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PLANT-HERBIVORE INTERACTION IN A FRAGMENTED LANDSCAPE: LOCAL ADAPTATION AND INBREEDING

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ABSTRACT

Reciprocal selection between interacting species is a major driver of biodiversity at both the genetic and the species level. This reciprocal selection, or coevolution, has led to the diversification of two highly diverse and abundant groups of organisms, flowering plants and their insect herbivores. In heterogeneous environments, the outcome of coevolved species interactions is influenced by the surrounding community and/or the abiotic environment. The process of adaptation allows species to adapt to their local conditions and to local populations of interacting species. However, adaptation can be disrupted or slowed down by an absence of genetic variation or by increased inbreeding, together with the following inbreeding depression, both of which are common in small and isolated populations that occur in fragmented environments.

I studied the interaction between a long-lived plant *Vincetoxicum hirsutum* and its specialist herbivore *Abrostola asclepiadis* in the southwestern archipelago of Finland. I focused on mutual local adaptation of plants and herbivores, which is a demonstration of reciprocal selection between species, a prerequisite for coevolution. I then proceeded to investigate the processes that could potentially hamper local adaptation, or species interaction in general, when the population size is small. I did this by examining how inbreeding of both plants and herbivores affects traits that are important for interaction, as well as among-population variation in the effects of inbreeding. In addition to bi-parental inbreeding, in plants inbreeding can arise from self-fertilization which has important implications for mating system evolution.

I found that local adaptation of the plant to its herbivores varied among populations. Local adaptation of the herbivore varied among populations and years, being weaker in populations that were most connected. Inbreeding caused inbreeding depression in both plants and herbivores. In some populations inbreeding depression in herbivore biomass was stronger in herbivores feeding on inbred plants than in those feeding on outbred ones. For plants it was the other way around: inbreeding depression in anti-herbivore resistance decreased when the herbivores were inbred. Underlying some of the among-population variation in the effects of inbreeding is variation in plant phenolic compounds. However, variation in the modification of phenolic compounds in the digestive tract of the herbivore did not explain the inbreeding depression in herbivore biomass. Finally, adult herbivores had a preference for outbred host plants for egg deposition, and herbivore inbreeding had a positive effect on egg survival when the eggs were exposed to predators and parasitoids.

These results suggest that plants and herbivores indeed exert reciprocal selection, as demonstrated by the significant local adaptation of *V. hirsutum* and *A. asclepiadis* to one another. The most significant cause of disruption of the local adaptation of herbivore populations was population connectivity, and thus probably gene flow. In plants local adaptation tended to increase with increasing genetic variation. Whether or not inbreeding depression occurred varied according to the life-history stage of the herbivore and/or the plant trait in question. In addition, the effects of inbreeding strongly depended on the population. Taken together, inbreeding modified plant-herbivore interactions at several different levels, and can thus affect the strength of reciprocal selection between species. Thus inbreeding has the potential to affect the outcome of coevolution.

TIIVISTELMÄ

Lajienväliset evolutiiviset vuorovaikutukset tuottavat ja ylläpitävät sekä lajinsisäistä että lajienvälistä monimuotoisuutta. Kun valintatekijänä on toinen laji, joka myös vastavuoroisesti sopeutuu ensimmäiseen lajiin vuorovaikutuksen seurauksena, on kyse koevoluutiosta. Koevoluutio on ollut keskeinen tekijä useissa lajiutumisprosesseissa ja sen arvellaan johtaneen mm. siemenkasvien ja niitä ravinnokseen käyttävien kasvinsyöjähyönteisten erilaistumiseen ja ylivertaiseen lajirunsauteen. Lajienväliseen keskinäiseen koevoluutiokehitykseen voivat vaikuttaa myös eloton ympäristö ja ympäröivä eliöyhteisö. Koska vuorovaikutteisten lajien genotyypit ja elinympäristöt yleensä eroavat populaatioiden välillä, koevoluutiivinen valinta voi johtaa erilaisten piirteiden yleistymiseen eri populaatioissa. Kyseessä oleva paikallissopeutuminen voi tuottaa piirteitä, jotka ovat eduksi ja parantavat kelpoisuutta lajin yhdessä populaatioissa, mutta eivät välttämättä saman lajin muissa populaatioissa. Sopeutumisen ja lajienvälisen koevoluutiivisen vuorovaikutuksen esteenä tai hidasteena voi kuitenkin olla perinnöllisen muuntelun puute tai pienestä populaatiokoosta johtuva sisäsiitos. Jos valinnan edellytystä, perinnöllistä muuntelua, ei ole riittävästi, lajin mahdollisuudet vastata toisen lajin tuottamaan valintapaineeseen ovat oleellisesti heikentyneet.

Väitöskirjassani tutkin monivuotisen kasvin, käärmeenpistoyrtin, (*Vincetoxicum hircundinaria*) ja yksinomaan käärmeenpistoyrttiä toukkavaiheessa ravinnokseen käyttävän harmosuomuyökkösen (*Abrostola asclepiadis*) välisiä vuorovaikutuksia Turun saaristossa. Aluksi tutkin lajien paikallissopeutumista toisiinsa. Paikallissopeutuminen olisi todiste vastavuoroisesta valinnasta lajien välillä ja siten myös merkki koevoluutiivisesta vuorovaikutuksesta. Sen jälkeen tutkin miten sekä kasvin että hyönteisen sisäsiitos muuttaa lajienvälistä vuorovaikutusta. Selvitin miten sisäsiitos vaikuttaa kasvin ja hyönteisen kelpoisuuteen, kasvin kemialliseen puolustukseen ja hyönteisen kykyyn sietää kasvin kemiallista puolustusta. Lopuksi perehdyin vielä miten kasvin sisäsiitos vaikuttaa hyönteisen munintapreferenssiin ja miten se yhdessä hyönteisen sisäsiitoksen kanssa vaikuttaa hyönteisen munien selviytymiseen munia saalistavien petohyönteisten läsnä ollessa.

Sekä kasvi että hyönteinen olivat paikallissopeutuneita joissain tutkimissani populaatioissa. Lisäksi hyönteisen paikallissopeutuminen vaihteli vuosien välillä. Koska hyönteisen paikallissopeutuminen oli yleisintä levinneisyysalueen rajoilla, arvelin että populaatioiden välinen liiallinen geenivirta oli ensisijainen syy sopeutumisen puuttumiseen. Kasvipopulaatioiden paikallissopeutumista puolestaan rajoitti perinnöllisen muuntelun määrä. Molemmilla lajeilla sisäsiitos johti sisäsiitosheikkouteen. Sen voimakkuus kuitenkin riippui siitä, oliko toinen osapuolista (eli kasvi tai hyönteinen) samanaikaisesti sisäsiitetty vai ei. Kasveilla sisäsiitosheikkous oli lievempää jos hyönteiset olivat myös sisäsiitettyjä. Hyönteisillä puolestaan vaikutus oli päinvastainen, eli sisäsiitosheikkous oli suurempaa sisäsiitetyillä kasveilla.

Tulosten perusteella vaikuttaa siltä, että tutkimani käärmeenpistoyrtti ja harmosuomuyökkönen ovat koevoluutiivisessa vuorovaikutuksessa keskenään. Niiden sopeutumista toinen toisiinsa ajavat ja rajoittavat eri tekijät, mikä on johtanut paikallissopeutumisen vaihteluun populaatioiden ja lajien välillä. Perinnöllisen muuntelun määrällä on kuitenkin molempien lajien sopeutumisessa keskeinen tekijä. Sisäsiitos muutti oleellisesti lajien välistä vuorovaikutusta. Koska sisäsiitoksen seuraukset vaihtelivat populaatioiden ja mitattujen piirteiden välillä, sisäsiitoksen ja pienenevän populaatiokoon vaikutukset ovat laji- ja populaatiokohtaisia. Tulokseni kuitenkin osoittavat, että pieni populaatiokoko ja sisäsiitos voivat heikentää koevoluutiivisen valinnan voimakkuutta. Elinympäristöjen pirstoutumisella ja sisäsiitoksella voi siis olla kauaskantoisia vaikutuksia paitsi yksittäisten lajien selviytymiseen, myös siihen miten ne ja niiden kanssa vuorovaikutuksessa elävät lajit pystyvät sopeutumaan koevoluutiivisen valintapaineen alla.

