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Reciprocal interactions between native and introduced populations of common milkweed, *Asclepias syriaca*, and the specialist aphid, *Aphis nerii*

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Abstract

Following its introduction into Europe (EU), the common milkweed (*Asclepias syriaca*) has been free of most specialist herbivores that are present in its native North American (NA) range, except for the oleander aphid *Aphis nerii*. We compared EU and NA populations of *A. nerii* on EU and NA milkweed populations to test the hypothesis that plant–insect interactions differ on the two continents. First, we tested if herbivore performance is higher on EU plants than on NA plants, because the former have escaped most of their herbivores and have perhaps been selected for lower defence levels following introduction. Second, we compared two *A. nerii* lines (one from each continent) to test whether genotypic differences in the herbivore may influence species interactions in plant–herbivore communities in the context of species introductions. The NA population of *A. nerii* developed faster, had higher fecundity and attained higher population growth rates than the EU population. There was no overall significant continental difference in aphid resistance between the plants. However, milkweed plants from EU supported higher population growth rates and faster development of the NA line of *A. nerii* than plants from NA. In contrast, EU aphids showed similar (low) performance across plant populations from both continents. In a second experiment, we examined how chewing herbivores indirectly mediate interactions between milkweeds and aphids, and induced *A. syriaca* plants from each continent by monarch caterpillars (*Danaus plexippus*) to compare the resulting changes in plant quality on EU aphid performance. As specialist chewing herbivores of *A. syriaca* are only present in NA, we expected that plants from the two continents may affect aphid growth in different ways when they are challenged by a specialist chewing herbivore. Caterpillar induction decreased aphid developmental times on NA plants, but not on EU plants, whereas fecundity and population growth rates were unaffected by induction on both plant populations. The results show that genetic variation in the plants as well as in the herbivores can determine the outcome of plant–herbivore interactions.

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Zusammenfassung

Nach ihrer Einwanderung nach Europa (EU) war die Seidenpflanze (*Asclepias syriaca*) frei von den spezialisierten Herbivoren, die in ihrer nordamerikanischen (NA) Heimat vorkommen, mit Ausnahme der Oleanderblattlaus *Aphis nerii*. Wir verglichen EU und NA Populationen von *A. nerii* auf EU und NA Seidenpflanzenpopulationen, um die Hypothese zu testen, dass sich die Insekt-Pflanze-Interaktionen zwischen den beiden Kontinenten unterscheiden. Zunächst prüften wir, ob die Performanz des Herbivoren auf den EU Pflanzen besser als auf NA Pflanzen ist, weil erstere den meisten ihrer Herbivoren entkommen sind und nach der Einfuhr Pflanzen mit reduzierter pflanzlicher Abwehr herangezüchtet worden sein könnten. Zum anderen verglichen wir zwei Stämme von *A. nerii* (einen von jedem Kontinent) um zu prüfen, ob genotypische Unterschiede beim Herbivoren die Interaktionen in Insekt-Pflanze-Systemen im Kontext von Einschleppungen beeinflussen könnten. Die NA Population von *A. nerii* entwickelte sich schneller, hatte eine höhere Fruchtbarkeit und erreichte höhere Wachstumsraten als die EU Population. Es gab keinen generellen signifikanten Unterschied in der Resistenz gegen Blattläuse zwischen den Pflanzen der beiden Kontinente. Indessen erlaubten Seidenpflanzen aus EU höhere Populationswachstumsraten und schnellere Entwicklung des NA Stammes von *A. nerii* als Pflanzen aus NA. Im Gegensatz dazu zeigten EU Blattläuse ähnliche (geringe) Performanz für die Pflanzenpopulationen von beiden Kontinenten.

