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**THE GEOGRAPHIC MOSAIC OF COEVOLVING
PLANT-HERBIVORE INTERACTIONS:
GENETIC VARIATION, LOCAL ADAPTATION
AND PLANT SECONDARY CHEMISTRY**

by

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Of coevolution,

“Thus I can understand how a flower and a bee might slowly become, either simultaneously or one after the other, modified and adapted in the most perfect manner to each other, by continued preservation of individuals presenting mutual and slightly favourable deviations of structure.”

Charles Darwin

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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** Muola, A., Mutikainen, P., Lilley, M., Laukkanen, L., Salminen, J.-P. & Leimu, R. 2010: Associations of plant fitness, leaf chemistry, and damage suggest selection mosaic in plant–herbivore interactions. – *Ecology* 91: 2650–2659.
- II** Muola, A., Mutikainen, P., Laukkanen, L., Lilley, M. & Leimu, R. 2010: Genetic variation in herbivore resistance and tolerance: the role of plant life-history stage and type of damage. – *Journal of Evolutionary Biology* 23: 2185–2196.
- III** Muola, A., Mutikainen, P., Laukkanen, L., Lilley, M. & Leimu, R.: The role of plant inbreeding and outbreeding on herbivore resistance and tolerance. *Submitted manuscript*.
- IV** Laukkanen, L., Leimu, R., Muola, A., Lilley, M. & Mutikainen, P.: Plant chemistry drives local adaptation in a coevolving plant-herbivore interaction. *Submitted manuscript*.
- V** Muola, A., Mutikainen, P., Laukkanen, L., Lilley, M. & Leimu, R.: Herbivore pressure drives divergence in plant chemistry in a coevolving plant-herbivore interaction. *Manuscript*.

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1. INTRODUCTION

Human induced environmental changes, such as habitat fragmentation, are rapidly reducing biodiversity. The scale of impact of these ongoing and, unfortunately also accelerating, processes ranges from genetic diversity within species to the species diversity of communities (e.g. Saunders *et al.* 1991, Young *et al.* 1996). Although the effects of fragmentation on a single species have been widely studied (e.g. Leimu *et al.* 2006), the impacts of fragmentation on species interactions are poorly understood. At the same time the growing body of evidence demonstrates that reciprocal evolutionary responses between interacting species, i.e. coevolution, is a major force generating biological diversity (Thompson 1994, 2005). Thus, understanding the factors affecting the process of coevolution has far-reaching implications for conservation of biodiversity. I conducted my research in the southwestern archipelago of Finland that consists of ca 10000 islands of varying size, age, and degree of isolation. Therefore, my study system provides a unique, naturally-fragmented geographic mosaic for investigating coevolutionary dynamics.

1.1. Coevolution

A large part of evolution is coevolution – the process of reciprocal evolutionary change between interacting species (Ehrlich & Raven 1964, Thompson 1994, 2005). Coevolution can shape the patterns of adaptation and speciation and is claimed to be a major force in organizing the diversity of life on earth (Janzen 1980, Thompson 1994, 2005). Indeed, many major events in the history of life are consequences of reciprocal evolutionary change, including the origin of eukaryotic cell and the origin of plants (Taylor 1970, Horiike *et al.* 2001).

Most species have spatially structured populations and these populations are commonly genetically differentiated. This genetic differentiation among populations may be caused by different processes, such as mutations, genetic drift, founder effects, isolation or population bottlenecks that affect the distribution of genetic variation within and among populations of a species (Lande 1976, Gomulkiewicz *et al.* 2007). Moreover, genetic differentiation may arise due to spatial variation in the selective pressure caused by environmental, both physical and biological, conditions. Variable selection pressure at a geographic scale is likely to cause adaptive divergence of populations of a species in heritable traits (Mayr 1947). As a consequence, the form of coevolving interaction often varies across geographic areas and leads to different evolutionary trajectories. Adaptive divergence is, thus, a consequence of natural selection and a key to understanding how coevolution maintains diversity within

species, how it affects the evolution of natural populations, and eventually, how divergent coevolutionary selection among populations may lead to speciation (Mayr 1947, Thompson 1994, 2005). To what extent coevolution of local populations of interacting species reflects the coevolution of these species across their distribution range is, however, not well understood (Thompson 1994, 2005).

The *geographic mosaic theory of coevolution* (GMTC) (Thompson 1994, 1999, 2005) states that coevolutionary processes are often driven by geographic variation in species interactions. According to this theory, variation in species interactions among local populations forms the raw material for the coevolutionary processes that take place over larger geographical scales. Variation in the average intensity of interactions is important because evolutionary trajectories will diverge only if trait correlations of interacting species differ among populations. Selection can be reciprocal only in those populations where the interacting species occur and they impose selection on each others, i.e., the coevolutionary *hot spots*. In coevolutionary *cold spots* the interacting species can at times be absent or the interaction between the species may not lead to reciprocal selection. Furthermore, the level of specialization and adaptation to local conditions is likely to vary among populations and communities (Thompson 1994, 2005). To investigate the assumptions of the GMTC we need to study one particular interspecific interaction across large spatial and temporal scales. Information on the relative roles of several factors, such as levels and distribution of genetic variation and environmental conditions that explain variation in the interactions among and within populations, is essential in understanding how persistent such variation in the interactions is over time (Thompson 1994, 2005).

Although examples of coevolutionary change in interacting species are accumulating, coevolution is difficult to measure (Gomulkiewicz *et al.* 2007, Laine 2009). In order to show that both interacting species are evolving as a result of reciprocal selection it is necessary to overcome the difficulties of studying more than one species simultaneously (Gomulkiewicz *et al.* 2007). Likewise, to measure evolutionary change for both of the interacting species over an evolutionary relevant time scale is often impossible (Gomulkiewicz *et al.* 2007). Furthermore, coevolution at the population level is difficult to link with the coevolution of species that occurs across the whole distribution range of the species. This is because much of the coevolution takes places below the species level, but above the level of local populations. In practice, a commonly used solution to overcome these difficulties is to collect snapshot data on traits that are relevant for the coevolving interaction and are closely linked with the fitness of the interacting species (Gomulkiewicz *et al.* 2007, Laine 2009).

1.2. Local adaptation

In spatially structured systems, studies of local adaptation provide one tool to detect the coevolutionary dynamics of interacting species (Thompson 2005, Laine 2009). For instance, in a plant-herbivore interaction, local adaptation is revealed by relatively higher mean fitness of an herbivore on its sympatric compared to its allopatric host plant (Kawecki & Ebert 2004). However, interacting species can be locally adapted in some populations, but they may not be locally adapted in other populations, or they may even show maladaptation, i.e., lower fitness in sympatry compared with allopatry, or no difference in fitness between sympatric and allopatric counterparts (Thompson *et al.* 2002). While eventual maladaptation may indicate that interacting species are not reciprocally coevolving, it may also reflect the dynamic nature of coevolutionary process (Lively 1999, Thompson 2005, 2009; Laine 2009), or result from gene flow from populations adapted to different conditions (Thompson *et al.* 2002). Local adaptation is, thus, central for creating and maintaining spatial variation in coevolving interactions (Thompson 1994; Kawecki & Ebert 2004; Laine 2009).