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ABSTRACT

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- I 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. *Journal of Ecology* 100: 1359–1372.
- II Kalske A, Leimu R, Scheepens JF & Mutikainen P. Isolation promotes persistent local adaptation of an herbivore to its host plant. *Manuscript*
- III Kalske A, Mutikainen P, Muola A, Scheepens JF, Laukkanen L, Salminen J-P & Leimu R (2014) Simultaneous inbreeding modifies inbreeding depression in a plant-herbivore interaction. *Ecology Letters* 17: 229–238
- IV Kalske A, Leimu R, Muola A, Salminen J-P & Mutikainen P. Is modification of phenolic compounds in an herbivore affected by plant or herbivore inbreeding? *Manuscript*
- V Kalske A, Muola A, Mutikainen P & Leimu R (2014) Preference for outbred host plants and positive effects of inbreeding on egg survival in a specialist herbivore. *Proceedings of the Royal Society of London: Biological Sciences* 281: 20141421

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1. INTRODUCTION

Biotic interactions and coevolution are the key to understanding the generation and maintenance of biological diversity both within and between species (Ehrlich & Raven 1964; Mitter *et al.* 1988; Thompson 1994; Marquis 2004; Thompson 2005; Bérénos *et al.* 2011; Schulte *et al.* 2013). Coevolution, i.e. reciprocal evolutionary change in interacting species, can occur in antagonistic host-enemy interactions as arms-race coevolution, where resistance in the host is counteracted by adaptations in the enemy that allow it to overcome the new resistance (Ehrlich & Raven 1964; Dawkins & Krebs 1979). An interacting species can be highly variable as a selective agent, as it is also changing due to evolution and adaptation. The focal species therefore needs to retain its ability to respond to selection in order to keep up in the arms race (e.g. Jaenike 1978; Hamilton 1993; Peters & Lively 1999). To retain the ability to respond to highly variable selection requires genetic variation in the particular traits that are important for the interaction. Accordingly, research on host-enemy coevolution has provided some of the most compelling evidence that helps to understand why genetic variation, and processes promoting its maintenance, are so widespread (Hamilton 1980; Lively 1987; Hamilton *et al.* 1990; Vergara *et al.* 2014). Furthermore, at the between-species level the coevolution of plants and insect herbivores has been the major driver of the vast macroevolutionary diversification of these groups of organisms (Farrell & Mitter 1994; Becerra 2003; Agrawal *et al.* 2009; Becerra *et al.* 2009)

The physical environment and/or the surrounding community can have an added impact on the outcome of interactions among coevolved species, and can create variation in these interactions in space (Orians & Fritz 1996; Agrawal *et al.* 2006; Tétard-Jones *et al.* 2007; Laine 2008). In his geographic mosaic theory of coevolution, Thompson (1994, 2005) describes this variation in fitness as a result of genotype \times genotype \times environment interaction ($G \times G \times E$). This theory postulates that species interactions and their ecological outcomes vary in space among the different populations of the species, which then provide the raw material for coevolution. The selection trajectories vary among species populations, and selection may be reciprocal at only some of the sites where species co-occur (i.e. coevolutionary hotspots). At other sites selection may be imposed on only one or neither of the species. In these coevolutionary coldspots the other species may be absent, or the interaction for some other reason does not lead to reciprocal selection (Thompson 1994, 2005). Coevolutionary interaction within a population is further modified by migration and

gene flow between the populations. All these processes add up to variation in coevolutionary interactions in space and time (Thompson 1994, 2005, 2013).

The purpose of this thesis has been to test some of the assumptions and possible limitations affecting the coevolution of interactions between plants and herbivores in a heterogeneous fragmented landscape. I first studied the mutual local adaptation of plants and herbivores, as this can provide evidence for reciprocal selection, a prerequisite for coevolution. I then studied factors that can potentially disrupt or slow down the coevolutionary process. I focused on the effects of inbreeding in both plants and herbivores; inbreeding is important both from the perspective of the evolution of plant mating systems and from that of conservation. Since inbreeding can modify traits that are important for $G \times G$ interaction, for example plant resistance to herbivory, it has the potential to influence the coevolutionary outcomes of species interactions. In the following I first explore in more detail why these topics are important for species interactions; I then elaborate on the factors that make plant-herbivore systems particularly relevant for such studies.

1.1 Species interactions in heterogeneous environments

In heterogeneous environments, the spatial structure and limited gene flow between populations may lead to adaptation to divergent environmental conditions in different parts of the distribution range of a species. This adaptation to divergent environments allows for the maintenance of more genetic variation at the species level, relative to a situation with highly connected populations that remain genetically uniform through gene flow (e.g. Nuismer *et al.* 1999). For example, populations of bees (*Bombus mori*) that occur on islands have less genetic diversity but higher private allelic richness than mainland populations, suggesting that some alleles that would not have been preserved in a mainland population are still present in that on an island (Jha 2015). Spatially variable selection and genotype \times environment interactions have been corroborated by numerous studies that have demonstrated local adaptation and adaptive divergence of populations in heterogeneous landscapes (Van Zandt & Mopper 1998; Reznick & Ghalambor 2001; Kawecki & Ebert 2004; Leimu & Fischer 2008; Hereford 2009).

While the spatial structure of populations can have a positive effect on species by creating and maintaining genetic heterogeneity at the species level, small population size and isolation can easily hamper evolution and may negatively affect population persistence (Ellstrand & Elam 1993; Verhoeven *et al.* 2011). When a population is small, its adaptive potential can be impaired by the lack of genetic variation, the fixation of deleterious alleles due to genetic drift, and/or inbreeding coupled with subsequent inbreeding depression (Kimura & Crow 1964; Lande 1995; Hedrick & Kalinowski 2000; Keller & Waller 2002; Ridley 2004; Pertoldi *et al.* 2007). In species with a mixed mating system, inbreeding may occur via self-fertilization if suitable mates are scarce, in addition to bi-parental inbreeding (Ellstrand & Elam 1993). Small and isolated populations are then less able to respond to natural selection, due to reduced genetic variation and the expression of deleterious alleles. Along these lines, it has been demonstrated that population size and genetic diversity correlate positively with fitness, and that small populations with low

genetic diversity thus have lower fitness relative to large populations (Reed & Frankham 2003; Leimu *et al.* 2006).

The effects of declining population size along with a declining area of suitable habitat in one species can have broader ramifications when we consider specialised species interactions (Valladares *et al.* 2006; Leimu *et al.* 2012b). For example, in plants and herbivores the population structure of the host species is likely to be reflected in the population size and genetic variation of its specialist herbivore as well (Kéry *et al.* 2001; Koh *et al.* 2004; Leimu *et al.* 2012b). Such tight interactions have already led to significant problems on a global scale: Koh *et al.* (2004) have estimated that up to 6300 affiliate species face the threat of extinction because their host species are endangered. Declining population size can also have implications for species interaction even before species or population persistence are at risk. As reduced genetic variation and increased inbreeding can compromise the ability of species to respond to natural selection, it can also affect elements of the coevolutionary process.

1.1.1 Local adaptation

In an environment that is heterogeneous, and in which that heterogeneity results in variable selection across space, species can become adapted to their local environmental conditions (Williams 1966). Natural selection can favour traits that increase individual fitness under specific local conditions, even though these same traits may prove disadvantageous under the conditions prevalent in different populations of the same species. The end result is local adaptation: the fitness of individuals is highest in their native, i.e. sympatric environment relative to a novel, i.e. allopatric environment (Langlet 1971; Schmid 1985; Linhart & Grant 1996; Kawecki & Ebert 2004). In addition to the physical environment, local adaptation can occur with respect to other species (Linhart & Grant 1996; Kaltz & Shykoff 1998; Lajeunesse & Forbes 2002; Thompson 2005). In plants, local adaptation has been studied and observed for example in relation to pollinators, hemiparasites, pathogens and herbivores (e.g. Kaltz *et al.* 1999; Mutikainen *et al.* 2000; Thrall *et al.* 2002; Crémieux *et al.* 2008; Gómez *et al.* 2009; Bischoff & Tremulot 2011; Garrido *et al.* 2012). Mutual local adaptation of interacting species is a result of reciprocal selection, and thus an indication of potential coevolutionary interaction (Thompson 2005).

In order to confirm that what underpins geographic variation in fitness is genetic adaptation rather than phenotypic plasticity, the fitness of particular genotypes needs to be compared across different environments (Kawecki & Ebert 2004; Blanquart *et al.* 2013). This can take the form of reciprocal transplant or cross-inoculation experiments in native and novel environments/hosts ('home vs. away' comparisons); alternatively, genotypes from different origins can be compared in a single environment/host ('local vs. foreign' comparison: Langlet 1971; Schmid 1985; Linhart & Grant 1996; Kawecki & Ebert 2004). Local adaptation can also be seen as a property of a metapopulation; local adaptation can then be confirmed if on average sympatric combinations outperform allopatric ones (Blanquart *et al.* 2013).

Local adaptation in species interactions is not expected to be the same in all populations across the species distribution (Gandon & Van Zandt 1998; Kaltz & Shykoff 1998; Lively

1999; Thompson 2005). In general, local adaptation is thought to be more likely between populations that are further apart from one another, since greater environmental divergence results in different selection pressures in these populations (Lande 1976; Hereford 2009). Populations that are further apart are also more likely to be genetically divergent, as gene flow is reduced at greater distances (Hanks & Denno 1994; Galloway & Fenster 2000; Becker *et al.* 2006; Cogni & Futuyma 2009). At short distances, gene flow can hamper local adaptation by introducing non-adapted genotypes into the population (Slatkin 1987; García-Ramos & Kirkpatrick 1997; Holt & Gomulkiewicz 1997; Lenormand 2002; Kawecki 2008; Tack & Roslin 2010). Some theoretical models, however, predict that moderate gene flow can provide populations with novel genetic variation, thus promoting local adaptation (Gandon *et al.* 1996; Holt & Gomulkiewicz 1997; Blanquart *et al.* 2012); this has been corroborated by empirical studies demonstrating local adaptation despite ongoing gene flow (e.g. Gonzalo-Turpin & Hazard 2009). Gene flow can be particularly important for small populations (Stockwell *et al.* 2003), as a lack of genetic variation can limit their ability to respond to local selection (Linhart & Grant 1996; Leimu & Fischer 2008).