In einem zweiten Experiment untersuchten wir, wie beißend-kauende Pflanzenfresser indirekt die Interaktionen zwischen Seidenpflanze und Blattlaus beeinflussen. Hierzu induzierten wir Seidenpflanzen von beiden Kontinenten mit Monarchraupen (*Danaus plexippus*), um den Effekt der resultierenden Änderungen in der Pflanzenqualität auf die Performanz von EU Blattläusen zu erfassen. Da spezialisierte beißend-kauende Herbivore der Seidenpflanze nur in NA vorkommen, erwarteten wir, dass Pflanzen unterschiedlicher Herkunft das Blattlauswachstum unterschiedlich beeinflussen würden, wenn sie von einem solchen Spezialisten angegriffen werden. Die Induktion mit Raupen verringerte die Entwicklungszeiten der Blattläuse auf NA Pflanzen, aber nicht auf EU Pflanzen, während Fruchtbarkeit und die Populationswachstumsraten auf beiden Pflanzenpopulationen nicht beeinflusst wurden. Diese Ergebnisse zeigen, dass genetische Variation sowohl bei den Pflanzen als auch bei den Herbivoren das Ergebnis von Insekt-Pflanze-Interaktionen bestimmen kann.

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Keywords: Intercontinental introduction; Milkweed; *Asclepias syriaca*; *Aphis nerii*; Plant–herbivore interactions; Plant quality; Enemy release hypothesis; Invasive species

Introduction

Intercontinental introductions of exotic species provide unique opportunities to investigate the evolution of interactions within communities. Several hypotheses have been formulated to predict how species interactions in novel environments may lead to rapid evolutionary changes. The ‘enemy release hypothesis (ERH)’ (Maron & Vila 2001; Keane & Crawley 2002) posits that in the absence of coevolved (specialist) enemies in the introduced range, plant reproductive success may increase (Schaffner et al. 2011). In turn, the ‘evolution of increased competitive ability hypothesis (EICA)’ posits that introduced plants need not endure the fitness costs of anti-herbivore defences, and thus more resources remain available for growth and competition (Blossey & Nötzold 1995; Müller-Schärer, Schaffner, & Steinger, 2004; Schaffner et al. 2011). Introduced plants are therefore expected to exhibit an increased susceptibility to their specialist natural enemies, because their coevolved defence traits are reduced (Zangerl & Berenbaum 2005; Berenbaum & Zangerl 2006; Zangerl, Stanley, & Berenbaum, 2008). Consequently, when exposed to their coevolved enemies, those plants that have less specialist herbivores in their

introduced range, are expected to support higher herbivore performance than plants in their native range (Wolfe, Elzinga, & Biere, 2004).

The outcome of plant–insect interactions in native and introduced ranges may not only depend on the genetic variation in the plant, but also on that in the herbivore. Most hypotheses on resource–herbivore interactions in the context of invasiveness (including the ERH and EICA), however, consider herbivores a static parameter, and as a consequence, comparisons of plant traits in native and exotic plant populations typically assay herbivores from a single genetic background (Carroll, Dingle, Famula, & Fox, 2001). Introduced plants however, are rarely devoid of attackers and soon after introduction are engaged in trophic interactions with herbivores that can potentially affect co-evolution (Verhoeven, Biere, Harvey, & van der Putten, 2009; Harvey, Bukovinszky, & van der Putten, 2010; Bezemer, Harvey, & Cronin, 2014). Introduced plants may be engaged in novel interactions with herbivores in different ways, which is a topic that to date has received limited attention (Parker, Burkepile, & Hay, 2006). When generalist herbivores attack introduced plants for example, the novel chemical plant defences may be effective in reducing herbivore performance and

increasing plant reproductive success. However, novel specialists may also attack plants in their introduced ranges, for example when a herbivore species is associated with relatives of the invading plant (Agrawal et al. 2005). As related native and invasive plants may share traits in defence chemistry, consumers may be permitted to efficiently switch to a novel host (Arnett & Louda 2002; Maron, Combs, & Louda, 2002; Louda, Pemberton, Johnson, & Follett, 2003; Agosta & Klemens 2008; Harvey, Ximénez de Embún, Bukovinszky, & Gols, 2012). Accordingly, predictions on herbivore performance in response to novel food plants are less straightforward, because the plant and herbivore do not necessarily share a history of adaptation and counter-adaptation in specific defence traits (Agosta 2006). Despite its potentially important implications for resource–consumer interactions in the context of species introductions, the role of genetic variation in the plant as well as in the herbivore have received limited attention (but see e.g. Schaffner et al. 2011). In the current study we argue that genetic variation in both plants and herbivores is important for understanding community interactions and potentially invasion success.