In general, local adaptation is predicted to be more likely with growing genetic, geographic, and phenotypic divergence among the populations (Becker *et al.* 2006; Hereford & Winn 2008; Hereford 2009). However, local adaptation can occur even within continuous populations or between physically connected populations, if gene flow among the patches or populations is not strong enough to counteract the forces of selection (Kawecki & Ebert 2004). Generation times of interacting species are likely to affect local adaptation. In the case of long-lived plants and their herbivores, the herbivore with shorter generation time is assumed to show stronger local adaptation than the host (Gandon 2002, Hoeksma & Forde 2008). Adaptation to the sympatric interacting species is predicted to be driven by variation in traits that are central for the coevolving interaction, such as host resistance and tolerance (Hoeksma & Forde 2008). The occurrence and degree of local adaptation in coevolving interactions is, thus, predicted to vary among populations and in time (Thompson 2005, 2009; Laine 2009). In accordance with these predictions, some studies on local adaptation in host-enemy interactions have demonstrated that enemies are locally adapted in at least some of the populations (Kaltz & Shykoff 1998; Koskela *et al.* 2000; Laine 2005) while other studies provide no evidence of local adaptation (Strauss 1997; Kaltz & Shykoff 1998; Kaltz *et al.* 1999; Mutikainen *et al.* 2000; Spitzer 2006).

1.3. Plant-herbivore interaction

Interactions between plants and herbivores have often been used as model systems for examining the patterns of coevolution (Ehrlich & Raven 1964, Berenbaum & Zangerl 1998, Pellmyr 2003, Stenberg *et al.* 2006, Pauw *et al.* 2009). In general, herbivory reduces plant growth, fecundity, and survival (Crawley 1989, Strauss 1991). Plants are

also usually consumed by several different herbivores. The fitness effects of multiple herbivores on a shared host plant may differ between the herbivores (Strauss 1991). On the other hand, variation in plant quality can influence herbivore populations through effects on herbivore fecundity, survival, movement, mortality due to natural enemies, and competition (Price *et al.* 1980, Denno *et al.* 1995, Larsson *et al.* 2000, Lill *et al.* 2002).

Plant-herbivore interactions often show significant spatiotemporal variation. Herbivore abundances can vary spatially and temporally due to abiotic (e.g. weather conditions) and biotic (e.g. abundance of pathogens and predators) factors (Schoonhoven *et al.* 1998). Host plant quality and quantity is also likely to vary spatially due to unevenly distributed resources and genetic variation (Karban 1992). Variation in plant quality can, in turn, affect the spatial distribution of herbivores (Hunter *et al.* 1996). Moreover, the reproductive output of plants often varies spatiotemporally producing variation in the amount of resources available for seed predators (Janzen 1971, Solbreck & Sillén-Tullberg 1986). In summary, variation in the strength of plant-herbivore interaction is important in shaping the life histories, defensive traits, resistance and tolerance of the interacting species.

1.3.1. Plant quality

Plant quality for herbivores is determined both by nutritive compounds and defensive chemicals in plant tissues (Schoonhoven *et al.* 1998). Plant quality reflects both genetic variation in the composition of compounds in plant tissues and resources available for the plant. Plant secondary metabolites play a major role in plant defence against herbivores (Fritz & Simms 1992, Schoonhoven *et al.* 1998). However, specialist herbivores are often adapted to the chemical composition of their host plant (Fox 1981, Kraft & Denno 1982, Bordner *et al.* 1983, Bowers & Puttick 1988) and can even use some compounds to find their host plants or exploit the plant compounds in their own defence (Blum 1983, Bowers 1983, Malcolm 1991). Therefore, unlike generalist herbivores, specialist herbivores maybe unaffected or even prefer high levels of a particular compound (Bowers & Puttick 1988).

1.3.2. Herbivore resistance and tolerance

In addition to plant quality, which can affect the level of herbivore damage, plants have evolved other defence strategies to reduce the detrimental effects of herbivores. Resistance traits reduce damage while tolerance traits reduce the negative fitness impacts of herbivore damage. Resistance to herbivory may be provided by physical structures or chemical mechanisms that reduce herbivore damage while tolerance mechanisms enable compensation for damage (Strauss & Agrawal 1999, Tiffin 2000). Resistance and tolerance are commonly genetically determined traits that show heritable genetic variation within a plant species, and are often under selection exerted by herbivores (Berenbaum *et al.* 1986, Mauricio 1998, Juenger *et al.* 2000, Baucom &

Mauricio 2004). Genetic variation in plant resistance and tolerance to herbivory, with which plants can respond to selection exerted by herbivores, is important for plant-herbivore dynamics, and more importantly, a prerequisite for plant-herbivore coevolution (Carr & Eubanks 2002).

Generally, traits that reduce the detrimental effects of herbivores on plant fitness, and thus offer a fitness benefit for the plant, are expected to be selected for and show reduced genetic variation. However, studies on herbivore resistance and tolerance have commonly observed genetic variation in these traits (Berenbaum *et al.* 1986, Mauricio 1998, Juenger *et al.* 2000, Baucom & Mauricio 2004). Several factors create and maintain genetic variation in resistance and tolerance traits. Firstly, genetic variation may result from recombination and mutations, while gene flow may introduce new alleles to populations. Secondly, abiotic or biotic conditions may create variable selection pressures on resistance and tolerance traits. For example, abiotic conditions, such as drought or shortage of nutrients, may alter plant's ability to resist or tolerate herbivores (Bergelson & Purrington 1996). Likewise, population dynamics of herbivores, pathogens, and predators create both spatial and temporal variation, which affect plant resistance and tolerance (Karban & Baldwin 1997, Schoonhoven *et al.* 1998, Stowe *et al.* 2002). Furthermore, in natural populations plants are often attacked by multiple natural enemies. For example, generalist and specialist herbivores may impose differential selection pressures on plant defence strategies, resulting in the maintenance of variation in both tolerance and resistance within a population (Stowe 1998, Tiffin 2000, Leimu & Koricheva 2006a). For example, tolerance traits are likely to decrease the detrimental effects of mammalian herbivores (Lennartsson *et al.* 1997) and specialists adapted to the chemical defences of their host plants (Jokela *et al.* 2000, Bowers & Puttick 1988). Resistance traits, such as a specific chemical compound, function, in turn, especially against generalist herbivores, but may also reduce the damage by specialist herbivores (Rosenthal & Berenbaum 1991, Leimu & Koricheva 2006b). Defensive traits have generally been assumed to involve fitness costs. Existence of such costs is demonstrated by negative genetic correlations, i.e., trade-offs, between resistance traits and plant fitness (Bergelson & Purrington 1996, Koricheva 2002, Strauss *et al.* 2002). Trade-offs between the levels of resistance and tolerance are expected to occur if plants have a limited amount of resources for allocation to defence, and these two strategies serve a fundamentally similar function for plants (Van Der Meijden *et al.* 1988, Rosenthal & Kotanen 1994, Fineblum & Rausher 1995, Tiffin & Rausher 1999). Although trade-offs do not directly create genetic variation in resistance and tolerance, they contribute to the maintenance of genetic variation in resistance and tolerance especially in environments where the amount of resources vary spatially. Trade-offs between resistance and tolerance have also been assumed to constrain the evolution of these two defence strategies (Rosenthal & Kotanen 1994, Tiffin & Rausher 1999). However, increasing amount of empirical evidence from natural populations suggests that plants rather allocate resources simultaneously to

resistance and tolerance, and maintain multiple defensive strategies against herbivores (for a review, Leimu & Koricheva 2006a, Núñez-Farfán *et al.* 2007).

