

# Variation and constraints of local adaptation of a long-lived plant, its pollinators and specialist herbivores

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## Summary

1. Due to geographically variable species interactions, plants may become locally adapted to their sympatric herbivores and pollinators. However, adaptation to the abiotic environment may significantly affect plant interactions with herbivores and pollinators. Local adaptation to the abiotic environment may constrain local adaptation to herbivores and pollinators under contrasting selection pressures, resulting in trade-offs in local adaptation.

2. We studied local adaptation of a perennial herb, *Vincetoxicum hirundinaria*, in a reciprocal transplant experiment among four populations and measured plant fitness, pollination success and resistance to two specialist herbivores. We also estimated local adaptation of these two herbivores and generalist pollinators to their sympatric plant populations. Local adaptation was compared with within-population genetic variation, genetic and geographical divergence, and with divergence in terms of population size, environmental conditions and plant secondary chemistry. We further compared local adaptation to the environment, to local adaptation to herbivores and pollinators to detect possible trade-offs in local adaptation.

3. The existence and degree of local adaptation varied among the plant populations. Plants from two populations were locally adapted to their sympatric leaf herbivores and plants from two populations were locally adapted to their abiotic environment. Herbivores from one population were locally adapted to their sympatric plant population. Local adaptation of *V. hirundinaria* to the seed predator increased with increasing among-population divergence in precipitation and temperature. Local adaptation to the seed predator and the environment increased with increasing population genetic variation. Local adaptation of *V. hirundinaria* in fitness and in herbivore resistance also correlated positively, suggesting lack of trade-offs in local adaptation.

4. *Synthesis.* These results demonstrate that species interactions can lead to a mosaic of locally adapted plant, herbivore and pollinator populations. In addition to natural enemies, genetic variation, the abiotic environment and mutualistic interactions contribute to the evolution of local adaptation in long-lived plants. These results provide new insights into the patterns and causes of variation in local adaptation and are among the first to demonstrate that conflicting selection pressures within a population do not constrain local adaptation in multiple traits.

**Key-words:** *Abrostola asclepiadis*, co-evolution, *Euphranta connexa*, genetic variation, plant-herbivore interactions, pollinators, reciprocal transplant experiment, resistance, trade-offs, *Vincetoxicum hirundinaria*

## Introduction

Spatially variable selection due to spatial heterogeneity in biotic and abiotic conditions may result in populations that diverge by adapting to their local environments (Williams

1966). Selection can favour different traits in each population of a species depending on local conditions regardless of the fitness consequences or adaptive value of these traits in other populations of the species. This may lead to local adaptation that is expressed as a higher fitness of individuals at their home site compared to another site inhabited by the same species (Kawecki & Ebert 2004). In a co-evolving species

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interaction, reciprocal selection may lead to local adaptation of the interacting species to one another (Thompson 2005). Because of the dynamic nature of the co-evolving interaction, local adaptation may occur in one or both of the interacting species. In a co-evolving antagonistic species pair (e.g. plants and their specialist insect herbivores), the species with shorter generation time is generally expected to be locally adapted due to its higher evolutionary potential (Dawkins & Krebs 1979; Gandon & Michalakakis 2002; Núñez-Farfán, Fornoni & Valverde 2007). In long-lived plants, it is likely that the abiotic environment causes more consistent selection pressure than antagonistic biotic interactions (Linhart & Grant 1996). Therefore, local adaptation of plants to their abiotic environment is usually stronger and more likely to occur than local adaptation to natural enemies (Linhart & Grant 1996; Bischoff & Trémulot 2011). The ability of plants to resist natural enemies may be limited by unfavourable abiotic environmental conditions, such as soil nutrient composition (Springer, Hardcastle & Gilbert 2007). Consequently, local adaptation of plants to their sympatric herbivores may be limited by local adaptation of the plants to their physical environment. Contrasting selection pressures from the physical environment and herbivores may therefore result in evolutionary constraints for local adaptation, which may prevent the otherwise never-ending arms race co-evolution (Thompson 1986; Brodie & Brodie 1999). Currently, we lack studies that look at plant local adaptation to physical environment and the herbivores at the same time.

Due to multiple or conflicting selection pressures, a population may become locally adapted in one trait but not in others. In other words, we suggest that local adaptation observed in one trait may occur at the expense of local adaptation in other traits. For example, if selection favours high herbivore resistance, a plant population can become locally adapted in terms of herbivore resistance. However, negative correlations of herbivore resistance with other fitness traits, such as pollinator attraction or reproductive success (e.g. Herms & Mattson 1992; Strauss *et al.* 1999; Koricheva 2002), may then constrain increase and local adaptation in these traits, even if other factors would select for them. Such trade-offs in local adaptation could arise due to resource limitation, allocation costs, or ecological and genetic constraints. The existence of trade-offs in local adaptation can be tested by comparing among-population pairwise local adaptation values measured in different traits, such as fitness and resistance traits. Negative correlation between local adaptation in different traits would indicate a trade-off in local adaptation.

Various factors are expected to affect the occurrence and strength of local adaptation (Linhart & Grant 1996; Galloway & Fenster 2000; Lenormand 2002; Hoeksema & Forde 2008; Leimu & Fischer 2008; Hereford 2009). Geographical distance among populations is commonly assumed to affect local adaptation, because gene flow decreases and environments become more differentiated with increasing distance (Galloway & Fenster 2000). Therefore, populations further apart are more likely to have diverged from one another compared to adjacent populations (Hanks & Denno 1994;

Galloway & Fenster 2000; Becker *et al.* 2006; Cogni & Futuyma 2009). Likewise, the magnitude of local adaptation will increase with greater environmental divergence between populations (Lande 1976; Hereford 2009). Gene flow is not only acting against local adaptation (Slatkin 1987), because moderate levels of migration and gene flow can promote local adaptation by introducing new genetic variation to populations, which can then become subject to natural selection (Gandon *et al.* 1996; Holt & Gomulkiewicz 1997; Lenormand 2002; Hoeksema & Forde 2008). Introduction of new genetic material via gene flow can be especially important for small populations (Stockwell, Hendry & Kinnison 2003) since their ability to locally adapt can be limited by lack of genetic variation (Linhart & Grant 1996; Stockwell, Hendry & Kinnison 2003; Leimu & Fischer 2008). In co-evolving species pairs, the species with relatively higher gene flow is expected to be locally adapted (Gandon *et al.* 1996; Gandon & Michalakakis 2002; Hoeksema & Forde 2008).

We studied the local adaptation of the perennial herb *Vincetoxicum hirundinaria* Medik. (= *Cynanchum vincetoxicum* (L.) Pers.) (Apocynaceae, formerly Asclepiadaceae) to the environment, to two specialist herbivores and generalist pollinators in a reciprocal transplant experiment in the southwestern archipelago of Finland. In here, 'local adaptation to the environment' refers to the combined effects of the abiotic (physical, chemical) and biotic aspects of the environment. We measured local adaptation to the environment in terms of the reproductive output of the plants. Local adaptation of the plants to the herbivores and pollinators was measured separately in terms of herbivore damage and pollen removal, respectively. We also estimated local adaptation of the two herbivores and the generalist pollinators to their sympatric plant populations. The two specialist herbivores included in this study are the folivorous larvae of the moth *Abrostola asclepiadis* (Lepidoptera, Noctuidae) Denis & Schiffermüller and the larvae of the seed predator fly *Euphranta connexa* (Diptera, Tephritidae) Fabricius. *Euphranta connexa* has a stronger negative effect on plant fitness and population growth than *A. asclepiadis*, although they both can have significant negative effects on population growth of *V. hirundinaria* (Leimu & Lehtilä 2006). In general, natural enemies that impose strong selection on their host species are more likely to be locally adapted than those imposing weaker selection on their host species (Lively 1999). Because *E. connexa* is likely to exert stronger selection on the plants than *A. asclepiadis*, it is more likely to become locally adapted to *V. hirundinaria* than *A. asclepiadis*. Local adaptation of plants to their insect herbivores has been studied to some extent (e.g. Sork, Stowe & Hochwender 1993; Crémieux *et al.* 2008; Ortigón-Campos *et al.* 2009; Bischoff & Trémulot 2011), but studies that look at local adaptation of both the plant and the herbivore simultaneously are scarce (but see Garrido, Andraca-Gómez & Fornoni 2012). We are also lacking studies that intent to quantify local adaptation to multiple selective agents, such as herbivores, pollinators and abiotic environment separately. In our study area, populations of *V. hirundinaria* form a naturally fragmented mosaic that

serves as an excellent setting for studying local adaptation. Herbivore pressure imposed by the specialist herbivores on *V. hirundinaria* varies spatially and temporally (Leimu & Syrjänen 2002; Leimu & Lehtilä 2006; Muola *et al.* 2010). In plant-herbivore co-evolution, herbivore local adaptation can be driven by variation in plant secondary chemistry (Ehrlich & Raven 1964; Berenbaum & Zangerl 1997, 2006). Accordingly, variation and associations of leaf chemistry, plant fitness and herbivore resistance suggest co-evolutionary dynamics between the host plant and the herbivores in our study system (Muola *et al.* 2010).

