

Local adaptation: simultaneously considering herbivores and their host plants

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Summary

- Although a major expectation of coevolutionary theory between plants and herbivores is the occurrence of reciprocal local adaptation, this has remained almost untested. Thus, we evaluated the presence and variation in the patterns of reciprocal local adaptation between an herbivorous insect and its host plant.
- Two four-by-four cross-infestation experiments were performed under similar abiotic conditions. The first one was done under laboratory conditions to estimate herbivore individual performance while the second one was performed in a common garden to simultaneously estimate herbivore population growth rate as well as seed production and plant defenses (resistance and tolerance to herbivory).
- The patterns of population differentiation for the herbivore and the plant were not independent of each other, showing all the possible outcomes from locally adapted to maladapted populations. These results indicate differences in the magnitude of local adaptation. While an association between resistance and herbivore performance was observed, there was no clear pattern between tolerance and herbivore local adaptation.
- Our results demonstrated the occurrence of reciprocal local adaptation following the pattern expected by theory: when the herbivores or the plants were adapted, the other species was non-adapted or even maladapted.

Introduction

How natural selection acts on traits to produce adaptations to different environments remains a central question in evolutionary biology. The biotic component of the environment experienced by every organism is usually variable in space and time (Thompson, 2005). This is particularly true for coevolutionary victim–exploiter systems, where each species constitutes an ever-changing environment to which its opponent has to adapt (Gandon & Michalakis, 2002). The geography of coevolutionary responses may lead to variation among populations in the patterns of local adaptation for both the victim and the exploiter (Futuyma & Slatkin, 1983; Thompson, 2005) because of the relative intensity of selection acting on each species (i.e. selection mosaics) and the differential rates of gene flow (Gandon *et al.*, 1996; Gomulkiewicz *et al.*, 2000; Gandon & Michalakis, 2002). Although the study of local adaptation has received much attention in both plants (Leimu & Fischer, 2008) and animals (Greischar & Koskella, 2007), there are few studies evaluating how the biotic component of the environment affects the patterns of reciprocal local adaptation among interacting species (see Ortégón-Campos *et al.*, 2009; Bischoff & Trémulot, 2011).

In a coevolutionary process, when natural enemies are locally adapted to their hosts, their performance is expected to be higher

than that achieved by ‘foreign’ enemies (Kawecki & Ebert, 2004). As a consequence of enemy adaptation, hosts would suffer higher levels of damage when consumed by their native enemies than when eaten by foreign ones because of reduced efficiency in host resistance (Lively & Dybdahl, 2000). Under this scenario, it has been suggested that hosts would defend themselves through a tolerance response to ameliorate the negative effect of their natural enemies (Jokela *et al.*, 2000). Conversely, when hosts are locally adapted to their enemies, they are expected to experience lower amounts of damage (i.e. higher levels of resistance) when exposed to their local enemies than when consumed by foreign ones. Thus, local natural enemies would perform less well when consuming their local hosts. Testing for patterns of local adaptation in both interacting species can help us evaluate how the ecological interactions shape the geographic patterns of population differentiation and the role of specific traits involved in the interaction in the extent of local adaptation. Although these expectations constitute a central tenet of coevolutionary theory, there have been no attempts to test them simultaneously in both interacting species.

Reciprocal transplant experiments have been the standard approach to test for local adaptation. This experimental design allows the examination of the relative importance of the interaction between genetic and environmental variation in

determining individual fitness (Antonovics & Primack, 1982). However, when individuals are transplanted to a different environment and their fitness is lower than that achieved by the native genotypes, such a pattern of local adaptation can be the result of differences in some abiotic component of the environment (e.g. temperature, altitude, humidity) between the native and the foreign site and/or differences in the genetic composition of the interacting species (Kawecki & Ebert, 2004). Thus, this experimental approach is not sufficient to determine the causes of adaptation because neither the physical nor the biotic environment are usually manipulated or controlled (Biere & Verhoeven, 2008; Crémieux *et al.*, 2008). Here, we were interested in evaluating if there is an effect of the biotic environment on the patterns of local adaptation, thus we controlled the physical conditions while measuring the responses of the interacting species under different combinations of native and foreign populations.

Using an herbivorous insect–plant system, we first determined if the herbivore and plant populations were genetically differentiated in fitness and in those traits involved in the interaction. Second, we evaluated the extent of local adaptation of the herbivore to its host plant. The reciprocal question of whether the host plant is adapted to its herbivore was also addressed. To answer these questions, two four-by-four cross-infestation experiments were performed under similar abiotic conditions to determine the possible effects of biotic interactions on the patterns of local adaptation.