1.3.3. Plant mating system

Plant mating systems (i.e. self-fertilization versus cross-fertilization) are a fundamental factor affecting genetic variation and genetic structure of plant populations. Self-fertilization reduces heterozygosity and the contribution of overdominance, and increases the expression of recessive alleles within individuals, which alter the distribution of genetic variation (Falconer 1981, Charlesworth & Charlesworth 1987). These genetic changes usually incur fitness costs to the offspring produced by self-pollination, referred to as inbreeding depression (Husband & Schemske 1996). Increased inbreeding is considered to be a major genetic consequence of habitat fragmentation and small population size because it can result in inbreeding depression, which reduces population viability (Keller & Waller 2002). On the other hand, cross-pollination between plants whose genomes are increasingly genetically dissimilar, e.g., plant individuals from different populations, may lead to fitness decline of the offspring (Waser & Price 1983, Lynch 1991, Waser 1993, Leimu & Fischer, 2010). This fitness decline, referred as outbreeding depression, may arise because of disruption of local adaptation, or disruption of allelic coadaptation within or across gene loci, or a combination of these mechanisms (Campbell & Waser 1987, Lynch 1991).

Because variation in plant responses to herbivores often has a genetic basis (Berenbaum *et al.* 1986, Simms & Rausher 1987, Marquis 1990), inbreeding and between-population outbreeding may indirectly affect plant fitness by altering the suitability of a plant to herbivores or by altering its capacity to resist or tolerate herbivore damage (Carr & Eubanks 2002, Ivey *et al.* 2004, Stephenson *et al.* 2004, Du *et al.*, 2008; Delphia *et al.*, 2009; Bello-Bedoy & Núñez-Farfán, 2010; Leimu & Fischer, 2010). In some studies inbreeding has been found to decrease herbivore resistance and/or tolerance (Carr & Eubanks 2002, Ivey *et al.* 2004, Stephenson *et al.* 2004, Du *et al.*, 2008; Delphia *et al.*, 2009; Bello-Bedoy & Núñez-Farfán, 2010) whereas in other studies no such effects have been found (Nunez-Farfán *et al.* 1996). The most probable explanation for these contrasting results is that complex interactions between genetic and environmental conditions, such as the history of inbreeding and levels of herbivory, influence how inbreeding affects plant resistance to herbivores (Leimu *et al.* 2008). Because the genetic and environmental conditions vary across populations, effects of inbreeding and within-population outbreeding may also vary among populations and, thus, have major effects on the spatial dynamics of coevolving species.

Compared to the effects of inbreeding on plant resistance, the effects of between-population outbreeding on plant resistance have been much less studied (but see Leimu

& Fischer 2010), and most studies on outbreeding effects have focused on studying hybrid plants. Studies examining the effects of hybridization between species on plant resistance have either reported no differences in resistance between the hybrids and parental plants, an additive effect, hybrid susceptibility or hybrid resistance that resembles that of the susceptible parent (Fritz *et al.* 1999). In theory, between-population cross-fertilization may also have positive effects on herbivore resistance as new gene combinations in the loci determining resistance may have a selective advantage over common local genotypes to which the local herbivores are adapted (Strauss & Karban 1994). However, spatial and temporal variation in gene flow and selection regimes as well as different population histories are likely to contribute to the variation in outbreeding depression in plant fitness and herbivore resistance (Waser & Price 1983, Waser 1993). Thus, plant mating system can affect the interactions of plants and their herbivores and these effects may vary among populations of interacting species.

1.3.4. The roles of gene flow and genetic drift

As stated earlier, significant variation in trait distribution among populations might arise from spatially variable reciprocal selection (Thompson 1994, 2005). Likewise, this variation may result from adaptive responses to spatially structured variation in habitat quality as well as from neutral genetic divergence or gene flow between geographically structured populations (Lande 1976, Gomulkiewicz *et al.* 2007). Unraveling all possible mechanisms that drive spatial variation in traits important for a given species interaction is crucial for understanding the dynamics of coevolving species, especially in rapidly changing landscapes. Human induced changes, for instance fragmentation of habitats, may take place really fast measured on an evolutionary timescale and, eventually, lead to small population sizes and increased isolation (Saunders *et al.* 1991, Young *et al.* 1996). This may lead to changes in gene flow, but may also affect the relative roles of selection and random genetic drift, since, especially in small populations selection might not be able to counteract the effect of genetic drift (Gandon & Nuismer 2009). Understanding the relative importance of selection, gene flow, and genetic drift for coevolutionary dynamics has only recently received some attention in theoretical studies, but has not been investigated in natural populations in the field (e.g. Gandon & Nuismer 2009).

1.4. Aims of the study

The aim of this thesis is to examine the factors affecting potential coevolution between a long-lived perennial herb *Vincetoxicum hirundinaria* (Asclepiadaceae) and its two specialist herbivores: a folivorous moth *Abrostola asclepiadis* (Lepidoptera) and a seed predator, *Euphranta connexa* (Diptera). In addition to the two specialist herbivores, I have studied the seed-predating bug, *Lygaeus equestris* (Heteroptera) that prefers *V.*

hirundinaria as a host plant, although it can feed and survive on alternative host plant species. In my study areas, *L. equestris* is, however, only found in sites where also *V. hirundinaria* occurs.

One key assumption underlying the process of plant-herbivore coevolution is that herbivores exert selection on their host plants. Furthermore, according to the geographic mosaic theory of coevolution this selection varies among plant populations. Therefore, in article **I**, I examined spatial variation in the existence and strength of phenotypic selection on host plant resistance exerted by the two specialist herbivores. Since plant resistance and tolerance to herbivores are important for the plant-herbivore interaction, genetic variation in these traits is a prerequisite for plant-herbivore coevolution. Thus, in article **II**, I studied genetic variation in herbivore resistance and tolerance. Linking plant-herbivore interactions and plant mating system is important, because variation in plant mating system is likely to lead to variation in plant-herbivore interactions, and, thus, herbivory is likely to exert selection on plant mating system. Therefore, in article **III**, I studied inbreeding and outbreeding depression in herbivore resistance and tolerance. Local adaptation is central for creating and maintaining spatial variation in coevolving plant-herbivore interactions. In my fourth (**IV**) article I investigated the impact of geographic variation in plant secondary chemistry of a host plant on among-population variation in local adaptation of a specialist folivore, *Abrostola asclepiadis*. Variation in coevolving interactions can also be driven by factors other than selection. Adaptive responses to spatially structured variation in habitat quality as well as genetic drift or gene flow between geographically structured populations may create variation in coevolving interactions. To disentangle the relative importance of different factors affecting divergence in traits important for coevolving plant-herbivore interactions, in article **V** I analyzed if herbivore pressure, genetic differentiation, and/or geographic distance explains the observed among-population variation in leaf chemicals of *V. hirundinaria*. The results of my thesis add to the understanding of coevolutionary dynamics in fragmented landscapes.

2. MATERIALS AND METHODS

2.1. Study species

Vincetoxicum hirundinaria (Asclepiadaceae) L. (Figure 1) is a long-lived perennial herb that grows mainly on sunny exposed cliffs and slopes, but also on more shaded habitats along forest margins and in deciduous woods below cliffs. It prefers calcareous substrate. The plant has a short, branched rootstock and forms a dense tussock of above-ground shoots. The flowers are hermaphroditic and arranged in inflorescences that grow from the leaf nodes of the shoots. *V. hirundinaria* is insect pollinated and the main pollinators are large flies, moths, and bees (Timonin & Savitskii 1997). Pollen is aggregated into pollen sacs (pollinia) and each flower has five pairs of pollinia. Pollination occurs when the pollinia are inserted into the stigmatic chambers from which the pollen tubes grow towards the ovaries. In my study populations *V. hirundinaria* has a mixed-mating system (Leimu 2004), i.e. plant individuals are capable of producing seeds through both self-fertilization and outcrossing. In my study area, flowering begins in the middle of June and lasts until the beginning of August. The fruits normally ripen from the end of August and into September. The ratio of pods produced to flowers (i.e. fruit set) is very low, which is characteristic for many species of Asclepiadaceae (Wyatt & Broyles 1994). Each pod contains approximately 20 wind-dispersed seeds (Leimu 2004).