We addressed the following specific questions and hypotheses:

1 Are *V. hirundinaria* populations locally adapted to their sympatric herbivores, pollinators and the physical environment? Is there among-population variation in local adaptation?

2 Are the specialist herbivores and generalist pollinators locally adapted to their sympatric *V. hirundinaria* plants? We expect the seed predator to show local adaptation more likely than the leaf herbivore because of its higher negative effects on host plant fitness.

3 Are populations with higher level of plant genetic variation ( $H_e$ ) more likely to display local adaptation? Is the among-population variation in plant local adaptation related to the geographical distance among populations, genetic differentiation ( $F_{ST}$ ) of the plants or divergence among the populations in terms of precipitation, temperature, population size or plant secondary chemistry?

4 Is local adaptation of the plants to their environment negatively related to local adaptation to herbivores, that is, are there trade-offs between local adaptations in different traits?

## Materials and methods

### STUDY SPECIES

*Vincetoxicum hirundinaria* is a long-lived perennial herb. It is native to Europe and western Asia and occurs as a non-native species in North America (Sheeley & Raynal 1996; Hämet-Ahti *et al.* 1998). In Finland, its distribution is limited to the coastal areas of the south-western mainland and to the south-western archipelago (Hämet-Ahti *et al.* 1998). *Vincetoxicum hirundinaria* grows on sunny exposed rocky slopes and cliffs and along forest margins (Hämet-Ahti *et al.* 1998). It prefers calcareous substrate but despite of being a habitat specialist, it is not rare or endangered. Each plant has a short, branched rootstock and a dense tussock with above-ground shoots. The shoots of a given plant individual flower more or less synchronously. In our study area, that is, the SW archipelago of Finland, flowering usually starts in the middle of June and lasts until the end of July. The flowers are perfect (i.e. cosexual) and arranged in inflorescences that grow from the leaf nodes of the shoots. Pollen is contained in pollen sacs (pollinia) that are arranged in five pairs (pollinarium) in each flower (Leimu & Syrjänen 2002). *Vincetoxicum hirundinaria* is specialized to insect pollination, and large flies, moths, and bees are believed to be its main pollinators (Leimu 2004; Ågren, Ehrlén & Solbreck 2008). *Vincetoxicum hirundinaria* has a mixed mating system with varying levels of self-fertilization and outcrossing (Leimu 2004). Seeds are wind dispersed.

In Finland, *V. hirundinaria* has three specialized herbivores: the folivorous moth *A. asclepiadis* and two seed predators, *E. connexa* and *Lygaeus equestris* (Heteroptera, Lygaeidae). *Abrostola asclepiadis* lays its eggs on the leaves of *V. hirundinaria* in July and, once hatched, the larvae consume the leaves (Förare 1995). The damage caused by the larvae has significant negative effects on population growth even though it usually does not completely decimate the reproductive success of individual plants (Leimu & Lehtilä 2006). *Euphranta connexa* lays its eggs into the developing pods where the larvae consume the soft developing seeds (Solbreck & Sillén-Tullberg 1986). In some years, seed predation by *E. connexa* results in the loss of all seed production in some populations (Solbreck & Sillén-Tullberg 1986) and, therefore, *E. connexa* may have a stronger negative effect on plant fitness and population growth of *V. hirundinaria* than *A. asclepiadis* (Leimu & Lehtilä 2006). Both *E. connexa* and *A. asclepiadis* are present in all of the populations included in this study. The bug *L. equestris* is an oligophagous pre- and post-dispersal seed predator and it is present and abundant in three of the studied populations, lacking in Ruissalo. *Vincetoxicum hirundinaria* contains high levels of alkaloids and other secondary compounds (Stærk *et al.* 2000; Muola *et al.* 2010) and is considered to be highly poisonous (Solbreck & Sillén-Tullberg 1990; Hämet-Ahti *et al.* 1998). Damage by vertebrates or other generalist herbivores is thought to be minimal (Leimu, Riipi & Stærk 2005).

### STUDY POPULATIONS

Our study area, the SW archipelago of Finland, forms a naturally fragmented landscape in which the islands and populations on the islands are physically isolated to a variable degree. The populations have been established relatively recently, due to the late formation of the archipelago as a result of land uplift after the last ice age (Ekman 1996). Despite the fragmented distribution, the level of within-population genetic variation is relatively high and there is some gene flow among the populations, which is most likely due to the characteristics of pollen and seed dispersal of *V. hirundinaria* (see above; Leimu & Mutikainen 2005).

We selected four populations of *V. hirundinaria* for the experiment. These populations represent a geographical gradient from the proximity of mainland to the outer archipelago. The populations are situated on the islands of Ruissalo (67° 02' 57" N, 15° 63' 73" E), Lammasluoto (66° 80' 66" N, 15° 52' 62" E), Ånskar (66° 76' 06" N, 15° 38' 90" E) and Utö (66° 29' 54" N, 15° 21' 28" E). The Ruissalo Island is adjacent to the mainland, and the other three islands are in the central or outer archipelago. The among-population distances range from 14 to 85 km (Fig. 1). The study populations vary in size (number of plants), and in biotic and abiotic conditions. The Ruissalo population is the smallest one with *c.* 100 individuals, and Lammasluoto and Ånskar are relatively large with *c.* 5000 and 2500 individuals, respectively, and Utö is the largest population with *c.* 10 000 individuals (Leimu & Syrjänen 2002; Leimu & Mutikainen 2005; Muola *et al.* 2010). The populations are genetically differentiated (Leimu & Mutikainen 2005): the reported pairwise  $F_{ST}$  values for the population pairs are -0.046 between Ruissalo and Ånskar, 0.076 between Ruissalo and Lammasluoto, and 0.0995 between Lammasluoto and Ånskar. Genetic analyses have not been conducted for the Utö population. The biotic and abiotic conditions in the study populations vary spatially and temporally (Leimu & Lehtilä 2006; Muola *et al.* 2010); here, we concentrated on the intensity of herbivory by the two specialist herbivores, pollination, precipitation and temperature.



**Fig. 1.** Map of the study area showing *Vincetoxicum hirundinaria* populations included in the reciprocal transplant experiment. Ruissalo (i) is situated adjacent to the mainland, Lammasluoto (ii) and Ånskär (iii) are in the central, and Utö (iv) is in the outer archipelago. Grey areas denote land.

#### EXPERIMENTAL DESIGN AND DATA COLLECTION

The experiment was established in June 2008. We randomly chose 20 large plant individuals from each of the four populations for the reciprocal transplant experiment. These plants were dug up with as complete rootstock as possible. Each rootstock was then divided in four in order to get four genetically identical replicates of each plant individual. The plants were then potted (pot size 7.5 L) with standard potting soil. One randomly chosen replicate of each genotype was assigned to each of the four transplant sites. Thus, we had four genetically identical experimental populations with 80 plants in each to be transplanted in their pots to the four study sites. The total number of plants in the beginning of the experiment was 320 (four populations  $\times$  20 genotypes  $\times$  four replicates/genotype). During the experiment, we lost altogether seven plants and, therefore, had 80, 79, 77 and 77 plants in the four populations (313 altogether). The pots were transported to the transplant sites and distributed haphazardly among the *V. hirundinaria* individuals growing naturally on the sites. In each study site, the areas in which the plants were placed were c. 50 by 50 m. *Vincetoxicum hirundinaria* is abundant at all sites, and all the experimental plants were placed among individuals in the natural populations. The nearest natural plant to each experimental plant was 1–2 m away. To reduce the effects of spatial autocorrelation, two plants from the same home site were never placed close to one another. The experiment lasted for 16 months, until the fall 2009. The plants were watered during the dry periods in both summers (2008 and 2009). *Vincetoxicum hirundinaria* can survive well in dry conditions, but watering was necessary, because the soil in the pots dried more easily than the natural soil.