Materials and Methods

Study system

Datura stramonium L. (Solanaceae) is a hermaphroditic annual plant that grows from tropical forests to xerophytic shrublands on areas where natural vegetation is highly disturbed (i.e. areas around garbage dumps, bare-soil spots within agricultural fields and along the roads). Within these modified environments the species interact with a small group of folivorous insect herbivores in Central Mexico: *Lema trilineata* and *Epitrix* sp. (both Chrysomelidae), *Manduca sexta* (Sphingidae) and *Sphenarium*

purpurascens (Pyrgomorphidae). Overall, *D. stramonium* experiences average levels of damage between 10–50% of total leaf area (Valverde *et al.*, 2001). A recent survey of 32 populations in the Central region of Mexico revealed that both beetle species were the only consumers observed (J. Hernández-Cumplido & J. Fornoni, pers. obs.). However, other reports indicate the presence of two less common enemies, *M. sexta* and *S. purpurascens* that are rarely observed (Núñez-Farfán, 1991). Further experimental manipulation of the presence of the two most common consumers indicated that *Epitrix* sp. consumed half the plant leaf area consumed by *L. trilineata* (D. Carmona & J. Fornoni, unpublished), indicating that, when present, this latter beetle is likely to be responsible for the majority of the foliar damage observed in the field. All but the pupa stage of *L. trilineata* occur on the leaf tissue of its host, where it can survive and reproduce for up to four generations per season (E. Garrido, pers. obs.). Previous studies indicate that herbivore damage reduces the fitness of *D. stramonium* (Valverde *et al.*, 2001) while plant resistance has a negative effect on the survival of *L. trilineata* (Espinosa & Fornoni, 2006).

Experimental design

Four populations in Central Mexico were chosen: Esperanza, Pedregal, Teotihuacan and Tula. These four populations are present within the same type of plant community (xerophytic shrubland), have similar climatic and soil conditions, and show high levels of herbivory by *L. trilineata* (J. Hernández-Cumplido & J. Fornoni, unpublished) (Table 1). Geographic distances between populations varied from 52 to 245 km. In addition, these populations present significant levels of genetic structure for both the herbivore ($F_{ST} = 0.09$; $P < 0.0001$; using 11 polymorphic enzymatic loci) and the plant ($F_{ST} = 0.39$; $P < 0.0001$; using five nuclear microsatellites) (G. Andraca-Gómez *et al.*, unpublished; see Table 2). To test for herbivore and plant local adaptation, two four-by-four cross-infestation experiments were performed during 2005 and 2006. The first experiment was done under laboratory conditions to estimate herbivore individual performance while the second experiment was performed in a

Table 1 Abiotic and biotic characteristics of the four populations included in the study

Population (State)	Geographic coordinates	Altitude above sea level (m asl)	Annual precipitation* (mm)	Mean annual temperature* (°C)	Soil pH [†]	Organic matter [‡] (%)	P [†] (kg ha ⁻¹)	K [†] (kg ha ⁻¹)	Herbivore damage [‡] (%)
Esperanza (Puebla)	18.85° N, 97.37° W	2278	801.0	13.4	7.4	0.2	18.2	19.5	37.51
Pedregal (Distrito Federal)	19.32° N, 99.19° W	2200	691.0	16.3	5.75	18.3	15.3	132.6	30.64
Teotihuacan (State of Mexico)	19.68° N, 98.86° W	2294	563.3	14.9	8.3	1.0	13.6	19.5	51.59
Tula (Hidalgo)	20.05° N, 99.35° W	2020	618.7	17.5	7.8	3.5	22.2	35.1	37.24

*Data taken from García (1988). Values were obtained from 9 to 53 yr of climatic records.

[†]Soil information was obtained from INEGI (2010) and J. Campo (unpublished).

[‡]Percentage of herbivore damage was calculated at the population level from a sample of 10 randomly selected leaves from 30 plants at each site. *Lema trilineata* was the main folivore insect observed in all populations (J. Hernández-Cumplido & J. Fornoni, unpublished).

Table 2 Pairwise F_{ST} estimates for four populations of the herbivore *Lema trilineata* (above diagonal) and its host plant *Datura stramonium* (below diagonal)

	Esperanza	Pedregal	Teotihuacan	Tula
Esperanza	–	0.16	0.13	0.14
Pedregal	0.56	–	0.04	0.07
Teotihuacan	0.29	0.47	–	0.03
Tula	0.39	0.27	0.25	–

All values had a $P < 0.0001$. All 11 polymorphic enzymatic loci used for the herbivore had between two and three alleles per loci. Of the five nuclear microsatellites used for the plant one had five alleles while the remaining four had two. All analyses were performed in F_{STAT} version 2.9 (Goudet, 1995).

common garden to simultaneously estimate herbivore population growth rate as well as seed production and resistance and tolerance to herbivory.