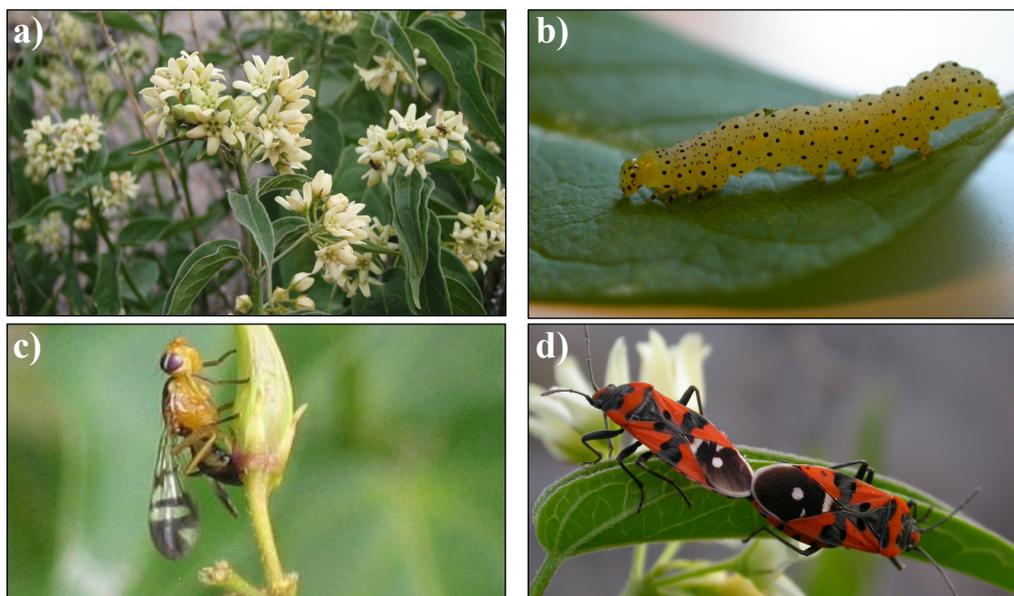


Figure 1. Study species. The host plant *Vincetoxicum hirundinaria* (a) and its herbivores, folivore *Abrostola asclepiadis* (b) and seed predators *Euphranta connexa* (c) and *Lygaeus equestris* (d). Photos by Anne Muola (1a), Kalle Rainio (1b & d) and Roosa Leimu (1c).

Vincetoxicum hirundinaria is highly toxic to mammals and many generalist insects (Solbreck & Sillén-Tullberg 1990). *V. hirundinaria* is known to contain for instance several types of alkaloids (Eibler *et al.* 1995, Staerk *et al.* 2000, Leimu *et al.* 2005). In the papers I, IV, and V, I studied the role of secondary metabolites of *V. hirundinaria* in different aspects of coevolving plant-herbivore interactions. I concentrated on antofine, a phenanthroindolizidine alkaloid, which is known for its cytotoxic activity (Staerk *et al.* 2000). I have also investigated chlorogenic acid, catechin derivatives and flavonoids that belong to phenolic compounds, because they have many ecological and physiological roles in plants, and they are traditionally believed to play an important role in interactions between plants and herbivores (Feeny 1976, Bennett & Wallsgrove 1994). I also chose to study lipophilic compounds, although they consist of many yet unidentified compounds. However, they form a relatively large group of chemical compounds including, for instance, chlorophylls and carotenoids and compounds found on leaf surface, and may, thus, have a potential role in plant herbivore resistance.

Despite its toxicity, *V. hirundinaria* is the host plant for the folivorous moth, *Abrostola asclepiadis* (Noctuidae) Denis & Schiffermüller and the pre-dispersal seed predator, the tephritid fly *Euphranta connexa* (Tephritidae) Fabricius, both of which are strict specialists on this plant species (Figure 1). The female *A. asclepiadis* oviposits on the leaves of *V. hirundinaria* in June and July (Förare & Engqvist, 1996). The eggs hatch about ten days after oviposition and the five larval instars are completed in about five to six weeks (Förare 1995). Damage levels vary among years and among populations from no damage to almost complete defoliation of the plants (Leimu & Lehtilä 2006). The larvae of the tephritid fly *E. connexa* are the main pre-dispersal seed predators of *V. hirundinaria* in my study populations. The female flies insert their eggs in the developing pods (Solbreck 2000). The larvae live within the developing pods and feed on the ripening seeds. In some years and some populations seed predation by *E. connexa* may destroy almost 100 % of the seeds (Solbreck 2000, Leimu & Syrjänen 2002, Leimu & Lehtilä 2006, Solbreck & Ives 2007). The third herbivore in this system is the pre- and post-dispersal seed predator, the bug *Lygaeus equestris* (Lygaeidae) L (Figure 1). *L. equestris* is relatively common in our study area (Leimu & Syrjänen 2002), but it is more generalist in its food use and can use several other species as food plants (Kugelberg 1973, Kugelberg 1974, A. Muola personal observations). However, when *L. equestris* feeds on species other than *V. hirundinaria* it performs less well (Laukkanen *et al.* unpublished data). The adults and larvae of *L. equestris* feed on green ovulae, and developing and mature seeds (Solbreck & Sillén-Tullberg, 1990) but they also suck sap from *V. hirundinaria*'s leaves (A. Muola personal observations). *L. equestris* usually feeds only on a single seed within a pod, before moving to another pod within the plant or to another plant. Thus the bug does not completely destroy the seed production of the pods, in contrast to *E. connexa* and its effect on the seed production is therefore less (see also Ågren *et al.* 2008).

2.2. Study populations

Vincetoxicum hirundinaria has a continental Eurasian distribution, but its natural range covers also the islands and coastal areas of the middle Baltic Sea. My study populations are mainly located in the southwestern archipelago of Finland, but populations situated on the southwestern mainland of Finland and one population from southeastern mainland of Sweden were also included in some of the studies. The archipelago forms a naturally fragmented landscape where islands, and the populations therein, are of different sizes and isolated to variable degree. The populations have been established relatively recently, due to late formation of the archipelago as a result of land uplift after the latest ice age (von Numers & van der Maarel 1998, Leimu & Mutikainen 2005). Although these areas are located close to the northern and western limits of the species' range, *V. hirundinaria* is relatively abundant in this archipelago occurring on over 700 islands (von Numers & van der Maarel 1998). The level of genetic variation in *V. hirundinaria* is relatively high (Leimu & Mutikainen 2005). Despite their fragmented distribution, the populations seem to have some gene flow among them due to the characteristics of pollen and seed dispersal (Leimu & Mutikainen 2005). *V. hirundinaria* is pollinated mainly by large flies, moths, and bees (Timonin & Savitskii 1997, A. Muola personal observations) that are typically capable of extended flight and thus, cause long-distance pollen dispersal. Seeds of *V. hirundinaria* are wind dispersed, but they can also survive and float for up to ten days in brackish water that is characteristic of my study area (Leimu unpublished data). I used a total of 19 populations in my experiments. Two of them are located on the coastal southwestern mainland of Finland, one on the coastal mainland of Sweden and 16 on separate islands of the nearby Archipelago Sea (Figure 2). The distances among the study populations vary from few hundred meters to 260 kilometres. The size of the study populations varies from small (< 100 individuals) to large (> 10000 individuals). This naturally fragmented archipelago together with my study system that is relatively simple with no generalist and only a very few specialized herbivores feeding on the study plant, provides an ideal opportunity for testing the spatial dynamics in and factors affecting coevolving plant-herbivore interactions in a fragmented landscape.