We collected the data in 2009, the year following the establishment of the experimental populations. We visited all study sites multiple

times during the growing season from the end of April–October. We quantified the level of leaf herbivory by the larvae of *A. asclepiadis* from all experimental plants by counting the number of damaged leaves. We also counted the number of undamaged leaves and calculated the proportion of leaves with any observed damage. The proportion of damaged leaves has been used previously as a measure of herbivore damage (e.g. Agrawal 2005; Muola *et al.* 2010). To investigate predispersal seed predation by the larvae of *E. connexa*, we collected and opened all pods and recorded the proportion of pods with seed predation. These measures of damage can be used as inverse measures of plants resistance against these specialist herbivores. Highly resistant plants sustain low damage, whereas plants with lower resistance against herbivores suffer from higher damage. We did not include the damage caused by *L. equestris* in this study, because its predation leaves no apparent evidence on the plant and thus it is difficult to reliably observe and quantify in the field.

We estimated plant fecundity by counting the number of inflorescences in the whole plant and the number of inflorescences and flowers in one randomly chosen average-sized stem per plant. The sampled stem contained on average 25% (SE 1.3%) of all inflorescences of a plant. To study male fitness, we recorded the proportion of pollinia removed for all of the flowers of the same average-sized stem of each plant. This measure can also be used for estimating pollination success and pollinator preference. In *V. hirundinaria*, the corpuscula of pollinia are visible in the flower, and the presence or absence of pollinia can be inspected without a microscope. We extrapolated the proportion of pollinia removed from the studied stem to the whole plant. We estimated female reproductive success by counting the number of initiating pods, matured intact pods, and aborted pods. Matured intact pods refer to those full-sized pods that were not attacked by the seed predator *E. connexa* and were thus contributing to the realized reproductive output of the plant. We collected the pods as they ripened and opened them later in the laboratory and counted the number of seeds in the pods.

#### ENVIRONMENTAL VARIABLES

Of the predominant environmental characteristics, we included precipitation and temperature in this study. These environmental factors are likely to impose selection on the *V. hirundinaria* populations in our study area. Firstly, the sites where *V. hirundinaria* occurs usually have only a thin layer of soil and little shade, which makes the plants prone to drought. Secondly, shortage of water is also known to limit fruit set of *V. hirundinaria* (Ågren, Ehrlén & Solbreck 2008). Information on the accumulation of rainfall and average daily temperature was obtained from the records of the meteorological stations run by the Finnish Meteorological Institute nearest to each study population. Lammasluoto and Ånskär share the same meteorological information, because they have the same nearest meteorological station. From the meteorological data, we calculated temperature and precipitation summations for the whole growing season, that is, from the end of April (daily mean temperature above 5 °C for five successive days) to October (daily mean temperature below 5 °C for five successive days). These data were used later for calculating the Euclidean distances between pairs of populations in precipitation and temperature.

#### DATA ANALYSIS

We tested for local adaptation of the plants to their sympatric herbivores, to pollinators and to the environment by comparing plant resistance, pollinia removal and fitness traits between plants in their native

and non-native sites (i.e. 'home versus away' comparison, Kawecki & Ebert 2004). We first studied local adaptation by comparing the performance of plants at their 'home' site versus their performance at the 'away' sites using the analysis of variance (ANOVA) including home site, sympatry/allopatry, and their interaction as fixed factors (Proc Mixed in SAS). In the sympatry/allopatry factor, plants transplanted at their home site were considered 'sympatric' and those transplanted at other sites were considered 'allopatric'. Local adaptation would be indicated by higher performance of plants at their home sites than at the away sites (i.e. a statistically significant main effect of the sympatry/allopatry factor). Among-population variation in local adaptation would be indicated by a significant interaction between the home site and the sympatry/allopatry factor. Due to the significant interaction suggesting among-population variation in local adaptation (see Results), we further examined local adaptation using a two-way ANOVA with the home site, transplant site and their interaction as factors. Using contrasts, we compared the trait values of each population at their home site to trait values in all three away sites combined. Furthermore, we compared the trait values of each population at their home site to the trait values at each away site (i.e. pairwise comparisons). The contrasts were calculated for those traits that fulfilled the criteria of local adaptation in the first analysis; that is, a statistically significant main effect of sympatry/allopatry factor or a significant interaction between home site and the sympatry/allopatry factor (see Results, Table 1).

We used the proportion of leaves damaged by *A. asclepiadis*, proportion of pollinia removed, number of all pods, number of mature intact pods and number of seeds per pod as response variables. We included two different measures describing reproductive success as number of pods in the analysis: the total number of pods describes the reproductive effort, whereas the number of mature intact pods better describes the realized reproductive output excluding aborted and damaged pods and, thus, it also includes a rough estimate of resistance to seed predation. We did not calculate the contrasts for the number of seeds per pod, because it did not fulfil the criteria for local adaptation by the first analysis (Table 1). Before the analysis, we tested for the assumptions of normality and homogeneity of variances by visual examination of the residuals and by Levene's test, respectively. We applied arcsine transformation to the proportion of pollinia removed and proportion of leaves damaged, and square root transformation to the number of pods to meet the assumptions of the parametric model. The assumption for homogeneity of variances was not met for the proportion of leaves damaged by *A. asclepiadis*,

proportion of pollinia removed, number of all pods and number of mature intact pods. For these variables, the heteroscedasticity was accounted for in the model by using the repeated/group option (Littell *et al.* 2006). Because a considerable proportion of the plants was not damaged by *E. connexa* and hence a lot of zeros appeared in the data set causing strong deviations from parametric assumptions, we transformed the seed predation into a binary variable. If there was any seed predation present in any of the pods of a given plant, it got the value 1; in the absence of seed predation, plants were given the value 0. We analysed the binary data with generalized linear models (Proc Genmod in SAS) with the same explanatory variables as in the first analysis. Because of a significant main effect of sympatry/allopatry factor suggesting local adaptation (see Results), we conducted another analysis in generalized linear models using home site, transplant site and their interaction as factors. This allowed us to use contrasts to compare the trait values of each population at their home site to trait values in all three away sites. Furthermore, we compared the trait values of each population at their home site to the trait values at each away site pairwise. Prior to the analysis, we also tested for independency of seed predation and leaf herbivory from one another with a generalized linear model with seed predation as the dependent variable and leaf herbivory as an explanatory variable. The results showed that these factors are not related in any of the populations (Lammasluoto  $\chi^2_1 = 0.23$ ,  $P = 0.635$ , Ruissalo  $\chi^2_1 = 0.65$ ,  $P = 0.419$ , Utö  $\chi^2_1 = 0.15$ ,  $P = 0.699$ , Ånskar  $\chi^2_1 = 0.07$ ,  $P = 0.789$ ) and can thus be considered independently in the analysis. All analyses were performed in SAS 9.2/Enterprise guide 4.3 (SAS Institute Inc., Cary, NC, USA).

We tested for local adaptation of the herbivores and pollinating insects to their sympatric plant populations in terms of plant preference. For each site, we compared leaf damage, seed predation and pollinia removal between the plants transplanted to their home site and plants from the other sites transplanted to that particular site (i.e. sympatric and allopatric plants, respectively, home versus away comparison) with contrasts in the two-way ANOVA with home site, transplant site and their interaction as factors. Local adaptation of the herbivores and pollinators would be demonstrated as higher damage and higher pollinia removal, respectively, in the sympatric compared to the allopatric plants.