Experiment 1 During the summer of 2005, seeds collected in 1999 from 30 maternal families per population were germinated. Two weeks after germination, 30 plants per population (one plant per family \times 30 families \times four populations, $n = 120$) were individually transplanted into 4-l pots, filled with potting soil, and placed in a glasshouse at the Instituto de Ecología (UNAM). Simultaneously, *c.* 100 adults of *L. trilineata* from each population were collected and taken to the laboratory. Herbivores were allowed to reproduce for 1 wk to obtain *c.* 30 clutches per population ($n = 827$). After eclosion (August 2005), each clutch was divided in four groups so that each group could be fed with leaves from each plant population. There were at least six larvae per clutch per plant population. All the larvae were individually reared and their survival was checked daily until adult emergence (for a description of the rearing technique see Espinosa & Fornoni, 2006). Mean survival time was calculated for each plant–herbivore combination. For each individual larva, the efficiency of food consumption, developmental time and adult mass was also recorded. Efficiency was estimated as weight gained relative to the proportion of leaf area consumed. Weight gain was estimated as the increment in mass between the second and fourth larval stages relative to the number of days between these two stages. Leaf area consumed by each larva was calculated using a Digital Image Analysis System (WinDias Basic; Delta-T Devices Ltd, Cambridge, UK). Developmental time was considered as the number of days between hatching and adult emergence. Adults were weighted to the nearest mg using a digital balance (OHAUS, Parsippany, NJ, USA). To obtain a more accurate fitness estimate, individual performance was calculated as the product of the relativized values of mean survival time, efficiency of food consumption, inverse of developmental time and adult mass. Therefore, high values of individual performance should be interpreted as larvae that, on average, survived more days, were more efficient, completed their development quickly and were bigger as adults. For all these variables, a significant interaction between herbivore and plant population was detected previously, supporting the assumption that all these variables could be responsible for the variation in performance (see the

Supporting Information, Table S1). Because herbivore performance (Table S2) was estimated as the product of four variables, it is possible that high correlations between these variables could overestimate the values of performance. In order to estimate the severity of multicollinearity, the variance inflation factors (VIF) were calculated for each variable (Neter *et al.*, 1996; p. 386). All VIFs were between 1.03 and 1.08, which means that there is no overestimation of the F ratio in the ANOVA for herbivore performance.

Experiment 2 The following year (August 2006), 60 plants per population (two plants per family \times 30 families \times four populations, $n = 240$) were obtained as already described. Two months after planting, all the plants were taken to a common garden located in the south part of Mexico City (*c.* 18 km from the nearest population). At the common garden, four plants from the same population were placed inside mesh-cages (60 cages of $65 \times 70 \times 75$ cm each). Simultaneously, herbivores were collected as described in the first experiment and allowed to reproduce in the laboratory. After hatching, larvae originating from each experimental population were randomly placed at a constant density (three larvae per leaf), which controlled for initial plant size and minimized intraspecific competition, on plants from all four populations. That is, within each cage plants were only exposed to larvae from one population. In addition, a control treatment (i.e. plants inside cages without herbivores) was included. All the treatments were replicated three times (three cages per plant–herbivore combination, including the control one). Because *D. stramonium* experiences inbreeding depression (Núñez-Farfán *et al.*, 1996) and variation in the extent of autonomous selfing owing to herkogamy, all the plants were manually outcrossed within populations. The outcrosses were performed within each cage after 18:00 h with the help of a brush containing pollen from four donors from the same cage. This procedure was done throughout the flowering period to avoid possible sources of variation in fitness among plants that were not related to our treatments. Two months later (October 2006), when all the adults from the initial larvae emerged and reproduced, the total number of eggs, the larvae from all instars and the adults per combination treatment were counted. Afterwards, when all the plants ceased flower production, all the leaves and fruits per plant were cut and stored in paper bags for 3 wk at 25°C. Plant damage was estimated using a Digital Image Analysis System (WinDias Basic; Delta-T Devices Ltd) and total seed number was counted. Given the high levels of herbivore mortality observed in the common garden, herbivore population growth rate was estimated for each plant–herbivore combination ($n = 16$) as the ratio between final and initial number of first-instar larvae. Because there were eggs and larvae from different instars at the end of the experiment, we expressed all these life stages in terms of first-instar larvae using the transition probabilities obtained in the laboratory. Resistance to herbivory was estimated per plant as one minus the proportion of leaf area damaged (Simms & Rausher, 1987). Tolerance to herbivory was estimated for each herbivore–plant combination treatment as the difference in seed production between damaged and undamaged

(control) plants divided by the seed production when damaged (Strauss & Agrawal, 1999). Thus, the values of tolerance represent the proportional increase/decrease in seeds as a result of herbivory and include both genetic and environmental effects.