Figure 2. A map showing the locations of the nineteen studied *Vincetoxicum hirundinaria* populations. 1. Naantali, 2. Ruissalo, 3. Källklot, 4. Ävensör, 5. Innamo, 6. Rilot, 7. Lammasluoto, 8. Seili, 9. Jäämaluoto, 10. Henrysaari, 11. Limskär, 12. Ånskär, 13. Lohm, 14. Killingholm, 15. Petsör, 16. Berghamn, 17. Stenskär, 18. Jurmo, 19. Mörkö. Populations 1 to 18 are located in southwestern mainland and archipelago of Finland. Population 19 is located in eastern coast of Sweden.

2.3. Associations of plant fitness, leaf chemistry, and herbivore damage

To investigate spatial variation in specialized plant-herbivore interactions in a naturally fragmented archipelago environment I collected data from 17 *V. hirundinaria* populations during summer 2005 (I). Two of the populations were located on the coastal southwestern mainland of Finland and 15 on separate islands of the nearby Archipelago Sea. The distances among these populations vary from few hundred meters to 50 kilometres. The size of the study populations varies from small (< 100 individuals) to large (> 2000 individuals). I haphazardly selected and marked 20 plant individuals from each population before the plants had been damaged by the herbivores. I was specifically interested in investigating spatial variation in pod production that serves as a food source for the seed predator, *Euphranta connexa*. I used the number of pods per plant individual and fruit set (number of pods per flowers) as estimates of plant fitness. While fruit set describes the ability of flowers to develop into fruits and can thus be considered a measure of reproductive resource allocation, the number of pods per plant individual is a more direct measure of plant reproductive output, i.e., fitness. Secondly, I examined if the association between damage by the seed predator, *E. connexa* and the folivore, *Abrostola asclepiadis*, and plant fitness varies spatially, potentially indicating variable selection pressure among the populations (*sensu* Lande & Arnold 1983). I estimated herbivore damage by these two herbivores separately. Herbivory by *A. asclepiadis* larvae was assessed by estimating visually the proportion of leaf area damaged by the larvae. Seed predation by *E. connexa* was estimated by counting the number of pods damaged by the larvae. Thirdly, I investigated spatial variation in the damage by both herbivores, and in constitutive leaf chemistry. Since leaf compounds can potentially function either as defensive traits or as attractants to specialist herbivores, I further examined spatial variation in the associations between herbivory and components of leaf chemistry. Finally, I tested for spatial variation in the associations between plant fitness and leaf chemistry, which would indicate variation in the potential costs of defence. Leaf samples for the chemical analyses were collected from the marked individuals before herbivores had damaged the plants. The contents of leaf compounds in *V. hirundinaria* were analyzed by using high-performance liquid chromatography (HPLC).

2.4. Genetic variation in herbivore resistance and tolerance

To investigate genetic variation in herbivore resistance and tolerance of *V. hirundinaria* I conducted a series of greenhouse experiments (II). I was specifically interested in whether the level of genetic variation in tolerance depends on plant life-history stage, the type of the damage, or the timing of estimating the tolerance relative to the occurrence of the damage. To find out if there is genetic variation in the responses of plants to herbivore damage for selection to act on, I first tested for genetic variation in tolerance to artificial leaf damage in adult plants and whether putative

genetic variation in tolerance was observable over time. I artificially damaged replicates of 23 plant genotypes from the Lammasluoto population by cutting all of the stems of the plants between the second and third lowest node with scissors. I counted the number of inflorescences per plant before damage, six weeks later and also once during the following growing season, and used this as a measure of plant reproductive output. The number of inflorescences in *V. hirundinaria* correlates strongly with the number of pods produced, and is thus a good fitness estimate (Leimu & Lehtilä 2006; Leimu *et al.*, unpublished data). Tolerance of each plant genotype to clipping was estimated by dividing the mean number of inflorescences of the clipped plants by the mean number of inflorescences of the control plants of the same plant genotype (Strauss & Agrawal 1999). I further tested for genetic variation in tolerance to artificial defoliation in seedlings. To gain wider understanding of the amount of variation in tolerance, I compared the levels of genetic variation in the tolerance of seedlings to artificial damage among four plant populations. I collected and germinated seeds of ten randomly selected individuals (maternal plants) from four different populations (Stora Limsjär, Henrysaari, Rilot, Ruissalo). Half of the twenty randomly chosen seedlings of each plant family were defoliated and the other half served as undamaged controls. In the artificial defoliation treatment I removed all leaves of the seedlings by cutting the petioles close to the stem. I determined seedling performance in terms of number of leaves before the treatment and five weeks after the treatment. Tolerance to defoliation was estimated by dividing the mean number of leaves of the defoliated seedlings by the mean number of leaves of the control seedlings of the same seedling family. To compare with the responses to the artificial damage, we tested for genetic variation in resistance and tolerance to natural damage by the specialist folivore, *Abrostola asclepiadis*, and in resistance to the seed predator, *Lygaeus equestris*. To inflict natural damage we used F₁ – offspring of laboratory reared *A. asclepiadis* to damage the replicates of 23 plant genotypes from Lammasluoto population. I used larval biomass as a measure of herbivore performance. Because herbivore performance is likely to correlate negatively with plant resistance, the inverse of herbivore performance was used as a measure of plant resistance (Tiffin 2000). To estimate plant reproductive output and plant tolerance to *A. asclepiadis*, I counted the number of inflorescences before exposing the plants to *A. asclepiadis* and five weeks after the larvae were removed. I counted the number of inflorescences also in the following year. Tolerance to *A. asclepiadis* was estimated as the slope of the reaction norm relating the reproductive output of the plants representing each genotype to the damage level they experienced (Strauss & Agrawal 1999). The resistance to the seed predator, *L. equestris*, was estimated by conducting a feeding experiment where one larva from each of ten full-sib bug families was fed on seeds from one plant of each of the twenty plant genotypes from the Lammasluoto population. I tested whether there was variation in the biomass of the larvae among plant genotypes, which indicates genetic variation in plant resistance or in plant nutritional quality. Finally, I examined potential trade-

offs between plant resistance and tolerance by analyzing genetic correlations between plant resistance and tolerance.

2.5. Inbreeding and outbreeding depression in herbivore resistance and tolerance

To examine the effects of both experimental inbreeding (self-fertilization) and outbreeding (within-population and between-population cross-fertilization) on plant performance and on resistance and tolerance against herbivory, I conducted a series of greenhouse experiments (III). More specifically, I first examined if there is inbreeding depression in plant performance and in plant resistance against a specialist folivore, *A. asclepiadis*, and whether this inbreeding depression varies among populations or among plant families. Secondly, I examined if there is inbreeding depression in plant tolerance to artificial defoliation and if this inbreeding depression in tolerance varies among plant families. Finally, I studied if there is outbreeding depression in plant performance and plant resistance against a specialist folivore, and whether there is among-population or among-family variation in this outbreeding depression.

