. asclepiadis* (Figure 1) is a strict specialist of *V. hirundinaria*. The female *A. asclepiadis* oviposit on the leaves of *V. hirundinaria* in June and July, and the five larval instars are completed in approximately five to six weeks (Förare 1995). *Abrostola asclepiadis* can be locally common, but its population sizes vary both spatially and between years (Förare 1995, L. Laukkanen pers. obs.). Thus, damage levels caused by feeding *A. asclepiadis* larvae vary among years and among populations from no damage to almost complete defoliation of the plants (Leimu & Lehtilä 2006). *Abrostola asclepiadis* can disperse up to 50 km under optimal conditions (Förare 1995).

In this study, I concentrated on the interactions of *L. equestris* and *A. asclepiadis* with their food plant. However, there is also third herbivore, the tephritid fly *E. connexa* that is specialized to *V. hirundinaria*. The female flies oviposit in the developing pods, and the larvae live within the pods and consume the ripening seeds (Solbreck 2000). The larvae are significant pre-dispersal seed predators of *V. hirundinaria* in my study populations. In fact, in some years and some populations seed predation by *E. connexa* may destroy almost all the seeds, and thus this herbivore may also have significant effects on other herbivores, especially on *L. equestris* (Solbreck 2000, Leimu & Syrjänen 2002, Leimu & Lehtilä 2006, Solbreck & Ives 2007).

In the food-plant specialization experiments (chapters **II-IV**) I used *Centaurea phrygia* L. (Asteraceae), *Crepis tectorum* L. (Asteraceae), *Helianthus annuus* L. (Asteraceae), *Tanacetum vulgare* L. (Asteraceae), and *Verbascum thapsus* L. (Scrophulariaceae) as alternative food-plant species for *L. equestris*. All of these species except *H. annuus* occur naturally in my study area, and *L. equestris* feeds on them (L. Laukkanen, R. Leimu & A. Muola pers. obs.). In addition, *L. equestris* is a common pest on extensively cultivated *H. annuus* fields in Central and Southern Europe (Horváth *et al*. 2004). The other plant species used in the experiments contain several, but different specific chemical compounds

that may confer resistance to herbivores (e.g. Brewer & Ball 1981, Schearer 1984, Kisiel & Kohlmünzer 1989, Hussain *et al*. 2009). All species used in the study are listed in Table 1.

2.2. Study populations

I conducted my study in the south-western Finland and eastern Sweden. The naturally fragmented archipelago with several islands provides an ideal opportunity for studying spatial variation in species interactions, as well as population genetics of herbivores and the factors affecting it. Moreover, the simple study system with only a couple of specialized herbivores feeding on a shared food plant is optimal to study plant-herbivore interactions, as only a few other herbivore species compete for food, or may have indirect effects on the studied herbivore species, for example, via induced changes in the food-plant chemistry. Spatial variation is essential in two of the chapters of my thesis: I examined spatial variation in herbivore population genetics in chapter **I**, and in plant-herbivore interaction in chapter **V**. In the three chapters I used only one study site (**II-IV**). I used a total of 23 study sites in my experiments, 15 sites from Finland and eight sites from Sweden (Figure 2). The study sites in Finland are mainly situated on separate islands of the Baltic Sea. The study sites in Sweden are mainly on mainland, but I also included one mainland site from Finland and two island sites from Sweden in one of the studies (chapter **I**).
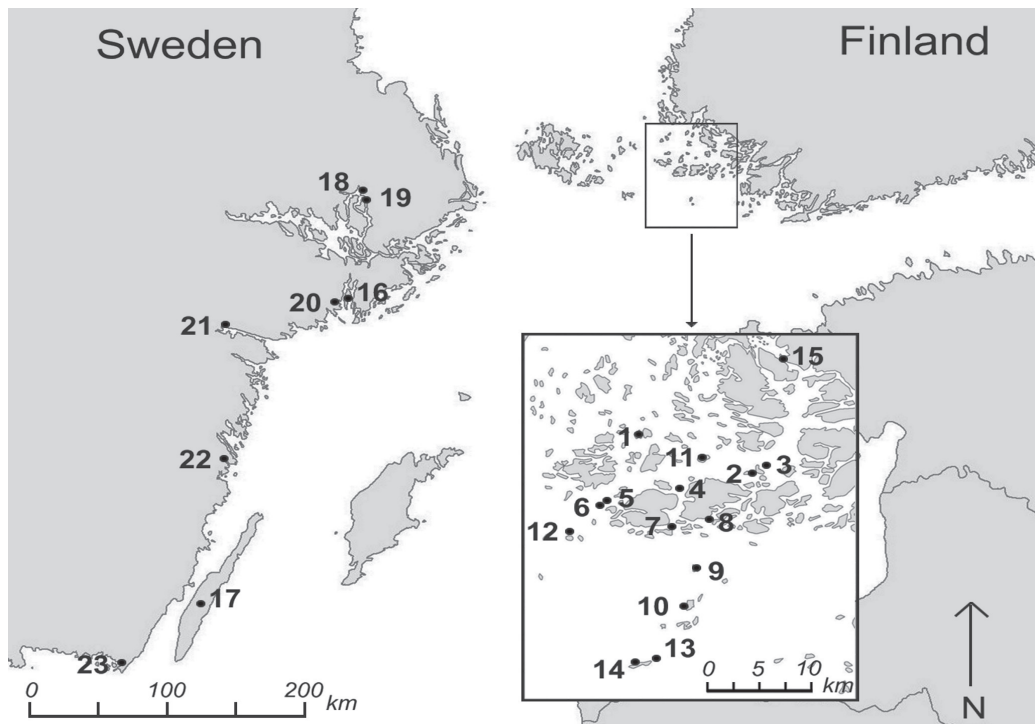

Figure 2. A map showing the locations of the 23 studied herbivore populations. All 23 *Lygaeus equestris* populations were used in paper **I**, and *L. equestris* population 2 in papers **II-IV**. *Abrostola asclepiadis* populations 2, 4, and 14 were used in paper **V**.

The archipelago forms a naturally fragmented landscape where several hundreds of islands are of different sizes and ages, and are isolated to variable degree. The islands have been established relatively recently, as the majority of Scandinavia was covered by ice only 12,000 years ago (Lundqvist 1986). After the ice retreated, land uplifting gradually exposed terrestrial habitats suitable for colonization by plants and insects. Therefore, in my research area the populations of *V. hirundinaria* and herbivores feeding on it also differ in age, size, and degree of isolation. There is plenty of previous information about the physical characteristics of this environment, insect fauna, and species interactions especially from the study sites located in Finland (e.g. Leimu & Lehtilä 2006, Muola *et al.* 2010b, Vesterinen 2010). In general, *V. hirundinaria* is relatively abundant in my research area, and there are over 700 known populations just in Finland (von Numers & van der Maarel 1998, von Numers pers. comm.). Population sizes of *V. hirundinaria* in this area range from tens to thousands of individuals and distances between the populations vary from less than one to tens of kilometres (Leimu 2004, Leimu & Mutikainen 2005). The distances between my study populations vary from one to 598 kilometres. The study populations in Finland form a transect from the mainland to the outer archipelago, and the populations in Sweden a transect from North to South (Figure 2).

2.3.   Population genetic variation and differentiation of a specialized seed predator

Here, I examined how characteristics of vegetation and other geographic and ecological factors relate to the genetic variation and differentiation of herbivore populations. I studied genetic variation and differentiation of 23 populations of *Lygaeus equestris* in southwestern Finland and in eastern Sweden (see Figure 2 and chapter **I**). I collected 628 *L. equestris* individuals for genetic analysis from 15 populations located in southwestern Finland and from eight populations in eastern Sweden (Figure 2), 9 – 30 individuals from each population. One Finnish and six Swedish populations are located on the coastal mainland, and the rest of the populations are located on separate islands of the Baltic Sea. I used amplified fragment length polymorphisms (AFLP) to assess the genetic variability.

First, because *L. equestris* largely depends on its primary food plant *V. hirundinaria* (Solbreck & Kugelberg 1972), and large plant populations may sustain larger and more stable herbivore populations than smaller plant populations (Colling & Matthies 2004, Sõber *et al.* 2009), I tested if genetic variation of *L. equestris* was higher within populations that occurred on large *V. hirundinaria* populations. Second, while *L. equestris* may use other plant species than *V. hirundinaria* as alternative food (Solbreck & Kugelberg 1972), and as a high number of alternative food-plant species may maintain stable *L. equestris* populations, I tested if there was more within-population genetic variation in *L. equestris* populations occurring in habitats with a high number of vascular plant species. I also tested if genetic differentiation between the *L. equestris* populations was related to the similarity of the vegetation in the habitats or to geographic distance between

the populations. Furthermore, I tested if large islands had more genetically variable *L. equestris* populations than small islands, as the size of the island may indirectly affect the viability of the *L. equestris* populations for instance via more diverse habitats occurring on large islands compared to smaller islands. Finally, as population size generally influences the level of genetic variation (Hartl & Clark 2007), I also tested the impact of population size on within-population genetic variation of *L. equestris*.

2.4. Food-plant specialization of a specialized seed predator

In chapters **II-IV**, I studied different aspects of food-plant specialization of the seed predator *L. equestris*. The considerable variation in seed production of the primary food plant, *V. hirundinaria*, may force *L. equestris* to feed occasionally on non-optimal food-plant species. In study **II**, I conducted a split-brood experiment in laboratory using one generation of *L. equestris* and four food-plant species. My aim was to study potential costs of alternative food-plant use and especially investigate whether there are genetic variation in food-plant utilization or trade-offs, i.e. negative genetic correlations in herbivore performance between different food plants that may influence specialization. I also conducted a multi-generation selection experiment with four food plant-species to evaluate the effects of long-term selection due to food plant (chapters **III** and **IV**), inbreeding (**IV**), and genetic drift (**IV**) on performance of *L. equestris* on different food plants. I further investigated the occurrence of trade-offs in performance between the different food-plant species in this experiment, as the possibility to find trade-offs might be higher in selection experiments compared to one-generation feeding trials (Fry 2003). As selection diminishes genetic variation in fitness-related traits (Willi *et al.* 2006), I investigated whether there was genetic variation present after the selection. *Lygaeus equestris* individuals (parental generation) for both the one-generation split-brood experiment (**II**), and multi-generation selection experiment (**III** and **IV**) were collected from one natural population in the south-western archipelago of Finland (population 2 in Figure 2).

