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Enhanced frugivory on invasive *Silene latifolia* in its native range due to increased oviposition

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Summary

1. Why some species become invasive is a timely question in ecology. Most hypotheses assume that changed ecological interactions, especially enemy release, allow species to become invasive. This paper investigates how the release from a specialist frugivore may lead to relaxed selection on avoidance and defence strategies in an invasive plant.
2. *Silene latifolia* was introduced from Europe to North America where its main enemies are absent. Reintroduced North American plants suffer greater damage than European plants by their specialist frugivore *Hadena bicruris*, which lays eggs in flowers. However, the mechanism is unclear: increased oviposition preference, decreased defence leading to higher egg and larval survival, or a closer phenological match.
3. We exposed plants from European and North American populations to this enemy by allowing natural oviposition and also by manually adding eggs. We recorded reproductive traits potentially associated with interactions with the seed predator and monitored the different stages of fruit attack (oviposition, larval growth and survival) to study the relative roles of increased attractiveness versus decreased defence.
4. North American plants received significantly more eggs (40.2 ± 2.1 eggs per plant) than European plants (28.4 ± 3.0 eggs per plant) and natural fruit predation was twice as high on North American than on European plants. Oviposition preference rates for North American over European plants persisted also after variation in flower size and phenology were accounted for.
5. European and North American plants did not differ significantly in larval survival, larval mass at emergence or in the rate of defensive fruit abscission, indicating that greater susceptibility of plants from the introduced range to the seed predator is largely due to an increased preference for oviposition rather than to changes in palatability or defence after oviposition.
6. *Synthesis*. Our results are consistent with the idea that introduced plants may evolve after introduction, if evolutionary constraints such as those imposed by plant–frugivore interactions, are broken down in the invasive range. For *S. latifolia,* release from its native predator *H. bicruris* may have allowed evolution of increased attractiveness to pollinators without the burden of attracting the frugivore.

Key-words: biological invasions, Caryophyllaceae, common garden, enemy release, floral morphology*, Hadena bicruris*, invasive plants, nursery pollination, pre-dispersal seed predation

Introduction

Invasive plants cause major environmental and economic damage throughout the world (Pimentel *et al.* 2005). An important explanation why plant species become invasive is the 'enemy release hypothesis' (ERH). Indeed, for many invasive plant species, major enemies such as specialized herbivores are absent in their introduced range, usually resulting in lower herbivore pressure (Wolfe 2002). However, the ERH alone cannot explain why many other introduced species that also experience less damage by natural enemies in the introduced area remain non-invasive (Parker & Gilbert 2007). In fact, when plants from the native and the invasive range are compared, genetic changes that have occurred in the invasive plants are often revealed. Common garden experiments using plants

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from the native and introduced range have shown that individuals from the introduced range often grow more vigorously (Leger & Rice 2003; Blair & Wolfe 2004; Joshi & Vrieling 2005; Güsewell *et al.* 2006; Zou *et al.* 2007) and that when plants are reintroduced from the invasive to their native range, they often experience more damage by enemies than the native plants (Wolfe 2002; Colautti *et al.* 2004; Liu & Stiling 2006; Liu *et al.* 2007). This suggests that genetic changes that favour enhanced growth and reproduction, but that also increase susceptibility to the original natural enemies, have taken place in the introduced range. Several non-exclusive explanations have been brought forward to explain these genetic differences. First, drift and founder effects might have led to a different genetic set-up of native versus introduced plants (Taylor & Keller 2007). Second, in the introduced range, plants from previously isolated populations could have hybridized and this might have led to genotypes exhibiting novel traits associated with invasiveness (Vila & D'Antonio 1998; Schierenbeck *et al.* 2005; Wolfe *et al.* 2007). The third explanation for genetic differences is that selection processes in the introduced range are different from those in the native range due to changed ecological interactions such as the release from their natural enemies. The most commonly cited example is the 'evolution of increased competitive ability' hypothesis which states that in the absence of natural enemies, selection favours plants that redirect resources from defensive traits, especially against specialist herbivores to invasive traits, such as increased seed production (Blossey & Nötzold 1995; Joshi & Vrieling 2005; Zangerl & Berenbaum 2005). Interestingly, such a change in selection pressure may also modify traits that *per se* are not defensive traits, but which would lead to the same effect, i.e. increased reproduction but higher susceptibility to enemies. An example of such a trait that would simultaneously increase reproductive rate and susceptibility to frugivores are larger floral displays.

The white campion, *Silene latifolia*, represents such an example of enemy release. Introduced from Europe to North America in the 1800s, it escaped two specialist enemies that are absent in North America (the noctuid seed predator *Hadena bicruris* and the aphid *Brachycaudus lychnidis*), and more rarely encounters the smut fungus *Microbotryum violaceum* (Wolfe 2002; Wolfe *et al.* 2004). In Europe *H. bicruris* has a dual role (Kephart *et al.* 2006), whereby the moth acts both as an important frugivore (i.e. the adult lays eggs in flowers and larvae feed on seeds) and as a pollinator for *S. latifolia* (Brantjes 1976b). Since its introduction the plant has spread across North America (Antonovics *et al.* 2003) and is considered a noxious weed (McNeill 1977; INVADERS Database System, 2009). Several studies have shown that phenotypic changes have occurred in the introduced range. In common garden experiments, North American plants developed faster and had higher reproductive output than European plants (Wolfe *et al.* 2004) but traits that could be involved in defence against the plant's different enemies had decreased (Blair & Wolfe 2004). Indeed, in Europe, North American plants are damaged more heavily by the seed predator *Hadena bicruris* than European plants (Wolfe *et al.* 2004). However, it remains unclear which mechanism leads to greater damage: increased preference by the herbivore resulting in higher oviposition, decreased defence leading to higher herbivore survival or a closer match of the phenology of North American plants with the peak in predator abundance.