To obtain selfed and outcrossed offspring of the 80 maternal plants from four different populations (Lammasluoto, Naantali, Seili and Mörkö) I conducted hand pollinations in the greenhouse in June 2007. All maternal plants received self-pollination and within-population cross-pollination. In addition, maternal plants from the Lammasluoto and Mörkö populations received between-population cross-pollination with pollen donors from three other populations (Mörkö, Naantali and Seili for Lammasluoto, and Lammasluoto, Naantali and Seili for Mörkö). To measure plant performance and herbivore resistance and tolerance, I randomly selected two to three seedlings per each maternal plant and pollination treatment from each population and planted them in separate pots in the following spring. I compared the performance of selfed and outcrossed offspring and used the length of the stems and the number of leaves as estimates of plant performance. To measure herbivore resistance, I used F₂-offspring of laboratory reared *A. asclepiadis* larvae to damage the plants. I used the larval biomass that is an estimate of the herbivore performance, and the proportion of damaged leaves as measures of plant resistance (Agrawal 2005, Muola *et al.* 2010a). I estimated tolerance to artificial defoliation by dividing the mean performance of the defoliated plants by the mean performance of the control plants of the same plant family for both selfed and outcrossed plants (Strauss & Agrawal 1999, Muola *et al.* 2010a).

2.6. Local adaptation of a specialist herbivore

To investigate if the specialist folivore, *Abrostola asclepiadis*, is adapted to its local host plant populations, I conducted a reciprocal feeding trial in laboratory with both

plants and larvae from three sites (Ånskär, Jurmo, Lammasluoto) (**IV**). In the reciprocal design, larvae from each population were randomly assigned for a particular plant from each of the populations immediately after hatching in July 2006, and later on fed with fresh leaves collected from the given plant. After the completion of the larval period, I measured pupal mass and determined the sex of each individual. Pupal mass was used as a measure of herbivore performance. Pupal mass has been shown to correlate with fitness in lepidopteran herbivores (Haukioja & Neuvonen 1985). Because the particular focus of the study was in whether the potential variation in local adaptation is driven by among-population variation in host plant chemistry, leaf samples were collected from all plants used in the reciprocal feeding trial to analyze the contents of leaf compounds. The content of leaf compounds was analyzed with high-performance liquid chromatography (HPLC) assisted with diode-array detection (Muola *et al.* 2010b). To examine if local adaptation is more pronounced between strongly diverged populations, I tested for correlations of local adaptation with geographic distance, genetic differentiation (Leimu & Mutikainen 2005), and phenotypic divergence in plant secondary chemistry among the plant populations. In addition, I studied the influence of among-population variation in plant chemistry on herbivore performance using data from two additional populations (Mörkö and Naantali).

2.7. The associations of genetic differentiation, geographic distance, and divergence in leaf chemicals and herbivory

In addition to selection, variation in coevolving interactions might arise from adaptive responses to spatially structured variation in habitat quality as well as from genetic drift or gene flow between geographically structured populations. To disentangle the relative importance of different factors affecting divergence in traits important for coevolving plant-herbivore interactions, I analyzed if herbivore pressure, genetic differentiation and/or geographic distance explain the observed among-population variation in leaf chemicals of *V. hirundinaria* (**V**). I used data collected from 17 *V. hirundinaria* populations that varied in their size and geographic distance to each others. Similarity in the amount of herbivory and in leaf chemical compounds between pairs of populations (total of 16 pairs) was calculated using Euclidean distance,

$$d_{(p,q)} = \sqrt{\sum_{i=1}^n (p_i - q_i)^2}$$

where p_i is the mean value of trait i for population p and q_i is the mean value of trait i for population q respectively. $d_{(p,q)}$ is, thus, the distance between populations p and q . I formed separate distance matrices for the percentage of plant individuals eaten by *A. asclepiadis*, the percentage of leaf area damaged by *A. asclepiadis*, the percentage of plant individuals eaten by *E. connexa*, and combined the percentage of plant

individuals eaten by the two herbivores. Furthermore, I formed separate distance matrices for flavonoid compounds, lipophilic compounds, and for the whole chemical profile by combining the concentrations of antofine, chlorogenic acid, catechin derivatives, total content of flavonoids, and total content of lipophilic compounds. I used F_{ST} -values to estimate the neutral genetic differentiation among populations. F_{ST} -values were based on isoenzyme electrophoresis (more detailed description of methods see Leimu & Mutikainen 2005). I compared the similarity in different geographic, genetic, chemical and herbivory matrices by conducting both simple and partial Mantel tests using the *zt* package (Bonnet & Van de Peer 2002).

3. RESULTS AND DISCUSSION

3.1. Associations of plant fitness, leaf chemistry and herbivore damage

The geographic mosaic theory of coevolution states that variation in species interactions forms the raw material for coevolutionary processes that take place over large geographic scales (Thompson 1994, 2005). I found extensive spatial variation in the levels of herbivory and in plant fitness (I). More importantly, the associations of plant fitness and leaf herbivory varied among the investigated populations: the fitness of plants damaged by leaf herbivores was lower than that of undamaged plants in some of the populations while in other populations the opposite was observed. This suggests that selection exerted by leaf herbivores for resistance varies among plant populations being stronger in populations with stronger negative association between leaf herbivory and plant fitness. In addition, leaf chemistry varied widely among plant populations reflecting spatial variation in plant defence and in plant quality as food for the herbivores. However, leaf compounds influenced folivory similarly in all the studied plant populations: plants damaged by the leaf herbivore contained less chlorogenic acid and more total lipophilic compounds. Interestingly, the content of catechin derivatives and the content of total flavonoids were associated with the intensity of seed predation. Finally, the contents of chlorogenic acid, catechin derivatives, and total lipophilic compounds were positively associated with plant fitness in some populations, but negatively or non-significantly in others.

The observed spatial variation in the strength of the interactions between *V. hirundinaria* and its specialist herbivores suggests a geographic selection mosaic. Similarly, in a recent review Laine (2009) reported that spatially divergent selection trajectories both in mutualistic and antagonistic interactions have generated variable outcomes in the traits important for coevolving interactions among populations. As my results also demonstrate, in order to find divergent selection trajectories, species interactions need not be compared across the species entire range. Divergent selection trajectories can be found among neighbouring populations located at scales smaller than 50 kilometres (Laine 2009). Because the occurrence and strength of spatial variation varied between the two studied specialist herbivores, my results further highlight the importance of considering multiple enemies when trying to understand evolution of interactions between plants and their herbivores.

3.2. Genetic variation in herbivore resistance and tolerance

To be able to respond to the selection imposed by herbivores, plants should have genetic variation in traits conferring herbivore resistance and tolerance. In my study **II**, I found that adult plants showed genetic variation in tolerance to artificial damage, but not in tolerance to damage by the specialist folivore, *Abrostola asclepiadis*. This difference might be explained by the pattern of selection imposed by the different types of damage on plant tolerance. Different types of herbivores and different causes of damage may be associated with different mechanisms of tolerance and thus cause variation in tolerance (Stowe *et al.* 2000). Moreover, plants are often differently tolerant to artificial and natural damage, because these damages may pose different types of stress and, therefore, result in different responses in the plants (Karban & Baldwin 1997, Lehtilä 2003). In addition, I found that the genetic variation observed in plant tolerance to artificial damage in the year of damage disappeared the following year, although the negative fitness effects of the damage did not disappear.