Common milkweed (*Asclepias syriaca* L.) is native to eastern North America (NA) and has been repeatedly introduced to southern and central Europe (EU) since the 17th century (Gaertner 1979). In NA, *A. syriaca* is attacked by a rich community of specialist chewing and phloem sucking insects and plants deploy defence traits, such as toxic cardenolides and latex to reduce herbivory (Malcolm 1991; Agrawal & Konno 2009). EU plants have escaped most of their herbivores attacking them in NA. One specialist herbivore, the cosmopolitan oleander aphid *Aphis nerii* B-F is present on both continents and attacks members of the dogbane family (Apocynaceae), including *A. syriaca*. Release from most of its specialist herbivores may have resulted in adaptive population differentiation in *A. syriaca* populations at the continental scale (Agrawal et al., submitted for publication). *A. nerii* displays variation in population growth in response to variation in defensive and nutritional traits of *Asclepias* species (Agrawal 2004; Smith, Mooney, & Agrawal, 2008), and accordingly differences in *A. nerii* growth across EU and NA populations of *A. syriaca* may be expected. We assumed that *A. nerii* genotypes from NA and EU may respond differently to intercontinental variation in plant nutritional quality of *A. syriaca*. *A. nerii* from NA have interacted and possibly co-evolved with *A. syriaca* over a longer evolutionary timescale, whereas aphids in EU have more frequently interacted with other members of Apocynaceae. Besides *A. syriaca* and *Nerium oleander*, both being present in both continents, *A. nerii* in NA may develop on a large number of *Asclepias* species. In contrast, European alternative hosts of *A. nerii* may include *Vinca* spp. possibly *Vincetoxicum* and *Calotropis procera* (North-Africa) (Blackman & Eastop 1984).

We examined the development, fecundity and population growth of *A. nerii* on *A. syriaca* populations of EU and NA

origins. To test whether aphid responses to host plant origin also depend on aphid genotype, two lines of aphids, one from each continent, were compared on each of the plant populations. Overall, we expected that *A. nerii* performance would be better on EU than on NA plants, because the EU plants may have lost defences towards specialists. We also expected that *A. nerii* would develop differently across plant populations from EU and NA, because these aphids in EU have interacted with different alternative host species than in NA. Specifically, we expected that aphids perform either better or worse, due to adaptation of herbivores to “home” plants or adaptation of plants to “home” herbivores, respectively. Additionally, as specialist chewing herbivores of *A. syriaca* are only present in NA, when challenged by the monarch butterfly (*Danaus plexippus* L.), plants from the two continents may affect aphid growth in different ways. Thus, in a second experiment, we used monarch larvae to induce plants and test if plant-mediated induced responses impact aphids differentially between a NA and a EU plant population.

Materials and methods

Plants & aphids

Seeds from six populations of plants, three from each continent (EU and NA) were collected in 2009. Within each plant population, seeds were collected from 3 to 5 maternal plants separated by a few meters. Experimental plants were grown from a subset of randomly selected seeds. NA populations originated from Fredericton (New Brunswick, Canada) and Quebec City (Quebec, Canada), and Ithaca (NY, USA). All three EU populations originated from Hungary (Neszűr, Kisszékely and Érd), with distances ranging from 80 to 150 km between populations. All populations were located along old fields and roadsides. Within each population, ripe seed pods from 5 to 10 mother plants were collected along transects of 5 to 10 m that were then pooled for each population. In order to maximize germination success, seeds were disinfected and the tip of the pericarp was excised before letting them germinate on glass beads. Seedlings were transplanted into 2L-pots containing peat-based potting soil (Lentse potgrond, Lent, The Netherlands) that contained a standardized initial mixture of nutrients and 7 weeks later plants were fertilized weekly with 100 ml of half-strength Hoagland solution. Two days before introducing herbivores into the bioassays, plants were individually encaged. All experiments took place in a greenhouse at a 21 °C (± 2 °C) and 16 h daylight and at 18 °C (± 2 °C) and 8 h darkness regimes. Aphids from NA (Ithaca, NY) and EU (Kisszékely, HU) were collected from the same locations as *A. syriaca* seeds, and were reared on their respective plant population for 2–3 months until the bioassay started.

