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Quantitative genetics of plant tolerance and resistance against natural enemies of two natural populations of *Datura stramonium*

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ABSTRACT

Plants can respond to damage from natural enemies through resistance and/or tolerance. Evolution of these traits among natural plant populations can be constrained by (1) the absence of genetic variation or (2) because of the presence of a trade-off between resistance and tolerance. This last hypothesis remains one of the main assumptions of evolutionary theory of plant–natural enemy interactions. Such a trade-off could represent a constraint for adaptive evolution because high levels of resistance or tolerance could be attained but not both. In this study, we examined the presence of genetic variation in tolerance and resistance, the existence of a negative genetic correlation between tolerance and resistance, and the extent of genetic differentiation in plant defensive strategies between two natural populations of *Datura stramonium* of Central Mexico. A reciprocal transplant experiment using paternal half-sib families was performed. The results of this experiment showed: (1) the presence of additive genetic variation for tolerance and resistance ($h^2_N = 0.41$–0.49) in both populations at their native site; (2) genetic differentiation in tolerance and resistance between populations; and (3) an environment-dependent genetic correlation between tolerance and resistance. The results support the hypothesis that a negative genetic correlation between tolerance and resistance can potentially constrain the simultaneous evolution of both traits within populations. Furthermore, genetic differentiation between populations supports the expectation that tolerance and resistance represent redundant alternatives against natural enemies. Thus, G × E interactions may represent an important causal factor promoting geographic variation in the outcome of the interaction between plants and their natural enemies.

Keywords: adaptive variation, *Datura stramonium*, environment-dependent trade-off, G × E interactions, herbivory, heritability, resistance, tolerance.

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INTRODUCTION

Antagonistic co-evolution is considered the main process behind the development and maintenance of adaptations that mediate trophic interactions between exploiters and victims (Ehlrich and Raven, 1964; van Valen, 1973). At the population level, co-evolutionary theory predicts that a dynamic process of adaptations and counter-adaptations of traits involved in the interaction between species will result in local adaptation (Seger, 1992; Thompson, 1994; Nuismer *et al.*, 1999; Buckling and Rainey, 2002). Furthermore, the direction and strength of back-and-forth genetic responses between interacting species may be altered along the geographic scale, provided there are selection mosaics in species' interactions (Thompson, 1994; Agrawal, 2001; Rausher, 2001; Schluter, 2001; but see Stinchcombe and Rausher, 2002). Although recent empirical evidence revealed the existence of selection mosaics for several interactions (Benkman *et al.*, 1999; Thompson, 1999; Gómez and Zamora, 2000; Gomulkiewicz *et al.*, 2000; Thompson and Cunningham, 2002), there is little information about the causal factors behind these patterns at the scale of local populations (Schluter, 2001). When both interacting species inhabit the same geographic area, the co-evolutionary process can be constrained by: (1) reduced genetic variation in traits involved in the interaction (e.g. Thompson, 1994); (2) trade-offs between resistance traits and other fitness-enhancing characters (e.g. Rausher, 2001); or (3) gene flow between populations experiencing different selection environments (e.g. Thompson, 1994).

Unlike most trophic interactions between animal species, plants have two basic strategies of defence against natural enemies (herbivores, pathogens) – namely, resistance and tolerance (Strauss and Agrawal, 1999). Resistance traits reduce the fitness of natural enemies (Underwood and Rausher, 2000) and can potentially affect genetic variation in enemy traits involved in the interaction with plants (Futuyma and Peterson, 1985; Jaenike, 1990; Rosenthal and Berenbaum, 1991). In contrast, plant tolerance does not promote a co-evolutionary response of their natural enemies because it does not have a negative effect on enemy populations (Rosenthal and Kotanen, 1994; Fay *et al.*, 1996; Strauss and Agrawal, 1999; Rausher, 2001). If fitness costs of herbivory can be reduced by tolerance, selection on resistance traits will be weak or even selected against when costly, given that levels of damage can be partially or almost fully compensated by tolerance (Abrahamson and Weis, 1997). This explanation assumes that both tolerance and resistance represent costly redundant alternatives to cope with natural enemies (Simms and Triplett, 1994; Fineblum and Rausher, 1995; Valverde *et al.*, 2003), and hence a negative genetic correlation between both strategies of defence has been expected within populations (Simms and Triplett, 1994; Fineblum and Rausher, 1995).