Since the species involved in an interaction are continuously affected by the changing genetic structures of the coevolving species, local adaptation between species is unlikely to remain constant over time (Lively 1999; Thompson 1999, 2005). A time-shift experiment with *Daphnia magna* and its parasite *Pasteuria ramosa* demonstrated that parasite infectivity was highest on contemporary hosts compared to past or future host genotypes, due to rapid changes in virulence (Decaestecker *et al.* 2007). New experimental approaches to coevolution between bacteria and phages have allowed researchers to examine local adaptation to enemies at different points in time, and have shown that local adaptation was most apparent when tested against genotypes from the recent past (Koskella 2014). In addition, species are always subject to selection from other agents, and the physical environment and/or surrounding biotic community can thus affect the strength of local adaptation and cause variation in local adaptation in both space and time (Nuismer *et al.* 1999; Thompson 2005; Laine 2008).

Where, when, and in which species local adaptation occurs depends on generation times, on the mating system, and on the level of gene flow. In antagonistic species interactions such as plant-herbivore interactions, the species with the shorter generation time is expected to exhibit local adaptation because of its higher evolutionary potential (Dawkins & Krebs 1979; Mopper & Strauss 1997; Kaltz & Shykoff 1998; Gandon & Michalakis 2002; Greischar & Koskella 2007). Shorter generation turnover together with a high reproductive potential will increase the evolutionary rate of the enemy relative to the host (Hafner *et al.* 1994). While empirical studies have confirmed that parasites are often locally adapted (Kaltz & Shykoff 1998; Greischar & Koskella 2007; Hoeksema & Forde 2008; Garrido *et al.* 2012), some studies have reported maladaptation of short-lived enemies and local adaptation of long-lived hosts to their short-lived enemies (Kaltz *et al.* 1999; Spitzer 2006; Crémieux *et al.* 2008; Adiba *et al.* 2010). Thus a short generation time is not always the key to evolutionary advantage, and other factors contribute to local adaptation in species interaction. First of all, differences in the mating system may mediate differences in the ability to respond to selection. Outcrossing species can have an advantage over species with mixed mating systems or complete self-fertilization (Kaltz *et al.* 1999; Morran *et al.* 2014), although the evidence is mixed (Leimu & Fischer 2008; Hereford 2010). Secondly, the species with the

higher gene flow is expected to be locally adapted when rates of gene flow are moderate (Gandon *et al.* 1996; Gandon 2002; Gandon & Michalakis 2002; Hoeksema & Forde 2008). Taken together, a lack of genetic variation can undermine the evolutionary advantage of the species with a shorter generation time and an apparent higher evolutionary potential.

1.1.2 *Inbreeding and plant mating system evolution*

Individuals in small and isolated populations can be constrained to reproduce with their own kin or by self-fertilization (Ellstrand & Elam 1993; Keller & Waller 2002; Aguilar *et al.* 2008). This typically leads to inbreeding depression, where the fitness of inbred individuals is inferior to that of outbred ones (Wright 1977; Charlesworth & Charlesworth 1987; Lynch & Walsh 1998). The reasons inbred individuals perform poorly arise primarily from the expression of deleterious recessive alleles, and to some extent from loss of heterozygote advantage, i.e. overdominance (Wright 1977; Lande & Schemske 1985; Charlesworth & Charlesworth 1987; Lynch & Walsh 1998; Charlesworth & Willis 2009). There is also evidence that epigenetic factors may contribute to inbreeding depression in plants. DNA methylation was greater in the inbred plant *Scabiosa columbaria* and when this methylation was removed, the inbreeding depression disappeared (Vergeer *et al.* 2012).

The various traits that have been reported to be reduced due to inbreeding in plants and insects include for example growth, fitness, adaptive plasticity, and the ability to tolerate stress (Jarne & Charlesworth 1993; Saccheri *et al.* 1996; Hull-Sanders *et al.* 2005; Leimu *et al.* 2012a; Campbell *et al.* 2013; Campbell *et al.* 2014; Prill *et al.* 2014). Evidence from meta-analyses strongly supports positive relationships between population size, fitness and genetic variation, suggesting that inbreeding and inbreeding depression commonly occur when populations become small and isolated (Reed & Frankham 2003; Leimu *et al.* 2006). The severity of inbreeding depression, however, can vary depending on the population history: with recurrent inbreeding the deleterious alleles may be purged from the population, leaving the following generations less likely to suffer from inbreeding depression than their predecessors (Husband & Schemske 1996; Charlesworth & Charlesworth 1999; Crnokrak & Barrett 2002; Leimu *et al.* 2008; Angeloni *et al.* 2011). Finally, abiotic factors can modify the strength of inbreeding depression, as it is typically more severe under harsh than benign environmental conditions (Dudash 1990; Armbruster & Reed 2005; Mena-Ali *et al.* 2008).

In self-compatible plants, even with a moderate level of inbreeding depression, self-fertilization has an inherent evolutionary advantage in transferring genes to the following generation, and yet these plants continue to reproduce by outcrossing (reviewed in Jarne & Charlesworth 1993; Goodwillie *et al.* 2005). One explanation suspected to contribute to this discrepancy is that herbivory often increases the expression of inbreeding depression in plants (Núñez-Farfán *et al.* 1996; Carr & Eubanks 2002; Steets *et al.* 2007; Carr & Eubanks 2014). These indirect effects of herbivory on plant mating systems act through reduced resistance in the inbred plants, and herbivores can thus strengthen selection for outcrossing (Carr & Eubanks 2014). Factors reported to mediate the negative effects of herbivory on inbred plants include plant volatiles (Ferrari *et al.* 2006; Delphia *et al.* 2009; Kariyat *et al.* 2012a), genetic control of anti-herbivore defence (Kariyat *et al.* 2012b), structural defences (Kariyat *et al.* 2013a), plant allelochemicals (Campbell *et al.* 2013), and adaptive plasticity (Campbell

et al. 2014). Recently the transition in plants from self-incompatibility to self-compatibility (i.e. acquiring the ability to reproduce by self-fertilization) at the macroevolutionary scale has been coupled with a shift from constitutive to induced herbivore resistance, providing further evidence for the importance of herbivory in the evolution of the plant mating systems (Johnson *et al.* 2009; Campbell & Kessler 2013; Johnson *et al.* 2014; Campbell 2015).

It is clear that inbreeding can have a significant negative effect on individual fitness, population viability and persistence, and that some of these negative effects in plants are mediated by their interactions with herbivores. Furthermore, inbreeding can have an effect on the coevolution of interacting species if it thwarts the ability of populations to respond to selection from the interacting species (Linhart & Grant 1996; Leimu & Fischer 2008). In nature, the populations of interacting species tend to have similar genetic and geographic population structures, particularly if the interactions are specialized (Kéry *et al.* 2001; Magalhaes *et al.* 2011). However, herbivore population size often fluctuates independently of plant population size (e.g. Ågren *et al.* 2008), and population bottlenecks are followed by increased inbreeding (Lynch *et al.* 1995; Saccheri *et al.* 1998). Alternatively, herbivore populations may be more connected and less inbred than their host plant populations due to their higher dispersal ability (Michalakis *et al.* 1993; Sallé *et al.* 2007). At a single site, inbreeding can thus occur in plant and herbivore populations independently or simultaneously in both species. The negative effects of inbreeding can be either enhanced or diminished depending on the occurrence and/or magnitude of inbreeding in the interacting species. Any or all of these scenarios can alter the premises of interaction between coevolving species, leading to changes in coevolutionary outcomes (Leimu *et al.* 2012b).

1.2 Plants and herbivores

Plants and their insect herbivores provide some of the classic examples of the coevolution of interacting species, and have been used to develop the theory (e.g. Ehrlich & Raven 1964; Mitter *et al.* 1988; Spencer 1988). They are important, firstly, due to their numbers: plants and insects make up half of the currently known eukaryotic species, and form some of the most important species interactions on the planet. Secondly, these interactions form the link between autotrophs and heterotrophs. Without plant-herbivore interactions, energy could not pass from autotrophic plants to higher trophic levels. Finally, herbivores are important from an applied perspective, as they consume over 10 % of crops intended for human consumption (Pimentel 1991). An evolutionary change in coevolving plants and insects can be rapid: insects can have a significant effect on plant evolution over just a few generations (Agrawal *et al.* 2012; Bode & Kessler 2012; Uesugi & Kessler 2013), and resistant plant genotypes can quickly select for counter-resistant herbivores, a process clearly demonstrated in agricultural practices (e.g. Devos *et al.* 2013). At the very core of plant herbivore interactions are thus the defences used by plants to deter herbivores, and the mechanisms used by herbivores to circumvent plant defences.