Plant and herbivore population origin and aphid performance

For each plant population we grew 10–14 plant individuals. Half of the plants of each population received the NA aphids, whereas the other half received the EU aphids. Individual plants were caged and assigned to one of 12 treatments (6 plant \times 2 aphid populations) and were distributed in the greenhouse compartment in a fully randomized design. Experimental animals were introduced onto plants as follows. The two first fully expanded leaves, facing opposite directions and originating from the same node were selected, and each received a clip-cage (2.5 cm \varnothing) with two late instar apterous aphids (i.e. generation G_0). When the first offspring (G_1) appeared, all other aphids were removed from the clip cage and the time until these aphids reached adulthood (first offspring appeared) was recorded. In order to measure aphid fecundity, from the moment of producing the first offspring (G_2), the number of subsequently produced offspring was recorded over a course of 72 h. Our approach to measure fecundity minimized maternal effects (Zehnder & Hunter 2007), because aphids were born onto the experimental plants and were of standardized age. After 72 h clip-cages were removed and aphids were counted on each plant twice, with a weekly interval, starting 20 days after introducing the first aphids (G_0) to the plants. Size and growth of plants were compared at the beginning and end of the bioassay by measuring plant height and the number of leaves on each plant.

Plant-mediated effects of caterpillar-induction on aphid performance

In this experiment, we used a NA population (Quebec, CA) and a EU (Neszúr, HU) population of *A. syriaca*, and a EU (Kisszékely, HU) line of aphids. Plants of each population were assigned to one of two groups. The treatment group received both aphids and larvae of the monarch butterfly (*D. plexippus*), a specialist chewing caterpillar of milkweeds; the control group of plants received aphids only. Plants were subjected to caterpillar damage as follows. After introducing aphids onto plants as described in the first experiment, a single early L2 *D. plexippus* caterpillar was placed in a clip cage on the nearest leaf and was removed 24 h later by when it consumed the available leaf tissue (1.8 cm²). Six days later, plants were once again subjected to caterpillar damage using the same method. Initially, ten plants were assigned to each of the four treatments, but aphids in this bioassay showed low success in settling behaviour possibly due to a pathogen infection so that the experimental treatments had between 4 and 10 replicates.

Statistics

When necessary, data was $\log(x)$ transformed in order to fulfil the assumption of normality. To compare aphid population growth, aphid count data was used to calculate the mean

specific population growth rates as $GR = \ln(X_n/X_0)/(t_n - t_0)$, where X is the number of aphid individuals per plant measured at time t , at the start of the experiment (0) and at $n = 1$ and 2 counting censuses. In the analysis, population growth rates were averaged across the two censuses for each plant. On a subset of experimental plants, one of the two aphid cohorts failed to establish (probably due to pathogen infection), resulting in varying initial aphid densities. Population growth of *A. nerii* depends strongly on initial densities (Agrawal 2004; Agrawal, Underwood, & Stinchcombe, 2004), which was also confirmed in our preliminary analysis. Therefore in the analysis of aphid population growth, only plants were considered on which both aphid cohorts established and reproduced. The effects of aphid and plant origin on development, fecundity and population growth of aphids were analysed using random effects mixed models with continental origins of the plant populations and those of the aphids as fixed effects. The three plant populations in each continent were considered a random factor (nested within continent) in all analyses. Furthermore, in the analysis of development time and fecundity of aphids, the relatedness between measurements on the two aphids developing on the same plant was taken into account by including plant identity as a random factor in the model. To test whether *A. nerii* performed better (i.e. adaptation of the herbivore to the “home” plant) or worse (i.e. adaptation of the plant to the “home” herbivore), we also carried out the above analysis with the six plant populations included as a fixed factor, and subsequently conducted linear contrasts. In the second experiment, a Generalized Linear Model for binary data was used to analyse data on aphid survival with plant population and caterpillar induction as fixed effects. Other data was analysed using General Linear Models and the residuals of the fitted models were tested for normality using the Shapiro–Wilk’s test. All analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Plant and herbivore population origin and aphid performance