2.4.1. One-generation split-brood experiment

In order to study how feeding on alternative food-plant species affects life-history characteristics of *L. equestris* and to especially investigate whether there are genetic variation in food-plant utilization or trade-offs in herbivore performance between different food plants, I conducted feeding trials in laboratory using one generation of *L. equestris* and seeds of the four plant species, *V. hirundinaria*, *Crepis tectorum*, *Tanacetum vulgare* and *Verbascum thapsus* (chapter **II**). Twenty *L. equestris* females and males were randomly paired, and the larvae of the first offspring generation were assigned to feed on seeds of the four plant species immediately after hatching. The larvae from each egg cluster were divided among the four plant species to control for the effect of genetic differences among the egg clusters. Since insect females and males may have different nutritional requirements (Lee 2010), I studied whether food-plant species affected

the sexes differently. I used mortality, developmental time from hatching to maturity, and adult biomass (fresh weight of adults at the day of the last moult) as estimates of performance of *L. equestris* (Eyles 1964, Kugelberg 1973b). See chapter **II** for details about establishing and conducting the experiment.

2.4.2. Multi-generation selection experiment

Selection experiments provide a tool to manipulate natural selection in a controlled way so that the evolutionary process behind adaptations possibly leading to specialization can be better understood. In a selection experiment, a base population of study organisms is subjected to a set of conditions, such as to different diets, and natural selection is allowed to proceed for several generations. Samples are then extracted from each of the selection lines, and the fitness traits of the study organisms are assayed across a range of conditions, preferably reciprocally the ones used during selection period (Kassen 2002). In quasi-natural selection experiments the experimental set-up resembles natural conditions, and the individuals' contribution to the next generation is not influenced by the researcher, but depends on the inherent quality and adaptation of the individuals to their respective selective regimes (Fry 2003). The effects of selection can be separated from random genetic drift by replicating the populations within the selection lines (Kassen 2002, Fry 2003). The influence of inbreeding can be demonstrated by conducting controlled crosses between the individuals from same replicate population, between the replicate populations within a selection line, and between the selection lines (Kassen 2002, Fry 2003). Selection experiments have been widely used to examine food-plant specialization of herbivorous insects (e.g. Gould 1979, Fry 1990, Agrawal 2000).

I conducted a multi-generation selection experiment (chapters **III** and **IV**) in the laboratory using the seed predator *L. equestris* and four different food-plant species. First, I examined if the selection for effective utilization of a certain food-plant species leads to increased fitness and adaptation of *L. equestris*, and if such adaptation to one food plant in turn affects the fitness on another food-plant species (**III**). Second, I evaluated the effects of selection for food-plant utilization and random drift on between-population differentiation, and whether inbreeding affected the outcome of this selection experiment (**IV**). In the selection experiment I used the primary food plant *V. hirundinaria*, a novel food plant, sunflower *Helianthus annuus*, and two alternative food-plant species occurring in the distribution area of *L. equestris* in Finland, *Centaurea phrygia* and *V. thapsus*.

The parental generation of *L. equestris* was fed with a mixture of *V. hirundinaria*, *H. annuus*, *C. phrygia* and *V. thapsus* seeds, and allowed to breed randomly. The larvae from each egg cluster (F1-generation) were first randomly subdivided into four groups to form the four selection lines (*V. hirundinaria*, *H. annuus*, *C. phrygia*, and *V. thapsus*). Three replicate populations were established within each selection line to separate the effects of selection from random drift, so that altogether twelve populations were established (see chapter **III** for experimental procedure). The *C. phrygia* selection line went extinct

after few generations, and *V. thapsus* selection line already after the first generation. The replicate populations in *V. hirundinaria* and *H. annuus* selection lines were reared in the laboratory for 26 months (approximately 20 overlapping generations). Population sizes and sex ratios were calculated after 22 months of selection for each of the six replicate populations (three *V. hirundinaria* and three *H. annuus* populations) surviving till the end of experiment (see Table 1 in chapter **III**).

Adaptation to the food-plant species, genetic variation and trade-offs in fitness after selection. In this kind of selection experiment, increases in fitness during selection may indicate either herbivore adaptation to the food plants, adaptation to laboratory conditions, or both (e.g. Kingsolver 2007). However, differential changes in fitness between the two selection lines would indicate adaptation of *L. equestris* to the food-plant species, and especially to that species on which the increase in fitness is relatively greater. I examined the fitness of *L. equestris* on *V. hirundinaria* and *H. annuus* before and after the selection period to investigate whether *L. equestris* adapted to the novel food plant (*H. annuus*) during the selection experiment (chapter **III**). Fitness of *L. equestris* was estimated as lifetime reproductive success (the number of fertile eggs per female), mortality, developmental time, and adult biomass.

In order to evaluate the reproductive success of *L. equestris* on *V. hirundinaria* and *H. annuus*, females and males were picked randomly from the each replicate population in *V. hirundinaria* and *H. annuus* selection lines before and after selection (F1-generation, i.e. the generation used to establish the selection lines, and approximately 20th generation) and paired randomly within the replicate populations. The numbers of fertile eggs were then counted from these pairs. I conducted two feeding trials (before and after selection) in which the larvae grew to adulthood to obtain data on mortality, developmental time, and adult biomass of *L. equestris* on *V. hirundinaria* and *H. annuus*. The larvae were fed on the seeds of the food plant of their original selection line. In the feeding trials I used F1-generation and approximately 20th generation. See chapter **III** for details about conducting the experiments.

In addition, I conducted an extensive split-brood experiment after approximately 20 generations of selection to investigate variation in the fitness of *L. equestris* between the selection lines, among larval food plants, between sexes, and among replicate populations and families. In this experiment, I used the larvae originating from two selection lines that persisted until the end of the selection experiment (*V. hirundinaria* and *H. annuus*), and three replicate populations per selection line. Plant species used in the feeding trials were the same as those in the four original selection lines: *V. hirundinaria*, *H. annuus*, *C. phrygia*, and *V. thapsus*. In this trial, significant effects of family would indicate the genetic variation in the fitness and in food-plant utilization. Furthermore, I wanted to investigate the occurrence of the negative genetic correlations especially, i.e. trade-offs in mortality, developmental time, and adult biomass between the four food-plant species separately within the two selection lines.

Effects of selection, random drift, and inbreeding on food-plant specialization. As a part of the multi-generation selection experiment I evaluated the effects of long-term selection for food-plant utilization, random genetic drift, and inbreeding on the performance of *L. equestris* on different food plants (chapter **IV**). The differentiation of replicate populations with initially identical genetic structure under similar selective forces, i.e. within the same selection line, indicates that genetic drift outweighs the effects of selection. Furthermore, small population size in replicate populations may affect the outcome of selection experiments by increasing the chance that inbreeding may reduce the adaptive potential of the replicate populations. If the populations suffer from inbreeding, i.e. there is inbreeding depression, the performance of offspring originating from crosses within a replicate population should be lower compared with the performance of offspring from crosses between two replicate populations.

In this study, I used individuals from approximately the twentieth experimental generation of *L. equestris* from two selection lines, the primary food plant *V. hirundinaria* and novel food plant *H. annuus*. Three replicate populations within both selection lines were used in the crosses. Thus, I randomly picked 40 – 60 fifth instar larvae from each replicate population. After reaching maturity the larvae were randomly assigned to three different crossing treatments. In the "within-population crosses", both the female and the male originated from the same replicate population. In the "between-population within the selection line crosses", the female and the male originated from different replicate populations within their respective selection line. The "between selection line crosses" were conducted by pairing females and males from the different selection lines. Since in the within-population crosses only a few pairs from one replicate population in *H. annuus* selection line laid fertile eggs, this population was excluded from other types of crosses. Further, one replicate population was randomly selected to be excluded from the between-population and between selection line crosses from *V. hirundinaria* selection line as well, to retain balanced data structure. Each mating pair was feeding on the seeds of the food plant of the selection line of the female. Total number of fertile eggs per female was used as a measure of the herbivore fitness. See chapter **III** for the details of establishing the selection lines, and chapter **IV** for the rest of the experimental procedure, number of replicates, and other details.

2.5.   Local adaptation of a specialist folivore

The spatial variation in the associations of *Vincetoxicum hirundinaria* leaf chemistry, levels of foliar damage by specialist folivorous moth, *Abrostola asclepiadis*, and plant fitness vary among the populations and reflect a selection mosaic in my study area (sensu Thompson 2005, Muola *et al.* 2010b). The associations of plant chemical compounds and damage by *A. asclepiadis* range from negative to positive (Muola *et al.* 2010b). Thus, a given chemical may be positively associated with the level of herbivore damage in one population, indicating that the chemical compound functions as an attractant for *A. asclepiadis*, and negatively in an another population suggesting a function of the

particular chemical compound in herbivore resistance (Muola *et al.* 2010b). I investigated the influence of geographic variation in plant secondary chemistry of *V. hirundinaria* on among-population variation in local adaptation of *A. asclepiadis*. I conducted a feeding trial using three populations located in the south-western archipelago of Finland (Ånskär, Jurmo and Lammasluoto; see Figure 2 and chapter **V**). In the reciprocal design, larvae from each of the three sites were fed with plants originating from each of the three sites. A significant interaction between plant population and herbivore population indicates local adaptation of the herbivores if herbivore performance is higher on plants from the sympatric plant population compared with that on plants from allopatric populations. This is the home-away comparison presented by Kawecki and Ebert (2004).

Abrostola asclepiadis larvae from field-collected egg clusters were randomly assigned to feed on a particular plant immediately after hatching. To control for the effect of variation among plant individuals, larvae from the three populations were assigned to feed on each plant individual. Furthermore, to control for the effect of genetic differences among the egg clusters, the larvae from each egg cluster were divided among the three plant populations. Pupal mass was used as a measure of herbivore performance together with survival (Haukioja & Neuvonen 1985). Therefore, at the end of the larval period I determined the sex of each individual and weighed the pupae. To examine if the variation in local adaptation is driven by among-population variation in food-plant chemistry, I collected leaf samples from all *V. hirundinaria* individuals used in the feeding trial to analyze the contents of leaf compounds. Leaf secondary chemistry was analyzed with high-performance liquid chromatography (HPLC, see Muola *et al.* 2010b). I focused on phenolic compounds (flavonoids, chlorogenic acid, and catechin derivatives), since they have several ecological and physiological functions in plants, and they are generally assumed be important in plant-herbivore interactions (Feeny 1976, Bennett & Wallsgrove 1994, Treutter 2006). I also included lipophilic compounds in the study, as they form a relatively diverse group of compounds including, for instance, chlorophylls, carotenoids, and leaf surface compounds, and may have a potential role in resistance to herbivores (Valkama *et al.* 2005). In addition, antofine, a phenanthroindolizidine alkaloid, was included in study. This specific compound is known for its cytotoxic activity (Staerk *et al*. 2000). The phenanthroindolizidine alkaloids are characteristic constituents in species belonging to the subfamily Asclepiadoideae and to the genus *Vincetoxicum* (Stærk *et al*. 2000).