The diversification of plant defences against insect herbivores is one of the main drivers that has led to the current success of flowering plants, making these defences essential in studies of plant herbivore interactions and coevolution (Ehrlich & Raven 1964; Spencer 1988;

Agrawal *et al.* 2009). Direct plant defences against herbivores can be roughly divided into two strategies – resistance and tolerance (reviewed in Núñez-Farfán *et al.* 2007). Resistance traits allow plants to minimize the damage caused by herbivores (Simms & Rausher 1987; Karban & Baldwin 1997; Strauss & Agrawal 1999; Boege & Marquis 2005). Different resistance traits include physical barriers, such as hairs, thorns and waxes, as well as chemical defences, such as alkaloids, phenolic compounds and latex (Schoonhoven *et al.* 2005). These resistance traits are directed against the feeding life-stages of the herbivores. Some plants also “attack” herbivores before the latter even start feeding, by desiccating the herbivore eggs when they are attached to the plant at oviposition (Hilker & Meiners 2011; Hilker & Fatouros 2015). The alternative or complementary defence strategy, that of tolerance, allows plants to minimize the fitness cost of the damage by compensatory growth and reproduction (Simms & Triplett 1994; Strauss & Agrawal 1999). Resistance traits are generally considered to be more important for coevolution, as they are more likely to exert selection on herbivores than tolerance traits (Núñez-Farfán *et al.* 2007). Then again, tolerance may become an important strategy when plants face specialist insect herbivores that can overcome their resistance (Núñez-Farfán *et al.* 2007; Agrawal & Fishbein 2008).

In addition to exerting direct defences against insect herbivores, plants can co-opt the help of the herbivores’ enemies (Price *et al.* 1980; Boege & Marquis 2005). In deploying these so-called indirect defences, plants release volatile organic compounds upon damage to attract the parasitoids or predators of the herbivorous insects feeding on the plants (Price *et al.* 1980; Dicke & Sabelis 1988; Kessler & Baldwin 2001; Dicke & Baldwin 2010). Some plants also produce extrafloral nectar and cellular food bodies to attract the herbivores’ enemies (Heil 2008; Kessler & Heil 2011). More recently plants have also been reported to release volatiles, or to alter their volatile blend, upon deposition of eggs on the plant, in order to attract predators and parasitoids of the insect eggs (Meiners & Hilker 2000; Hilker & Meiners 2011; Hilker & Fatouros 2015).

Plant resistance in turn is counteracted by mechanisms in the herbivore that allow it to circumvent or neutralise the negative effects of plant resistance (generally referred to as herbivore counter-defences; Després *et al.* 2007; Núñez-Farfán *et al.* 2007). Different adaptations include behavioural mechanisms, such as that of vein-cutting used by the monarch butterfly (*Danaus plexippus*) to avoid coming into contact with latex in its host plants, the milkweeds (*Asclepias* sp.; Després *et al.* 2007). Chemical defences in plants are often counteracted by various physiological adaptations in herbivores, including rapid excretion of the compounds and enzymatic detoxification (Self *et al.* 1964; Schuler 1996; Després *et al.* 2007; Barbehenn *et al.* 2012). The detrimental effects of many compounds result from their pro-oxidant capacities, which are neutralized by the production or recycling of antioxidants in the insects’ midgut (Appel 1993; Barbehenn *et al.* 2003; Barbehenn & Stannard 2004). Some insects are even able to sequester and store the components produced by their host plant and utilise them for their own defence against predators and pathogens (Isman & Duffey 1983; Peterson *et al.* 1987; Simmonds 2003; del Campo *et al.* 2013; Kumar *et al.* 2014).

1.3 Aims of the study

The aim of this thesis was to examine geographical variation in a potentially coevolving interaction between a host plant and its specialist herbivore, together with the factors that disrupt this interaction and the reciprocal selection that accompanies it. The study system used in the thesis consisted of the long-lived plant *Vincetoxicum hirundinaria* and its two specialist herbivores, although I predominantly focused on only one of the herbivores, the noctuid moth *Abrostola asclepiadis*. In the study area, the southwestern archipelago of Finland, these species occur on islands that restrict population size and create a highly fragmented population structure. Geographically structured populations with limited gene flow can promote adaptive divergence, given the presence of sufficient standing genetic variation. Accordingly, the populations of *V. hirundinaria* show different associations between defensive compounds, leaf damage and fitness, which is suggestive of variable selection across the populations (Muola *et al.* 2010). To examine this variable selection in more detail, I first studied local adaptation in the plants and herbivores to one another (I, II). I studied geographical variation in local adaptation as well as variation in the local adaptation of one of the herbivores, over three successive generations. This also allowed me to test one of the prerequisites of coevolution, as mutual local adaptation is a sign that reciprocal selection in the interacting species has occurred in the past.

In the second part of this thesis, I focused on one process that can disrupt the coevolutionary process, namely inbreeding. When populations become small and disconnected because of limitations of the physical environment or adaptive divergence, inbreeding due to mating with relatives or self-fertilization can increase in the population (Ellstrand & Elam 1993; Lynch *et al.* 1995; Verhoeven *et al.* 2011). The populations of *V. hirundinaria* vary both in the frequency of individuals capable of reproducing by self-fertilization (Leimu 2004) and in the level of actual inbreeding (Leimu & Mutikainen 2005). Experimental inbreeding results in inbreeding depression in growth and resistance against leaf herbivory (Muola *et al.* 2011). In specialized plant-herbivore interactions, the sizes of species populations are likely to be linked: small plant populations cannot support large populations of herbivores (Zabel & Tscharntke 1998; Kéry *et al.* 2001; Schoonhoven *et al.* 2005). I therefore determined what happens when inbreeding occurs in plants and herbivores at the same time (III–V), at the same time taking into account the potential variation among populations in the effects of inbreeding (III, IV). I specifically focused on traits that are important for the interaction: direct and indirect plant defences, herbivore preferences, plant secondary metabolites, and the modification of plant secondary chemicals in the herbivores.

The different articles deal with the following questions:

- (I) Are plants locally adapted to their sympatric herbivores, pollinators and the physical environment? Are there trade-offs between local adaptation measured in different traits?
- (II) Are herbivores locally adapted to their sympatric plants, and is local adaptation persistent between generations of herbivores?

- (III) Does simultaneous plant and herbivore inbreeding modify the level of inbreeding depression in either one of the species?
- (III, IV) Does inbreeding affect plant defences and/or herbivore counter-defences?
- (V) Does plant inbreeding affect herbivore oviposition preference?
- (V) Does plant and/or herbivore inbreeding affect the interactions of herbivores with their natural enemies?

2. MATERIALS AND METHODS

2.1 Study species

Vincetoxicum hirundinaria Med. (= *Cynanchum vincetoxicum* (L.) Pers.) (Apocynaceae, formerly Asclepiadaceae) is a long-lived perennial herb (Hämét-Ahti *et al.* 1998; Fig. 1a). It occurs most frequently on calcareous soils in rocky, open habitats and along forest margins. Plants produce multiple above-ground shoots from overwintering root buds in the branched rootstock. In the study area, flowering usually starts in mid-June and lasts until the end of July. The flowers are arranged in inflorescences that sprout from the leaf nodes. Pollen is contained in pollen sacs (pollinia), arranged in five pairs (pollinarium) in each flower (Leimu & Syrjänen 2002). The plant is specialized to insect pollination: its main pollinators are considered to be large flies, moths, and bees (Leimu 2004; A. Muola, personal observation). In the study area *V. hirundinaria* has a mixed mating system; the proportion of individuals capable of self-fertilization varies among populations (Leimu 2004). The plant is toxic to generalist insects and small mammals (Pavela 2011; Hess 2014) due to its high concentrations of phenolic compounds and other plant secondary metabolites (Stærk *et al.* 2000; Muola *et al.* 2010).

Despite its toxicity, there are some insect herbivores that are specialized to feeding on *V. hirundinaria*. In this thesis I focused on two specialist herbivores that occur in the study area: the leaf-chewing larva of the moth *Abrostola asclepiadis* Denis & Schiffermüller (Lepidoptera, Noctuidae; I–V; Fig. 1b), and the seed-feeding larva of the fruit fly *Euphranta connexa* Fabricius (Diptera, Tephritidae; I; Fig. 1c). The moth *A. asclepiadis* lays its eggs under the leaves of *V. hirundinaria* in late June and July, in eggs clusters containing from one to twenty eggs (Förare 1995). The damage caused by the larvae varies significantly from year to year and among populations, from nearly zero damage to severe defoliation of most of the plants within a population during local outbreaks. The second herbivore, *E. connexa*, lays single eggs inside the developing pods of *V. hirundinaria*. Once hatched, the larvae consume the soft developing seeds inside the pod (Solbreck & Sillén-Tullberg 1986). The damage caused by *E. connexa* is also highly variable among populations and between years: in some years seed predation by *E. connexa* results in the loss of all seed production within a population (Solbreck & Sillén-Tullberg 1986). The damage caused by both herbivores reduces the fitness and population growth of *V. hirundinaria*, but the effect of *E. connexa* may be greater than that

of *A. asclepiadis* (Leimu & Lehtilä 2006). In addition to the two herbivores included in this thesis, the oligophagous pre- and post-dispersal seed predator *Lygaeus equestris* (Heteroptera, Lygaeidae) L. occurs in all but one of the study populations (Ruissalo; Fig. 2).