We studied if plant populations with higher levels of within-population genetic variation are more locally adapted compared to populations with lower levels of within-population genetic variation and if local adaptation more likely occurs between populations that are more diverged environmentally, chemically, genetically,

**Table 1.** Results ( $F$ -values) of analysis of variance testing for local adaptation of *Vincetoxicum hirundinaria* in resistance to leaf herbivory and in plant fitness measured in pollinator attraction and reproductive output. Local adaptation in resistance to seed predation was tested with a generalized linear model ( $\chi^2$ -values)

Source	Home site		Sympatry		Home site $\times$ sympatry	
	d.f.	$F$	d.f.	$F$	d.f.	$F$
Leaf herbivory	3,82.6	7.18**	1,100	0.03	3,82.6	14.79***
Proportion of pollinia removed	3,59.5	11.47***	1,105	0.97	3,59.5	20.96***
Number of pods/plant	3,77.5	1.43	1,128	0.55	3,77.5	6.22**
Number of mature intact pods/plant	3,89.5	1.58	1,90.2	0.02	3,89.5	6.90**
Number of seeds per pod	3,100	0.33	1,100	0.17	3,100	0.14
	d.f.	$\chi^2$	d.f.	$\chi^2$	d.f.	$\chi^2$
Seed predation	3	6.54	1	5.39*	3	2.97

d.f. = numerator, denominator d.f. for  $F$ -values.

\* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ .

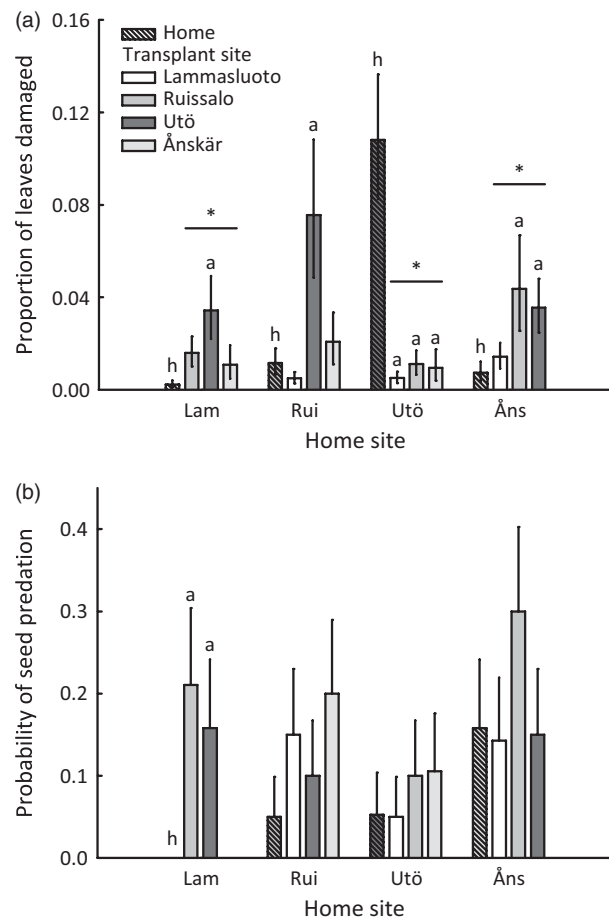
geographically or in terms of population size. We included only those traits that displayed local adaptation in these analyses (see Results, Table 1). We calculated a new variable describing plant resistance to herbivores by subtracting the proportion or probability of damage from value one. These new resistance variables are similar to the fitness variables, that is, the values increase with increasing resistance, which makes comparisons between variables more straightforward. We calculated a value for local adaptation in all the traits for all pairs of populations (1). We divided the mean value of a trait at the home site with the mean value of the trait of plants from the same home site in each transplant site (Hereford 2009). Values above one suggest local adaptation, whereas values below one indicate lack of local adaptation. We then calculated the population mean of all pairwise local adaptation values of a given trait (three pairwise values) for each population (2). We correlated (Spearman rank correlation) the mean local adaptation values (2) with the genetic variation of the population ( $H_e$ ). Utö was not included in this analysis because we have no genetic data from this population. We also correlated (Spearman rank correlation) all the pairwise local adaptation values (1) of different traits with the among-population geographical and genetic distances ( $F_{ST}$  values), with among-population divergence in terms of environment (precipitation and temperature) and in plant secondary leaf chemicals (antofine, chlorogen acid, catechin derivatives, flavonoids and lipophilic compounds), and with the differences in population sizes. Among-population divergence between pairs of populations in terms of environment and plant leaf chemical composition was calculated using Euclidean distance,  $d(p, q) = \sqrt{[\sum (p_i - q_i)^2]}$ , where  $p_i$  stands for the mean value of the trait in  $i$  for population  $p$  and  $q_i$  for the mean value of  $i$  for population  $q$ .  $d_{(p,q)}$  is the distance between populations  $p$  and  $q$  for the trait  $i$ . Data on within-population genetic variation ( $H_e$ ), genetic distances ( $F_{ST}$ ) and plant secondary chemistry were obtained from previous studies (Leimu & Mutikainen 2005; Muola *et al.* 2010).

To test for trade-offs between local adaptation in different traits, we correlated (Spearman rank correlation) the local adaptation values calculated for fitness, pollination and herbivore resistance. In here, local adaptation to the environment includes both the abiotic and biotic environments; our experimental design does not allow us to separate the effects of these two environmental components on fitness. For this analysis, we used the pairwise local adaptation values of traits that displayed local adaptation (pollinia removal, number of mature intact pods, resistance to leaf herbivory and seed predation; see Results). Local adaptation trade-off would be indicated by a negative correlation between local adaptation values in different traits.

## Results

### LOCAL ADAPTATION OF THE PLANTS

We found evidence for among-population variation local adaptation of the plants to both of the specialist herbivores measured in terms of herbivore damage. Local adaptation to leaf damage by *A. asclepiadis* varied among the populations, suggested by the statistically significant interaction between home site and the sympatry/allopatry factor for leaf damage (Table 1, Fig. 2a). Comparisons between the home site and the mean of all away sites for each home site separately revealed that two of the plant populations (Lammasluoto and Ånskärr) were locally adapted to their sympatric leaf herbivore and had the lowest damage at their home sites (contrasts:



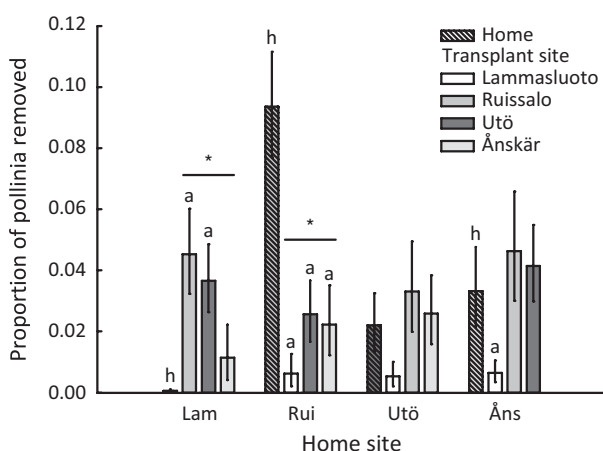
**Fig. 2.** Proportion of leaves damaged by *Abrostola asclepiadis* (a) and probability of seed predation by *Euphranta connexa* (b) in *Vincetoxicum hirundinaria* in the four study sites in a reciprocal transplant experiment. Bars represent the mean ( $\pm$ SE). Standard errors for the probability of seed predation were calculated using equation  $\sqrt{[p(1-p)/n]}$ , where  $p$  is the proportion of plants with damage. One bar cluster represents the plants that originate from the same population. Plants transplanted to their original site ('home') are placed first in each bar cluster and are depicted by striping. Letters 'a' and 'h' indicate a significant pairwise difference between 'home' plants (h) and the transplant site plants (a). An asterisk (\*) and a line indicate a significant contrast testing the difference between the 'home' plants and the mean of all 'away' plants combined. If 'home' plants have the lowest values, the population can be considered locally adapted in terms of resistance to herbivory.