The main goal in this study was to address the mechanisms of greater frugivore damage of North American plants, in particular to distinguish between the relative roles of avoidance of oviposition versus defences acting after oviposition. We exposed *S. latifolia* plants from different populations of both continents to the specialist seed predator *H. bicruris* in a common garden in the native range (Switzerland). We simultaneously performed two experiments to study the relative roles of increased attractiveness (decreased avoidance) versus changed palatability (defences after oviposition) for differences in the level of attack. First, we monitored the natural oviposition rate on a subset of plants to test whether the moth oviposits preferentially on North American plants compared with European plants. Second, we manually added eggs to a subset of plants to test whether differences in larval growth and survival through the different larval stages (i.e. differences in palatability between North American and European plants) could account for the differences in the level of frugivore attack. Since most aspects of the interaction between the plant and the moth involve floral and fruit structures, we measured floral traits potentially associated with oviposition (time to flower, total number of female and male flowers, corolla depth, petal width and stigma protrusion) as well as fruit traits associated with larval feeding and survival (fruit abscission, individual seed mass, number of seeds per fruit and fruit size). Furthermore, comparing reproductive traits between North American and European plants allowed us to detect differences which could indicate whether selection on such traits had changed in the invaded area. We discuss whether relaxed selection on oviposition avoidance or on defensive mechanisms, after enemy release, has influenced trait evolution in invasive *S. latifolia*.

Materials and methods

STUDY SYSTEM

Silene latifolia Poiret [= *S. alba* (Miller) Krause = *Melandrium album* (Miller) Garcke] is a short-lived perennial occurring in open, disturbed habitats. It is common in its native range Eurasia, and has been introduced into North America in the early 1800s (McNeill 1977), where it is now a widespread weed (McNeill 1977; Royer & Dickinson 1999). *Silene latifolia* is dioecious and flowers from April to October. Pollination in Europe and North America is mainly performed by nocturnal moths, although some diurnal insects such as hoverflies also visit the flowers (Shykoff & Bucheli 1995; Van Putten *et al.* 2007; Young 2002). After pollination the ovary expands and develops into a seed capsule containing several hundreds of seeds (Jürgens *et al.* 1996; Jolivet & Bernasconi 2007).

In its native range, *S. latifolia* is heavily attacked by the specialist fruit predator *Hadena bicruris* Hufn. (Lepidoptera: Noctuidae). Larvae were found in over 90% of investigated *S. latifolia* populations in Europe where they destroyed 25–70% of the fruits (Elzinga *et al.* 2005). By contrast, in North America *H. bicruris* was absent and virtually no seed damage through other species occurred (Wolfe 2002). Both male and female moths visit flowers and act as pollinators (Brantjes 1976b). Female moths lay a single egg in or on the female flowers after nectar feeding (Brantjes 1976a). The first-instar larva enters the developing fruit from the side and feeds on the seeds. It will stay in this fruit ('primary' fruit) until it has consumed all the seeds, i.e. usually until its fourth or fifth instar. At this stage it leaves and feeds on additional fruits ('secondary' fruit). On secondary infested fruits, larvae start feeding from the top (hence primary and secondary attack can be distinguished).

Several avoidance and defence strategies against *H. bicruris* have been suggested for *S. latifolia*. A smaller floral display or smaller flowers may reduce attractiveness, reducing the risk of receiving eggs (Wright & Meagher 2004; Biere & Honders 2006). A thick fruit wall may reduce attack by the larvae (Wolfe *et al.* 2004; but see Jolivet & Bernasconi 2006), and selective abscission of infested fruits (see Shapiro & Addicott 2004) may reduce survival of larvae and reduce secondary infestation (Jolivet & Bernasconi 2006; Burkhardt *et al.* 2009) as well as allow the plants to reallocate resources (Wright & Meagher 2003). Finally, eggs placed outside the flowers may have lower success through higher predation or desiccation risk than those laid on the ovary; however, it is unknown whether floral traits can influence the site of oviposition.

COMMON GARDEN EXPERIMENTS

We tested whether European and North American plants differed in the risk of oviposition or larval performance by exposing plants from both continents in a common garden (15 × 30 m grassland plot) in Lausanne, Switzerland (46°31′18.60″ N, 6°34′48.84″ E). In this area, *S. latifolia* and *H. bicruris* occur naturally. Plants were grown in 2006 from seeds collected between 1999 and 2005 at different sites in Europe and North America (Fig. 1). We germinated approximately 50 seeds from three maternal families per population in Petri dishes filled with glass pearls (diameter 1 mm), water and a 0.05 mM giberrellic acid solution (25/15 °C; 60% relative humidity; 16 h/8 h L/D). A maximum of 20 germinated seeds per family were transplanted separately into Jiffy-7 peat pellets (Jiffy International AS, Kristiansand, Norway). We repotted plants individually at the eight-leaf stage into 5.2 L pots with a soil mixture (65% peat and turf, 10% perlite, 15%