In contrast to the adult plants, I found that, statistically, seedlings showed only marginally significant genetic variation in tolerance to artificial defoliation. Herbivores may exert different selection pressures on plant traits at different plant life-history stages (Watson 1995, Boege & Marquis 2005). It has been predicted that plant tolerance to herbivory is likely to be lower in juveniles compared to adult plants, because of resource allocation constraints and shortage of stored reserves in young plants (Strauss & Agrawal 1999, Haukioja & Koricheva 2000, Kelly & Hanley 2005). A recent meta-analysis, however, did not find tolerance to differ between juveniles and adult plants (Barton & Koricheva 2010). Contrary to the results of Barton & Koricheva (2010), I found that seedlings compensated for artificial damage much better (tolerance as damaged / undamaged: 0.75 ± 0.03 , mean \pm stderr) than adult plants (0.06 ± 0.02). However, because the artificial damage used and especially the biomass removed in the artificial damage treatments differed between adults and seedlings, I cannot directly compare them. Nevertheless, my results suggest that the average level of tolerance and the level of genetic variation in tolerance differed between the life-history stages.

I also found genetic variation in plant resistance to the two different herbivores, the folivore *Abrostola asclepiadis* and the seed predator *Lygaeus equestris*. The evolution of resistance against one type of enemy may constrain, enhance, or be independent of the evolution of resistance to another type of enemy that shares the same host plant species (Rausher 1996). Because I did not use the same plant genotypes in measuring the resistance to *A. asclepiadis* and *L. equestris*, I was not able to test for genetic correlations between resistances to these two herbivores.

Furthermore, I did not find negative genetic correlation between plant resistance and tolerance, which indicates lack of a trade-off between the two defence strategies and suggests that they can evolve independently. This is in line with the increasing amount

of empirical evidence reporting that trade-offs between resistance and tolerance are not that common (Leimu & Koricheva 2006a) and suggesting that herbivory can select independently for both resistance and tolerance (Mauricio *et al.* 1997, Valverde *et al.* 2003, Núñez-Farfán *et al.* 2007).

Taken together, my results suggest that selection can act on genetic variation in plant defence against herbivory and that herbivory can select for increased tolerance and resistance in this plant species. Furthermore, I found that the level of genetic variation in tolerance and resistance depended on plant life-history stage, type of damage, and timing of estimating the tolerance relative to the occurrence of the damage. These findings contribute to our understanding of ecology and evolution of herbivore defence of long-lived perennial plants. They also highlight the importance of taking plant life-history stage and several natural herbivores sharing the host plant into account when studying plant resistance and tolerance.

3.3. Inbreeding and outbreeding depression in herbivore resistance and tolerance

As stated earlier, variation in plant responses to herbivores often has a genetic basis. Variation in plant mating system may affect plant fitness by increasing the vulnerability of plants to natural enemies by altering plant quality or defence, which in turn is likely to lead to variation in plant-herbivore interaction. Linking plant-herbivore interactions and plant mating system is, thus, important. Overall, I found that inbreeding affected plant fitness both directly by decreasing the performance of selfed offspring, and indirectly by affecting the ability of plants to resist the specialist herbivore (III). I found that selfed plants produced fewer pods and seeds compared to plants from within-population outcrosses whereas there were no significant differences in the relative germination rates between the selfed and outcrossed offspring. Likewise, I found that offspring originating from within-population outcrossing were taller and had more leaves than selfed offspring indicating inbreeding depression. Interestingly, in a previous study on the same plant species, Leimu (2004) found no clear signs of inbreeding depression in pod and seed production. My results indicate that *V. hirundinaria* exhibits inbreeding depression both early and later in its development.

I found that selfed plants were more damaged by the specialist folivore, *A. asclepiadis*, than outcrossed ones indicating inbreeding depression in plant resistance. My results also show that feeding on selfed or outcrossed plants did not affect herbivore performance measured as larval biomass, although *A. asclepiadis* larvae consumed more leaf material of the selfed plants compared to the outcrossed plants to gain the same biomass on selfed and outcrossed plants. This indicates that the herbivore was able to compensate for any possible changes in plant defence or nutritive quality that could have altered the quality of the food for the herbivores.

The effects of inbreeding on plant performance and resistance against specialist folivore were similar in all studied populations and plant families. Previous studies investigating inbreeding effects on plant resistance in multiple populations have found that the severity of inbreeding depression in plant resistance varies among host-plant populations (Carr & Eubanks 2002, Ivey *et al.* 2004, Leimu *et al.* 2008). Likewise, the severity of inbreeding depression in plant performance (Dudash *et al.* 1997, Mutikainen & Delph 1998, Waller *et al.* 2008) and in plant resistance (Ouborg *et al.* 2000, Ivey *et al.* 2004, Bello-Bedoy & Núñez-Farfán 2010) has been found to vary among plant families. My failure to detect among-population or among-family variation in the effects of inbreeding might be explained by the past levels of inbreeding in the *V. hirundinaria* populations. The frequency of self-fertile *V. hirundinaria* individuals within populations is relatively high and does not differ among populations in my study area (Leimu 2004). Interestingly, the amount of genetic variation in *V. hirundinaria* is also high in the investigated populations (Leimu & Mutikainen 2005), which might indicate that the negative effects of selfing have been at least partly purged from these populations. Environmental stress is known to exacerbate the effects of inbreeding (Dudash 1990, Heschel & Paige 1995). It is, therefore, possible that my failure to detect among among-family and among-population variation was caused by the stable and benign greenhouse conditions while in field conditions such variation might be more pronounced.

Inbreeding did not affect tolerance to artificial defoliation. In my study system, herbivore tolerance may be such an important defence strategy for plant juvenile stages that are often heavily consumed by *A. asclepiadis* that selection has acted to purge inbreeding depression in it. Alternatively, in my greenhouse experiment plants did not suffer from drought or lack of nutrients and were thus more capable of compensating for the artificial damage.

Between-population outcrosses did not cause outbreeding depression in plant performance or resistance. The lack of outbreeding depression is likely explained by the low level of genetic differentiation and relatively high levels of gene flow among the *V. hirundinaria* populations (Von Numers & van der Maarel, 1998; Leimu & Mutikainen, 2005). Moreover, *V. hirundinaria* populations in our study area seem not to be strongly locally adapted (Kalske A, Muola A, Mutikainen P, Laukkanen L, Leimu R, unpublished data). Taken together, my findings suggest that, due to inbreeding depression, inbreeding plays an important role in the evolution of the interaction between *V. hirundinaria* and its specialist folivore, *A. asclepiadis*. The effects of cross fertilization between populations seem, in turn, not to play such an important role. My results together with the growing body of evidence suggest that the effects of inbreeding on the mating system evolution of the host plants and the dynamics of plant-herbivore interactions should not be ignored when coevolving plant-herbivore interactions are studied.