Lammasluoto  $F_{1,296} = 5.75$ ,  $P = 0.0171$ , Ånskärr  $F_{1,296} = 4.99$ ,  $P = 0.0263$ ), whereas Utö plants had the highest leaf damage at their home site ( $F_{1,296} = 39.66$ ,  $P < 0.0001$ ) (Fig. 2a). In general, the plants were locally adapted to their sympatric seed predators, as the probability of seed predation of plants at their home sites was significantly lower ( $0.064 \pm 0.028$ , mean  $\pm$  SE) than at the away sites ( $0.139 \pm 0.023$ ; main effect of sympatry/allopatry in Table 1). However, when the home sites were tested separately, we found evidence for local adaptation to seed predation by *E. connexa* only in the Lammasluoto population: none of the pods were attacked when transplanted at the home site, whereas about 21.1% and 15.8% of the Lammasluoto plants

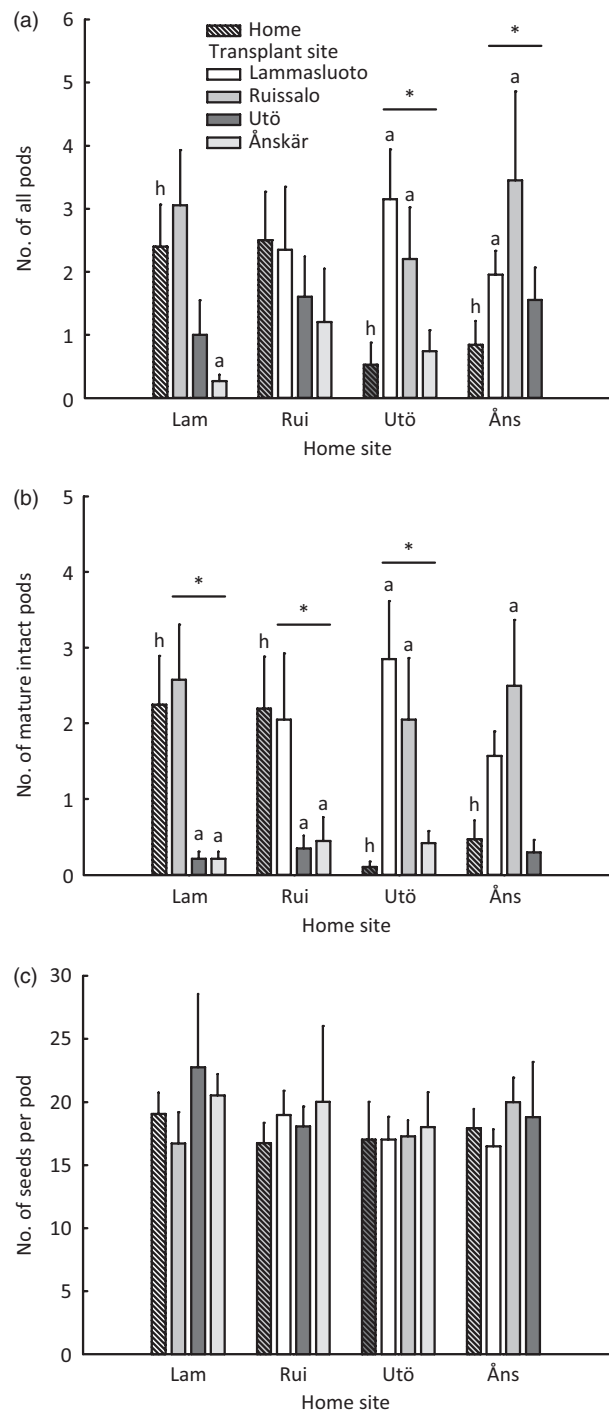


were attacked when transplanted to Ruissalo and Utö, respectively (contrasts: Ruissalo  $\chi^2_1 = 6.24$ ,  $P = 0.0125$ , Utö  $\chi^2_1 = 4.58$ ,  $P = 0.0324$ , Fig. 2b).

We also found among-population variation in local adaptation of *V. hirundinaria* to its physical environment, measured in terms of plant reproductive success. Pollinia removal, number of all pods and number of mature intact pods displayed variation in local adaptation among the populations, suggested by the significant interactions between the home site and the sympatry/allopatriy factor for these traits (Table 1, Figs 3 and 4a,b). The contrasts showed that when grown at their home site, the plants from Ruissalo had the highest proportion of pollinia removed ( $F_{1,245} = 21.19$ ,  $P < 0.0001$ ) (Fig. 3) suggesting that the plants from Ruissalo were locally adapted in terms of pollinator attraction/male fitness. On the other hand, plants from Lammasluoto had the lowest proportion of pollinia removed when grown at their home site ( $F_{1,245} = 16.42$ ,  $P < 0.0001$ ) (Fig. 3). Plants from Lammasluoto and Ruissalo were locally adapted in terms of fitness; they had more mature intact pods at their home sites compared to the away sites combined (Lammasluoto  $F_{1,299} = 4.03$ ,  $P = 0.0455$ , Ruissalo  $F_{1,299} = 4.09$ ,  $P = 0.0441$ ) (Fig. 4b). On the contrary, for Utö and Ånskar, the pod production at home site was lower than that at the transplant sites (number of pods: Utö  $F_{1,299} = 6.22$ ,  $P = 0.0132$ , Ånskar  $F_{1,299} = 4.74$ ,  $P = 0.0303$  and number of mature intact pods: Utö  $F_{1,299} = 6.97$ ,  $P = 0.0087$ ) (Figs 4a and b). The number of seeds per pod did not differ among the home sites or between the sympatric and allopatric transplant sites (Table 1, Fig. 4c).



**Fig. 3.** Proportion of pollinia removed in four sites in a reciprocal transplant experiment of local adaptation of *Vincetoxicum hirundinaria*. Bars represent the mean ( $\pm$ SE). One bar cluster represents the plants that originate from the same population. Plants transplanted to their original site ('home') are placed first in each bar cluster and are depicted by striping. Letters 'a' and 'h' indicate a significant pairwise difference between 'home' plants (h) and the transplant site plants (a). An asterisk (\*) and a line indicate a significant contrast testing the difference between the 'home' plants and the mean of all 'away' plants from the same population combined. If 'home' plants have highest values, the population can be considered locally adapted in terms of male fitness.



**Fig. 4.** Number of pods per plant (a), number of mature intact pods per plant (b) and number of seeds per pod (c) in four sites in a reciprocal transplant experiment of local adaptation of *Vincetoxicum hirundinaria*. Bars represent the mean ( $\pm$ SE). One bar cluster represents the plants that originate from the same population. Plants transplanted to their original site ('home') are placed first in each bar cluster and are depicted by striping. Letters 'a' and 'h' indicate a significant pairwise difference between 'home' plants (h) and the transplant site plants (a). An asterisk (\*) and a line indicate a significant contrast testing the difference between the 'home' plants and the mean of all 'away' plants from the same population combined. If 'home' plants have highest values, the population can be considered locally adapted in terms of that trait.