Statistical analyses

All the variables except seed production were analysed with a two-way ANOVA including herbivore population, plant population and their interaction as sources of variation. Because there was only one replicate per combination treatment for the variable of herbivore population growth rate, we followed the procedure developed by Tukey (Kutner *et al.*, 2005) for a two-way analysis of variance with a non-replicated design (Type III sum of squares, CATMOD, SAS Institute, 1999). This method allows for the estimation of the *F*-statistics corresponding to the interaction term based on the comparison of the residual sum of squares that results from adjusting the interaction term vs the total squares sum (Milliken & Johnson, 1989; Kutner *et al.*, 2005). Finally, seed production was analysed with an ANCOVA including the same factors mentioned earlier and initial plant size as a covariate. All the variables measured followed a normal distribution and the analyses were performed in JMP 7.0 (SAS, 2007). Differences between sympatric and allopatric plant and herbivore responses were compared using a Tukey test.

Patterns of local adaptation Both the home-away and the local-foreign criteria were used to test for local adaptation (i.e. differentiation) as these answer complementary questions regarding local adaptation (Thrall *et al.*, 2002; Kawecki & Ebert, 2004). The home-away criterion compares the response of a focal population across habitats, that is, it tests in which habitat a given genotype or population perform better. When organisms have higher performance when growing under sympatric than allopatric conditions, maternal effects can be involved. By contrast, the local-foreign criterion emphasizes the comparison between herbivores or plants of different provenances within the habitat of the native resident. Therefore, it is a test of the hypothesis that herbivores or plants performed better at their native site relative to foreign genotypes, as expected under divergent patterns of local adaptation (Kawecki & Ebert, 2004). Thus, because local adaptation results from natural selection in response to local conditions (Sork *et al.*, 1993), the local-foreign criterion provides a direct demonstration of local adaptation (D. Ebert, pers. comm.).

Coefficient of local differentiation To describe the variation in the extent of local adaptation among the herbivore and plant populations, we estimated the degree of local differentiation for each population with the following equation

$$\phi = \frac{1}{n} \sum_{a=1}^n 1 - \frac{\bar{x}_a}{\bar{x}_s} \quad \text{Eqn 1}$$

where \bar{x}_a and \bar{x}_s represent the mean response of an herbivore or plant population in allopatric and sympatric combinations,

respectively, and *n* stands for the number of allopatric combinations. This coefficient is basically an average of all the deviations between the responses achieved in sympatric treatments relative to allopatric ones. The appropriateness of our coefficient depends on the number of populations used because extreme high values of performance on any combination could bias the result of the equation. That is, the effect of high values of performance achieved under any combination treatment could be diluted as more populations are being compared. Our coefficient ϕ can be used to estimate local differentiation (i.e. adaptation) following the both criteria mentioned above. For both criteria, \bar{x}_s is the mean performance of a given population in its native site (sympatry). For the home-away criteria, \bar{x}_a values correspond to the mean performance of a single population across different habitats. Conversely, for the local-foreign criteria the \bar{x}_a values represent the mean performance of different populations growing within a single habitat (see the Supporting Information, Note S1 for a detailed explanation). Positive values denote that organisms had higher performance on sympatric than on allopatric treatments (i.e. locally adapted), negative ones indicate the opposite (i.e. maladaptive) and values near zero indicate no differences in performance between sympatric and allopatric combinations (i.e. not adapted). In order to estimate the confidence intervals (95%) of ϕ , a standard jackknife procedure was performed.

Results

There was population differentiation for all the variables measured in both the herbivores and the plants (Table 3). Herbivores from Esperanza had the highest individual performance compared with the performance achieved by herbivores from Teotihuacan and Tula while herbivores from Pedregal had an intermediate value (Table 4). Herbivores from Pedregal showed the highest population growth rate, although this value was only different from the population growth rate achieved by herbivores from Tula (Table 4). However, plants from Esperanza and Tula produced more seeds than plants from the other populations (Table 4). Interestingly, plants from Tula expressed higher levels of both resistance and tolerance to herbivory (Table 4), and no evidence of a possible negative correlation between both defensive strategies among populations was observed. In addition, we detected an effect of the biotic environment on all the traits measured except herbivore population growth rate. That is to say, plant population had a significant effect on herbivore individual performance while herbivore population had an effect on all the plant traits measured (Table 3). Specifically, when herbivores consumed plants from Teotihuacan their individual performance was higher compared with the performance achieved by herbivores consuming plants from Tula (Table 5). By contrast, plants eaten by herbivores from Tula produced more seeds than plants eaten by herbivores from Esperanza and Pedregal (Table 5). This result is probably related to the fact that herbivores from Tula seem to induce both greater resistance and tolerance in their host plants (Table 5), thus reducing the amount of damage and the negative impact that damage has on seed production. These results indicate that both resistance and