Although the existence of a negative genetic correlation is relevant to understanding the simultaneous evolution of tolerance and resistance (Fineblum and Rausher, 1995; Rausher, 1996; Tiffin, 2000), few studies have detected it (Fineblum and Rausher, 1995; Stowe, 1998). Hence several studies have failed to detect this type of trade-off within natural populations (Simms and Triplett 1994; Mauricio *et al.*, 1997; Tiffin and Rausher, 1999; Roy and Kirchner, 2000; Stinchcombe and Rausher, 2002; Strauss *et al.*, 2002). Moreover, selection studies suggest that combined levels of tolerance and resistance may be favoured, corresponding to mixed strategies of defence (Mauricio *et al.*, 1997; Tiffin and Rausher, 1999; Pilson, 2000; Medel, 2001; Koskela *et al.*, 2002; Valverde *et al.*, 2003). In this sense, the presence of this constraint remains to be explored.

Although several studies have found within-population genetic variation for resistance and tolerance simultaneously (Mauricio *et al.*, 1997; Agrawal *et al.*, 1999; Tiffin and Rausher, 1999; Koskela *et al.*, 2000), none of these were conducted using more than one population (but see Juenger *et al.*, 2000). The aim of the present study was to assess the presence/absence of a negative genetic correlation between resistance and tolerance, and the presence of genetic variation in both defensive strategies, in two populations of the annual plant *Datura stramonium*. Using paternal half-sib families from two natural populations, we specifically searched for: (1) the presence of additive genetic variation in tolerance and resistance; (2) the existence of a negative genetic correlation between tolerance and resistance; and (3) the occurrence of local differentiation in defensive strategies against herbivores.

MATERIALS AND METHODS

Plant species and study sites

Datura stramonium L. (Solanaceae) is an annual plant that occurs in a wide variety of plant communities throughout Mexico and North America (Weaver and Warwick, 1984). This species is commonly found inhabiting open, cultivated and disturbed habitats (Weaver and Warwick, 1984; Núñez-Farfán and Dirzo, 1994). In Central Mexico, leaves of *D. stramonium* are frequently eaten by two specialist herbivorous insects, *Epitrix parvula* (Coleoptera: Chrysomelidae) and *Lema trilineata* (Coleoptera: Chrysomelidae) (Núñez-Farfán and Dirzo, 1994). Also, the generalist grasshopper *Sphenarium purpurascens* (Othoptera: Acrididae) consumes foliar tissue of this plant species in Central Mexico (Núñez-Farfán and Dirzo, 1994; Núñez-Farfán *et al.*, 1996).

Datura stramonium possess secondary compounds (trophane alkaloids) and trichomes as components of resistance against herbivory damage (Shonle and Bergelson, 2000; Valverde *et al.*, 2001). Natural selection has been detected acting on these defensive characters (Shonle and Bergelson, 2000; Valverde *et al.*, 2001, 2003). In addition, artificial defoliation studies have revealed that this species can tolerate foliar damage around 10% of total leaf area without showing fitness reductions (Fornoni and Núñez-Farfán, 2000).

Based on a previous survey of several natural populations of *D. stramonium* in Central Mexico (see Figure 1 in Valverde *et al.*, 2001), we selected two extreme populations in the observed range of resistance. These populations showed significant differences in average levels of total leaf area damaged (Ticumán: 10.9 ± 4.0%; Sto. Domingo: 44.1 ± 1.4%; mean ± standard error) (Valverde *et al.*, 2001). Furthermore, common garden experiments have shown that both populations are genetically differentiated in their response to herbivory, Ticumán being the most resistant (see Table 1 in Valverde *et al.*, 2003). These populations grow in contrasting habitats and were selected for a reciprocal transplant experiment. The two populations are separated by about 30 km and differ in altitude by approximately 900 m. The Ticumán population (18°45′N and 99°07′W) is located in a tropical dry forest at 961 m, where average annual precipitation and temperature are 802.1 mm and 23.1°C, respectively. The population of Sto. Domingo (19°00′N and 99°03′W) occurs in a temperate pine-oak forest at 2050 m, with an average annual precipitation and temperature of 1463.2 mm and 19.9°C, respectively. In both transplant sites, plants start their growing season with the first rains at the beginning of July (J. Núñez-Farfán, personal observation).