# LOCAL ADAPTATION OF LEAF HERBIVORES, SEED PREDATORS AND POLLINATORS

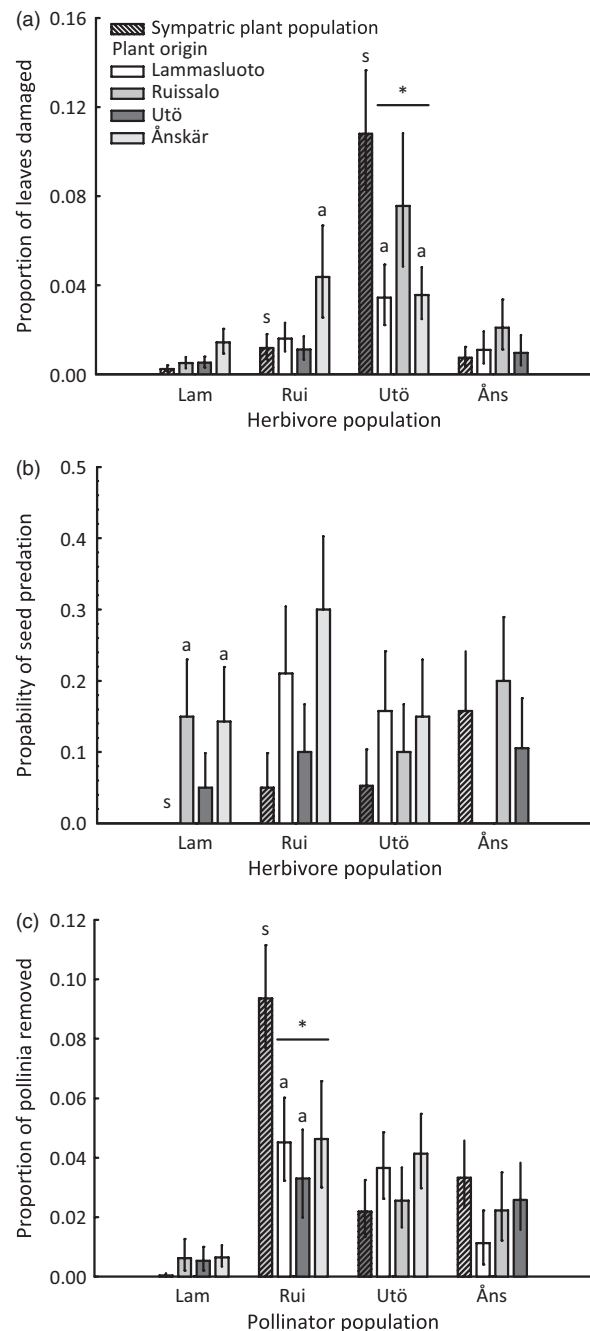
The leaf herbivore showed among-population variation in local adaptation: the interaction between home site and the sympatry/allopatry factor was statistically significant ( $F_{3,82.6} = 14.79$ ,  $P < 0.0001$ ). Results of contrasts show that in Utö, the larvae of the leaf herbivore *A. asclepiadis* caused more damage to their sympatric compared to the allopatric plants which indicates local adaptation of the leaf herbivore to its sympatric plants (contrasts:  $F_{1,296} = 9.07$ ,  $P = 0.0028$ ) (Fig. 5a). The differences in leaf damage between sympatric and allopatric plants were not significant in the other herbivore populations ( $P > 0.05$ ; Fig. 5a).

On average, the seed predator *E. connexa* was locally mal-adapted as seed predation was less likely on sympatric ( $0.064 \pm 0.028$ , mean  $\pm$  SE) than on allopatric plants ( $0.139 \pm 0.023$ ; main effect of sympatry/allopatry  $\chi^2_1 = 5.39$ ,  $P = 0.0203$ ). Despite the significant main effect of the sympatry/allopatry factor, contrasts did not find statistically significant differences in probability of seed predation between sympatric and allopatric plants in any of the sites when tested separately ( $P > 0.05$ ; Fig. 5b).

Local adaptation of the pollinators varied among populations, as suggested by the significant interaction between home site and the sympatry/allopatry factor for proportion of pollinia removed ( $F_{3,59.5} = 20.96$ ,  $P < 0.0001$ ). However, when the populations were tested separately, only in Ruissalo, the pollinators were locally adapted to their sympatric plants, that is, sympatric plants had the highest proportion of pollinia removed (contrasts:  $F_{1,245} = 7.21$ ,  $P = 0.0078$ ) (Fig. 5c).

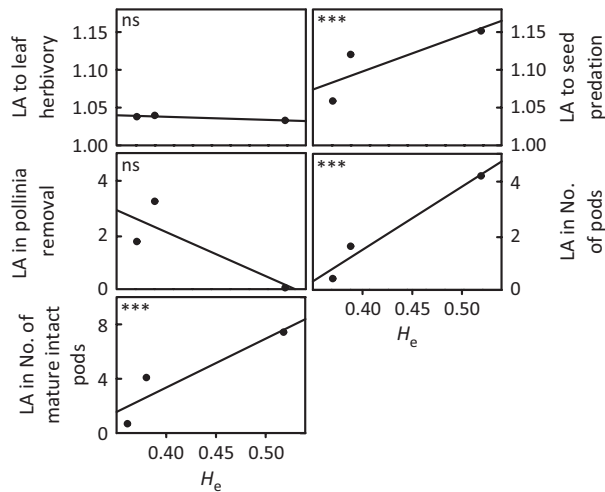
## VARIATION IN LOCAL ADAPTATION AND TRADE-OFFS IN LOCAL ADAPTATION

The magnitude of local adaptation varied among the studied traits: local adaptation values varied from 0.04 to 10.7. The highest value (10.7) was observed for Lammasluoto plants that had over 10 times more fruits when grown at their home site compared to two of the transplant sites. The lowest value (0.04) was observed for plants from Utö that produced 96% less pods when grown at their home site compared to when grown in Lammasluoto. Local adaptation values in the resistance measures were close to one, which is the threshold for local adaptation. In three of the five studied traits (resistance to seed predation, number of all pods and number of mature intact pods), local adaptation values were higher in populations with higher levels of within-population genetic variation (Fig. 6). For two traits (resistance to leaf herbivory and proportion of pollinia removed), the correlation was statistically nonsignificant (Fig. 6). Local adaptation in resistance to seed predation correlated positively with population divergence in precipitation and temperature (Table 2, Fig. 7). The observed variation in the magnitude of local adaptation in other traits did not correlate with the variables describing among-population divergence (precipitation and temperature, genetic and geographical distance, and difference in population size and

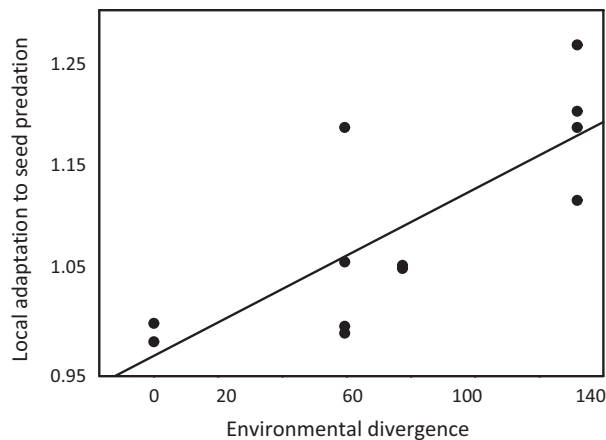


**Fig. 5.** Proportion of leaves damaged by *Abrostola asclepiadis* (a), probability of seed predation by *Euphranta connexa* (b) and proportion of pollinia removed (c) in *Vincetoxicum hirundinaria* in the four study sites in a reciprocal transplant experiment. Bars represent the mean ( $\pm$ SE). Standard errors for the probability of seed predation were calculated using equation  $\sqrt{[p(1-p)/n]}$ , where  $p$  is the proportion of plants with damage. One bar cluster represents the plants grown in the same transplants site during the experiment. First bar in each bar cluster corresponds to the plants that are sympatric to the herbivores and pollinators (also depicted by striping). Letters 's' and 'a' indicate a significant pairwise difference between the sympatric and allopatric plants transplanted at each site. An asterisk (\*) and a line indicate a significant contrast testing the difference between the sympatric plants and the mean of all allopatric plants combined. The herbivores (a and b) and pollinators (c) can be considered to prefer their sympatric plants if the sympatric plants have highest damage (a and b) or pollination success (c).





**Fig. 6.** Local adaptation (LA) of *Vincetoxicum hirundinaria* measured in different traits in relation to within-population genetic variation ( $H_e$ ) of the plants. Asterisks in the top left corner of each figure indicate a significant correlation (Spearman rank correlation). The scale on x-axis is the same for all figures, but y-axis is different between figures in different rows.