Table 3 Results from the ANOVAs and ANCOVA for all the traits measured to test for herbivore and plant local adaptation. *F*-values and degrees of freedom are shown along with statistical significance

Herbivore traits	Sources of variation			Initial plant size
	Herbivore population	Plant population	Herbivore population × plant population	
Individual performance	21.01 _{3,320} ***	15.05 _{3,320} ***	16.68 _{9,320} ***	–
Population growth rate	4.14 _{3,9} *	1.02 _{3,9}	0.01 _{1,8} †	–
Plant traits				
Seed production	8.57 _{3,175} ***	8.22 _{3,175} ***	1.15 _{9,175}	64.18 _{1,175} ***
Resistance	82.14 _{3,176} ***	6.09 _{3,176} ***	3.59 _{9,176} ***	–
Tolerance	6.90 _{3,32} **	19.65 _{3,32} ***	1.23 _{9,32}	–

Initial plant size was only included as a covariate in the analysis of seed production.

*, *P* < 0.05; **, *P* < 0.005; ***, *P* < 0.0001.

†This *F*-value was calculated following a Tukey test for a two-way ANOVA without replication, *n* = 16.

Table 4 Population differentiation in those traits measured to test for local adaptation

Population	Herbivore traits		Plant traits		
	Individual performance	Population growth rate	Seed production	Resistance	Tolerance
Esperanza	0.15 ± 0.011 A	1.15 ± 0.78 AB	846.90 ± 32.69 A	0.62 ± 0.03 B	–0.20 ± 0.07 B
Pedregal	0.13 ± 0.012 AB	3.68 ± 0.79 A	664.19 ± 31.43 B	0.58 ± 0.03 B	–0.05 ± 0.07 B
Teotihuacan	0.07 ± 0.006 C	2.68 ± 0.64 AB	686.63 ± 28.63 B	0.58 ± 0.04 B	–0.15 ± 0.04 B
Tula	0.10 ± 0.007 BC	0.60 ± 0.52 B	878.04 ± 42.16 A	0.69 ± 0.03 A	0.26 ± 0.03 A

Mean values ± SE are shown for each population. Letters indicate differences following a Tukey–Kramer test. Individual performance was estimated as the product of mean survival time, efficiency of food consumption, inverse of developmental time and adult mass. Therefore, high values of individual performance should be interpreted as larvae that survived more days, were more efficient, completed their development quickly and were bigger as adults (see the Supporting Information, Table S1). Population growth rate was estimated as the ratio between final and initial number of first-instar larvae. Resistance was estimated as the inverse of foliar damage while tolerance was calculated as the difference in seed production between damaged and undamaged plants.

Table 5 Effects of the biotic component of the environment on the herbivore and plant traits measured to test for local adaptation

Population	Effect of plant population on:		Effect of herbivore population on:		
	Individual performance	Population growth rate	Seed production	Resistance	Tolerance
Esperanza	0.12 ± 0.011 AB	2.71 ± 0.84 A	731.79 ± 37.05 B	0.63 ± 0.02 B	–0.07 ± 0.07 AB
Pedregal	0.12 ± 0.008 AB	1.75 ± 0.96 A	691.90 ± 35.80 B	0.40 ± 0.03 C	–0.17 ± 0.10 B
Teotihuacan	0.15 ± 0.019 A	1.17 ± 0.62 A	770.23 ± 36.55 AB	0.58 ± 0.02 B	–0.01 ± 0.06 AB
Tula	0.08 ± 0.011 B	2.47 ± 1.19 A	881.83 ± 31.57 A	0.85 ± 0.01 A	0.12 ± 0.05 A

Mean values ± SE are shown. Letters indicate differences following a Tukey–Kramer test. A description of how the variables were estimated can be found in the legend of Table 4. Individual performance was estimated as the product of mean survival time, efficiency of food consumption, inverse of developmental time and adult mass. Therefore, high values of individual performance should be interpreted as larvae that survived more days, were more efficient, completed their development quickly and were bigger as adults (see the Supporting Information, Table S1). Population growth rate was estimated as the ratio between final and initial number of first-instar larvae. Resistance was estimated as the inverse of foliar damage while tolerance was calculated as the difference in seed production between damaged and undamaged plants.

tolerance levels are influenced not only by the amount of damage plants experience but also by the herbivore provenance exerting the damage.