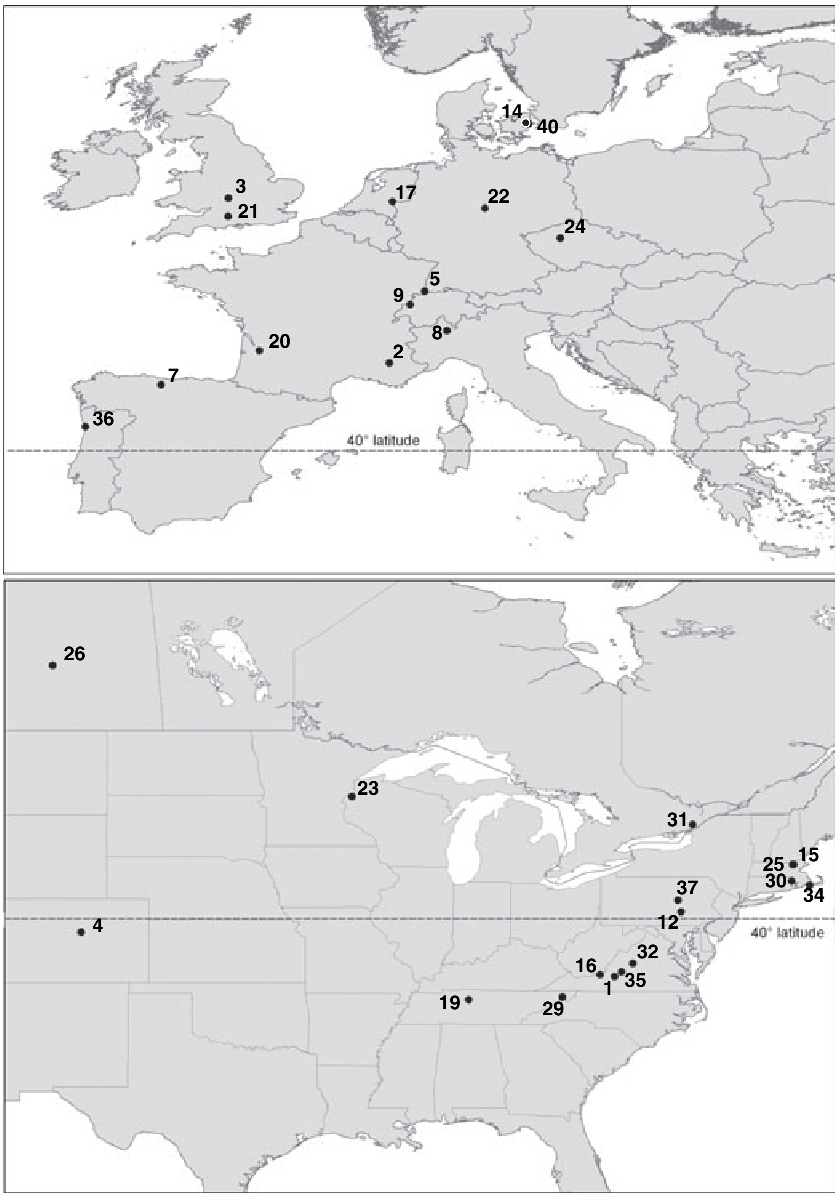

Fig. 1. Collection sites of *Silene latifolia* plants used in the common garden experiments (geographic map projection). Note that populations 14 and 40, as well as 25 and 15, are closely located and symbols overlap. Both maps have the same scale.

sand and 10% clay) and placed them outdoors; in total 11–12 plants from each of three maternal families from 14 European and 12 North American populations were repotted. We also had plants from four additional North American populations with only one or two maternal families. For each plant we recorded the time it took from repotting to appearance of the first open flower.

The four female and the two male plants per maternal family that were first to flower were placed in the common garden between 8 May 2006 and end of June (median: 22 May 2006). By selecting such relatively homogenous subsets of early flowering plants we were able to reduce potential effects of differential flowering phenology caused by differences in development time between the two continents (Table 2). Indeed, in the experiments, no obvious differences in phenology were observed between North American and European plants (Fig. 3). Average dates of first flowering were not significantly different between the continents in the separate experiments (nested ANOVA, $P_{\text{continent}} > 0.1$), nor did we detect any significant differences in the number of flowers produced by either the male or the female plants (Table 2). Each plant was placed at a randomized position in a grid of 16 × 33 places with 75 cm interplant distance. The remaining flowering plants were kept separately to collect eggs of *H. bicruris*, which were added manually to flowers (see below).

The four female plants per maternal family were assigned to either of the two experiments. The *Oviposition preference* experiment was an observational study used to detect differences in moth oviposition rate and to measure flower size. The *Larval performance* experiment was used to measure differences in larval growth and survival after infestation (by natural oviposition and manually adding eggs) and to measure fruit traits. All experimental plants were censused every 3 days from 6 June 2006 until the end of September and weekly thereafter until December. Censuses were performed at the same intervals in both experiments but on alternate days. On all plants, we counted the number of flowers at each census (either before 10 AM or after 9 PM when flowers were open).

Oviposition preference experiment

In order to determine whether flowers in European and North American plants differed in the probability of receiving an egg, at each census day we removed and examined all wilted flowers on this subset of plants ($n = 76$ and 73 for European and North American plants, respectively) for the presence of *H. bicruris* eggs. We recorded the number of eggs per flower and the position (i.e. inside the flower on the ovary or between the stigmas, or outside the flower on the petals), assuming that floral traits may constrain position and that position may influence egg survival. On one flower per plant per census day, we measured traits that we hypothesized to influence oviposition preference: *corolla depth, petal width* and *stigma protrusion* (Fig. 2).