Experimental design

Experimental plants were obtained in the greenhouse following a North Carolina I design of crosses to produce paternal half-sib families (Lawrence, 1984). This design, in which each male parent (sire) is mated to several female parents (dams), enables estimation of unbiased additive genetic variances free from possible maternal and common environmental effects (Falconer and Mackay, 1996). Because *D. stramonium* is a hermaphroditic species, plants were randomly assigned to function either as a male or as a female parent. Paternal half-sib families were obtained by mating two female parents to one male parent plant (Núñez-Farfán and Dirzo, 1994). Controlled crosses were performed between January and May 1999, obtaining 25 and 23 complete paternal half-sib families for the populations of Ticumán and Sto. Domingo, respectively. More than 100 experimental seeds per sire were germinated in the greenhouse. When the first two true leaves appeared, half of the first germinated plants from each paternal family (8–24) were transplanted to each experimental plot following a reciprocal transplant design. However, because 10 paternal families of Ticumán and 8 of Sto. Domingo had a low number of replicates, these families were only planted at their corresponding native site. In total, 1159 plants were planted at both experimental plots.

Both experimental plots were ploughed and disked before transplant. Experimental seedlings were randomly placed 1 m apart in a regular grid within experimental plots in a complete randomized design. During the experiment, both plots were periodically weeded by hand to avoid interference from other plants.

Resistance estimates

At plant maturity (4 months after transplanting), half of the leaves of each individual plant were randomly collected to estimate the proportion of leaf area damaged by folivorous insects. The proportion of damage was calculated using a Color Windows Image Analysis System (WinDIAS-Basic, Delta-T Devices Ltd, Cambridge, UK). For each individual plant, we directly measured the proportion of leaf area damaged for all sampled leaves. Resistance to herbivores was calculated as (1 − relative damage) (Stinchcombe and Rausher, 2001; Valverde *et al.*, 2001, 2003). Although plant size may influence the probability of attack by natural enemies in other plant species (Sork *et al.*, 1993; Roy, 1998), a previous study with *D. stramonium* indicated that the amount of damage experienced by plants is not conditioned by plant size (Valverde *et al.*, 2003).

Additive genetic variation on resistance

Nested analyses of variance (Model II; Sokal and Rohlf, 1995) were performed using the module PROC GLM (Type III SS) with the RANDOM option of SAS (SAS, 1999) to calculate the among-sire, among-dam within-sire, and error components of variance for resistance to herbivorous insects (Roff, 1997; Lynch and Walsh, 1998). These analyses were performed for each population grown at their native and alien site. Before statistical analyses, relative resistance was arcsine-square root transformed. The North Carolina I cross-breeding design allows the estimation of the additive genetic variance (σ^2_A) because the among-sire component of variance approximates $1/4\sigma^2_A$, corresponding to the intraclass correlation coefficient (Falconer and Mackay, 1996; Lynch and Walsh, 1998). Narrow-sense heritability (h^2_N) was estimated as $h^2_N = 4\ (\sigma^2_S/\sigma^2_T)$, where σ^2_S is the among-sire component of variance and σ^2_T is the total phenotypic variance (Falconer and Mackay, 1996; Lynch and Walsh, 1998). Standard errors and *t*-tests of heritability were estimated using a jackknife resampling procedure (Han and Lincoln, 1994; Sokal and Rohlf, 1995, p. 821).

Tolerance estimates

At the end of the reproductive season, all mature fruits on each individual plant were harvested. Individual plant fitness was estimated as the total number of seeds per plant corresponding to maternal fitness. Since fruit volume is highly correlated with the total number of seeds contained by a fruit (seed number = 0.026 × (volume); $r^2 = 0.984$, $P < 0.0001$, $n = 35$), the total number of seeds per plant was estimated by measuring the volume of all fruits produced by a plant. Tolerance for each paternal family was estimated as the slope of the relationship between relative fitness and natural damage (Mauricio *et al.*, 1997; Tiffin and Rausher, 1999; Stinchcombe, 2002).