Patterns of herbivore local adaptation

A significant interaction between herbivore and plant population was detected for the variable of herbivore individual performance

(Table 3). Although we also estimated herbivore population growth rate, we did not find an interaction between herbivore and plant population for this variable (Table 3). Following the home-away criterion for individual performance we found no evidence that herbivores from Esperanza, Pedregal and Tula had higher performance when fed with their sympatric rather than allopatric plants (Fig. 1a). Accordingly, our estimations of the extent of local differentiation (ϕ) for each herbivore population

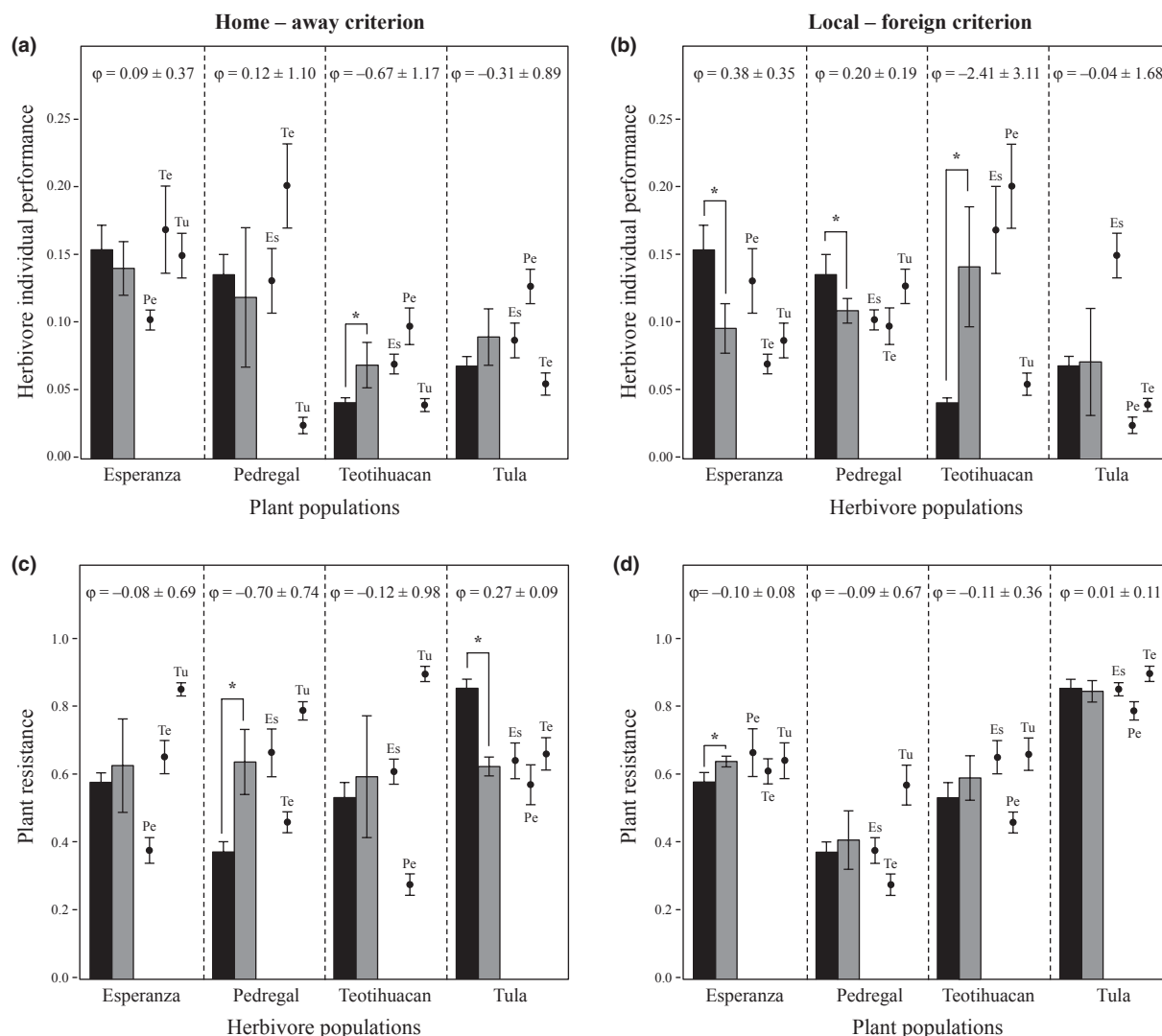


Fig. 1 Means \pm 1 SE for herbivore individual performance (*Lema trilineata*) and plant resistance (*Datura stramonium*) after two cross-infestation experiments using four pairs of interacting populations. Each panel shows the responses in sympatric (closed bar), average allopatric (tinted bar) and each allopatric combination (closed circles). Within each panel, asterisks show significant differences ($P < 0.05$ after Tukey test) between the sympatric and average allopatric combination, and the value for the coefficient of local differentiation (ϕ) is shown for each population. Positive values of $\phi \pm 95\%$ CI denote a higher response on sympatric treatments than on allopatric treatments (local adaptation), negative ones indicate the opposite (maladaptation) and values near zero indicate no differences in the response between sympatric and allopatric combinations (no adaptation). (a,c) Contrasts following the home-away criterion; (b, d) contrasts following the local-foreign criterion. Insect individual performance was estimated as the product of mean survival time, efficiency of food consumption, inverse of developmental time and adult mass. Therefore, high values of individual performance should be interpreted as larvae that survived more days, were more efficient, completed their development quickly and were bigger as adults. Conversely, plant resistance was estimated as the inverse of foliar damage.