To examine if local adaptation was more pronounced between strongly diverged populations, I tested for correlations of local adaptation with geographic distance, genetic differentiation of food plant, and phenotypic divergence in plant secondary chemistry among the plant populations. Here, I calculated values for local adaptation, i.e. the ratio of pupal mass between herbivores grown on plants from their sympatric population and herbivores grown on plants from allopatric population, reciprocally for each pair of populations resulting in six datapoints on local adaptation. Values above one indicate local adaptation of the herbivore to its sympatric food-plant population. The geographic

distances among the populations varied between 15.6 km and 49.6 km. I used the F_{ST} values presented by Leimu and Mutikainen (2005) as measures of genetic differentiation among the *V. hirundinaria* populations. The *V. hirundinaria* populations are genetically differentiated, which indicates restricted gene flow between the populations. This may promote local adaptation of herbivores if gene flow of the herbivore is greater than gene flow of the plant (Gandon *et al.* 1996, Lively 1999). To determine phenotypic differentiation in plant secondary chemistry I calculated Euclidean distances among the three populations using the two individual chemicals (chlorogenic acid, antofine) and the three groups of chemicals analysed (lipophilic compounds, flavonoids, and catechin derivatives).

3. RESULTS AND DISCUSSION

3.1. Population genetic variation and differentiation of a specialized seed predator

In the first chapter (**I**), I investigated how different geographic and ecological factors explain the genetic structure of populations of the oligophagous seed predator *Lygaeus equestris*. I found significant genetic differentiation among the 23 studied *L. equestris* populations in south-western Finland and in eastern Sweden. In general, genetic differentiation among the populations was rather low, but increased with the increasing geographic distance between the populations. The somewhat low level of differentiation observed among the *L. equestris* populations may be explained by high migration and relatively short history of the populations in the area of the Baltic Sea (Lundqvist 1986). In general, populations that have persisted over a long time are expected to be more differentiated than younger populations (Hartl & Clark 2007). Genetic differentiation between the *L. equestris* populations was not related to the similarity of vegetation between the sites. Thus, my results are consistent with the recent view of Wang *et al.* (2013), who stated that geographical isolation explains genetic differentiation more than ecological isolation.

In addition to low, but significant genetic differentiation among *L. equestris* populations, I found high level of genetic variation within the populations. It seems that sexual reproduction and migration together sustain genetic variation. Furthermore, my results suggest that both geographic and ecological factors affect the level of genetic variation. Firstly, island size seems to matter, as the larger the island the higher was the level of genetic variation within *L. equestris* populations. In my study area, large islands may have more stable microclimates or more diverse habitats for feeding, ovipositioning, and hibernation, and thus, *L. equestris* populations on large islands might be less susceptible to population bottlenecks and extinction-recolonisation processes, both of which can reduce within-population genetic variation (Saunders *et al*. 1991, Hartl & Clark 2007). In addition, island size seemed to modify the effect of population size of *L. equestris* on within-population genetic variation. When a small population was located on a small island, the level of within-population genetic variation was on the average low, whereas small populations on larger islands seemed to maintain a higher level of genetic variation. The reason for this may lie in the positive correlation between island size and island age in my study area (L. Laukkanen unpubl. data), suggesting that *L. equestris* populations on large islands are likely to be older than those on smaller islands. The deleterious recessive alleles may have been purged in the small but older populations on larger islands reducing the probability of extinction (Hartl & Clark 2007, Pekkala *et al.* 2012). Thus, extinction-colonisation processes may lead to a reduced level of within-population genetic variation especially in the small and young populations on smaller islands. Secondly, the more species in the plant community the higher was the

level of genetic variation within *L. equestris* population. The high number of potential alternative food-plant species seems to maintain more stable *L. equestris* populations. Unexpectedly, the level of within-population genetic variation was not influenced by population size of primary food plant *V. hirundinaria*. This result may be a consequence of the great spatiotemporal variation in *V. hirundinaria* seed production, or impact of contemporary migration.

3.2.   Food-plant specialization of a specialized seed predator

3.2.1. *Genetic factors affecting food-plant specialization*

I studied two principal genetic factors that have been shown to affect herbivore specialization to food: genetic variation in herbivore performance on different food plants for selection to act on (e.g Futuyma *et al*. 1995, Forister *et al*. 2007) and the occurrence of trade-offs, i.e. negative genetic correlations, in the performance of the herbivores between different food-plant species. Both high and low levels of heritable variation have been found in the use of alternative food-plant species by herbivores (Karowe 1990, Futuyma *et al*. 1995, Thompson 1996, Keese 1998, Ueno *et al*. 2003, García-Robledo & Horvitz 2011). The lack of genetic variation in performance on different food-plant species may potentially affect the evolution of diet breadth of insect herbivores (Futuyma *et al*. 1995, Keese 1998). In the one-generation experiment I did not find significant genetic variation in fitness of *L. equestris* on its primary food plant *V. hirundinaria*, and especially not on suboptimal, alternative food-plant species (**II**). Thus, the low level of genetic variation may have constrained adaptation of *L. equestris* especially to these low-quality food-plant species. As selection in general diminishes genetic variation in fitness-related traits (Wasserman & Futuyma 1981, Willi *et al.* 2006), I investigated whether there was significant genetic variation left in the food-plant use after the selection (**III**). Surprisingly, I found that significant genetic variation still existed after the selection experiment in mortality, larval developmental time, and adult biomass regardless of the larval food plant. The simplest and most likely reason for the contrasting results might be the different sizes of the parental generations used in these two experiments (**II** and **III**). The number of individuals in parental generation was higher in the selection experiment than in the one-generation experiment, and thus, more of the within-population genetic variation that was present in natural population was captured. Because I estimated genetic variation only after but not before the selection experiment, we do not know if the level of genetic variation in fitness traits changed during the experiment. However, preliminary results from microsatellite data suggest that the level of within-population genetic variation decreased during the selection experiment: on average 38 % of alleles were lost during the selection (L. Laukkanen unpubl. data).

Trade-offs may promote the evolution of specialization (Fry 2003, Scheirs *et al*. 2005). The possibility to find trade-offs might be higher in selection experiments compared with one-generation feeding trials (Fry 2003). However, I did not find any trade-offs in mortality, developmental time, or adult biomass of *L. equestris* between the studied food-plant species

neither in the one-generation study (chapter **II**), nor in the multi-generation selection experiment (**III**). This suggests that fitness trade-offs do not constrain the use of alternative food-plants of *L. equestris*. My results are thus in agreement with most previous studies that have found no evidence for trade-offs in performance of insect herbivores on different food plants (e.g. Thompson 1996, Keese 1998, Agosta & Klemens 2009). The significance of trade-offs in specialization and coevolution has recently been much disputed, and the lack of significant negative genetic correlations in food-plant use of insect herbivores has been explained by numerous methodological problems and confounding genetic factors (e.g. Rausher 1988, Scheirs *et al*. 2005). However, it is good to remember that the mechanisms producing food-plant specialization might differ from those maintaining it. Thus, the importance of trade-offs may change when the process of specialization proceeds (Thompson 1996). Trade-offs might be essential in the early phases of the process, i.e. when a generalist herbivore utilizes many food-plant species of equal importance. When herbivore is specialized to feed on one, primary plant species, fitness reaches the maximum value only on this food-plant species. If the availability of the primary food plant remains high, the herbivore prefers this plant species regardless of the trade-offs in fitness between the primary and alternative, suboptimal food plants. Due to selection, the alleles that produce the best fitness on the primary food-plant species may be fixed and the trade-off purged. Of course, food-plant specialization may also arise when trade-offs are not detected or do not exist, for example, when the level of herbivore predation by natural enemies differs between food-plant species (Rausher 1988, Fry 2003). Moreover, specialization may evolve if feeding on different food plants is not equally common, for instance due to differences in species abundance, and the correlation in herbivore fitness between different food plants is positive, but less than one (Fry 1996). This is due to the accumulation of harmful mutations that may create lower average fitness on the less frequently used food-plant species (Fry 1996). However, I am not able to address this issue with the relatively short-term selection experiment in which the herbivore fed only on a single food plant. Nevertheless, in natural populations this mechanism may contribute to the specialization of *L. equestris* on *V. hirundinaria*, as specialization seems to exist even though trade-offs in food-plant use have not been found (**II** and **III**).

Instead of trade-offs, I found some significant, positive correlations in the performance of the herbivores between the different food-plant species (**II** and **III**). A positive correlation may indicate a generalist strategy in food-plant utilization, i.e. "a master of all trades" genotype that has high fitness on several host species (Ueno *et al*. 2003, Forister *et al*. 2007, García-Robledo & Horvitz 2011). Positive correlations in performance between different food-plant species may further suggest the evolution of generalization by inclusion of sets of plant species to the diet rather than by independent adaptation to single plant species (Ueno *et al*. 2003, Forister *et al*. 2007).

Besides the level of heritable variation and trade-offs in performance on different food plants, we also need to consider ecological factors, such as predator refuge and variation in nutritional quality or secondary chemistry of the different food-plant species in order to

understand the food-plant breadth of an herbivore. Spatiotemporal variation in availability of the food is probably one major factor behind the evolution of food-plant utilization in *L. equestris*. Seed production of *V. hirundinaria* varies highly geographically and between years due to abiotic factors and seed predation by the specialist fly *Euphranta connexa* (Solbreck & Sillén-Tullberg 1986ab, Ågren *et al*. 2008). In some populations and certain years nearly 100 % of seeds may be destroyed (Leimu & Lehtilä 2006, Solbreck & Ives 2007). Given this variation in food availability, strict specialization to the primary food plant *V. hirundinaria* might not be an evolutionary feasible strategy for *L. equestris*.