Additive genetic variation on tolerance

Additive genetic variation on tolerance to herbivory was evaluated using a nested analysis of covariance (Type III SS) for each population at each transplant site (PROC GLM module of SAS) (SAS, 1999). According to the operational definition of tolerance as a norm of reaction of fitness in relation to damage (Abrahamson and Weis, 1997), relative fitness was entered as the dependent variable and the proportion of natural damage was entered as a covariate in the analysis. For this analysis, relative fitness was log-transformed to ensure normality (Mitchell-Olds and Shaw, 1987). Sire and dams nested within sire were considered random effects. A significant damage × sire interaction would indicate the existence of additive genetic variation on tolerance (i.e. differences among paternal half-sib families in the slope of the relationship between relative fitness and proportion of leaf area damaged) (Pilson, 2000; Stinchcombe, 2002). The RANDOM option of SAS was used to perform *F* estimates. When the interaction damage × dam (sire) was significant, the *F*-test for the interaction damage × sire was corrected using the mean squares (MS) of damage × dam (sire) as the denominator of the *F*-test (see Appendix B in Hougen-Eitzman and Rausher, 1994; Tiffin and Rausher, 1999). After regressing relative fitness against plant size (total number of leaves), estimates of tolerance using relative fitness and residuals of relative fitness (without size effects) were evaluated (Tiffin and Rausher, 1999). Rank correlations between both estimates of tolerance revealed that these correlations were highly significant and positive (average pairwise correlation coefficient was 0.80, range 0.70–0.86, $P < 0.005$), indicating that plant size did not differentially alter our estimates of tolerance among paternal families. Thus, the final analyses of genetic variation on tolerance were performed without considering plant size.

Correlation between tolerance and resistance

To explore the possible existence of an association between tolerance and resistance, we performed an analysis of covariance of resistance as a function of site, population and tolerance (covariable). For this analysis, only those paternal families that were completely replicated at both transplant sites were used. The presence of a significant site × tolerance interaction would indicate that the association between tolerance and resistance differs between sites (see Roff and Gélinas, 2003). After this analysis, pairwise paternal half-sib family-mean correlations between tolerance and resistance for each population in each site were calculated using the product–moment correlation coefficient (Via, 1984). The significance of genetic correlations was obtained using jackknife resampling methods (see Roff and Preziosi, 1994).

Population differentiation in fitness, resistance and tolerance

To determine if populations at their native site outperform the alien population in terms of fitness, and are locally differentiated in tolerance and resistance, separate two-way analyses of variance were performed for fitness, resistance and tolerance. In these analyses, site and population were entered as fixed factors. These analyses were carried out using paternal family mean values of fitness and resistance. Values of tolerance corresponded to the slopes of the relationship between fitness and damage for each paternal family. A significant population effect indicates the presence of genetic differentiation in plant defensive strategies between populations. All these analyses were performed using only those paternal families that were completely replicated at both transplant sites.

RESULTS

Plants growing at Ticumán performed better than those growing at Sto. Domingo (Table 1). Plants were 20 times larger in terms of total number of leaves in Ticumán than in Sto. Domingo (Table 1). Leaf area damaged per plant was on average very similar at the two sites (Ticumán: 23.5 ± 1.10; Sto. Domingo: 22.6 ± 1.4; mean ± standard error). In the locality of Ticumán, the native population received less damage (21.65 ± 0.7) than the alien population (25.3 ± 1.0). In the locality of Sto. Domingo, mean damage was 25.7 ± 0.8 and 19.5 ± 1.1 for the native and alien population, respectively. In other words, at both sites, plants from Sto. Domingo showed more damage than those from Ticumán. In Ticumán

Table 1. Mean plant traits of two populations of *Datura stramonium* grown in a reciprocal transplant experiment (standard error in parentheses)