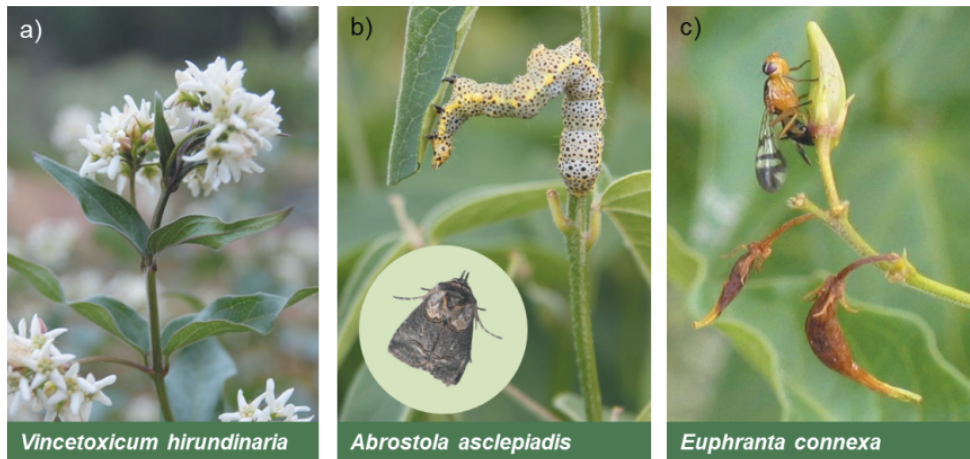


FIGURE 1. The study species: a) flowering shoot of *Vincetoxicum hirundinaria*; b) larva of the moth *Abrostola asclepiadis* feeding on leaves and adult moth in lower left hand corner; c) the adult fly *Euphranta connexa* ovipositing into a developing pod of *V. hirundinaria*. Photographs: Anne Muola and Roosa Leimu.

The eggs of *A. asclepiadis* are parasitized by egg parasitoids from the genera *Telenomus* and *Trichogramma* (Förare 1995). In the study area the rate of eggs being parasitized within a population ranges from nearly zero to two thirds. In addition to attacks by parasitoids the eggs are predated by ants, spiders, mites and larvae of net-winged insects. The larvae are primarily attacked by the same predators as are the eggs, and mainly during the first two instars (Förare 1995). The larvae of *E. connexa* are parasitized by *Scampus brevicornis* Gravenhorst (Inchneumonidae) and *Bracon picticornis* Wesmael (Braconidae) in Sweden (Janzon 1982; Solbreck & Sillén-Tullberg 1986), but I have only encountered a Braconid species in the study area.

2.2 Study area

I conducted the studies in the southwestern archipelago of Finland, with one additional population on an island off the east coast of Sweden (Fig. 2). This area is located at the northern limit of the species' native range, which extends from western Asia and Central Europe northwards up to the southwest Finland and the Åland Islands. The archipelago forms a fragmented landscape, where the population sizes of *V. hirundinaria* are limited by the amount of suitable habitat within an island, and ultimately by the size of the island. While the water that forms the matrix between populations is uninhabitable, the populations are connected to some extent by gene flow (Leimu & Mutikainen 2005). The pollinators of *V. hirundinaria* are large insects, able to fly long distances and cause gene flow. In addition, since the seeds of *V. hirundinaria* are wind-dispersed and can survive in the brackish water of the Baltic Sea for up to ten days (R. Leimu, unpublished data), gene flow can also occur via seed dispersal.

In the studies, I used a total of eleven populations of *V. hirundinaria*: ten located on islands and one on the coast of mainland Finland (Naantali; Fig. 2). The distances between the populations range from a few hundred meters to nearly 300 km. The maximum distance between populations in the Finnish archipelago is 85 km. The plant populations varied in size from c. 100 to 10000 individuals. All *V. hirundinaria* sites are populated also by both *A. asclepiadis* and *E. connexa*.

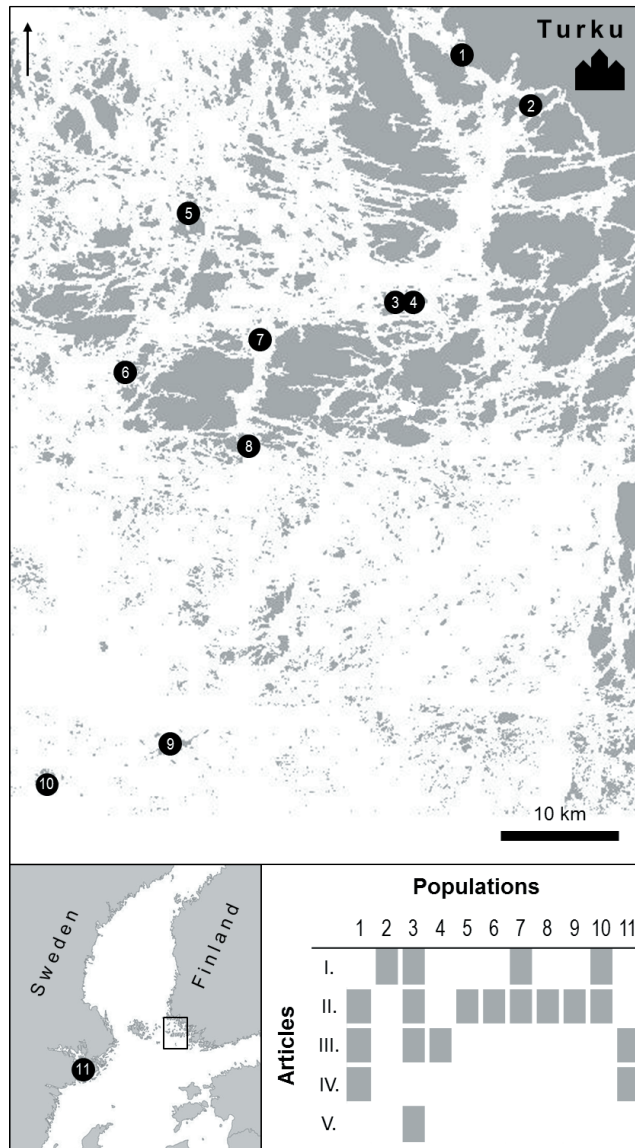


FIGURE 2. Map of all populations of *V. hirundinaria* included in the thesis and table listing populations included in each article. Names of populations are as follows: 1=Naantali, 2=Ruissalo, 3=Lammasluoto, 4=Seili, 5=Kälklot, 6=Limskär, 7=Ånskär, 8=Killingholm, 9=Jurmo, 10=Utö, and 11=Mörkö. Base map by Timo Rantanen.

2.3 Traits studied

I measured traits that were the best possible proxies of plant or herbivore fitness, and that are relevant to the particular species interaction in question. In the plant I measured resistance in terms of damage level, i.e. the reverse of resistance. I mainly used the proportion of damaged leaves (I, III), but I also estimated the damage percentage visually, which correlated positively with the proportion of damaged leaves. In two articles (III, IV) I adopted a more direct measure of resistance by quantifying the phenolic compound content of the leaves and the herbivores frass. I also measured several traits that indicate plant size or reproductive output (flowers, pod production, and removal of pollinia); this, however, was not always possible, as some of the plants were still small and not yet flowering. In the herbivores I assessed performance by measuring larval biomass (III, IV), and pupal mass (II). I estimated the proportions of sterile, fertile, predated and parasitized herbivore eggs as well as eggs lost (V). Parasitized eggs are easily recognized because they turn black. Predation was confirmed with a microscope to identify eggs that had a broken shell, which was indicative of predation.

2.4 Local adaptation

I studied the local adaptation of both the plant (I) and the herbivore *A. asclepiadis* (II). I first conducted a reciprocal transplant experiment in the field in four *V. hirundinaria* populations to observe local adaptation of the plant to the environment and to the two specialist herbivores (I; Fig. 2). I used full-grown plants; these were removed from their populations of origin and split into four equal-sized parts, so as to establish four genetically identical common gardens. These common garden plants were planted in pots and transplanted back in the four populations. The following year the performance of the plants was measured over the course of one growing season. I considered separately the resistance to the two specialist herbivores (*A. asclepiadis* and *E. connexa*), pollination success, and reproductive output of the plants. I compared local adaptation values in different traits across populations to establish the possible presence of conflicting selection pressures, leading to local adaptation in one aspect of performance but not in others. Finally, I compared differences among populations in local adaptation to genetic variation within populations, to genetic and geographical distance, and to differences in population size, abiotic environmental conditions, and plant secondary metabolites. The data on population genetics and plant secondary metabolites were obtained from Leimu & Mutikainen (2005) and Muola *et al.* (2010).

I studied local adaptation of *A. asclepiadis* in eight populations in the laboratory under controlled conditions over three consecutive years (II; Fig. 2). This experimental design allowed me to extract the effect of local adaptation of the herbivore to the plant by controlling the abiotic environment. I collected the herbivores from the field as eggs and brought them to the lab. Once the larvae hatched, they were reared individually in vials and fed *ad libitum* with leaves of plants from a common garden that included plants from all the study populations. Each larva was always fed on plants from a single population, and siblings (larvae hatched from eggs from the same egg cluster) were assigned to different plant populations. Pupal mass was used as a proxy of fitness.