Development time of aphids ranged from 8.5 (± 0.4 SE) to 10.8 (± 0.6) days across the six *A. syriaca* populations. There were differences in developmental time of aphids both between aphid lines and between plant populations of different continental origin. Aphids from the NA (Ithaca) line developed 6.8% faster than those of the EU (Kisszékely) line (Fig. 1A, B, $F_{1, 73.8} = 5.31$, $P = 0.024$). Although continental origin of the plant populations had no overall effect on aphid developmental time ($F_{1, 1.76} = 4.37$, $P = 0.189$), NA aphids developed 13.7% faster on EU than on NA plant populations, whereas the developmental time of EU aphids was not influenced by the continental origin of plant populations (Fig. 1A, aphid line \times plant origin interaction: $F_{1, 74.2} = 5.22$,

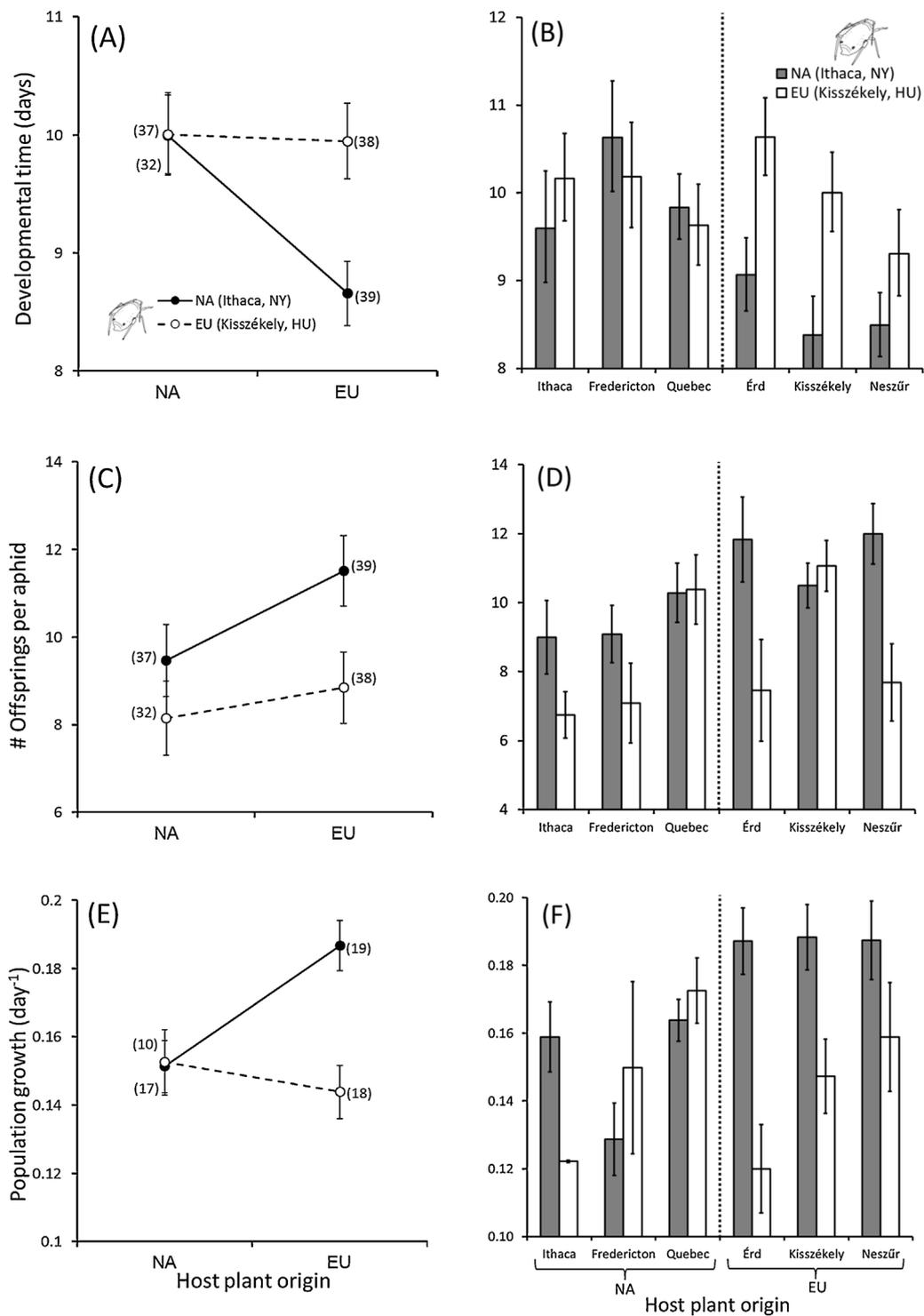


Fig. 1. Performance of two lines of aphids (*Aphis nerii*), originating from North America (Ithaca, NY, closed circles) and from Europe (Kisszékely, Hungary, open circles), and developing on milkweed (*Asclepias syriaca*) plants originating from NA (solid line) or EU (dashed line) populations. (A and B) Time (days, \pm 1SE) until first reproduction, (C and D) offspring production of aphids 72 h after reaching adulthood, and (E and F) population growth rate (individuals individuals⁻¹ day⁻¹ \pm SE) of aphids. Values are (A, C and E) least square mean estimates from random effects mixed models (with number of experimental animals in parentheses), and (B, D and F) are observed means shown separately for the six populations of *A. syriaca*.

$P=0.025$). Three days after reaching adulthood, the fecundity of NA aphids was 19% higher than the fecundity of the EU line (Fig. 1B, $F_{1,77}=9.85$, $P=0.002$, Fig. 1C). Although fecundity showed considerable variation across the six populations ranging from 6.8 (± 0.7) to 12 (± 0.9) offspring produced per individual after 72 h (Fig. 1D), there was no overall effect of continental origin ($F_{1,2.1}=1.98$, $P=0.289$) or an interaction between aphid line and plant origin ($F_{1,77.2}=1.13$, $P=0.290$).