3.2.2. *Selection for food-plant utilization and adaption to food-plant species*

I examined if long-term selection for utilization of a certain food-plant species leads to adaptation of a specialized herbivore (chapter **III**). In here, I assume that the higher herbivore fitness is a consequence of an increased ability to utilize the food plant. My results suggest adaptation of *L. equestris* to the novel food plant *H. annuus*: the relatively greater increases in the number of eggs produced per female and in adult biomass on *H. annuus* compared to those on *V. hirundinaria* suggest adaptation of this seed predator to a novel food plant during approximately 20 generations of selection. Moreover, the effect of larval food plant on developmental time differed between the two selection lines after the selection. This suggests divergence of lines during the experiment in the ability of *L. equestris* to utilize different food plants, which is in accordance with the idea of emergence of differential evolutionary trajectories within the two selection lines. Multi-generation selection experiments have demonstrated that rapid evolution is possible in plant-herbivore interactions (Gould 1979, Wasserman & Futuyma 1981, Thompson 1998). For example, the fitness of spider mites differed between two selection lines only after five generations of selection for food plant (Agrawal 2000), the reproductive success of the cowpea weevil increased and oviposition preference changed as a response to selection for diet in just 11 generations (Wasserman & Futuyma 1981), and a mite population adapted to an alternative food plant in 50 generations indicated by reduced mortality and increased reproductive success (Gould 1979).

Three of the four fitness traits studied changed significantly during the selection experiment: the number of fertile eggs and adult biomass increased, and larval developmental time decreased. As laboratory conditions often select for large size and fast development of insects (e.g. Kingsolver 2007), a part of the change in fitness in the *H. annuus* selection line may be due to adaptation to laboratory conditions. Correspondingly, the change in fitness observed in the *V. hirundinaria* selection line may also indicate further adaptation of the herbivores to this primary food plant, adaptation to laboratory conditions, or both. It is highly probable that natural populations of *L. equestris* were unable to survive without occasionally using alternative food-plant species. Obviously, the natural population from where individuals were collected for the selection experiment contained significant genetic variation in food-plant utilization,

which then enabled adaptation of *L. equestris* to the primary and novel food plants when only a single plant species was available.

3.2.3. Effects of random drift and inbreeding on food-plant specialization

In selection experiments, controlled crosses within and between the replicate populations from same selection line reveal whether the replicate populations suffer from inbreeding. In my study (chapter **IV**), the within-population crosses resulted to on average 45 % lower number of fertile eggs per female than the between-population crosses within the selection lines. Thus, although the mating within populations was random, inbreeding depression decreased the number of viable eggs produced when the parents originated from the same population. This indicates that individuals within each replicate population were likely to be related to each other at least to some extent due to the relatively small population sizes and isolation with no gene flow during the selection experiment. The higher female fitness in the between-population crosses may also result from heterosis, i.e. from the positive effects of hybridization of genetically different individuals. Heterosis increases genetic diversity within populations and generates novel, viable phenotypes as a result of new allelic combinations (Lynch 1991, Lynch & Walsh 1998, Whitlock 2000). Hybridization between genetically differentiated populations can alleviate the genetic problems of small populations. The *L. equestris* males choose the mating partner without preliminary courtship (Sillén-Tullberg 1981). My result suggests that in natural populations *L. equestris* would benefit from the ability of the male to be able to select an unrelated female when given the choice.

When comparing the between-population within the selection line crosses and the crosses between the selection lines, female fitness did not differ between the crosses indicating that the differentiation between the selection lines was not significant enough to result in reduced female fitness following the between-selection line crosses. If locally adaptive combinations of genes are disrupted because of crossing individuals adapted to different conditions, such crosses may lead to outbreeding depression expressed as reduction in fitness (Frankham 2005, Frankham *et al.* 2011). In my experiments, it seems that there was no outbreeding depression, and the level of heterosis did not depend on whether outbreeding between populations happened within the selection line or between the selection lines. Of course, the origin of the female and male may still have importance in natural populations, for instance, if the male originates from a population that is strongly differentiated from the population of female in terms of food-plant utilization. All Scandinavian *L. equestris* populations depend on *V. hirundinaria* (Solbreck & Kugelberg 1972, Solbreck & Sillén-Tullberg 1990a, Rintala & Rinne 2010), but in other parts of distribution area the primary food plants and the level of food-plant specialization may vary more among populations (Solbreck *et al.* 1989).

In the multi-generation selection experiment, the three replicate populations within each selection line were established particularly to separate the effects of selection from random, stochastic processes, such as random genetic drift. Indeed, besides selection for effective food-plant utilization, random processes seemed to influence the ability

of *L. equestris* to use the food plants, as developmental time and adult biomass after the selection differed significantly among the replicate populations within the two selection lines (chapter **III**). Furthermore, in between-population crosses I found that the amount of variation in the number of fertile eggs was bigger in replicate populations in *H. annuus* selection line compared to populations in *V. hirundinaria* selection line indicating that especially replicate populations feeding on the novel food plant *H. annuus* were affected by random processes (**IV**). The populations feeding on the primary food plant, *V. hirundinaria*, were larger than those feeding on *H. annuus* (see Table 1 in chapter **III**), and thus, the relative importance of random genetic drift potentially driving differentiation of populations should have been lower in *V. hirundinaria* selection line (Frankham 2005, Pekkala *et al.* 2012). However, because the fitness increased in both selection lines during the experiment (**III**), and because in the within-population crosses female fitness did not differ between the replicate populations in either selection line, my results suggest that the effect of selection was stronger than those of random processes.

According to the preliminary results from microsatellite data, the level of genetic variation remained higher in the replicate populations of the *V. hirundinaria* selection line compared with the *H. annuus* selection line: on average 23 % of alleles were lost in *V. hirundinaria* selection line and 53 % of alleles in the *H. annuus* selection line during approximately 20 generations of selection (L. Laukkanen unpubl. data). The loss of alleles was quite similar within the replicate populations of the *V. hirundinaria* selection line. In contrast, in the *H. annuus* selection line the loss was over 60 % in one of the populations and about 50 % in the other two. Accordingly, the populations varied in size especially in the *H. annuus* selection line (Table 1 in chapter **III**), and the loss of alleles was highest in the smallest population. Presumably population sizes initially declined due to the lower reproductive success when fed on the novel food and were likely to be further reduced by inbreeding (Frankham 2005). Thus, due to the lower population sizes, random drift and inbreeding presumably had stronger negative effects on the level of neutral genetic variation within the replicate populations of the *H. annuus* selection line. The negative effects of random drift and inbreeding may restrict the adaptive potential of populations and, thus, affect their food-plant use and potential specialization (Berlocher & Feder 2002, Pekkala *et al.* 2012).

3.3.   Local adaptation of a specialist folivore

To investigate if the specialist folivorous moth, *Abrostola asclepiadis,* is adapted to its sympatric *Vincetoxicum hirundinaria* populations, and if geographic variation in secondary chemistry of *V. hirundinaria* influenced on among-population variation in local adaptation, I conducted a reciprocal feeding trial in laboratory with both plants and larvae from three sites (chapter **V**). I found significant quantitative variation in secondary chemistry among the three plant populations studied. Plants from the Lammasluoto population differed from the other two populations in their levels of flavonoids and antofine. I also found among-population variation in herbivore local adaptation to the

food-plant populations, when pupal mass was used as an indicator of herbivore fitness. *Abrostola asclepiadis* larvae from the Ånskär population performed significantly better on their sympatric food-plant population compared with allopatric food-plant populations suggesting local adaptation of the herbivores. Similar pattern, although not statistically significant, was found for another herbivore population, Lammasluoto. Herbivores from the third population, Jurmo, were not locally adapted to their sympatric food-plant population, as their fitness seemed to be higher on plants from the allopatric Ånskär population. In accordance with my results, Kalske *et al.* (2013) recently demonstrated spatial variation in the existence and degree of local adaptation of both *A. asclepiadis* and *V. hirundinaria*. However, my results on survival suggest lack of local adaptation, since survival did not differ between larvae fed on sympatric and allopatric plants.

Lipophilic compounds and flavonoids seemed to modify the observed variation in pupal mass of *A. asclepiadis* among the herbivore and plant populations as indicated by statistically significant interactions between herbivore population, plant population, and the total concentrations of these chemical compounds. The effects of lipophilic compounds and flavonoids on pupal mass varied among *A. asclepiadis* populations and also depended on the origin of the food plant. For example, I found that an increase in total lipophilic compounds in plants from the sympatric food-plant population increased the pupal mass of the herbivores from the locally adapted Ånskär population. By contrast, when fed on plants from the two allopatric populations the pupal mass of the herbivores from Ånskär was either negatively or only weakly influenced by the increased lipophilic compounds. This result reflects the qualitative variation in lipophilic compounds observed among plant populations by Muola *et al.* (2010b) and suggests that *A. asclepiadis* from the Ånskär population tolerate or detoxify especially those lipophilic compounds that their sympatric food plants contain. In addition to the effects of lipophilic compounds and flavonoids, the effect of chlorogenic acid on herbivore pupal mass varied among the *A. asclepiadis* populations regardless of the origin of food plant. To conclude, several different secondary chemicals were related to herbivore pupal mass, but the effect varied among herbivore populations and also depended on plant population.