We calculated the oviposition per plant as the total number of eggs that a plant received during the experimental period (until 19 September when the last eggs were found). We calculated the *observed oviposition rate* as the proportion of flowers that received at least one egg over the total number of flowers that the plant produced in the experimental period. However, the density of moths varied greatly over time (Fig. 3) and different plants did not produce their flowers simultaneously, creating variation between plants in the risk to receive eggs. To correct for this variation and to increase the power of the comparison between European and North American plants, we calculated the *expected oviposition rate* the focal plant would have if all eggs in the experiment were distributed uniformly over all plants and flowers on each census day. From this, we obtained the oviposition preference index calculated for each plant as: oviposition preference index = observed oviposition rate − expected oviposition rate. Expected oviposition rate was calculated as:

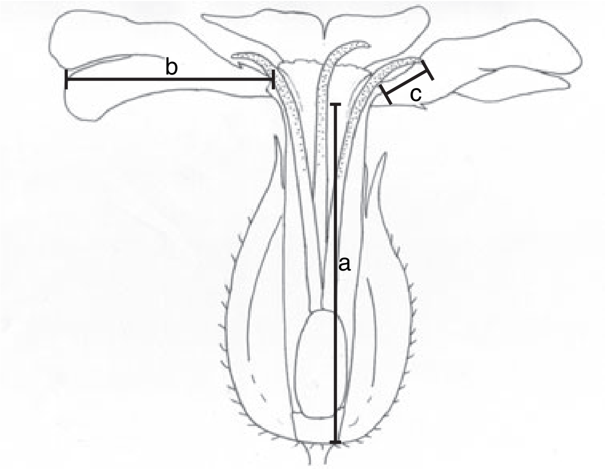

Fig. 2. Measured traits of *Silene latifolia* female flowers: a = corolla depth, b = petal width, c = protrusion of the stigma.

$$\text{Expected oviposition rate(focal plant)} = \frac{\sum_{i=1}^{i_{\max}} (\text{Oviposition rate(all plants)}_i \times \text{Flowers(focal plant)}_i)}{\sum_{i=1}^{i_{\max}} \text{Flowers(focal plant)}_i}$$

where i is the census day ($i = 1$ to $i_{\max}$, the last census day eggs were found), *Oviposition rate*(*all plants*)$_i$ is the proportion of flowers that had at least one egg summed over all plants in the whole experiment at census day i, and *Flowers(focal plant)*$_i$ is the number of flowers the focal plant had on census day i. A negative index value indicates that the plant received fewer eggs than expected based on a uniform distribution of eggs over all experimental *Oviposition preference* plants and flowers.

The same reasoning applies to the probability that an egg is laid inside or outside the flower; on different nights, different moths may be present with an individual propensity to lay eggs inside or outside of flowers. To account for this, we calculated the index for the proportion of eggs that were deposited inside flowers as:

$$\text{Oviposition site index} = \text{Observed deposition(focal plant)} - \frac{\sum_{i=1}^{i_{\max}} (\text{Deposition(all plants)}_i \times \text{Eggs(focal plant)}_i)}{\sum_{i}^{i_{\max}} \text{Eggs(focal plant)}_i}$$

where *Observed deposition*(*focal plant*) is the proportion of eggs deposited inside flowers, *Deposition*(*all plants*)$_i$ is the total proportion of eggs deposited inside flowers over all plants in the experiment on census day i and *Eggs(focal plant)*$_i$ is the number of eggs deposited on census day i on the focal plant.

Larval performance experiment

To test whether North American and European plants differed in their palatability (i.e. defences acting after oviposition) as measured through survival and growth of *H. bicruris* larvae, we manually added moth eggs to a large number of randomly selected flowers ($n = 1226$ flowers on $n = 77$ European and 80 North American plants). We monitored survival through the different larval stages and weighed the larvae at emergence from the primary infested fruits. Eggs were

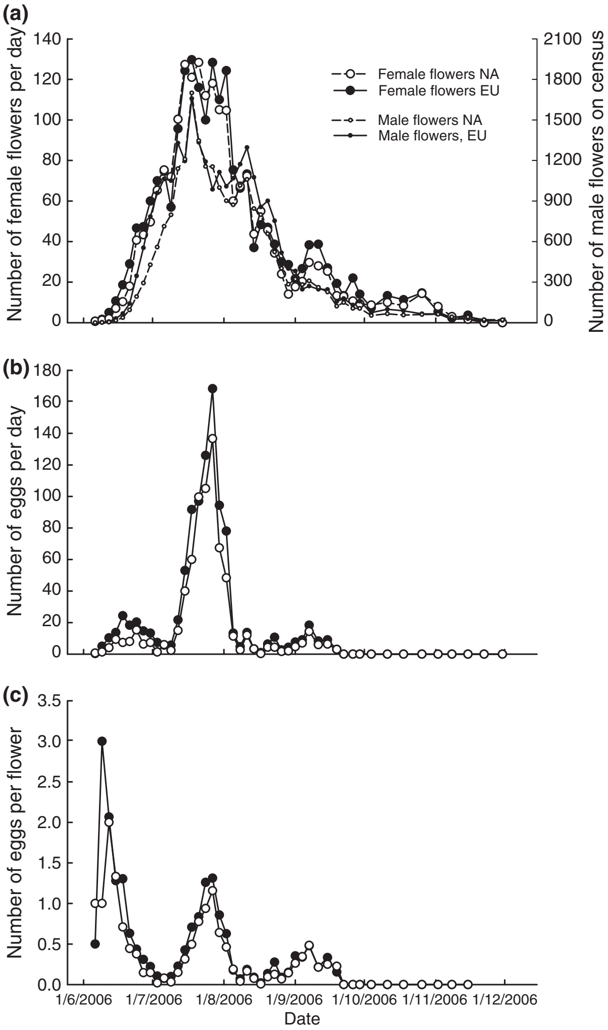

Fig. 3. Temporal variation in (a) the number of male and female *Silene latifolia* flowers for North American (NA) and European (EU) plants, (b) the total number of *Hadena bicruris* eggs per day and (c) the average number of eggs per flower per day. All values are calculated from data collected at three-day intervals in the *Oviposition preference* experiment.