	Site Sto. Domingo		Site Ticumán	
	Pop. Sto. Domingo	Pop. Ticumán	Pop. Sto. Domingo	Pop. Ticumán
Plant size (leaf number)	7.3738 (0.7719)	11.0803 (0.9643)	113.2590 (23.8743)	223.6414 (27.2289)
Fitness	190.3225 (24.9956)	183.5789 (20.8196)	3858.9670 (857.5163)	8398.292 (1004.042)
Resistance	0.7425 (0.0180)	0.8046 (0.0109)	0.7468 (0.0105)	0.7835 (0.0108)
Tolerance (range)	−0.0250 (−0.2733 to 0.4537)	0.0221 (−0.0825 to 0.4885)	−0.1060 (−0.3241 to 0.0337)	−0.3380 (−1.5948 to 0.0852)

Note: Means were obtained using untransformed values of paternal family means.

damage was caused mainly by the flea beetle *Epitrix parvula*, whereas in Sto. Domingo it was caused by the grasshopper *Sphenarium purpurascens* (Valverde, 2001).

Genetic variation in resistance and tolerance

The analysis of variance revealed additive genetic variation in resistance for both populations, as indicated by the existence of a significant sire component of variation (Table 2). No difference in the magnitude of heritability was detected between populations as estimated from their confidence intervals ($h^2_N = 0.41 \pm 0.35$ *vs* $h^2_N = 0.49 \pm 0.28$ for Ticumán and Sto. Domingo, respectively). Only for the population of Ticumán grown at its native site was the estimate of heritability using the dam component of variance significant ($h^2_N = 0.44$). Additive genetic variation in tolerance was detected for both populations (Table 3). At both sites, a significant damage × sire interaction was detected indicating differences among paternal families in the slope of the relationship between fitness and the amount of damage (i.e. tolerance) (Table 3). No significant genetic variation in tolerance and resistance was detected for the alien population at each locality.

Additionally, a mixed-model analysis of variance of resistance as a function of sire, dam and site supports the previous pattern of undetected additive genetic variation at the alien sites. Sire and dam were considered random factors and site was considered fixed. In this analysis, a significant sire (overall) effect ($F = \mathrm{MS}_{\mathrm{sire}} / \mathrm{MS}_{\mathrm{dam(sire)}}$) would indicate the presence of additive genetic variation for resistance across environments. A significant sire (G × E) effect $[F = \mathrm{MS}_{\mathrm{sire}} / (\mathrm{MS}_{\mathrm{dam(sire)}} + \mathrm{MS}_{\mathrm{sire \times site}} - \mathrm{MS}_{\mathrm{dam(sire) \times site}})]$ would indicate the presence of an additive genetic covariance (positive) between environments. A significant sire × site interaction ($F_{\mathrm{sire \times site}} = \mathrm{MS}_{\mathrm{sire \times site}} / \mathrm{MS}_{\mathrm{dam(sire) \times site}}$) would indicate the presence of a negative genetic covariance between environments (Fry, 1992; Roff and Gélinas, 2003). The results of this analysis showed that neither an additive genetic component across environments ($F_{30,31} = 1.08$, $P = 0.4157$) nor an additive covariance component between environments ($F_{30,13.4} = 0.93$, $P = 0.5758$) was significant. The interaction sire × site was also not significant ($F_{30,31.06} = 1.13$, $P = 0.3638$). The same analysis for tolerance revealed the absence

Table 2. Nested analyses of variance for resistance to herbivorous insects in two populations of *Datura stramonium* (Ticumán and Sto. Domingo) grown at their native sites

Population	Source of variation	d.f.	MS	F	P	$\%\sigma^2$	h^2_N
Ticumán	Sire	24	0.0385	2.03	0.0417	10.65	0.41 ± 0.20*
	Dams (sire)	25	0.0190	2.09	0.0019	10.72	0.44 ± 0.16**
	Error	392	0.0090			78.12	
Sto. Domingo	Sire	22	0.4258	2.32	0.0250	12.36	0.49 ± 0.16**
	Dams (sire)	23	0.1834	1.57	0.0484	6.79	0.28 ± 0.23
	Error	289	0.1166			80.83	

Note: Sire and dam nested within sire were considered random effects. Jackknifes were performed to estimate standard errors of narrow-sense heritabilities. $*P < 0.05$, $**P < 0.01$ correspond to heritabilities significantly different from zero. $\%\sigma^2$ indicates the percentage of phenotypic variance explained by each source of variation. Sums of squares Type III were used. No significant sire main effect was detected for the populations of Ticumán and Sto. Domingo grown at their respective alien site (Ticumán: $F_{14,15.03} = 1.06$, $P = 0.4516$; Sto. Domingo: $F_{14,14.13} = 0.94$, $P = 0.5370$).