Theoretical models on coevolving interactions between hosts and their natural enemies predict that local adaptation of the enemy is more likely to occur if the enemy has shorter generation time and higher migration rate than the host (e.g. Gandon *et al.* 1996, Greischar & Koskella 2007). In my study system, *A. asclepiadis* has a considerably shorter generation time than its food plant *V. hirundinaria*. Moreover, it seems probable that *A. asclepiadis* has higher migration rates than *V. hirundinaria*, which fits the predictions of the model of Gandon *et al.* (1996). Therefore, *A. asclepiadis* should, in general, show local adaptation. Furthermore, local adaptation is also predicted to be more likely among strongly differentiated populations located in clearly divergent environments (Becker *et al.* 2006, Hereford & Winn 2008, Hereford 2009). In line with these predictions, I found that the strength of local adaptation of *A. asclepiadis* increased with increasing geographic distances among the populations, genetic differentiation of *V.*

hirundinaria, and phenotypic divergence in food-plant secondary chemistry (Figure 3). However, when generalizing from my results one needs to take into account that I only studied three populations. On the other hand, given that I had only three populations, the tested correlations were surprisingly strong.
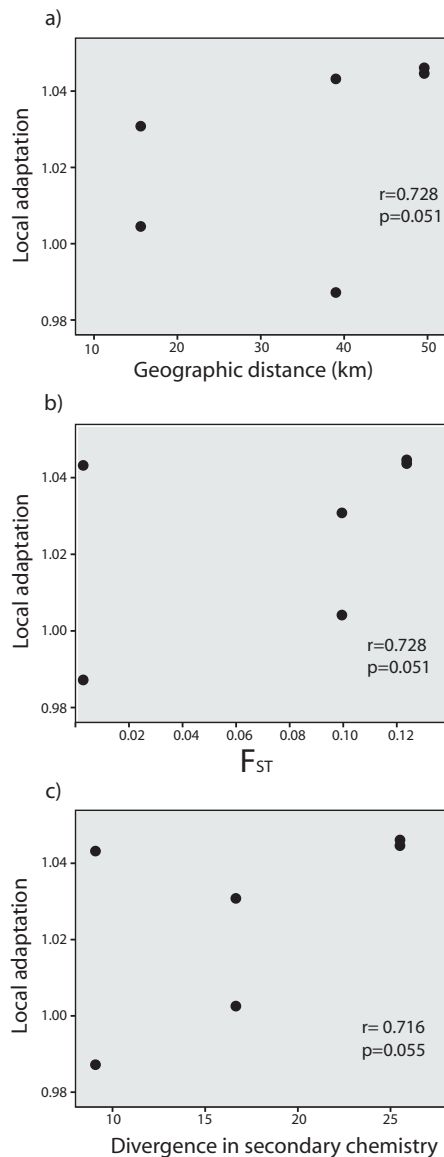
Figure 3. Correlations of local adaptation of *Abrostola asclepiadis* with (a) geographic distance, (b) genetic differentiation of the food plant *Vincetoxicum hirundinaria*, and (c) phenotypic divergence in plant secondary chemistry. The local adaptation was defined as the ratio of pupal mass between herbivores grown on plants from their sympatric population and herbivores grown on plants from allopatric population. Values above one indicate local adaptation of the herbivore *A. asclepiadis* to its sympatric food-plant population.

Although it is known that plant chemistry has the potential to influence the abundance and performance of herbivores (Berenbaum & Zangerl 2006, Zangerl *et al.* 2008), the selective factors that cause variation in local adaptation are not yet thoroughly explored. My results suggest that variation in herbivore local adaptation may be driven by the qualitative and quantitative among-population divergence in host-plant chemistry.

4. CONCLUSIONS

My aim was to address several essential questions related to the evolution of plant-herbivore interactions from the herbivore's point of view. To begin with, my findings provide valuable information about the role of geographic and ecological factors as modifiers of the population genetic structure of insect herbivore. Several studies have shown that the species diversity of herbivores increases with plant-species diversity and plant genetic diversity (e.g. Crutsinger *et al.* 2006, Cook-Patton *et al.* 2011). However, to my knowledge, my study is the first to suggest that plant-species diversity is related to population genetics of a herbivore species: the more diverse plant community, the higher level of within-population genetic variation of the specialized seed predator. Whether the higher genetic variation of the seed predator is related to the damage the predators induce on their host plants would be worth further studies. As the diversity of food organisms is likely to affect predator genetic variation in other plant-herbivore and predator-prey systems as well, I believe that my results have important implications for studies of trophic interactions, habitat fragmentation, and landscape genetics.

Divergent reciprocal selection plays undoubtedly a central role in generating diversity in nature. Food-plant specialization accelerates co-evolutionary arms race between a plant and its herbivores, as specialist species evolve faster than generalist species (Whitlock 1996). Overall, spatially divergent food-plant specialization of herbivores and adaptation to novel food plants may ultimately lead to speciation, and thus, they are considered essential factors behind the enormous diversity of herbivorous insects (Berlocher & Feder 2002, Matsubayashi *et al.* 2011). My results on the relative importance of *V. hirundinaria* and the alternative food plants for the fitness of *L. equestris* and on the factors that affect specialization or utilization of alternative food-plant species are central for understanding the evolution of relationship between *L. equestris* and *V. hirundinaria*. Furthermore, such knowledge is necessary in order to understand the potential coevolution between *V. hirundinaria* and the other herbivore species, because selection and evolutionary changes due to one of the herbivores are likely to affect the other herbivores using the same food resource. My results suggest that trade-offs, i.e. negative genetic correlations, do not drive food-plant specialization of the *L. equestris*. The occurrence of trade-offs is much disputed, but previously rarely studied using somewhat specialized herbivores and multi-generational selection experiments (Wasserman & Futuyma 1981, Fry 2003). One of the major findings of this thesis is that not just generalist herbivores, but even a herbivore specialized on its primary food plant may still have potential to rapid evolution on other food-plant species. Moreover, my results indicate that random processes, such as genetic drift, should not be ignored when processes of resource specialization and adaptation are studied, especially in small and fluctuating populations. However, the relative roles of selection, random genetic drift, and inbreeding in rapid evolution, resource specialization, and adaptation of herbivores to novel food are worth further studies. As my results suggest that the inbreeding depression and mate choice of *L.*

equistris males may have impacts on fitness, it would be interesting to test mate choice of *L. equestris*: are males able to identify genetically related females, and is this ability better in small populations compared to the larger populations, where the mating between relatives is less probable?

Local adaptation is central for creating and maintaining spatial variation in antagonistic interactions, such as plant-herbivore interactions (Thompson 1994, Kawecki & Ebert 2004, Laine 2009). Studies identifying the mechanisms that drive geographic variation in local adaptation in spatially structured systems provide one tool to understand the coevolutionary dynamics of interacting species (Thompson 2005, Laine 2009). I demonstrated that the occurrence and degree of local adaptation of *A. asclepiadis* to food plant may vary among populations and correlate with qualitative and quantitative variation in plant chemistry among *V. hirundinaria* populations. These findings contribute to our understanding of how spatial variation in specific defensive traits of the host may drive local adaptation of a potentially coevolving antagonist.

Although in many study systems it is impossible to observe the coevolutionary process in action, studies on these systems help us to recognize the factors modifying species interactions (e.g. Zangerl *et al.* 2008, Laine 2009, Toju 2009, Garrido *et al.* 2011). This knowledge is needed to further develop empirical and experimental approaches to detect coevolutionary dynamics and to recognize natural systems that may currently undergo coevolution. My study and previous studies from this study system have not found anything that would exclude coevolution between *Vincetoxicum hirundinaria* and the specialized herbivores. *Lygaeus equestris* and *A. asclepiadis* reduce together the fitness of their shared host plant, and *V. hirundinaria* individuals vary in their quality as food for *A. asclepiadis* and *L. equestris* (Muola *et al.* 2010ab, R. Leimu unpubl. data). The results from studies on local adaptation also support the view of coevolutionary relationship between these species (see above, and Kalske *et al.* 2013). Local adaptation is traditionally seen as the first step towards coevolution, as local adaptation of one of the interacting species to another species may affect traits that are central for the species interaction, and might, thus, lead to local adaptation of the other species (Crémieux *et al.* 2008).

In my studies, I used two herbivore species, *Lygaeus equestris* and *Abrostola asclepiadis,* that seem to differ from each other in many ways. These two herbivores differ in their feeding habit and in the type of damage they cause to their shared food plant, *Vincetoxicum hirundinaria*. However, as both these herbivores have strong negative effects on fitness of *V. hirundinaria*, and as the resistance of the plant against these herbivores appears to vary among populations (Muola *et al.* 2010b, R. Leimu unpubl. data), adaptation of these herbivore species to local food-plant populations seems to be likely. Furthermore, *L. equestris* and *A. asclepiadis* differ in the degree of interspecific resource competition they experience. Besides *A. asclepiadis*, there are no other folivores feeding on *V. hirundinaria* in my study area, but seed predation by the tephritid fly *Euphranta connexa* may be close to 100 % in some populations and certain years (Solbreck & Ives 2007),

which is likely to influence *L. equestris.* Interspecific competition for the primary food plant may affect food-plant specialization of herbivores in two ways: it may either force the herbivore to specialize on alternative food-plant species, or expand the variety of food plants used (Futuyma & Moreno 1988). Thus, competition may either increase or decrease the level of specialization. The herbivore species studied here differ in their degree of diet specialization: while *A. asclepiadis* is a strict specialist, *L. equestris* is able to utilize alternative food when the seeds of primary food plant are scarce. Thus, it seems possible that *L. equestris* has responded to the competition of *E. connexa* by expanding the diet breadth. The interactive effects of the herbivore species sharing *V. hirundinaria* as their food plant on each other, and on the host plant, are clearly worth further studies (Linhart & Grant 1996, Wise & Rausher 2013). As these herbivores are so different from each other in many ways, and I have studied the questions related to evolution of plant-herbivore interactions only using one herbivore species, it would be interesting to study the same questions also using the other herbivore species as study organism.

I conducted part of my research on plant-herbivore interactions in a naturally fragmented geographic mosaic, in which the size, age, and degree of isolation vary among the islands and populations. The archipelago provides a unique framework for studying the spatial variation in the evolution of plant-herbivore interactions. Ultimately, my studies add to the knowledge on how the genetic diversity and species diversity are created and maintained in a landscape via the evolution of interactions. As human activities, such as climate change, deforestation, and habitat fragmentation, are rapidly changing the biological landscapes and consequently altering evolutionary trajectories, it is becoming increasingly important to understand the factors affecting the evolution of interactions among species. Likewise, novel information about spatial variation and rapid evolution of species interactions may have essential implications for conservation biology, and applied ecology dealing with multitude of questions having great importance to human society, such as management of introduced species and agricultural pests.

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REFERENCES

Abdala-Roberts, L. & Marquis, R. J. 2007. Test of local adaptation to biotic interactions and soil abiotic conditions in the ant-tended *Chamaecrista fasciculata* (Fabaceae). *Oecologia* 154: 315–326.

Agosta, S. J. & Klemens, J. A. 2009. Resource specialization in a phytophagous insect: no evidence for genetically based performance trade-offs across hosts in the field or laboratory. *J. Evol. Biol.* 22(4): 907–912.

Agrawal, A. A. 2000. Host-range evolution: Adaptation and trade-offs in fitness of mites on alternative hosts. *Ecology* 81: 500–508.

Agrawal, A. A., Lau, J. A. & Hambäck, P. A. 2006. Community heterogeneity and the evolution of interactions between plants and insect herbivores. *Q. Rev. Biol.* 81(4): 349–376.