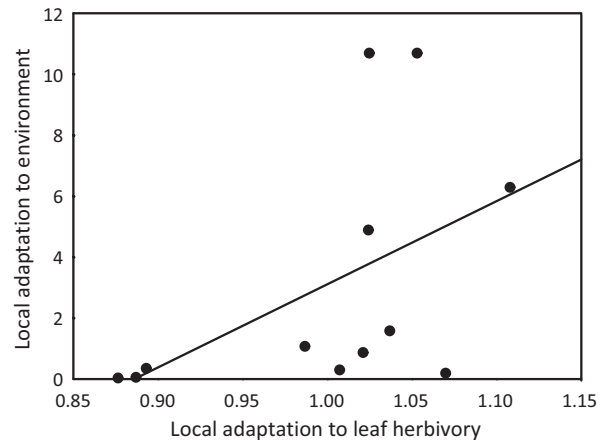


**Fig. 7.** Local adaptation of *Vincetoxicum hirundinaria* to seed predation in relation to environmental divergence in precipitation and temperature between populations.

plant leaf chemistry) (Table 2). Local adaptation in the number mature intact pods correlated positively with local adaptation to leaf herbivory (Table 2, Fig. 8) and local adaptation in the number of all pods (Table 2). None of the traits correlated negatively suggesting that there were no trade-offs in local adaptation (Table 2).

## Discussion

We found significant variation in local adaptation of *V. hirundinaria* to its specialist herbivores, generalist pollinators and the environment. In two of the studied populations, plants were locally adapted to their sympatric leaf herbivores. By



**Fig. 8.** Local adaptation of *Vincetoxicum hirundinaria* to environment measured in plant fitness (number of mature intact pods per plant) in relation to local adaptation in resistance to leaf herbivory.

contrast, plants from one of the populations suffered from higher leaf damage at home site compared to when grown at away sites, which indicates local maladaptation of the plants. One plant population was locally adapted to their sympatric seed predators. Two of the studied plant populations were locally adapted to their environment, which was displayed as higher fitness of the plants grown at their home site compared to when grown at the away sites. Our correlation analysis suggests that there were no trade-offs between local adaptation to the environment and local adaptation to the sympatric herbivores: populations that had higher fitness at home compared to when grown at the away sites had also relatively higher resistance against their sympatric herbivores. These results imply that local adaptation to the environmental conditions does not limit local adaptation to sympatric herbivores, or *vice versa*. However, since the level of local adaptation both to sympatric herbivores and to the environmental conditions correlated positively with genetic variation, our results suggest that local adaptation in this system is limited by the level of within-population genetic variation. These results provide new insights into the patterns and causes of variation in local adaptation, and are among the first to demonstrate that conflicting selection pressures within a population do not constrain local adaptation in multiple traits.

## LOCAL ADAPTATION BETWEEN PLANTS AND THEIR HERBIVORES

Local adaptation of long-lived plants to their short-lived insect herbivores may seem unlikely because of the higher evolutionary potential of the herbivores that have short generation times (Dawkins & Krebs 1979; Gandon & Michalakakis 2002; Núñez-Farfán, Fornoni & Valverde 2007). However, we found that in two populations (Lammasluoto and Ånskär), the plants had lower damage, that is, they were more resistant against the leaf herbivores, at their home site compared to when grown at the away sites, which indicates local adaptation of the plants to their sympatric leaf herbivores. In the

**Table 2.** Correlation coefficients ( $r_s$ ) for local adaptation values and among-population divergence in geographical and genetic distance, environment (precipitation and temperature), plant leaf chemicals and plant population size. Significant negative correlation coefficients in the upper part of the table would indicate trade-offs in local adaptation measured in different traits

	Local adaptation in				
	Leaf herbivory	Seed predation	Pollinia removal	All pods	Mature intact pods
Local adaptation in					
Seed predation	0.284				
Pollinia removal	−0.084	−0.382			
All pods	0.559	0.235	−0.119		
Mature intact pods	0.644*	0.160	−0.158	0.963***	
Population divergence					
Distance	0.014	−0.028	−0.057	−0.297	−0.127
Population size	−0.155	−0.304	−0.085	−0.240	−0.057
Environment	0.109	0.766**	0.109	−0.044	−0.120
Plant chemistry	−0.717	0	0	0.120	0.120
$F_{ST}$	−0.359	−0.717	0	0.239	0.239

\* $P < 0.05$ ; \*\* $P < 0.01$  and \*\*\* $P < 0.001$ .

Lammasluoto population, seed predators attacked none of the sympatric plants, which suggests that plants in this population were also locally adapted to their sympatric seed predators. Higher resistance in long-lived plants against their sympatric herbivores has been documented before (Sork, Stowe & Hochwender 1993; Crémieux *et al.* 2008). Shorter generation time does not always cause an advantage in the arms race co-evolution (Kaltz *et al.* 1999; Lively 1999; Gandon & Michalakakis 2002). If mutation and migration are not effective in providing the population with novel genetic variation, the repeated selection can lead to loss of genetic variation in the herbivore population and subsequently to lack of local adaptation (Gandon & Michalakakis 2002). On the other hand, local genetic differentiation may be swamped by high levels of migration in mobile insects (Michalakakis *et al.* 1993). It is surprising that in our study, local adaptation to seed predation was less widespread than local adaptation to leaf herbivory. One would assume that *V. hirundinaria* is more likely to locally adapt to the sympatric seed predators, because the negative effect of seed predation on population growth is stronger than the effect of leaf herbivory by *A. asclepiadis* and, thus, seed predation is likely to exert stronger selection on the plants (Lively 1999; Leimu & Lehtilä 2006). However, there is significant variation in the intensity of seed predation among years, and in some years, the seed predator *Euphranta connexa* can destroy almost all seed production. If all seeds are destroyed, selection on resistance may be diminished. On the other hand, our results suggest that local adaptation to seed predation may be present in Ruissalo and Utö populations as well (Fig. 2b), but these results were not significant, probably due to the low overall levels of seed predation in the study year; on average, only 12% of the plants suffered from seed predation.

To gain more thorough understanding of the co-evolutionary dynamics in a species pair, one should look at local adaptation of both the plant and the herbivore simultaneously (Garrido, Andraca-Gómez & Fornoni 2012). Local adaptation is assumed to vary across populations in the geographical

mosaic, and local adaptation may shift from one of the co-evolving antagonistic species to another (Thompson 1994, 2005). In one of our study populations (Utö), leaf herbivores caused more damage on their sympatric host plant than on allopatric host plants. This can infer local adaptation of the herbivores to their sympatric plant population expressed as host preference. Host preference is known to correlate with herbivore fitness (Rank *et al.* 1998; Gripenberg *et al.* 2010), although this is not always the case (Thompson & Pellmyr 1991). Differences in plant secondary compounds may have led to the herbivores favouring their sympatric plants when laying their eggs, which could explain the higher damage of the sympatric plants (Förare & Engqvist 1996; Leimu, Riipi & Stærk 2005; Muola *et al.* 2010). Plant populations in the study area in general vary in their leaf secondary compounds, but we do not know whether plants in the Utö population differ from the other populations investigated in this study. In here, we did not measure the performance of the herbivore larvae that would have been a more comprehensive fitness trait to test for local adaptation. However, herbivore preference is likely to correlate with their fitness, as adult herbivores oviposit preferably on plants on which the survival or growth of their larvae is increased (e.g. Rank *et al.* 1998; Prudic, Oliver & Bowers 2005; Gripenberg *et al.* 2010). We also know that the performance of *A. asclepiadis* larvae increases with increased amount of leaf area eaten (A. Kalske, unpubl. data). Therefore, the herbivore preference is a reliable estimate of herbivore fitness and can be used to examine local adaptation of the herbivore. In addition, our previous investigations have demonstrated that some of the *A. asclepiadis* populations are locally adapted to their sympatric host plant populations, while others are not when local adaptation was measured in terms of herbivore performance (L. Laukkanen, unpubl. data). This variation in local adaptation appears to be driven by among-population variation in plant secondary chemistry (L. Laukkanen, unpubl. data).

Local adaptation to seed predators was more likely to occur between populations that were more diverged in terms of

precipitation and temperature. Abiotic conditions are often more persistent than biotic conditions and are, therefore, thought to affect plant evolution and local adaptation more than biotic conditions (Linhart & Grant 1996; Bischoff & Trémolot 2011). If plants are transplanted to an environment that is similar to their own, their performance may not differ so much from that observed at their home site (Grøndahl & Ehlers 2008). Then again, when plants are transplanted to a site where abiotic conditions differ from their site of origin, the plants may suffer from relatively higher stress. Higher stress in turn may decrease the level of herbivore defences of plants, thereby resulting in higher damage at the away transplant site with different environmental conditions. This then appears as local adaptation of the plants to the sympatric herbivores. Unfavourable abiotic conditions are known to decrease host plant's ability to resist natural enemies (Springer, Hardcastle & Gilbert 2007). Geographical, genetic or chemical distances among the populations did not explain the observed among-population differences in local adaptation. Therefore, our results do not support the assumption that populations further apart geographically are more likely to display local adaptation (Hereford 2009).