The differences observed in aphid development times and fecundity were reflected in the observed differences in aphid population growth. The specific growth rate of the EU aphid line was on average 15.4% lower than that of the NA (Ithaca) line (Fig. 1E; $F_{1,53.6}=7.9$, $P=0.007$). Aphid population growth showed variation across the six populations, ranging from 0.11 (± 0.01) to 0.19 (± 0.01 , individuals individual⁻¹ day⁻¹, Fig. 1F). Although continental origin of plants had no overall effect on aphid population growth ($F_{1,2.05}=2.29$, $P=0.267$), the response to *A. syriaca* continental origin depended on the *A. nerii* line (Fig. 1E, aphid line \times plant origin interaction: $F_{1,53.8}=8.91$, $P=0.004$).

We also tested whether aphids performed better or worse on their “home” than on “away” plant populations. Both the development time ($F_{5,67.9}=2.38$, $P=0.048$) and growth rates ($F_{5,49}=2.58$, $P=0.038$) of aphids showed significant variation among populations (Fig. 1B and F), whereas the differences in fecundity between populations were marginal ($F_{5,72.8}=2.34$, $P=0.05$, Fig. 1D). Furthermore, the patterns of differences in the growth rates across the six plant populations were different for the two *A. nerii* lines (interaction, $F_{5,49}=3.26$, $P=0.013$). However, aphid performance was neither the highest, nor lowest on the “home” plant populations (i.e. population of origin, Fig. 1B, D and F).

At the onset of the experiment, the average plant height was similar across the populations. The number of leaves per plant was different, with populations from Ithaca and Quebec having 1–2 leaves less than plants from the other populations. Neither of these variables however, differed across the six populations at the end of the experiment ($P>0.05$). In the course of the experiment, however, aphids had a significant effect on plant growth. The number of leaves recorded at the end of the experiment showed a negative correlation (Pearson corr. = -0.56) with an increase of aphid population size ($F_{1,75.4}=22.27$, $P<0.001$). This relationship was unaffected by the origin of aphid line ($F_{1,75.1}=0.03$, $P=0.862$) or plant population ($F_{1,2.23}=0.07$, $P=0.814$).