Awmack, C. S. & Leather, S. R. 2002. Host plant quality and fecundity in herbivorous insects. *Annu. Rev. Entomol.* 47: 817–844.

Ayres, M. P. & Scriber, J. M. 1994. Local adaptation to regional climates in *Papilio canadensis* (Lepidoptera: Papilionidae). *Ecol. Monogr.* 64(4): 465–482.

Bach, C. E. 1980. Effects of plant density and diversity on the population dynamics of a specialist herbivore, the striped cucumber beetle, *Acalymma vittata* (Fab.). *Ecology* 61(6): 1515–1530.

Becerra, J. X. 1997. Insects on plants: macroevolutionary chemical trends in host use. *Science* 276: 253–256.

Becker, U., Colling, G., Dostal, P., Jakobsson, A. & Matthies, D. 2006. Local adaptation in the monocarpic perennial *Carlina vulgaris* at different spatial scales across Europe. *Oecologia* 150: 506–518.

Bennett, R. N. & Wallsgrove, R. M. 1994. Secondary metabolites in plant defence mechanisms. *New Phytol.* 127(4): 617–633.

Berenbaum, M. R. & Zangerl, A. R. 1998. Chemical phenotype matching between a plant and its insect herbivore. *Proc. Natl. Acad. Sci. USA* 95: 13743–13748.

Berlocher, S. H. & Feder, J. L. 2002. Sympatric speciation in phytophagous insects: moving beyond controversy? *Annu. Rev. Entomol.* 47: 773–815.

Bernays, E. & Graham, M. 1988. On the evolution of host specificity in phytophagous arthropods. *Ecology* 69(4): 886–892.

Brewer, G. J. & Ball, H. J. 1981. A feeding deterrent effect of a water extract of tansy (*Tanacetum vulgare* L. Compositae) on three lepidopterous larvae. *J. Kans. Ent. Soc.* 54(4): 733–736.

Carroll, S. P., Dingle, H. & Klassen, S. P. 1997. Genetic differentiation of fitness-associated traits among rapidly evolving populations of the soapberry bug. *Evolution* 51(4): 1182–1188.

Colling, G. & Matthies, D. 2004. The effects of plant population size on the interactions between the endangered plant *Scorzonera humilis*, a specialised herbivore, and a phytopathogenic fungus. *Oikos* 105: 71–78.

Collinge, S. K. 2000. Effects of grassland fragmentation on insect species loss, colonization, and movement patterns. *Ecology* 81: 2211–2226.

Cook-Patton, S. C., McArt, S. H., Parachnowitsch, A. L., Thaler, J. S. & Agrawal, A. A. 2011. A direct comparison of the consequences of plant genotypic and species diversity on communities and ecosystem function. *Ecology* 92(4): 915–923.

Crawley, M. J. 1989. Insect herbivores and plant-population dynamics. *Annu. Rev. Entom.* 34: 531–564.

Crémieux, L., Bischoff, A., Šmilauerová, M., Lawson, C. S., Mortimer, S. R., Doležal, J., Lanta, V., Edwards, A. R., Brook, A. J., Tscheulin, T., Macel, M., Lepš, J., Müller-Schärer, H. & Steinger, T. 2008. Potential contribution of natural enemies to patterns of local adaptation in plants. *New Phytol.* 180: 524–533.

Crutsinger, G. M., Collins, M. D., Fordyce, J. A., Gompert, Z., Nice, C. C. & Sanders, N. J. 2006. Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science* 313(5789): 966–968.

Del Campo, M. L., Via, S. & Caillaud, M. C. 2003. Recognition of host-specific chemical stimulants in two sympatric host races of the pea aphid *Acyrthosiphon pisum*. *Ecol. Entomol.* 28: 405–412.

Dempster, J. P. 1991. Fragmentation, isolation and mobility of insect populations. In: The conservation of insects and their habitats (eds. Collins N. M. & Thomas J. A.). Pp. 143–154, Academic Press, San Diego.

Desurmont, G. A., Hérard, F. & Agrawal, A. A. 2012. Oviposition strategy as a means of local adaptation to plant defence in native and invasive populations of the viburnum leaf beetle. *Proc. R. Soc. B* 279: 952–958.

Dlugosch, K. M. & Parker, I. M. 2008. Founding events in species invasions: genetic variation, adaptive

evolution, and the role of multiple introductions. *Mol. Ecol.* 17(1): 431–449.

Ehrlich, P. R. & Raven, P. H. 1964. Butterflies and plants: a study in coevolution. *Evolution* 18: 586–608.

Eyles, A. C. 1964. Feeding habits of some Rhyparochrominae (Heteroptera: Lygaeidae) with particular reference to the value of natural foods. *Trans. R. Ent. Soc. Lond.* 116(5): 89–114.

Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. *Annu. Rev. Ecol. Evol. Syst.* 34: 487–515.

Feeny, P. 1976. Plant apparency and chemical defence. *Recent Adv. Phytochem.* 10: 1–40.

Forister, M. L., Ehmer, A. G. & Futuyma, D. J. 2007. The genetic architecture of a niche: variation and covariation in host use traits in the Colorado potato beetle. *J. Evol. Biol.* 20(3): 985–996.

Fornoni, J. 2011. Ecological and evolutionary implications of plant tolerance to herbivory. *Funct. Ecol.* 25(2): 399–407.

Fox, C. W. & Caldwell, R. L. 1994. Host-associated fitness trade-offs do not limit the evolution of diet breadth in the small milkweed bug *Lygaeus kalmii* (Hemiptera: Lygaeidae). *Oecologia* 97: 382–389.

Fox, L. R. & Morrow, P. A. 1981. Specialization: species property or local phenomenon? *Science* 211: 887–893.

Frankham, R. 1996. Relationship of genetic variation to population size in wildlife. *Conserv. Biol.* 10(6): 1500–1508.

Frankham, R. 2005. Genetics and extinction. *Biol. Cons.* 126: 131–140.

Frankham, R., Ballou, J. D., Eldridge, M. D. B., Lacy, R. C., Ralls, K., Dudash, M. R. & Fenster, C. B. 2011. Predicting the probability of outbreeding depression. *Conserv. Biol.* 25: 465–475.

Fry, J. D. 1989. Evolutionary adaptation to host plants in a laboratory population of the phytophagous mite *Tetranychus urticae* Koch. *Oecologia* 81: 559–565.

Fry, J. D. 1990. Trade-offs in fitness on different hosts: evidence from a selection experiment with a phytophagous mite. *Am. Nat.* 136: 569–580.

Fry, J. D. 1996. Are the trade-offs overrated? *Am. Nat.* 148: S84–S107.

Fry, J. D. 2003. Detecting ecological trade-offs using selection experiments. *Ecology* 84(7): 1672–1678.

Funk, D. J. & Bernays, E. A. 2001. Geographic variation in host specificity reveals host range evolution in *Uroleucon ambrosiae* aphids. *Ecology* 82: 726–739.

Futuyma, D. J. 1976. Food plant specialization and environmental predictability in Lepidoptera. *Am. Nat.* 110: 285–292.

Futuyma, D. J., Keese, M. C. & Funk, D. J. 1995. Genetic constraints on macroevolution: the evolution of host affiliation in the leaf beetle genus *Ophraella*. *Evolution* 49(5): 797–809.

Futuyma, D. J. & Moreno, G. 1988. The evolution of ecological specialization. *Annu. Rev. Ecol. Syst.* 19: 207-233.

Futuyma, D. J. & Peterson, S. C. 1985. Genetic variation in the use of resources by insects. *Ann. Rev. Entomol.* 30: 217–38.

Förare, J. 1995. The biology of the noctuid moth *Abrostola asclepiadis* Schiff. (Lepidoptera, Noctuidae) in Sweden. *Ent. Tidskr.* 116: 179–186.

Gandon, S., Capowiez, Y., Dubois, Y., Michalakis, Y. & Olivieri, I. 1996. Local adaptation and gene-for-gene coevolution in a metapopulation model. *Proc. R. Soc. Lond. B* 263: 1003–1009.

Gandon, S. & Michalakis, Y. 2002. Local adaptation, evolutionary potential and host-parasite coevolution: interactions between migration, mutation, population size and generation time. *J. Evol. Biol.* 15: 451–462.

García-Robledo, C. & Horvitz, C. C. 2011. Jack of all trades masters novel host plants: positive genetic correlations in specialist and generalist insect herbivores expanding their diets to novel hosts. *J. Evol. Biol.* 25: 38–53.

Garrido, E., Andraca-Gómez, G. & Fornoni, J. 2011. Local adaptation: simultaneously considering herbivores and their host plants. *New Phytol.* 193: 445–453.

Gayathri Samarasekera, G. D. N., Bartell, N. V., Lindgren, B. S., Cooke, J. E. K., Davis, C. S., James, P. M. A., Coltman, D. W., Mock, K. E. & Murray, B. W. 2012. Spatial genetic structure of the mountain pine beetle (*Dendroctonus ponderosae*) outbreak in western Canada: historical patterns and contemporary dispersal. *Mol. Ecol.* 21(12): 2931–2948.

Gols, R., Wagenaar, R., Bukovinszky, T., van Dam, N. M., Dicke, M., Bullock, J. M. & Harvey, J. A. 2008. Genetic variation in defense chemistry in wild cabbages affects herbivores and their endoparasitoids. *Ecology* 89: 1616–1626.

Gomulkiewicz, R., Drown, D. M., Dybdahl, M. F., Godsoe, W., Nuismer, S. L., Pepin, K. M., Ridenhour, B. J., Smith, C. I. & Yoder, J. B. 2007.

Dos and don'ts of testing the geographic mosaic theory of coevolution. *Heredity* 98: 249–258.

Gould, F. 1979. Rapid host range evolution in a population of the phytophagous mite *Tetranychus urticae* Koch. *Evolution* 33(3): 791–802.

Greischar, M. A. & Koskella, B. 2007. A synthesis of experimental work on parasite local adaptation. *Ecol. Lett.* 10(5): 418–434.

Hanks, L. M. & Denno, R. F. 1994. Local adaptation in the armored scale insect *Pseudaulacaspis pentagona* (Homoptera: Diaspididae). *Ecology* 75(8): 2301–2310.

Hartl, D. L. & Clark, A. G. 2007. Principles of population genetics. Sinauer Associates Inc., Sunderland.

Haukioja, E. & Neuvonen, S. 1985. The relationship between size and reproductive potential in male and female *Epirrita autumnata* (Lep., Geometridae). *Ecol. Entomol.* 10(3): 267–270.