A recent meta-analysis found that population size and, therefore, presumably population genetic variation is the single most important factor limiting local adaptation in plants (Leimu & Fischer 2008). Firstly, high levels of heritable variation will allow populations to respond to selection more efficiently compared to populations with less genetic variation (Hill 1982). Secondly, populations that have low levels of genetic variation may suffer from inbreeding depression (Keller & Waller 2002), which may conceal local adaptation (Leimu & Fischer 2008). In line with these ideas, we found that the strength of plant local adaptation tended to increase with increasing genetic variation of the plant population. Although our results need to be interpreted with caution due to the low number of populations, they suggest a pattern that is worth further studies. It is also possible that local adaptation, in fact, leads to a higher level of within-population genetic variation. In a population where selection imposed by specialist herbivores is strong, new rare genotypes arising through mutation may be selected for more intensely via frequency-dependent selection compared to populations, where selection by specialist herbivores is not strong (Dybdahl & Lively 1998). This would lead to higher within-population genetic diversity in populations where plants are under co-evolutionary selection, because new genotypes would establish themselves more often than in populations with no selection. However, the data we have on genetic variation of the plant populations are based in neutral markers, which means that the genetic variation under selection is not necessarily included in the measure (Leimu & Mutikainen 2005).

#### LOCAL ADAPTATION OF PLANTS TO THEIR ENVIRONMENT

Because environmental conditions are likely to vary considerably during a plant's lifetime, especially for long-lived plants,

and plants cannot escape these conditions due to their sessile growth form, phenotypic plasticity is considered to have a major role in plants adapting to their environment. Despite the major role of plasticity in plants in general, we did find that plants from two populations (Lammasluoto and Ruissalo) had more mature intact pods when grown at their home site compared to when grown away, which indicates local adaptation to their home environment in terms of fitness. Similarly to local adaptation of the plants to their herbivores, local adaptation of the plants to their environment also increased with increasing genetic variation of the population. The relatively lower pod production in plants at the away transplant sites could also be due to between-population outbreeding depression. Plants transplanted to foreign sites were more likely to receive pollen from individuals of the foreign population. If this was the case, possible outbreeding depression would appear as reduced female fitness due to early-acting outbreeding depression (Price & Waser 1979; Lynch 1991; Waser & Price 1993; Waser, Price & Shaw 2000). Outbreeding depression for plant size or herbivore resistance was not detected in Lammasluoto population in a previous study (Muola *et al.* 2011), but it has been observed for fitness in this system (R. Leimu, unpubl. data).

In addition to the physical environment and antagonistic interactions, mutualistic interactions, such as those between plants and their pollinators, are essential in shaping plant populations and communities. Plants from the Ruissalo population had the highest proportion of pollinia removed at their home site, which may indicate local adaptation of the plants to their sympatric pollinators. Our results also suggest that the pollinators in Ruissalo were locally adapted to their sympatric plant population, because the pollinators removed the highest proportion of pollinia from the sympatric plants compared to the allopatric plants. Local adaptation to the pollinators was not observed in the other populations. In fact, in one of the populations, Lammasluoto, the sympatric plants had the lowest proportion of pollinia removed. This among-population variation in local adaptation may be explained by variation in the pollinator assemblage among the populations. Since various generalist insects (Leimu 2004) pollinate *V. hirsutaria*, it is more likely that the plants adapt to the local composition of pollinating insects than *vice versa*. Differences in the preferences of pollinating insect assemblages can cause spatially divergent selection in plants (Gómez *et al.* 2008, 2009). Co-evolution and thereby adaptation is thought to be more likely between plants and their specialist pollinators than between plants and more generalist pollinators (Lajeunesse & Forbes 2002; Gómez *et al.* 2009). Our results support those of Gómez *et al.* (2009) in that plants may be able to adapt locally even to their generalist pollinators and the generalist pollinator assembly, although they also indicate among-population variation in local adaptation to pollinators.

The 'home versus away' contrasts used in this study compare the performance of a given genotype in its native and in novel environments. If natural selection has acted on genotypes in the population resulting in local adaptation, individuals perform better at their 'home' site compared to

when transplanted to another site, that is, the 'away' site. However, problems may arise in detecting local adaptation if the sites differ significantly in their resource availability. In such a case, local adaptation may not be detected if plants originating from an environment with limited resources are transplanted into an environment with higher resource level, leading to better performance in the away site (Kawecki & Ebert 2004). If the transplant sites differ in their resource availability and this is reflected in the performance of the individuals, this should be displayed as a statistically significant main effect of the 'home site' in the analysis of variance. In this study, the effect of home site was statistically nonsignificant for the fitness traits and for seed predation (Table 1). Therefore, it seems that the variation in resource levels among the sites did not cause problems in detecting local adaptation in terms of fitness or seed predation. However, for resistance to leaf herbivory, the main effect of home site was statistically significant (Table 1), suggesting that better environmental conditions (in here, lower level of leaf herbivory) in the away sites could have impaired the detection of local adaptation. This might be a problem for two of the populations: Ruissalo that was found not to be locally adapted and Utö that was found to show maladaptation to local leaf herbivores. Therefore, the fact that we did not detect local adaptation to leaf herbivores in these two populations needs to be interpreted with caution, as it might be obscured by among-site variation in the level of leaf herbivory. Excluding the results of leaf herbivory, the among-site variation in resource levels did not affect the detection of local adaptation in this study, and therefore, this inherent problem of the 'home versus away' contrast does not affect the interpretation and reliability of our results.

#### CONSTRAINTS OF LOCAL ADAPTATION

Plant evolution and adaptation is strongly affected by the physical environment, and local adaptation to the abiotic conditions may constrain the evolution of local adaptation to the biotic environment. Studying multiple traits simultaneously during the experiment allowed us to compare local adaptation in different traits and test for constraints between local adaptation in different traits, that is, trade-offs in local adaptation. We found a positive relationship between local adaptation to the environment (higher fitness at home compared to away) and local adaptation to the herbivores (higher resistance at home compared to away). Contrary to our expectations, it seems that there are no trade-offs between local adaptation to herbivores and local adaptation to the environment. This is unexpected because trade-offs between plant antiherbivore defences and growth or reproduction are relatively common (e.g. Herms & Mattson 1992; Koricheva 2002) and ecological costs between resistance and pollinator attraction have been reported (Strauss *et al.* 1999). Because selection pressures on these traits may be conflicting within a population and limiting resources can restrict the ability to respond to these multiple selection pressures, one would expect that a trade-off could also be observed between local adaptation measured in

these traits. Local adaptation could, therefore, be limited to few traits, and trade-offs between local adaptation in different traits may arise. However, according to our results, local adaptation in one trait seems not to exert evolutionary, ecological or fitness cost or constraints to local adaptation in other traits. To the best of our knowledge, this is the first investigation into local adaptation trade-offs and, thus, further studies are needed.

#### Conclusions

We found local adaptation of *V. hirundinaria* in the study area to its specialist herbivores, generalist pollinator insects and the environment. These results imply that long-lived plants may be able to locally adapt to their enemies, despite the high evolutionary potential of the short-lived insects. There was significant variation in local adaptation of the plants across the study area, and in some populations, it was the herbivores and pollinators that were locally adapted to their sympatric plant population. Local adaptation of the plants to the seed predators was more pronounced when plants originated from populations that were more diverged in terms of precipitation and temperature. Local adaptation was not constrained by trade-offs between local adaptations to the herbivores and local adaptations to the environment. It seems that local adaptation is more likely limited by genetic variation of the plant populations. These results provide novel information on patterns and causes of spatial variation in local adaptation and they are the first to demonstrate that conflicting selection pressures within a population, resource limitation or ecological constraints do not constrain local adaptation in multiple traits.

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