collected from natural oviposition on the previous day and kept on a moist filter paper in a Petri dish at 4 °C prior to use (Elzinga *et al.* 2002). To simulate natural egg laying and to allow undisturbed pollination of the flowers, we removed one tip of the calyx and pushed the egg with a moist toothpick into the flower close to the ovary. These manipulated flowers could receive also eggs from natural oviposition either before or after manipulation. All flowers were labelled, allowing the distinction between manipulated and unmanipulated flowers.

We followed the fate of each flower produced in the *Larval performance* experiment, i.e. whether a fruit formed but was abscised, whether it showed signs of primary infestation, of secondary infestation, or whether it escaped attack and developed into a fully developed, intact fruit. We tried to retrieve abscised fruits in the grass to establish the presumed cause of abscission: primary or secondary infestation or incomplete development, possibly due to insufficient pollination. Non-abscised fruits with signs of primary infestation (a small hole with protruding frass) were bagged with a small mesh bag to be able to collect the larva when it emerged. We weighed emerged larvae and measured width and length of these fruits. Wherever possible, we measured the growth of at least two larvae per plant that emerged from a primary fruit. For most plants we obtained larval mass data ($n = 238$ and 153 larvae on 73 North American and 67 European plants, respectively); in the remaining cases, larvae failed to develop. We estimated fruit volume as $4/3 \times \pi \times (\frac{1}{2}$ fruit width$)^2 \times \frac{1}{2}$ fruit length (i.e. assuming ellipsoid shape), as potential correlate of larval mass, for each fruit from which a larva emerged. In addition we estimated fruit volume from all fruits that were ripe and determined the number of seeds per fruit, on an additional fruit that was ripe but was not yet open. Seeds were counted on a seed counter (Elmor C3, Schwyz, Switzerland). Seed mass was estimated by weighing a group of 10 seeds per fruit on a microbalance (MX5, Mettler Toledo International Inc., Columbus, OH, USA).

We calculated the establishment rate of the first-instar larva as the proportion of manipulated flowers that developed into a non-abscised primary fruit or were abscised. Examination of retrieved abscised fruits revealed that fruit abscission was highly associated with primary infestation, in agreement with experimental evidence that *S. latifolia* responded to *H. bicruris* by selective fruit abscission (Jolivet & Bernasconi 2006; Burkhardt *et al.* 2009). Ninety per cent of the abscised manipulated fruits that were retrieved ($n = 220$, 69% of the abscised manipulated fruits) showed signs of primary infestation. Also, for non-manipulated flowers, primary infestation was five times more frequent among abscised fruits (51% of 142 retrieved fruits) than among non-abscised fruits (11%, 2092 fruits). Abscission rate was estimated as the proportion of abscised fruits out of the total number of fruits with signs of primary predation. We calculated the survival rate of established larvae as the proportion of non-abscised primary infested fruits from which a larva emerged.

DATA ANALYSIS

To analyse differences between European and North American plants we performed nested ANOVA with type III sum of squares with continent as a fixed factor and population (nested within continent), family (nested within population), and, if multiple measurements per plant were available, plant (nested within family) as random factors. For proportion data we used a binomial error structure with a logit link function. Additionally, we performed ANCOVA to test whether effects of continent on oviposition rate and larval performance could be attributed to differences in covariates, i.e. respectively, flower traits and fruit traits. Where applicable, flower and fruit traits were entered as plant averages. To improve normality and homoscedasticity of the residuals, we transformed response variables before the analysis where required (Zar 1996). Data were analysed in SPSS v 15.0 (SPSS Inc., Chicago, IL, USA) and in SAS v 9.1(SAS Institute Inc., Cary, NC, USA). Averages are given ± SE. Average values for continents are always based on mean values per population, calculated, where applicable, from family means, with $n = 14$ populations for European plants and $n = 16$ for North American plants, unless stated otherwise.

Results

DIFFERENCES IN OVIPOSITION PREFERENCE BETWEEN CONTINENTS

North American plants were more heavily attacked by *H. bicruris* than European plants. North American plants received a significantly higher number of eggs, summed over the entire duration of the experiment, than European plants (about 40% more, Table 1). This difference could not be fully explained by differences in floral display between North American and European plants or by temporal variation in moth abundance. Indeed, on average, 55 ± 4% of the flowers produced by North American plants received an egg, compared to 46 ± 4% of flowers on European plants. When corrected for variation in floral display and in moth abundance (oviposition preference index, see Materials and methods) this difference was still marginally significant (Table 1, $P = 0.059$). Taking into account the positive correlations between oviposition preference and flower size (corolla depth and petal width), the differences between the continents became more pronounced with oviposition preference being significantly higher on North American than European plants (ANCOVA with corolla depth or petal width as covariate and continent as fixed factor, both $P_{\text{continent}} = 0.02$, Fig. 4). This indicates that oviposition preference on North American plants persists, also after taking into account floral display, variation in moth abundance and flower size. Oviposition was not correlated with the length of the protruding stigma (ANCOVA, $P_{\text{stigma}} > 0.1$).