Plant-mediated effects of caterpillar-induction on aphids

Aphids developed 17.6% faster on EU (Neszűr) than on NA (Quebec) plants ($F_{1,26}=6.22$, $P=0.019$, Fig. 2) and had a higher survival rate ($\chi^2_1=8.85$, $P=0.003$) and higher population growth rates ($F_{1,14}=5.394$, $P=0.036$) on EU

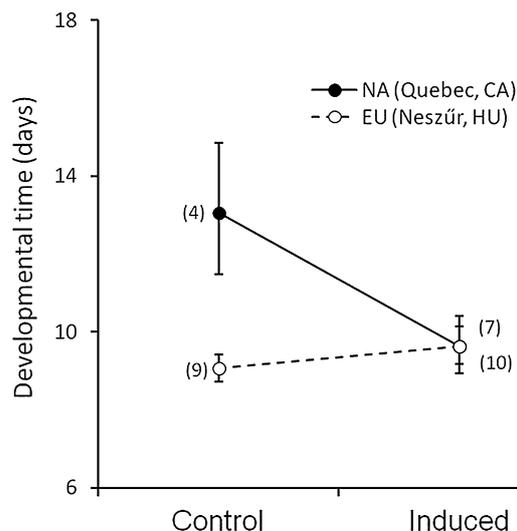


Fig. 2. Average number of days (back-transformed, $\pm 1SE$) that aphids (*Aphis nerii*) originating from Europe needed to reach maturity on milkweed (*Asclepias syriaca*) plants that originated from populations in North America (Quebec, CA) and Europe (Neszűr, HU). Plants were either previously subjected to induction by larva of the monarch butterfly (*Danaus plexippus*) or were uninduced by caterpillars. The number of replicates given in parentheses.

plants. Induction had contrasting effects on the development time of aphids; caterpillar induction accelerated aphid development on plants from the Quebec population by 26%, whereas it did not affect development on Neszűr plants (Fig. 2, Plant population \times Induction interaction: $F_{1,26}=6.2$, $P=0.019$). Caterpillar induction, however, neither influenced aphid fecundity ($F_{1,22}=1.142$, $P=0.297$) nor aphid population growth ($F_{1,14}=0.901$, $P=0.359$). Although aphid fecundity tended to be higher on EU plants, this effect was not significant ($F_{1,22}=3.37$, $P=0.08$).

Discussion

Our results show variation in the development, fecundity and population growth of *A. nerii* on European (EU) and North American (NA) populations of *A. syriaca*. Both aphid lines showed differences in these performance measures across milkweed populations, indicating geographic variation in plant quality. Population growth of *A. nerii* is influenced by variation in several plant traits such as defence chemistry, nutrient content and leaf hairiness (Agrawal 2004). These plant traits may explain variation in aphid population growth between milkweed species and between *A. syriaca* populations (Agrawal 2004; Smith et al., 2008; Agrawal et al. 2009).

We found that development and population growth of *A. nerii* were related to the continental origin of *A. syriaca* populations. Faster development and higher population growth of NA *A. nerii* was observed on EU plants. A recent intercontinental analysis of *A. syriaca* populations, which

included some of the populations used in this study, found continental divergence in plant nutritional traits between EU and NA plants, such as leaf nitrogen content, and inducibility of defences in response to monarch feeding (Agrawal et al., submitted for publication). It is thus plausible that the observed effects of continental origin of *A. syriaca* on NA *A. nerii* performance reflected intercontinental differences in plant traits. The pattern observed in the performance of the NA line of *A. nerii* here is consistent with the enemy release hypothesis, where plant populations that are exposed to lower herbivore pressure in introduced range invest less into costly defences that were active against the enemies that they left behind. In that case we would indeed expect that the enemies from the original (NA) range would experience increased performance when tested on plants from the introduced range (Zangerl & Berenbaum 2005; Berenbaum & Zangerl 2006; Zangerl et al., 2008). We did not find evidence of local adaptation. When comparing aphid performance on plant populations from their place of origin compared to other populations, we did not find that aphids exhibited the highest or lowest performance on plants from their home populations. Ultimately, a larger set of aphid clones should be tested to explain whether the observed genotypic differences between the aphid lines reflect consistent intercontinental differences between aphid populations, or the observed differences originate from clonal variation between aphid colonies, such as founder effects.