3.4. Local adaptation of a specialist herbivore

Since local adaptation is central for creating and maintaining spatial variation in coevolving interactions, studies of local adaptation provide a commonly used tool to detect coevolutionary dynamics of interacting species (Thompson 1994, Kawecki & Ebert 2004, Laine 2009). I found among-population variation in local adaptation of the specialist folivore, *A. asclepiadis*, to the sympatric populations of its host plant, *V. hirundinaria* (IV). The herbivores from one of the populations (Anskär) performed significantly better on their sympatric host plant population compared to allopatric host plant populations. Similar pattern, although not statistically significant, was found for another population (Lammasluoto). Herbivores from the third population (Jurmo) were not locally adapted to their sympatric host plant population. My findings are in line with theoretical predictions and several studies that have found spatial variation in local adaptation of herbivores to their host plant populations (e.g. Hanks & Denno 1994, Ortegón-Campos *et al.* 2009). The drivers of spatial variation in local adaptation are, however, less studied. In a quantitative survey on local adaptation studies on animals and plants, Hereford (2009) found that environmental divergence among populations correlated with the magnitude of local adaptation. I used the difference in secondary chemical composition to quantify the divergence of the plant populations. From the herbivore's viewpoint, the plant characteristics are essential environmental variables. Interestingly, I found that both quantitative and qualitative variation in plant chemistry was linked to herbivore local adaptation. Furthermore, I found that the more differentiated the sympatric host plant population was in terms of secondary chemistry from the allopatric plant populations the higher the level of local adaptation of the herbivores to their sympatric host plant population. My results suggest that local adaptation of this folivore is modified both by the quantitative and qualitative composition of secondary chemicals of its host plant, and that the divergence of the host plant populations in plant chemistry explains the degree of local adaptation observed between pairs of host populations. I also found that the strength of local adaptation of *A. asclepiadis* increased with increasing genetic and geographic distances among the host plant populations. Taken together, I demonstrated how spatial variation in specific defensive traits drives local adaptation of a specialist herbivore among geographically structured host plant populations. These findings provide new insights into the mechanisms driving variation in local adaptation in coevolving plant-herbivore interactions.

3.5. The associations of genetic differentiation, geographic distance, and divergence in leaf chemicals and herbivory

The relative importance of factors other than selection that affect divergence in traits important for coevolving plant-herbivore interactions should not be ignored in studies of coevolution. Geographic distance affects the dispersal of plant and its herbivores,

and may, thus, affect gene flow between populations. Neighbouring populations are often considered to be more similar because of supposedly more gene flow between them, or because abiotic conditions are often more similar in closely located populations than in populations further apart from each other. However, I found that geographically close populations were not more similar in leaf chemicals or herbivory than populations that were further apart (**V**). This might be due to diverged selection pressure among the populations or good dispersal abilities of species in question. Likewise, genetic differentiation among populations, measured with neutral genetic markers, was not associated with geographic distance, divergence in leaf chemicals, or divergence in herbivory. Variation in neutral genetic markers is caused by random drift and gene flow, and may be used as a neutral expectation against which the spatial pattern of trait variation can be compared (Merilä & Crnokrak 2001, McKay & Latta 2002). If trait values within populations result from drift rather than selection, levels of divergence among populations of traits important to interaction should resemble levels observed in selectively neutral traits (Gomulkiewicz *et al.* 2007). My results also showed that the divergence in the percentage of plant individuals damaged by *A. asclepiadis* was negatively associated with the divergence in the contents of flavonoids, and that the divergence in the content of lipophilic compounds was positively associated with the combined percentage of plant individuals eaten by the seed predator and the folivore. These results suggest that flavonoids might not play an important role in the interaction of the folivore and the host plant, while the role of lipophilic compounds might be more central. Taken together, my results suggest that neutral processes (i.e. drift or gene flow) seem not to solely drive the among-population divergence in leaf chemistry. Even if these results do not unequivocally indicate that herbivory has caused population divergence in plant chemistry, they suggest that herbivory might contribute to this divergence, and that its role is worth further studies.

4. CONCLUSIONS AND FUTURE PROSPECTS

The increasing amount of studies on coevolutionary dynamics has shown that in several different between-species interactions, such as plant-pollinator, plant-herbivore, and plant-pathogen interactions, there is potential for coevolution (e.g. Laine 2005, Andersson & Johnson 2008, Parchman & Benkman 2008, Toju 2008). Although all of these studies have not been able to detect coevolution *per se*, they provide us tools for understanding the factors affecting species interactions. This knowledge is necessary in order to further develop empirical approaches to detect coevolutionary dynamics and to recognize empirical systems that may currently undergo coevolution. Ultimately, these studies add to our knowledge on how the diversity of life is created and maintained via the process of reciprocal evolutionary change between interacting species. This is essential not only for a more precise understanding of evolution species interactions, but also for conservation and applied biology.

One of the major findings of this thesis is the diversity of factors that can affect coevolutionary dynamics in plant-herbivore interactions. I observed qualitative and quantitative variation in plant chemistry among host-plant populations. Furthermore, this variation was associated with plant fitness and local adaptation of the specialist folivore indicating that plant chemistry plays an important role in mediating the interaction. In addition to plant quality that can affect the level of herbivore damage, plants have evolved other defence strategies, such as resistance and tolerance traits, to reduce the detrimental effects of herbivores. My results suggest that variation in plant mating system may alter the ability of plants to resist herbivores and thus affect the evolution of the interaction between plant and its herbivores. Furthermore, I found that the level of genetic variation in tolerance and resistance depended on plant life-history stage, type of damage, and timing of estimating the tolerance relative to the occurrence of the damage. These findings together with the observed differences in the occurrence and strength of spatial variation between the two specialist herbivores highlight the importance of studying multiple natural enemies when trying to understand coevolving plant-herbivore interactions.

As a future direction, in order to deepen the knowledge of the factors affecting the coevolutionary process, I have studied local adaptation of the host plant to its physical and biotic environment. In an interaction between a long-lived host plant and its herbivores with much shorter generation times, the herbivores are likely to show stronger local adaptation than their host plant (Gandon & Michalakis 2002, but see also **IV**). However, local adaptation of the host to its physiological or biotic environment may affect traits that are central for the species interaction, and may, thus, lead to local adaptation of the long-lived host to its enemy (Crémieux *et al.* 2008). My preliminary results indicate among-population variation in local adaptation of the host plant in

herbivore resistance against their sympatric herbivores and in pollinators present in their sympatric population. However, my findings may also indicate that the herbivores were maladapted to their sympatric host plants. Furthermore, an additional future direction is to study the potential impacts of gene flow, spatially variable selection, and genetic drift on coevolutionary dynamics. These factors have not yet been thoroughly studied (Gomulkiewicz *et al.* 2007, Gandon & Nuismer 2009), especially in combination with ongoing human-induced environmental changes, such as habitat fragmentation. Habitat fragmentation is known to reduce population sizes and increase their isolation, thereby altering the structure and diversity of communities (Saunders *et al.* 1991, Young *et al.* 1996). Likewise, small population size may influence the relative importance of selection and random genetic drift and cause spatial variation in traits central for the coevolving interactions (Gandon & Nuismer 2009). Isolation of populations may decrease gene flow between them and, thus, increase the genetic differentiation among populations and possibly also adaptation to local conditions. However, although gene flow has previously been thought of as the solely counteracting force of adaptation to local conditions, introduction of new genetic material via gene flow is especially important for small populations (Stockwell *et al.* 2003). Lack of genetic variation may disturb the ability of populations to become locally adapted (Gandon & Michalakis 2002, Hoeksma & Forde 2008). Furthermore, fragmentation of host plant populations may affect the abundances and population dynamics of herbivores (Hanski & Gilpin 1997). Recolonization of habitats by herbivores after a local extinction might take longer especially if host-plant populations are isolated. Furthermore, inbreeding is more common in small populations, which may lead to inbreeding depression in both fitness traits and traits that central for the coevolving interaction (e.g. Carr & Eubanks 2002, Leimu *et al.* 2008, see also **III**), and subsequently alter the coevolutionary dynamics between host plant and its herbivores. Habitat fragmentation may, thus, have strong impact on coevolution of interacting species. Taken together, while there is still many unanswered question about coevolution, divergent coevolutionary selection plays undoubtedly a central role in generating diversity in nature, especially when considering the amount of different types of interactions among living organisms.

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