In accordance with an oviposition preference for North American plants, in the *Larval performance* experiment, significantly more non-manipulated flowers were attacked on North American plants than on European plants (Table 1). In total, 28% of North American flowers with natural oviposition suffered primary attack or abscission versus 14% for the European flowers.

There were no significant differences between continents in the proportion of eggs laid inside the corolla tube ($n = 2864$ eggs, European plants: 42% and North American plants: 49% of eggs laid inside the flowers, respectively; Table 1), also not after correcting for the negative correlation with corolla depth and petal width (ANCOVA, $P_{\text{continent}} > 0.1$).

NO DIFFERENCES IN LARVAL PERFORMANCE BETWEEN CONTINENTS

European and North American plants did not differ significantly in any of the larval performance measurements: egg establishment rate, fruit abscission, larval survival to emergence, larval mass at emergence, or the number of secondarily attacked fruits per emerged larva (Table 1). In 61% out of the 1226 flowers in which we experimentally deposited an egg, the young larva established itself but this percentage was neither affected by continent (Table 1) nor after correcting for the positive correlation with the number of flowers a plant produced (ANCOVA, $P_{\text{continent}} > 0.1$). On flowers where we observed a naturally oviposited egg on the petals ($n = 138$), the establishment rate (50%) was indeed significantly lower

Table 1. Averages (±SE) per continent (based on population means) and results of nested ANOVA for the effect of continent [Europe (EU) vs. North America (NA)], population within continent, family within population, and where applicable, plant within family on attack by *Hadena bicruris* on *Silene latifolia*

	Number of eggs†‡			Oviposition preference‡			Oviposition site‡			Egg establishment (%)§		
NA	40.2 ± 2.1			0.055 ± 0.020			−0.033 ± 0.031			61.5 ± 2.8		
EU	28.4 ± 3.0			−0.012 ± 0.025			−0.027 ± 0.050			62.3 ± 2.7		
Source	d.f.	MS	*F*	d.f.	MS	*F*	d.f.	MS	*F*	d.f.	MS	*F*
Continent	1	0.677	4.914*	1	0.128	3.815$^{(*)}$	1	0.005	0.045	1	0.008	0.134
Population	28	0.142	1.995*	28	0.034	5.583***	28	0.111	3.618***	28	0.06	1.704*
Family	55	0.073	0.881	55	0.007	1.264	55	0.031	1.222	50	0.035	0.762
Residual	64	0.079		64	0.006		64	0.025		77	0.046	

	Larval survival (%)§			Larval mass (mg)†,§			Natural attack (%)§			Fruit abscission (%)§		
NA	58.4 ± 4.9			130.0 ± 8.1			30.1 ± 7.0			24.7 ± 6.5		
EU	55.0 ± 5.1			138.4 ± 7.9			22.8 ± 6.2			28.1 ± 4.8		
Source	d.f.	MS	*F*	d.f.	MS	*F*	d.f.	MS	*F*	d.f.	MS	*F*
Continent	1	0.02	0.14	1	0.072	0.671	1	0.493	4.605*	1	0.105	0.393
Population	28	0.144	1.426	28	0.119	1.245	28	0.109	2.743***	28	0.278	4.365***
Family	50	0.102	1.464$^{(*)}$	45	0.104	1.222	48	0.04	0.853	50	0.064	1.082
Plant	–	–	–	65	0.087	1.716***	–	–	–	–	–	–
Residual	64	0.031		251	0.051		68	0.047		77	0.059	

See Materials and methods for definition of the variables and the calculation of the oviposition preference and oviposition site indices. Significance levels: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, $(^{*})P < 0.1$.

†Log10-transformed for ANOVA.

‡Measured in the *Oviposition preference* experiment.

§Measured in the *Larval performance* experiment.

(chi-squared test, $P = 0.013$), confirming that oviposition site might affect egg and larval survival, although we could not exclude that the experiment itself led to lower egg mortality in the experimental flowers. Survival rate of larvae (51% of all 836 primary attacked, non-abscised fruits) was not significantly different between continents (Table 1).

Abscission rate of fruits was not significantly different between continents (Table 1), also not after taking into account the positive correlation with flower number (ANCOVA, $P_{\text{continent}} > 0.1$) and the negative correlation with average fruit volume (ANCOVA, $P_{\text{continent}} > 0.1$). In total, 22% of the manipulated flowers were abscised, but populations varied greatly in abscission rate from 0% in some populations to almost 100% in others.

There were no significant differences between continents (Table 1) in larval mass at emergence, also not after correcting for the positive correlation with the size of the fruit it emerged from (ANCOVA, $P_{\text{continent}} > 0.1$). On average, for each larva that emerged from a primary infested fruit, another 1.92 ± 0.15 fruits were secondarily attacked on the same plant, with no significant differences between North American and European plants (nested ANOVA, $P_{\text{continent}} > 0.1$), also not after correcting for the negative correlation with average fruit size (ANCOVA, $P_{\text{continent}} > 0.1$). The relatively low secondary attack rate may be explained by mortality induced by the parasitoid *Bracon variator* (Elzinga *et al.* 2007b) which we often observed in this experiment.

DIFFERENCES IN FLORAL AND PLANT TRAITS BETWEEN CONTINENTS

Out of all traits measured (Table 2), four differed significantly between continents. Time from repotting to appearance of the first open flower was significantly shorter (on average 3.5 days) for North American plants than for European plants (Table 2). Stigma protrusion was significantly larger for North American plants (on average 1 mm) than for European plants (Table 2), but corolla depth and petal width did not differ significantly between continents (Table 2).