For both local adaptation studies I used mixed models approach where a significant interaction between plant population by sympatry factor (I) or plant population by herbivore population by year factor (II) was considered an indication of local adaptation, and variation between years in local adaptation respectively (Kawecki & Ebert 2004). Further specifics of which populations were locally adapted I determined by using contrasts (I) and effects sizes (II; Parachnowitsch & Lajeunesse 2012). In addition, I calculated indices for comparison of local adaptation in different populations (Hereford 2009). I considered the “home *versus* away” comparison (*sensu* Kawecki & Ebert 2004) to be a more relevant test for local adaptation. In home-away comparison the performance of the plants or herbivores in the native site/host is compared to the performance of the plants or herbivores that originate from the same population in novel sites/hosts (Kawecki & Ebert 2004).

2.5 Effects of inbreeding on plant herbivore interactions

In the last three articles (III–V) I studied the effects of inbreeding in both the plants and the leaf-chewing herbivore *A. asclepiadis*. In all the studies I obtained inbred plants and herbivores by self-fertilization and brother sister mating, respectively. Outbred plants and herbivores came from random within population pairings. I specifically did not cross individuals from different populations in order to avoid outbreeding depression (Hufford & Mazer 2003; Leimu *et al.* 2008; Muola *et al.* 2011).

In the first part of the experiment reported in article (III) I allowed inbred and outbred neonate larvae to feed on inbred and outbred plants. The plants and herbivores originated from four populations (Fig. 2), and the experiment was fully reciprocal, including all combinations of plant and herbivore cross and origin (a total of 64 combinations). After the larvae had grown on the plant for nine days, I weighed them to obtain an estimate of performance; I also measured the damage inflicted on the plant (as the proportion of damaged leaves), so as to obtain an estimate of plant resistance.

In the second part of the experiment I studied the variation in plant resistance (III) and herbivore counter-defence (IV) more directly, by quantifying the phenolic compound content from plant leaves and herbivore frass. Following the first part of the third experiment (above), I removed the larvae from the plants and continued to rear them in individual vials. Once the larvae were big enough (third and fourth instar) I sampled frass and collected leaf samples from a subset of the plants (all four populations included) and herbivores (two populations included). I used ultra-performance liquid chromatography with an electrospray triple quadrupole mass spectrometry detector (UPLC-MS/MS) to quantify the phenolic compound content of the leaves and frass.

In the last article (V), I focused on the effects of inbreeding on adult herbivores and eggs. To determine whether adult *A. asclepiadis* prefer to oviposit on inbred or outbred plants, I placed pairs of herbivores in a cylindrical mesh cage with an inbred and an outbred *V. hirsutaria* plant. I inspected the number of eggs on each plant daily until each plant acquired more than ten eggs, which took on average three to four days. At this point I took the plants with eggs on them out to the field to observe the effect of plant and herbivore inbreeding on interactions with the herbivores’ enemies. Half of the pairs in the preference

test had been siblings and the other half not, to produce the inbred and outbred eggs for the latter part of the experiment. I inspected the eggs daily or every other day to account for eggs that were parasitized, predated (shell broken, i.e. perforated), lost (due to predation and plant hypersensitive responses), sterile or fertile.

I tested the effects of plant and herbivore inbreeding with a mixed models approach (III–V), with plant and herbivore cross included as fixed factors. In studies with multiple populations (III, IV), plant and herbivore population were also included, as well as all the interactions among the fixed factors. I tested the modification of phenolic compounds in the digestive tract of the larvae and oviposition preference with a repeated measures model (VI, V).

3. RESULTS AND DISCUSSION

3.1 Local adaptation of plants

In the first article (I), I studied local adaptation of the plant *V. hirundinaria*. I found that local adaptation in resistance and reproductive traits varied across the study populations. Plants were locally adapted to their sympatric leaf herbivores (*A. asclepiadis*) in two of the four populations (Fig. 3), and to seed predators (*E. connexa*) in one population. At one site the plants suffered the highest leaf damage when replanted at their original site, which is indicative of local maladaptation in resistance to the leaf-chewing herbivore *A. asclepiadis* (I). In contrast, it appeared that the herbivores in this population were locally adapted to their sympatric host plants (II).

Finding local adaptation of long-lived plants to herbivores is somewhat surprising; it is generally considered that herbivores, with their shorter life-span, would be more likely to be adapted to their local host plants due to their higher evolutionary potential (Dawkins & Krebs 1979; Mopper & Strauss 1997; Gandon & Michalakis 2002; Núñez-Farfán *et al.* 2007; Garrido *et al.* 2012). However, this is not the first time long-lived plants have been reported to have highest resistance against sympatric herbivores. Seedlings of oak (*Quercus rubra*) and *Plantago lanceolata*, for example, both suffered less leaf damage when transplanted to their native site in comparison with foreign genotypes (Sork *et al.* 1993; Crémieux *et al.* 2008). Provided that migration and mutation are not effectively providing novel genetic variation in the herbivore population, a lack of genetic variation in the herbivore can allow for local adaptation by the plant (Kaltz *et al.* 1999; Gandon & Michalakis 2002). Alternatively, local genetic divergence in the herbivore may be swamped by high migration and gene flow (Michalakis *et al.* 1993; Hanks & Denno 1994); this is also suggested by my further results on herbivore local adaptation (II).

Two of the plant populations were locally adapted in terms of reproductive output (I), which I (liberally) interpreted as local adaptation to the environment. The correlation between local adaptation in reproductive output and resistance was positive, indicating the absence of trade-offs between local adaptation in these two measures of performance. Populations that are locally adapted to their sympatric herbivores (i.e. had the lowest damage “at home”) are also likely to have the highest fitness at their home site. This suggests that local adaptation to herbivores does not restrict or trade off with local adaptation to the environment. It seems

rather than the level of local adaptation correlates positively with within-population genetic variation (H_e), which may then be the main driver of local adaptation in all the traits studied. The results of a recent meta-analysis also support this interpretation: plant population size and, hence presumably genetic variation, was the most important factor limiting plant local adaptation (Leimu & Fischer 2008). Populations with high levels of genetic variation will be able to respond to selection more efficiently than those with low ones (Hill 1982). In addition, small populations may suffer from inbreeding depression (Ellstrand & Elam 1993; Keller & Waller 2002), which can conceal local adaptation (Leimu & Fischer 2008).

3.2 Local adaptation of herbivores

In general, local adaptation of herbivores is assumed to be widespread and continuous due to their higher evolutionary potential (Morand *et al.* 1996; Mopper & Strauss 1997; Lively 1999). I found persistent local adaptation over the three study years in only two of the eight herbivore populations (Utö and Naantali) (II; Fig. 3). In three populations local adaptation occurred in one of the three years, while the remaining three populations never showed local adaptation.

Comparison of local adaptation with mean distance from the other populations indicated that more peripheral populations were more likely to be locally adapted than central ones (II; Fig. 3). In central, well-connected populations, gene flow from neighbouring populations can swamp local adaptation by introgression of non-adapted genes or alleles (Slatkin 1987; Hanks & Denno 1994; Tack & Roslin 2010). The locally adapted populations in this study also occur on the borders of the species distribution range in Finland, lending support to the idea that they are likely to receive fewer migrants from adjacent populations than do the central populations. Alternatively, local adaptation is more likely and more easily detectable when comparing populations that are further apart (Ebert 1994; Galloway & Fenster 2000; Thrall *et al.* 2002; Raabová *et al.* 2007). The host plants in more distant populations are also likely to be more genetically divergent and therefore genetically less similar compared to plants in the “home” population. However, since geographical distance does not correlate with genetic distance among populations in the host plant *V. hirundinaria* (Leimu & Mutikainen 2005), simple divergence due to geographical distance in the host may not be the main cause of the observed pattern of local adaptation in the herbivore.

Abiotic factors such as temperature can cause significant variation in the strength of local adaptation (Kawecki & Ebert 2004; Laine 2008). However, as the herbivores were reared in the laboratory under controlled conditions, variation in temperature could not have directly contributed to variation in local adaptation. As the plants were grown outside, some of the variation could also be due to indirect effects of the abiotic environment, mediated by changes in plant quality (i.e. effects of UV radiation on plant secondary metabolites; Hofmann *et al.* 2000). For example, local adaptation of the herbivores might be more apparent when the host plants are of poor quality. Even so, this is unlikely to be the main explanation for the differences in local adaptation observed between years, and it certainly would not explain the variation between herbivore populations.

In two populations the herbivores were locally maladapted in one year. Here the plants were the same for the duration of the experiment, and maladaptation could thus not be due

to changes in the host populations. For maladaptation to arise in this situation requires the presence of trade-offs in traits that confer counter-defence of the herbivore to its sympatric host plants between populations (Spitzer 2006; Hereford 2009). Trade-offs in traits conferring fitness benefits between different populations, together with strong gene flow, may result in maladaptation if deleterious alleles are introduced effectively into the population (Gandon & Michalakis 2002; Kawecki 2008; Tack & Roslin 2010; Blanquart *et al.* 2012). In the absence of such trade-offs, gene flow could result in loss of local adaptation, but not in maladaptation. Alternatively, trade-offs can occur within populations between adaptation to the host and enemies (e.g. Zovi *et al.* 2008). However, maladaptation only appeared sporadically and does not appear to be a stable state for the herbivores in this system.