were not significantly different from zero (Fig. 1a). Although herbivores from Teotihuacan showed lower performance on their sympatric than allopatric plants, our estimate of ϕ was not significantly different from zero after the jackknife (Fig. 1a), suggesting that this contrast should be considered with caution. The local-foreign approach asked whether sympatric herbivores showed better performance than herbivores from allopatric populations within their native habitat. When this criterion was followed, we found that herbivores from Esperanza ($\phi = 0.38$, jackknifed 95% CI = 0.345, $P = 0.0433$) and Pedregal ($\phi = 0.20$, jackknifed 95% CI = 0.196, $P = 0.0497$) were locally adapted to their plants (Fig. 1b). Herbivores from Tula showed

no evidence of local adaptation to their native plants ($\phi = -0.04$, jackknifed 95% CI = 1.679, $P = 0.4718$) while herbivores from Teotihuacan showed a tendency to be maladapted to their plants ($\phi = -2.41$, jackknifed 95% CI = 3.106, $P = 0.0756$) (Fig. 1b).

Patterns of plant local adaptation

We did not detect a significant interaction between herbivore and plant population for plant seed production; however, a significant interaction between herbivore and plant population for resistance was found (Table 3). These results indicate that plant resistance

could be involved in the reciprocal process of population differentiation, although we were unable to detect a significant interaction for fitness. In order to evaluate the expected association between the efficiency of plant resistance (i.e. changes in resistance against native vs foreign herbivores) and herbivore performance we estimated ϕ values for plant resistance. In this sense, the home-away criterion asks whether plants are better able to resist attack from their sympatric than herbivores than from their allopatric herbivores. Following this approach, plants from Tula were better able to resist the attack of their local herbivores ($\phi = 0.27$, jackknifed 95% CI = 0.094, $P = 0.0071$) (Fig. 1c). Plants from Esperanza and Teotihuacan presented similar levels of resistance when eaten by native vs foreign herbivores (i.e. ϕ values not different from zero) (Fig. 1c) and plants from Pedregal expressed marginally lower resistance when eaten by their native vs foreign herbivores ($\phi = -0.70$, jackknifed 95% CI = 0.742, $P = 0.0547$) (Fig. 1c). Conversely, the local-foreign criterion addresses whether native plants show greater resistance than allopatric plants when consumed by their native herbivores. Plants from Tula, Pedregal and Teotihuacan showed no differences in resistance (i.e. ϕ values not different from zero) while plants from Esperanza expressed significantly lower levels of resistance vs other plant populations when eaten by Esperanza herbivores ($\phi = -0.10$, jackknifed 95% CI = 0.080, $P = 0.0319$) (Fig. 1d).

Discussion

Gene flow and the patterns of local adaptation

The distribution of genetic variation, and specifically the pattern and extent of migration between interacting species, could determine the geographic scale at which local adaptation and coevolution may occur (Gandon & Michalakis, 2002). Here we found more evidence for herbivore adaptation than for its host plant, although the four populations studied were genetically isolated (Table 2). This result suggests that the four pairs of populations can be considered as potential units for the occurrence of a coevolutionary process (but see Toju *et al.*, 2011). Because the plant represents the most significant element of the herbivore environment, and the reciprocal condition is not necessarily true, the lower intensity of plant local adaptation to its herbivore reflects the fact that the abiotic component of the environment may exert stronger selection pressures on the plants than on the herbivores. Genetic analyses showed higher levels of population differentiation among the plant ($F_{ST} = 0.39$) than among the herbivore populations ($F_{ST} = 0.09$) (Table 2). That is, there seems to be more gene flow among herbivore populations, which could limit a process of local adaptation. Despite low population differentiation in neutral molecular markers, herbivore populations showed patterns consistent with local adaptation, suggesting that extensive gene flow does not reduce adaptation of herbivores to local plant populations. This is expected to be favored because the herbivore has a faster life cycle than its host plant. Although the plant showed lower levels of gene flow, the absence of adaptation in terms of fitness to its specialist herbivore suggest that other

evolutionary processes are probably affecting the distribution of genetic variation (Lande & Shannon, 1996; Gandon, 2002). Overall, the patterns detected in this study support previous observations that natural enemies are usually more easily adapted to their hosts than vice versa (Mopper & Strauss, 1998).