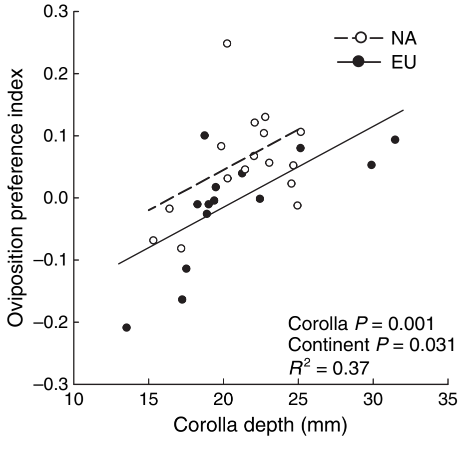

Fig. 4. Correlation of flower size (corolla depth, population averages based on plant means) with the oviposition preference index for *Silene latifolia* plants originating from Europe (EU) and North America (NA). Negative values of the index indicate that fewer eggs were oviposited than would have been expected if all plants had the same chance of receiving eggs. See Materials and methods for the calculation of the index. Lines represent the linear regressions for each continent based on ANCOVA.

Although North American plants produced significantly smaller (Table 2), and for a given fruit volume, significantly more seeds (Fig. 5), there were no significant differences between continents in either fruit volume, total number of seeds per fruit, or total seed mass per fruit (Table 2).

Discussion

Our common garden experiment in the native range of *Silene latifolia* showed that North American plants received proportionally more (40% more) eggs of the specialist fruit predator *Hadena bicruris* than European plants, leading to a higher primary attack rate. This suggests a difference in the preference by female moths for oviposition. However, we found no differences in survival of the larvae of this frugivore, suggesting that increased susceptibility is mostly affected by traits that affect oviposition preference rather than by defence traits acting after oviposition. Although we did not identify the actual trait variation causing this difference, this study revealed significant differences between continents for three traits (i.e. time to flower, individual seed mass and stigma length). These results suggest that the absence of a specific selection pressure (such as predation by *H. bicruris*) or a change in the relative importance of concurrent selection pressures has driven reduced pre-oviposition resistance in invasive North American populations. In the following sections, we discuss the findings and possible mechanisms.

EUROPEAN AND NORTH AMERICAN PLANTS DIFFER IN SUSCEPTIBILITY TO *HADENA BICRURIS* ATTACK

On a per-flower level *H. bicruris* laid more eggs on North American plants compared with European plants. Oviposition was positively correlated with flower size, but this correlation alone did not explain the increased oviposition we observed on North American plants. First, flower size was not significantly different between North American and European plants in our study. Second, for a given flower size, North American plants still received more eggs. Therefore the proximate mechanism of oviposition preference is not known and we can only speculate why North American plants could be preferred by female *H. bicruris* moths. Total floral display might be important, but we did not detect any significant differences in total number of flowers produced, in contrast with other studies (Blair & Wolfe 2004; Wolfe *et al.* 2004). North American and European *S. latifolia* showed only minor changes in scent composition (Dötterl *et al.* 2005), and it is not known whether this could influence oviposition. Greater risk of oviposition in North American plants might arise if flowers on North American plants were open and attractive for a longer time than on European plants. Because *S. latifolia* flowers wilt rapidly after pollination (Lankinen *et al.* 2006), if North American plants experienced lower pollinator visitation they might remain open for longer, thereby increasing the time of exposure of individual flowers. It would thus be interesting to compare pre- and post-pollination flowering periods between North American and European plants. Finally, oviposition choice might depend on floral traits that we did not measure, such as calyx width (Brantjes 1976b) or the quality and amount of nectar.

Irrespective of the mechanism, such a change in susceptibility is consistent with an evolutionary change following the breakdown of constraints on traits involved in biotic interactions. In the native range *S. latifolia* faces a trade-off between attractiveness to pollinators and enemies (Shykoff & Bucheli 1995; Biere & Honders 2006). If within the native range there is a cost to maintaining traits that reduce enemy attack such as pre-oviposition resistance to *H. bicruris* predation, this cost may drive reduced resistance in the invasive range. Indeed, in the invasive range, *S. latifolia* may attract more and novel pollinators without the added burden of increased seed predation. However, our findings are also consistent with the idea that other sources of selection (for instance, competition for pollinators, resource allocation and dispersal ability) and their relative importance may differ between the native and invasive environment.

Table 2. Averages (±SE) per continent (based on population means) and results of nested ANOVA of plant, floral and fruit traits of *Silene latifolia* (traits that are potentially associated with interactions with pollinators and seed predators) as a response to the effect of continent [Europe (EU) vs. North America (NA)], population within continent, family within population, and, where applicable, plant within family

	Time to flower (days)†			Female flowers‡			Female flowers§			Male flowers¶		
NA	37.8 ± 1.5			77.1 ± 7.8			22.7 ± 3.2			279 ± 30		
EU	41.2 ± 1.2			73.8 ± 9.2			22.6 ± 2.6			279 ± 25		
Source	d.f.	MS	*F*	d.f.	MS	*F*	d.f.	MS	*F*	d.f.	MS	*F*
Continent	1	0.345	4.397*	1	0.012	0.145	1	0.066	0.621	1	3398	0.065
Population	27	0.093	3.132***	28	0.088	1.834*	28	0.109	2.104**	28	55106	4.468***
Family	53	0.030	4.566***	55	0.048	1.021	50	0.052	1.425(*)	52	12122	0.617
Residual	793	0.007		64	0.047		77	0.037		79	19655	