Hereford, J. 2009. A quantitative survey of local adaptation and fitness trade-offs. *Am. Nat.* 173: 579–588.

Hereford, J. & Winn, A. A. 2008. Limits to local adaptation in six populations of the annual plant *Diodia teres*. *New Phytol.* 178: 888–896.

Hoeksema, J. D. & Forde, S. E. 2008. A meta-analysis of factors affecting local adaptation between interacting species. *Am. Nat.* 171: 275–290.

Horváth, Z., Hatvani, A. & Škorić, D. 2004. New data on the biology of the red spotted bug (*Spilostethus* [=*Lygaeus*] *equestris* L., Het., *Lygaeidae*) causing the achene greening in confectionery sunflower hybrids. *Helia* 27(41): 181–188.

Hussain, H., Aziz, S., Miana, G. A., Ahmad, V. U., Anwar, S. & Ahmed, I. 2009. Minor chemical constituents of *Verbascum thapsus*. *Biochem. Syst. Ecol.* 37: 124–126.

Hämet-Ahti, L., Suominen, J., Ulvinen, T. & Uotila, P. 1998. Retkeilykasvio (Field Flora of Finland). Finnish Museum of Natural History, Botanical Museum, Helsinki.

Janzen, D. H. 1980. When is it coevolution? *Evolution* 34: 611–612.

Kalske, A., Muola, A., Laukkanen, L., Mutikainen, P. & Leimu, R. 2012. Variation and constraints of local adaptation of a long-lived plant, its pollinators and specialist herbivores. *J. Ecol.* 100: 1359–1372.

Karowe, D. N. 1990. Predicting host range evolution: colonization of *Coronilla varia* by *Colias philodice* (Lepidoptera: Pieridae). *Evolution* 44(6): 1637–1647.

Kassen, R. 2002. The experimental evolution of specialists, generalists, and the maintenance of diversity. *J. Evol. Biol.* 15: 173–190.

Kawecki, T. J. & Ebert, D. 2004. Conceptual issues in local adaptation. *Ecol. Lett.* 7: 1225–1241.

Keese, M. C. 1998. Performance of two monophagous leaf feeding beetles (Coleoptera: Chrysomelidae) on each other's host plant: Do intrinsic factors determine host plant specialization? *J. Evol. Biol.* 11(4): 403–419.

Kelley, S. T., Farrell, B. D. & Mitton, J. B. 2000. Effects of specialization on genetic differentiation in sister species of bark beetles. *Heredity* 84: 218–227.

Kingsolver, J. G. 2007. Variation in growth and instar number in field and laboratory *Manduca sexta*. *Proc. R. Soc. B.* 274: 977–981.

Kisiel, W. & Kohlmünzer, S. 1989. A sesquiterpene lactone clycoside from *Crepis tectorum*. *Phytochemistry* 28(9): 2403–2404.

Kugelberg, O. 1973a. Laboratory studies on the effects of different natural foods on the reproductive biology of *Lygaeus equestris* (L.) Het. Lygaeidae. *Ent. Scand.* 4: 181–190.

Kugelberg, O. 1973b. Larval development of *Lygaeus equestris* (Heteroptera, Lygaeidae) on different natural foods. *Ent. Exp. Appl.* 16: 165–177.

Kugelberg, O. 1974. Laboratory studies on the feeding preference and feeding behaviour in *Lygaeus equestris* (L.) Het. Lygaeidae. *Ent. Scand.* 5: 49–55.

Labandeira, C. 2007. The origin of herbivory on land: initial patterns of plant tissue consumption by arthropods. *Ins. Sci.* 14: 259–275.

Laine, A.-L. 2009. Role of coevolution in generating biological diversity: spatially divergent selection trajectories. *J. Exp. Bot.* 60: 2957–2970.

Lande, R. 1976. Natural selection and random genetic drift in phenotypic evolution. *Evolution* 30: 314–334.

Lande, R. & Shannon, S. 1996. The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution* 50(1): 434–437.

Larsson, S., Ekbom, B. & Björkman, C. 2000. Influence of plant quality on pine sawfly population dynamics. *Oikos* 89: 440–450.

Lee, K. P. 2010. Sex-specific differences in nutrient regulation in a capital breeding caterpillar, *Spodoptera litura* (Fabricius). *J. Insect Physiol.* 56: 1685–1695.

Leimu, R. 2004. Variation in the mating system of *Vincetoxicum hirundinaria* (Asclepiadaceae) in

peripherial island populations. *Ann. Bot.* 93: 107–113.

Leimu, R. & Lehtilä, K. 2006. Effects of two types of herbivores on the population dynamics of a perennial herb. *Basic Appl. Ecol.* 7: 224–235.

Leimu, R. & Mutikainen, P. 2005. Population history, mating system and fitness variation in a perennial herb with a fragmented distribution. *Conserv. Biol.* 19(2): 349–356.

Leimu, R. & Syrjänen, K. 2002. Effects of population size, seed predation and plant size on male and female reproductive success in *Vincetoxicum hirundinaria* (Asclepiadaceae). *Oikos* 98: 229–238.

Lill, J. T., Marquis, R. J. & Ricklefs, R. E. 2002. Host plants influence parasitism of forest caterpillars. *Nature* 417: 170–173.

Linhart, Y. B. & Grant, M. C. 1996. Evolutionary significance of local genetic differentiation in plants. *Annu. Rev. Ecol. Syst.* 27: 237–277.

Lively, C. M. 1999. Migration, virulence, and the geographic mosaic of adaptation by parasites. *Am. Nat.* 153: S34–S47.

Lowe, A., Harris, S. & Ashton, P. 2004. Ecological genetics. Design, analysis and application. Blackwell Science Ltd., Padstow.

Lundqvist, J. 1986. Late Weichselian glaciation and deglaciation in Scandinavia. *Quat. Sci. Rev.* 5: 269–292.

Lynch, M. 1991. The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution* 45: 622–629.

Lynch, M. & Walsh, B. 1998. Genetics and analysis of quantitative traits. Sinauer Associates, Sunderland.

Mackenzie, A. 1996. A trade-off for host plant utilization in the black bean aphid, *Aphid fabae*. *Evolution* 50(1): 155–162.

Magalhaes, I. S., Gleiser, G., Labouche, A.-M. & Bernasconi, G. 2011. Comparative population genetic structure in a plant–pollinator∕seed predator system. *Mol. Ecol.* 20: 4618–4630.

Matsubayashi, K. W., Kahono, S. & Katakura, H. 2011. Divergent host plant specialization as the critical driving force in speciation between populations of a phytophagous ladybird beetle. *J. Evol. Biol.* 24: 1421–1432.

Mayr, E. 1947. Ecological factors in speciation. *Evolution* 1: 263–288.

McCauley, D. E. 1991. The effect of host plant patch size variation on the population structure of a specialist

herbivore insect, *Tetraopes tetraophthalmus*. *Evolution* 45(7): 1675–1684.

Mopper, S., Beck, M., Simberloff, D. & Stiling, P. 1995. Local adaptation and agents of selection in a mobile insect. *Evolution* 49(5): 810–815.

Mopper, S., Stiling, P., Landau, K., Simberloff, D. & Van Zandt, P. 2000. Spatiotemporal variation in leafminer population structure and adaptation to individual oak trees. *Ecology* 81(6): 1577–1587.

Muola, A., Mutikainen, P., Laukkanen, L., Lilley, M. & Leimu, R. 2010a. Genetic variation in herbivore resistance and tolerance: the role of plant life-history stage and type of damage. *J. Evol. Biol.* 23(10): 2185–2196.

Muola, A., Mutikainen, P., Lilley, M., Laukkanen, L., Salminen, J.-P. & Leimu, R. 2010b. Associations of plant fitness, leaf chemistry, and damage suggest selection mosaic in plant-herbivore interactions. *Ecology* 91(9): 2650–2659.

Nevo, E. 1978. Genetic variation in natural populations: patterns and theory. *Theor. Popul. Biol.* 13: 121–177.

Núñez-Farfán, J., Fornoni, J. & Valverde, P. L. 2007. The evolution of resistance and tolerance to herbivores. *Annu. Rev. Ecol. Evol. Syst.* 38: 541–566.

Ortegón-Campos, I., Parra-Tabla, V., Abdala-Roberts, L. & Herrera, C. M. 2009. Local adaptation of *Ruellia nudiflora* (Acanthaceae) to counterparts: complex scenarios revealed when two herbivore guilds are considered. *J. Evol. Biol.* 22: 2288–2297.

Pauw, A., Stofberg, J. & Waterman, R. J. 2009. Flies and flowers in Darwin's race. *Evolution* 63: 268–279.

Pekkala, N., Knott, K. E., Kotiaho, J. S. & Puurtinen, M. 2012. Inbreeding rate modifies the dynamics of genetic load in small populations. *Ecol Evol* 2(8): 1791–1804.

Peterson, M. A. & Denno, R. F. 1998. The influence of dispersal and diet breadth on patterns of genetic isolation by distance in phytophagous insects. *Am. Nat.* 152(3): 428–446.

Price, P. W., Bouton, C. E., Gross, P., McPherson, B. A., Thompson, J. N. & Weis, A. E. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annu. Rev. Ecol. Syst.* 11: 41–65.

Rank, N. E. 1992. Host plant preference based on salicylate chemistry in a willow leaf beetle (*Chrysomela aeneicollis*). *Oecologia* 90: 95–101.

Rasmann, S. & Agrawal, A. A. 2011. Evolution of specialization: a phylogenetic study of host

range in the red milkweed beetle (*Tetraopes tetraophthalmus*). *Am. Nat.* 177(6): 728–737.

Rausher, M. D. 1988. Is coevolution dead? *Ecology* 69(4): 898–901.

Rich, K. A., Thompson, J. N. & Fernandez, C. C. 2008. Diverse historical processes shape deep phylogeographical divergence in the pollinating seed parasite *Greya politella*. *Mol. Ecol.* 17(10): 2430–48.

Rintala, T. & Rinne, V. 2010. Suomen luteet. 1st edn. Hyönteistarvike TIBIALE Oy, Helsinki.

Roininen, H., Vuorinen, J., Tahvanainen, J. & Julkunen-Tiitto, R. 1993. Host preference and allozyme differentiation in shoot galling sawfly, *Euura atra*. *Evolution* 47(1): 300–308.