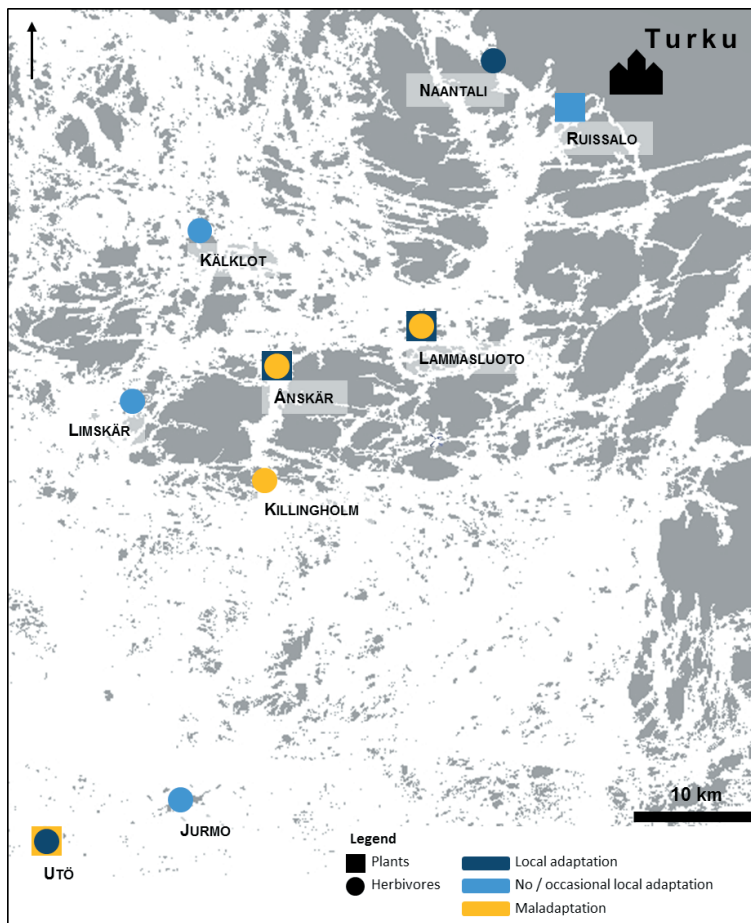


FIGURE 3. Map depicting the occurrence of local adaptation of a plant (*V. hirsutaria*) in resistance to a leaf-chewing herbivore (*A. asclepiadis*; squares) and local adaptation of this herbivore to the host plant (circles). For the herbivores “occasional local adaptation” refers to populations where local adaptation appeared in only one of the study years. “Maladaptation” in herbivores refers to populations where maladaptation was observed in one of the study years, or that were never either locally adapted nor maladapted. Note that local adaptation of herbivores was also studied in populations where plant local adaptation was not, and vice versa. Base map by Timo Rantanen.

3.3 Inbreeding depression in resistance and performance

Increased inbreeding can compromise the ability of species to respond to natural selection, and can thus also affect the outcome of coevolutionary interactions. In interactions involving a specialist species, inbreeding can occur in the plant and the herbivore simultaneously (Magalhaes *et al.* 2011), or in one but not the other species/population (Michalakis *et al.* 1993; Sallé *et al.* 2007). It is therefore important that the effects of plant and herbivore inbreeding on this interaction are not considered independent of each other. In the third article, I found that plant inbreeding modified the intensity of inbreeding depression in herbivores and vice versa (III). Comparison of the performance of herbivores that fed on outbred and inbred plants showed that inbreeding depression, i.e. the difference in performance between inbred and outbred herbivores, was stronger when the herbivores fed on inbred plants. However, this was the case for only two of the four herbivore populations of the study.

In general, the effects of inbreeding are to a large extent determined by the history of inbreeding in the population: in large populations one generation of inbreeding may not yet have a severe negative effect, or, alternatively, small populations with a history of inbreeding may have purged the deleterious alleles that cause inbreeding depression (Husband & Schemske 1996; Charlesworth & Charlesworth 1999; Crnokrak & Barrett 2002; Keller & Waller 2002). The differences among herbivore populations in the extent to which plant inbreeding modifies inbreeding depression may also partly stem from differences in their sympatric plant populations: if herbivores are adapted to their sympatric plant populations, and if the traits that are important for the interaction are modified differently by inbreeding in the different plant populations, this may lead to the variation observed. Alternatively, the explanation could lie in the variable effects of inbreeding on phenotypic plasticity among populations. Inbred individuals of snails exhibited lower phenotypic plasticity (Auld & Relyea 2010); inbreeding has also resulted in reduced adaptive plasticity in plants (Campbell *et al.* 2014). Phenotypic plasticity could have allowed the outbred herbivores from some populations to adjust and increase their performance on high-quality inbred plants, but this adjustment may have been restricted in the inbred herbivore, leading to stronger inbreeding depression.

Herbivore inbreeding reduced inbreeding depression in plant resistance. The effect was thus opposite to that found in the herbivores, nor did it vary between populations. Plants displayed considerable inbreeding depression in resistance when the herbivores feeding on them were outbred, but with inbred herbivores inbreeding depression was not significant. Inbreeding depression in plants has been reported to increase in the presence of herbivores for several study systems (Carr & Eubanks 2002; Hayes *et al.* 2004; Ivey *et al.* 2004; Campbell *et al.* 2013; Carr & Eubanks 2014), making herbivores important in promoting plant outcrossing (Ashman 2002; Steets *et al.* 2006; Carr & Eubanks 2014). My results suggest that the intensity of herbivore-induced selection for outcrossing in plants can decrease if the herbivores are inbred. Finally, the strength of inbreeding depression varied between plant populations, as well as – more importantly – between different combinations of plant and herbivore origin.

3.4 Effects of inbreeding on plant defence and herbivore counter-defence

In order to understand the reasons for the observed interactive effects of plant and herbivore inbreeding on inbreeding depression in plant resistance and herbivore performance, I investigated the effects of inbreeding on plant defences and herbivore counter-defences directly by determining phenolic compounds in the leaves (III, IV) and in the herbivore frass (IV). I found that the concentrations of phenolic compounds in the leaves of *V. hirundinaria* were modified by inbreeding of the plant (III). I quantified the water-soluble parts of the plant secondary metabolites in the leaves, which included three different types of phenolic compounds: quercetin and kaempferol glycosides (both belonging to the flavonoids) and caffeoyl quinic acids. A total of 25 different compounds were detected from the leaves and frass combined. Inbred plants had lower concentrations of seven compounds, and higher concentrations of one of them. These reductions following inbreeding were more subtle than those observed in phenolic compounds due to inbreeding in *Solanum carolinense* (Campbell *et al.* 2013), but were nevertheless significant. In *S. carolinense* many of the compounds were absent in the inbred plants (Campbell *et al.* 2013), whereas in *V. hirundinaria* the changes were purely quantitative. There was also significant variation in the concentrations of many of the compounds, as well as in the effect of inbreeding on the concentrations of the compounds among plant populations. These results also imply that the effects of inbreeding are highly dependent on the genetic background of the population and on the population history of inbreeding.

In the fourth article, I showed that the phenolic compounds found in the leaves of *V. hirundinaria* were modified during the passage through the digestive tract of *A. asclepiadis*. When the leaf mass passed through the larval intestine, the compound concentration was drastically reduced (IV). The most notable modifications occurred in caffeoyl quinic acids, which were isomerised with two novel compounds appearing in the frass. Chlorogenic acid (one of the caffeoyl quinic acid isomers) can have pro-oxidant properties when it is introduced into the alkaline environment of the Lepidopteran gut (Appel 1993; Salminen & Karonen 2011). High reductions in these compounds may suggest that phenolic oxidation has taken place in the gut, which also translates to high oxidative stress for the insect. Inbred insects can have lowered tolerance for oxidative stress (Okada *et al.* 2011), which could partly explain the slower growth of inbred herbivores compared to outbred ones (III). The flavonoids (quercetin and kaempferol glycosides) also showed reduced concentrations in the larval frass, but less so than caffeoyl quinic acids.

In all compound groups, the greatest part of the variation in modification of compounds in the digestive tract was accounted for by differences in the chemical composition between the two plant populations (IV). Plant and herbivore inbreeding also slightly affected the degree to which caffeoyl quinic acid and total concentrations of phenolics were reduced in the insect gut. Even then, the greater part of the differences observed were accounted for by variation in the compound concentrations in the leaves. A comparison of the quantitative differences between leaf and frass showed that the largest reductions of caffeoyl quinic acids occurred in outbred herbivores when they were fed on inbred plants. This does not correspond to the herbivores with the smallest biomass; it thus appears that causes other

than the modification of phenolics in the digestive tract may better explain inbreeding depression and effects of plant inbreeding in the herbivore. It is possible that the observed differences in herbivore performance are rather due to differences in the ways inbred and outbred herbivores cope with the oxidative stress caused by their food plant or to reduced feeding.