	Corolla depth (mm)			Petal width (mm)			Stigma protrusion (mm)			Individual seed mass (mg)		
NA	21.5 ± 0.7			9.7 ± 0.1			3.5 ± 0.2			0.65 ± 0.03		
EU	20.9 ± 1.3			10.1 ± 0.3			2.6 ± 0.2			0.79 ± 0.04		
Source	d.f.	MS	*F*	d.f.	MS	*F*	d.f.	MS	*F*	d.f.	MS	*F*
Continent	1	44.640	0.72	1	31.270	1.272	1	211.600	6.914*	1	83.130	12.05**
Population	28	803.100	17.484***	28	30.760	2.656***	28	37.050	0.914	28	7.027	2.601***
Family	55	50.410	2.732***	55	12.610	2.059**	55	43.230	2.676***	50	2.717	1.216
Plant	65	19.140	6.237***	65	6.320	3.698***	65	16.770	7.152***	–	–	–
Residual	1603	3.070		1603	1.709		1603	2.345		66	2.234	

	Seeds per fruit			Fruit volume (mm^3)		
NA	357 ± 21			298 ± 12		
EU	319 ± 26			329 ± 26		
Source	d.f.	MS	*F*	d.f.	MS	*F*
Continent	1	52721	1.404	1	123068	1.465
Population	28	40549	3.068***	28	159173	5.467***
Family	55	13436	1.362	51	35853	1.041
Plant	–	–	–	70	39849	4.662***
Residual	67	9868		1144	8621	

Significance levels: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, (*)$P < 0.1$.

†Log10-transformed for ANOVA; one outlier removed.

‡Log10-transformed and residuals for flower start date for ANOVA; measured in *Oviposition preference* experiment.

§Log10-transformed and residuals for flower start date for ANOVA; measured in *Larval preference* experiment.

¶Residuals for flower start date for ANOVA

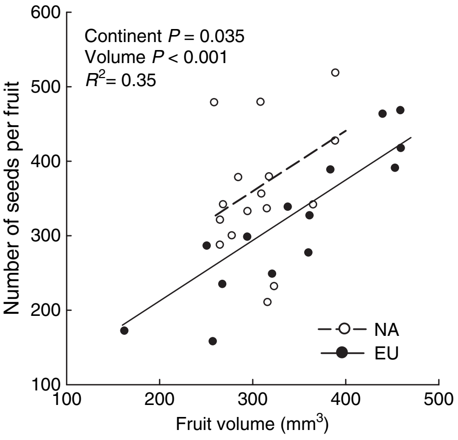

Fig. 5. Correlation between the estimated fruit volume and the number of seeds per fruit (population averages) in *Silene latifolia* plants from North America (NA) and Europe (EU). Lines represent the linear regressions by continent based on ANCOVA.

EUROPEAN AND NORTH AMERICAN PLANTS DO NOT DIFFER IN DEFENCE AGAINST HADENA BICRURIS

Although there were significant differences in oviposition preference, we did not detect any difference between European and North American plants in *H. bicruris* establishment rate, larval survival, larval growth and in the rate of subsequent secondary attack. A potential important defence in this system which occurred in about one fifth of attacked fruits was fruit abscission (Jolivet & Bernasconi 2006; Burkhardt *et al.* 2009). Despite remarkable variation among populations (in some populations none of the attacked fruits were abscised, in others almost all), no differences in abscission rate were observed between North American and European plants. Altogether, this suggests that greater susceptibility to attack of North American compared with European plants reported in previous studies (Wolfe *et al.* 2004) may be largely due to the greater risk of oviposition.

DIFFERENCES IN PLANT TRAITS

In agreement with previous studies (Blair & Wolfe 2004; Wolfe *et al.* 2004), several traits were significantly different between North American and European plants. The greater protrusion of the stigma in North American plants which may change the receptive surface exposed to pollinators is consistent with the idea that North American plants have adapted to a different pollinator community in the invasive range (Goulson & Jerrim 1997; Altizer *et al.* 1998; Young 2002; Barthelmess *et al.* 2006; Van Putten *et al.* 2007). It would be particularly interesting to compare flower visitation patterns and pollen loads between North American and European plants in both continents for this system involving a specialist pollinator (Stout 2007).

The faster development to the first flower observed for North American *S. latifolia* (see also Blair & Wolfe 2004; Wolfe *et al.* 2007) could reflect a response to release from opposing selection by *H. bicruris*. In Europe, selection for an earlier flowering start date by selective pressures other than from *H. bicruris* might be in conflict with selection to avoid frugivory as the phenology of the plant is related to the risk of attack by *H. bicruris* (Biere & Honders 1996; Elzinga *et al.* 2007a; Van Putten *et al.* 2007). However, because in our experiment we selected plants with similar flowering phenology, a test of this idea requires further study. Other potential selection pressures that have been suggested to play a larger role in plants in the invaded range include selection for increased dispersal capacity (Mason *et al.* 2008). Indeed, North American plants produced significantly smaller seeds.

In conclusion, our experiment indicates that *S. latifolia* plants from North American populations, which have been released from the main seed predator for over 100 generations, differ from the plants in the native range where the main seed predator is very abundant. These differences were detectable as a substantial and significant increase in the rate of oviposition preference by the seed predator. By contrast, components of larval success after oviposition did not differ significantly between continents. Although the trait divergence causing the difference in frugivore oviposition remains to be identified, our results were consistent with a breakdown of evolutionary constraints allowing for selection for increased attractiveness to pollinators (and thus, when exposed to it, also the seed predator, *H. bicruris*). These findings strengthen previous results which indicated that these constraints play a major role within the plant's native range for this plant pollinator–seed predator system.

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