Ruhnke, H., Schädler, M., Matthies, D., Klotz, S. & Brandl, R. 2006. Are sawflies adapted to individual host trees? A test of the adaptive deme formation hypothesis. *Evol. Ecol. Res.* 8: 1039–1048.

Saunders, D. A., Hobbs, R. J. & Margules, C. R. 1991. Biological consequences of ecosystem fragmentation: a review. *Conserv. Biol.* 5: 18–32.

Schearer, W. R. 1984. Components of oil of tansy (*Tanacetum vulgare*) that repel Colorado potato beetles (*Leptinotarsa decemlineata*). *J. Nat. Prod.* 47(6): 964–969.

Scheirs, J., Jordaens, K. & De Bruyn, L. 2005. Have genetic trade-offs in host use been overlooked in arthropods? *Evol. Ecol.* 19: 551–561.

Schoonhoven, L. M., van Loon, J. J. A. & Dicke, M. 2005. *Insect-plant biology.* 2nd edn. University Press, Cambridge.

Sillén-Tullberg, B. 1981. Prolonged copulation: a male 'postcopulatory'strategy in a promiscuous species, *Lygaeus equestris* (Heteroptera: Lygaeidae). *Behav. Ecol. Sociobiol.* 9(4): 283–289.

Singer, M. S. 2008. Evolutionary ecology of polyphagy. In: Specialization, speciation and radiation: The evolutionary biology of herbivorous insects (ed. Tilmon K. J.). Pp. 29–42, University of California Ltd. Berkeley, Los Angeles and London.

Singer, M. S., Rodrigues, D., Stireman, J. O., III & Carrière, Y. 2004. Roles of food quality and enemy-free space in host use by a generalist insect herbivore. *Ecology* 85(10): 2747–2753.

Solbreck, C. 2000. Ecology and biology of *Euphranta connexa* (Fabr.) (Diptera: Tephritidae) – a seed predator on *Vincetoxicum hirundinaria* Med. (Asclepiadaceae). *Ent. Tidskr.* 121: 23–30.

Solbreck, C. & Ives, A. R. 2007. Density dependence vs. independence, and irregular population dynamics of a swallow-wort fruit fly. *Ecology* 88: 1466–1475.

Solbreck, C. & Kugelberg, O. 1972. Field observations on the seasonal occurrence of *Lygaeus equestris* (L.) (Het., Lygaeidae) with special reference to food plant phenology. *Ent. Scand.* 3(3): 189–210.

Solbreck, C., Olsson, R., Anderson, D. B. & Förare, J. 1989. Size, life history and responses to food shortage in two geographical strains of a seed bug *Lygaeus equestris*. *Oikos* 55: 387–396.

Solbreck, C. & Sillén-Tullberg, B. 1986a. Seed production and seed predation in a patchy and time-varying environment. Dynamics of a milkweed – tephritid fly system. *Oecologia* 71: 51–58.

Solbreck, C. & Sillén-Tullberg, B. 1986b. The role of variable weather for the dynamics of a seed-seed predator system. *Oecologia* 71: 59–62.

Solbreck, C. & Sillén-Tullberg, B. 1990a. Population dynamics of a seed feeding bug, *Lygaeus equestris*. 1. Habitat patch structure and spatial dynamics. *Oikos* 58: 199–209.

Solbreck, C. & Sillén-Tullberg, B. 1990b. Population dynamics of a seed feeding bug, *Lygaeus equestris*. 2. Temporal dynamics. *Oikos* 58: 210–218.

Sork, V. L., Stowe, K. A. & Hochwender, C. 1993. Evidence for local adaptation in closely adjacent subpopulations of northern red oak (*Quercus rubra* L.) expressed as resistance to leaf herbivores. *Am. Nat.* 142(6): 928–936.

Sotka, E. E., Wares, J. P. & Hay, M. E. 2003. Geographic and genetic variation in feeding preference for chemically defended seaweeds. *Evolution* 57(10): 2262–2276.

Spitzer, B. 2006. Local maladaptation in the soft scale insect *Saissetia coffeae* (Hemiptera: Coccidae). *Evolution* 60: 1859–1867.

Stærk, D., Christensen, J., Lemmich, E., Duus, J. Ø., Olsen, C. E. & Jaroszewski, J. W. 2000. Cytotoxic activity of some phenanthroindolizidine N-oxide alkaloids from *Cynanchum vincetoxicum. J. Nat. Prod.* 63: 1584–1586.

Stireman, J. O., III, Nason, J. D. & Heard, S. B. 2005. Host-associated genetic differentiation in phytophagous insects: general phenomenon or isolated exceptions? Evidence from a goldenrod-insect community. *Evolution* 59(12): 2573–2587.

Storfer, A., Murphy, M. A., Spear, S. F., Holderegger, R. & Waits, L. P. 2010. Landscape genetics: where are we now? *Mol. Ecol.* 19: 3496–3514.

Strauss, S. Y. 1991. Direct, indirect, and cumulative effects of three native herbivores on a shared host plant. *Ecology* 72(2): 543–558.

Strauss, S. Y. 1997. Lack of evidence for local adaptation to individual plant clones or site by a mobile specialist herbivore. *Oecologia* 110: 77–85.

Sõber, V., Teder, T. & Moora, M. 2009. Contrasting effects of plant population size on florivory and pollination. *Basic Appl. Ecol.* 10(8): 737–744.

Tack, A. J. M. & Roslin, T. 2010. Overrun by the neighbors: landscape context affects strength and sign of local adaptation. *Ecology* 91(8): 2253–2260.

Tahvanainen, J. O. & Root, R. B. 1972. The influence of vegetational diversity on the population ecology of a specialized herbivore, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). *Oecologia* 10(4): 321–346.

Thompson, J. N. 1994. The coevolutionary process, 1st edn. University of Chicago Press, Chicago.

Thompson, J. N. 1996. Trade-offs in larval performance on normal and novel hosts. *Entomol. Exp. Appl.* 80: 133–139.

Thompson, J. N. 1998. Rapid evolution as an ecological process. *Trends Ecol. Evol.* 13(8): 329–332.

Thompson, J. N. 2005. The geographic mosaic of coevolution. The University of Chicago Press, Chicago and London.

Thompson, J. N. 2009. The coevolving web of life. *Am. Nat.* 173: 125–140.

Thompson, J. N., Nuismer, S. L. & Gomulkiewicz, R. 2002. Coevolution and maladaptation. *Integr. Comp. Biol.* 42 (2): 381–387.

Timonin, A. C. & Savitsky, M. Y. 1997. Pollination efficiency of *Vincetoxicum hirundinaria* (Asclepiadaceae s. str.). *Bot. Zh.* 82: 45–51.

Toju, H. 2009. Natural selection drives the fine-scale divergence of a coevolutionary arms race involving a long-mouthed weevil and its obligate host plant. *BMC Evol. Biol.* 9: 273.

Treutter, D. 2006. Significance of flavonoids in plant resistance: a review. *Environ. Chem. Lett.* 4: 147–157.

Ueno, H., Fujiyama, N., Yao, I., Sato, Y. & Katakura, H. 2003. Genetic architecture for normal and novel host-plant use in two local populations of the herbivorous ladybird beetle, *Epilachna pustulosa*. *J. Evol. Biol.* 16: 883–895.

Valkama, E., Koricheva, J., Ossipov, V., Ossipova, S., Haukioja, E. & Pihlaja, K. 2005. Delayed induced responses of birch glandular trichomes and leaf surface lipophilic compounds to mechanical defoliation and simulated winter browsing. *Oecologia* 146(3): 385–393.

Van Zandt, P. A. & Mopper, S. 1998. A meta-analysis of adaptive deme formation in phytophagous insect populations. *Am. Nat.* 152(4): 595–604.

Vesterinen, E. 2010. Arthropod diversity in *Vincetoxicum hirundinaria* populations in south-western Finland – Comparing "true" diversity between higher level taxa and species level taxa. *Pro gradu* thesis, University of Turku.

Via, S. 1991. The genetic structure of host plant adaptation in a spatial patchwork: demographic variability among reciprocally transplanted pea aphid clones. *Evolution* 45(4): 827–852.

Via, S. & Hawthorne, D. J. 2002. The genetic architecture of ecological specialization: correlated gene effects on host use and habitat choice in pea aphids. *Am. Nat.* 159: S76–S88.

von Numers, M. & van der Maarel, E. 1998. Plant distribution patterns and ecological gradients in the Southwest Finnish archipelago. *Global Ecol. Biogeogr.* 7: 421–440.

Wang, I. J., Glor R. E. & Losos, J. B. 2013. Quantifying the roles of ecology and geography in spatial genetic divergence. *Ecol. Lett.* 16: 175–182.

Waring, G. L., Abrahamson, W. G. & Howard, D. J. 1990. Genetic differentiation among host-associated populations of the gallmaker *Eurosta solidaginis* (Diptera: Tephritidae). *Evolution* 44(6): 1648–1655.

Wasserman, S. S. & Futuyma, D. J. 1981. Evolution of host plant utilization in laboratory populations of the southern cowpea weevil, *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae). *Evolution* 35(4): 605–617.

Whitlock, M. C. 1996. The red queen beats the jack-of-all-trades: the limitations on the evolution of phenotypic plasticity and niche breadth. *Am. Nat.* 148: S65-S77.

Whitlock, M. C. 2000. Fixation of new alleles and the extinction of small populations: drift load, beneficial alleles, and sexual selection. *Evolution* 54: 1855–1861.

Willi, Y., van Buskirk, J. & Hoffmann, A. A. 2006. Limits to the adaptive potential of small populations. *Annu. Rev. Ecol. Evol. Syst.* 37: 433–458.

Wise, M. J. & Rausher, M. D. 2013. Evolution of resistance to a multiple-herbivore community: genetic correlations, diffuse coevolution, and constrains on the plant's response to selection. *Evolution* 67(6): 1767–1779.

Young, A., Boyle, T. & Brown, T. 1996. The population genetic consequences of habitat fragmentation for plants. *Trends Ecol. Evol*. 11: 413–418.

Zangerl, A. R., Stanley, M. C. & Berenbaum, M. R. 2008. Selection for chemical trait remixing in an invasive weed after reassociation with a coevolved specialist. *Proc. Natl. Acad. Sci*. 105: 4547–4552.

Ågren, J., Ehrlén, J. & Solbreck, C. 2008. Spatio-temporal variation in fruit production and seed predation in a perennial herb influenced by habitat quality and population size. *J. Ecol*. 96: 334–345.