3.5 Effects of inbreeding on herbivore oviposition preference and tri-trophic interactions

Finally, the magnitude of inbreeding depression can vary among traits or life-history stages (Keller & Waller 2002; Angeloni *et al.* 2011), and can be further modified by the presence of the herbivores' enemies (Kariyat *et al.* 2012a). To gain a more thorough understanding of the possible effects of inbreeding on populations in nature, I investigated how inbreeding modifies herbivore preference and egg survival in the field. I found that adult *A. asclepiadis* deposited more eggs on outbred *V. hirundinaria* plants (V), which is a suboptimal choice, given that the larvae grow better on inbred plants (III). This is not highly unusual behaviour; ovipositing on sites that do not maximise the performance of the offspring has been previously reported for other Lepidopteran species (Rausher 1979; Underwood 1994; Gripenberg *et al.* 2007). It is possible that outbred *V. hirundinaria* plants are more vigorous than inbred ones, and that herbivores therefore perceive them as more suitable for egg deposition (Hull-Sanders & Eubanks 2005). In addition, chemical cues emitted by the plants may be important for host recognition (Simmonds 2001; Hilker & Meiners 2011; Knolhoff & Heckel 2014). Outbred plants are likely to be chemically more apparent due to their higher concentrations of some of the phenolic compounds (III), which may make them more attractive for herbivores in comparison to inbred plants. High apparency and vigour have worked against outbred plants in other plant-herbivore systems as well, leading to higher infestation rates by herbivores and pathogens in *Cucurbita pepo* ssp. *texana* and *Datura stramonium* (Ferrari *et al.* 2007; Bello-Bedoy *et al.* 2011).

When the plants with herbivore eggs under their leaves were in the field, only 8 % of them produced viable larvae. The rest of the eggs were either sterile or were lost to parasitism, predation, or due to plant hypersensitive responses (V). Inbred eggs were more likely to be sterile than outbred ones. However, since outbred eggs were more likely to be lost and detached than inbred ones (due either to plant hypersensitive responses or to predation), the number of eggs that in the end produced a larva did not differ between the inbred and outbred treatments. These results suggest that natural enemies, together with plant hypersensitive responses, may reduce inbreeding depression in herbivore fecundity. Contrary to my expectations and to the results of previous studies with *Solanum carolinense* (Kariyat *et al.* 2012a), inbreeding of the host plant did not affect the rate of parasitism and predation on herbivore eggs. It is possible that plant volatiles in this plant are not altered by egg deposition, as induction following egg deposition does not occur ubiquitously in plants (Fatouros *et al.* 2005; Hilker & Meiners 2011). Furthermore, if parasitoids and predators use other cues to find suitable eggs, such as the scales or

sex pheromones left behind by adults, or the adult moths themselves (Lewis *et al.* 1972; Noldus *et al.* 1991; Fatouros *et al.* 2008), plant inbreeding would not be expected to affect the rate of egg parasitism.

TABLE 4. Effects of inbreeding on different traits in the plant *V. hirundinaria* and its herbivore *A. asclepiadis*. Signs indicate benefit (+) or disadvantage (-) of inbreeding or outbreeding in the plant or herbivore relative to the other cross in terms of benefit for the plant/herbivore in which the trait was measured. In some cases the benefit was only observed in some populations or in interaction with either inbred or outbred plant/herbivore.

	Traits	Herbivore		Plant	
		Outbred	Inbred	Outbred	Inbred
Herbivore	Larval biomass	+	-	-	+
	Egg survival	-	+	ns.	ns.
Plant	Resistance to damage	-	+	+	-
	Herbivore preference			-	+
	Indirect defence against herbivore eggs	+	-	ns.	ns.

4. CONCLUSIONS & FUTURE DIRECTIONS

My results show that both the host plant *V. hirundinaria* and its herbivore *A. asclepiadis* exert reciprocal selection on one another, as indicated by their mutual local adaptation (**I, II**). The results demonstrate that this plant-herbivore interaction functions according to the predictions of the geographic mosaic theory of coevolution: there is variation in coevolutionary outcomes in space ($G \times G \times E$), leading to local adaptation that varies in strength among populations (Thompson 1994, 2005). Some of the evidence suggests that in the case of the herbivores it is not a shortage of genetic variation but rather an excess of novel, redundant or even disadvantageous genetic variation, provided by gene flow, that can confound local adaptation. For *V. hirundinaria*, local adaptation seems to be more likely in populations that have more genetic variation. Interestingly, at those sites where *V. hirundinaria* was locally adapted the herbivore was maladapted and vice versa. Taken together, the evidence for the mutual local adaptation of these species suggests that they are exerting reciprocal selection and are thus coevolving.

These results add to our understanding of plant-herbivore interactions as well as of host-enemy interactions in general, by corroborating that local adaptation of even long-lived hosts to their short-lived enemies is possible. In accordance with my studies (**I, II**), experimental coevolutionary studies on bacteria and their phages as well as meta-analysis of local adaptation studies have revealed that the higher evolutionary potential of species with shorter generation times does not necessarily lead to their local adaptation (Morgan & Buckling 2006; Greischar & Koskella 2007; Hoeksema & Forde 2008). In fact, gene flow and genetic variation are more important determinants of local adaptation in species interactions in general than the relative length of the life-span (Greischar & Koskella 2007; Hoeksema & Forde 2008).

As highlighted by studies on local adaptation, genetic variation is essential for adaptation in general, as well as specifically in traits that confer adaptation in species interactions. In small and isolated populations, the ability to adapt can also erode the following inbreeding. The negative effects of inbreeding can be intensified through species interactions: previous studies have demonstrated that for plants inbreeding depression is stronger when herbivores are present (reviewed in Carr & Eubanks 2014). Here I present the first evidence that the negative effects of herbivores on inbreeding depression in plants also depend on whether or not the herbivore too is inbred (**III**). The evolution of the plant mating systems is predicted to favour self-fertilization, especially at range margins and in novel areas where herbivore

pressure is relaxed (Baker 1955; Kéry *et al.* 2001; Torchin *et al.* 2003). Furthermore, at range margins the populations of co-occurring specialist herbivores are likely to at least start out small (Valladares *et al.* 2006), and hence also be inbred. The inbreeding depression in plant resistance caused by inbred herbivores was less severe than that due to outbred ones; thus herbivore inbreeding may contribute to the fact that self-fertilization is more frequently observed in plants at their range margins (e.g. Pujol *et al.* 2009; Campbell & Kessler 2013). Finally, since in general herbivores occur more abundantly in plant populations that are large (Valladares *et al.* 2006), the negative effect of herbivores on plants and plant inbreeding is likely to be stronger in core areas compared to the margins of the plant species range.

Far-reaching conclusions concerning the evolution of the plant mating systems need to be drawn with caution, as the direction (negative or positive) of the effect of inbreeding varied depending on which plant trait was measured (Table 4). Even though inbred plants retained higher leaf damage, and the larvae fare better on them (III), adult herbivores preferred to oviposit on outbred plants (V). Inbred plants may thus escape damage more effectively than outbred ones, which would result in lower inbreeding depression. However, this effect can be species dependent: *Manduca sexta* moths preferred to oviposit on inbred *Solanum carolinense* host plants (Kariyat *et al.* 2013b). Similarly to the plant, inbreeding in the herbivores led to diminished larval performance, whereas in the eggs the negative effects of inbreeding were levelled off by the enemies of the herbivore (V). To be able to explicitly evaluate the consequences of inbreeding in general, for both plants and herbivores, we need to take into account the full life cycle of the herbivore as well as several different plant traits when feasible.

Finally, how does inbreeding affect coevolutionary interactions? Although I did not measure selection or plant or herbivore fitness directly, I will here offer some predictions based on my results. For herbivores and plants alike, an inbred enemy/host may cause weaker selection than an outbred one. In herbivores this is due to the relaxation of plant defences, while in plants it is due to a weaker herbivore performance, leading to lesser damage (III). Weak selection due to an inbred host/enemy, or on the other hand a lower ability to respond to selection due to reduced genetic variation under inbreeding, can weaken the link in the $G \times G$ interactions, decelerate the arms race and slow down coevolution. Evidence from snails and their pathogens points in the same direction: host populations with a higher rate of self-fertile individuals were less likely to locally adapt (Morran *et al.* 2014), although a meta-analysis of the effect of the plant mating system on local adaptation yielded non-significant results (Hereford 2010). However, it is important to keep in mind that in nature the effects of host or enemy inbreeding may be further modified by the natural enemies of herbivores, the potential additional contributors to the E in the $G \times G \times E$ interactions (V).

My thesis may not unambiguously answer the question of how coevolution alters under inbreeding, but it provides an important opening for future investigation of the factors that can limit the adaptive potential in coevolving interactions. In general, this thesis highlights the necessity to take more than one trophic level into account in estimating the negative effects of declining habitat area, population size and increased inbreeding on a particular species. We should adopt a more holistic approach, considering how species interactions and coevolution are affected by these processes. This could take the form for example, of comparing populations that vary in their level of inbreeding, population size, or proportion

of self-fertile individuals. Such an approach would allow direct observation of what happens to interactions in a natural setting, with variable levels of inbreeding or self-fertilization, and whether coevolutionary interactions are strengthened or weakened by inbreeding. More solid evidence for the effect of inbreeding on plant-herbivore coevolution could also be obtained using experimental plant populations with variable levels of genetic variation or self-fertile individuals and infested with herbivores at different densities. Ideally these populations would be studied over multiple generations in order to document potential changes in genotype frequencies and the effect of herbivory on these changes. Through widespread field studies, combined with rigorous, manipulative experimental work that tests the questions arising from theoretical assumptions, we can expand our understanding of the impact of habitat loss and fragmentation on species interactions and coevolution.

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