We did find that the effect of plant population origin on aphid performance depended on the aphid line tested. In contrast to NA (Ithaca) aphids, aphids from EU (Kisszékely) performed similarly on plant populations originating from the two continents. Thus our data show that it is not only the genetic variation in the plant that determines the outcome of plant–insect interactions, but also that in the herbivore. An interesting venue for future research is to determine whether and how the genetic variation in the herbivore can be a factor that interacts with and perhaps shapes genetic differentiation in native and introduced populations of invasive plants (Garrido, Andraca-Gómez, & Fornoni, 2012; Suwa & Louda 2012; Bischoff & Hurault 2013). At present, we may only speculate on the possible importance of genetic variation in herbivores for the evolution of plant–insect interactions. Besides the presence or absence of alternative food plants, an important factor in this process may be the details in the biology and population genetics of the herbivore that limits, or enables, the possibility for co-evolutionary interactions. However, *A. nerii* populations show geographic conservatism (Groeters 1992) and local populations in temperate habitats are often transient (Groeters 1989, 1992). Also, the genetic diversity across *A. nerii* populations is low (Harrison & Mondor 2011), all are factors that make rapid evolutionary adaptation unlikely. Nevertheless, clonal variation and population differences in morph production have been found in *A. nerii* (Groeters 1989), indicating that genotypic differences between colonies

may occur. Genotypic differentiation in obligate parthenogenetic aphids then may occur through mutation, heritable epigenetic variation, or changes in endosymbiotic microbial communities (Ferrari & Godfray 2003; Wilson, Sunnucks, & Hales, 2003; Ferrari, Godfray, Faulconbridge, Prior, & Via, 2006; Mandrioli, Azzoni, Lombardo, & Manicardi 2011).

In the second experiment, we tested the hypothesis that plants, having co-evolved with different herbivore communities, will mediate indirect interactions between herbivores in different ways. In comparison with control plants that contained aphids only, induction by *D. plexippus* caterpillars accelerated aphid development on NA (Quebec) plants, whereas no effect of caterpillar induction was observed on *A. nerii* development on EU (Neszúr) plants. A recent study showed intercontinental divergence in defence chemistry of *A. syriaca* populations, and showed that upon *D. plexippus* induction, changes in salicylic acid and cardenolide concentrations were related to plant continental origin (NA or EU) (Agrawal et al., submitted for publication). Salicylic acid is a plant hormone involved in the regulation of defence chemistry (not only cardenolides) against pathogens and sucking insects (Thaler, Humphrey, & Whiteman, 2012; Ponzio, Gols, Pieterse, & Dicke, 2013). *A. nerii*, as a specialist, copes well with high concentrations of cardenolides, as it is able to sequester these compounds (Groeters 1993; Mooney, Jones, & Agrawal, 2008). Consequently, variation in cardenolide concentrations within *A. syriaca* may not always affect *A. nerii* performance (Helms, Connelly, & Hunter, 2004; Mooney et al., 2008). Although the differences in aphid development might reflect differences in defence chemistry between induced and control plants of NA (Quebec) and EU (Neszúr) origin, the exact traits that determine plant quality for *A. nerii* across these *A. syriaca* populations remain to be elucidated. Moreover, the differential effect of caterpillar induction on aphids of these two populations of plants should be considered weak, because the differences in developmental time were not reflected in differences in aphid population growth and fecundity. A possible reason for this observed weak effect on aphid development may be that *A. nerii* is little influenced by interspecific competition with other herbivores (Mooney et al., 2008; Smith et al., 2008). Thus the effects observed here may be stronger when measured on other (generalist) aphids on *A. syriaca*, which are less dominant competitors and respond stronger to changes in secondary chemistry.

Recent studies suggest that changes in the composition of herbivore communities associated with plants in native and introduced ranges may affect biological control of invasive species (Maron & Vila 2001; Harvey et al., 2010; Suwa & Louda 2012). Our study highlights the role of genotypic differences in maintaining variation in plant–herbivore interactions across native and introduced plant populations. Future studies of plant invasions should not only consider the genetic variation across plant populations, but also across the populations of the associated herbivores.

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