Patterns of reciprocal local adaptation

To examine whether the extent of local differentiation between both interacting species expressed reciprocal patterns, the key comparison is between the local-foreign contrast for the herbivore against the home-away contrast for the plant and vice versa. For example, when the herbivore is locally adapted (local-foreign) it is expected that the native plant should express lower levels of resistance against its native herbivores than against foreign herbivores (home-away). Thus we found that when the herbivores from Esperanza, Pedregal and Tula were locally adapted (positive values of ϕ) (Fig. 1b), their native plant populations had similar or lower levels of resistance (negative values of ϕ) (Fig. 1c). These results are in accord with a coevolutionary process between herbivore and host resistance. That is, at a given point in time we should find adaptation in either the herbivore or the plant but not in both because of the time-lagged cycles typical of the coevolutionary process (Gandon & Michalakis, 2002). The presence of no adaptation or even maladaptation for the population of Teotihuacan suggests that the interaction is likely to be younger than at the other sites studied, or high levels of genetic drift and/or migration from other locations could have swamped the effect of natural selection.

Although we have predicted that tolerance could be higher whenever the herbivores were locally adapted to the resistance levels of their native plants, we did not find any association between tolerance and herbivore adaptation. It is possible that even when the herbivore become locally adapted, host resistance still provides fitness benefits thus reducing the opportunity for the evolution of tolerance. While one study has detected a negative association between tolerance and resistance among populations (Roy & Kirchner, 2000), there is still no empirical study exploring the relationship between the variation in tolerance, the effectiveness of resistance and the degree of local adaptation of natural enemies. It is also likely that we did not detect any association with tolerance because we only evaluated the effects of a specialist herbivore. Thus, it is probable that the presence of other generalist enemies could affect both the expression of plant tolerance and the extent of adaptation of specialist herbivores. Overall, our results support the expectation that unlike resistance, tolerance is not involved in a coevolutionary process (Rosenthal & Kotanen, 1994; Strauss & Agrawal, 1999; Stinchcombe, 2002; Espinosa & Fornoni, 2006).

The reciprocal expression of plant defenses and herbivore performance can be influenced by several factors. In this study, the use of a single replicate at the level of a common-garden did not allow disentanglement of the relative role of abiotic vs biotic factors affecting the patterns of local adaptation, so our response variables could have been affected by a population-by-environment interaction. The presence of maternal effects has been

repeatedly reported in both plants and herbivores, and these result in higher performance of individuals growing in sympatric vs allopatric conditions (Galloway, 2005; van Asch *et al.*, 2010). However, in the present study we did not find a consistent pattern of higher sympatric performance in the home-away contrast, indicating that the patterns of local adaptation following the local-foreign approach are less likely to be overestimated by maternal effects. Despite our efforts to control for the initial herbivore density, the final density within the cages could have been affected by intraspecific competition reducing the chances of detecting a significant herbivore per population interaction for the insect population growth rate. Finally, as we only manipulated the presence of the main consumer in our experiment, the interaction with other natural enemies under field conditions could have affected the intensity of reciprocal local adaptation. Future studies should explore whether reciprocal patterns of local adaptation can be influenced by diffuse selection imposed by other community members.

In general, we found that the patterns of population differentiation for the herbivore and the plant were not independent from each other, supporting a necessary condition for the occurrence of reciprocal local adaptation. While an association between resistance and herbivore performance was observed, there was no clear pattern between tolerance and herbivore local adaptation. Whereas the expression of resistance can be highly specific (Kessler & Baldwin, 2002), tolerance has been viewed as a more general mechanism of defense (Fornoni, 2011). However, we found for the first time that within a pair of interacting species, the extent of tolerance depends not only on the amount of damage but also on the population provenance of the herbivore consuming the plant. In turn, in our experiments, the plasticity in tolerance among populations could have obscured the effect of damage on plant fitness.

Previous studies have independently reported plant local adaptation to biotic factors such as herbivores or pathogens (Sork *et al.*, 1993; Abdala-Roberts & Marquis, 2007; Crémieux *et al.*, 2008), and insect local adaptation to their host plants (van Zandt & Mopper, 1998). However, we are still far from determining whether reciprocal local adaptation driven by herbivory governs the evolution of interacting species. In the present study we showed a significant effect of the biotic environment on patterns of reciprocal local adaptation while controlling for abiotic conditions, thus supporting a major assumption of coevolutionary theory.

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Supporting Information

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Table S1 Results from the univariate ANOVAs for those traits used to estimate the individual performance of the herbivore *Lema trilineata*

Table S2 Mean values \pm 1 SE for those traits used to estimate the individual performance of the herbivore *Lema trilineata*

Note S1 The coefficient of local differentiation (ϕ).

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