Table 3. Results of analysis of variance to detect additive genetic variation on tolerance for two populations of *Datura stramonium* (Ticumán and Sto. Domingo) grown at their native sites

Population	Source of variation	d.f.	MS	*F*	*P*
Ticumán	Sire[a]	24	0.0524	1.59	0.1246
	Dams (sire)	25	0.0334	1.77	0.0140
	Damage	1	0.0247	1.31	0.2534
	Damage × sire	24	0.0399	2.11	0.0002
	Damage × dams (sire)	25	0.0283	1.20	0.0606
	Error	342	0.0188		
Sto. Domingo	Sire[b]	22	0.0425	2.67	0.0100
	Dams (sire)	23	0.0159	1.02	0.4404
	Damage	1	0.0007	0.05	0.8235
	Damage × sire	22	0.0290	1.86	0.0129
	Damage × dams (sire)	23	0.0225	1.38	0.1184
	Error	244	0.0156		

Note: A significant damage × sire interaction indicates significant differences among paternal families in the slope of the relationship between fitness and damage (i.e. tolerance). No significant damage × sire interaction was detected for either population when grown at their respective alien site (Ticumán: $F_{14,18.03} = 0.78$, $P = 0.6699$; Sto. Domingo: $F_{14,16.23} = 0.57$, $P = 0.8502$). Sums of square Type III were used.

[a] *F* estimated following Satterthwaite approximation, d.f. = 24,25.91.

[b] *F* estimated following Satterthwaite approximation, d.f. = 22,24.64.

of additive genetic variation across environments ($F_{30,31} = 1.22$, $P = 0.2924$, estimated as $F_{\text{damage}\times\text{sire(overall)}} = \text{MS}_{\text{damage}\times\text{sire}}/\text{MS}_{\text{damage}\times\text{dam(sire)}}$), additive genetic covariance between environments [$F_{30,5.51} = 1.76$, $P = 0.2610$, estimated as $F_{\text{damage}\times\text{sire(G}\times\text{E)}} = \text{MS}_{\text{damage}\times\text{sire}}/(\text{MS}_{\text{damage}\times\text{dam(sire)}} + \text{MS}_{\text{damage}\times\text{site}\times\text{sire}} - \text{MS}_{\text{damage}\times\text{site}\times\text{dam(sire)}})$], and an additive genetic interaction between environments ($F_{30,31} = 0.78$, $P = 0.7508$, estimated as $F_{\text{damage}\times\text{site}\times\text{sire}} = \text{MS}_{\text{damage}\times\text{site}\times\text{sire}}/\text{MS}_{\text{damage}\times\text{site}\times\text{dam(sire)}}$). All these analyses were performed using only those paternal families that were completely replicated at both sites ($n = 30$).

Genetic correlations between tolerance and resistance

The analysis of covariance for resistance revealed a significant tolerance × site interaction indicating site differences in the association between tolerance and resistance ($F_{1,979} = 4.77$, $P = 0.0291$). Specifically, at the locality of Ticumán we found a significant negative genetic correlation between tolerance and resistance for the populations of Ticumán ($r_g = -0.41$, $P = 0.0340$, $n = 25$; Fig. 1A) and Sto. Domingo ($r_g = -0.56$, $P = 0.0047$, $n = 15$; Fig. 1B). Conversely, at the locality of Sto. Domingo there was a positive correlation between resistance and tolerance for the population of Ticumán ($r_g = 0.44$, $P = 0.0252$, $n = 15$; Fig. 1C), whereas no correlation was detected for the population of Sto. Domingo ($r_g = -0.15$, $P = 0.2500$, $n = 23$; Fig. 1D).

Population differentiation in fitness, resistance and tolerance

The results of a two-way analysis of variance revealed significant differences in fitness (seeds/plant) between sites and populations (Tables 1 and 4). Plants at Sto. Domingo

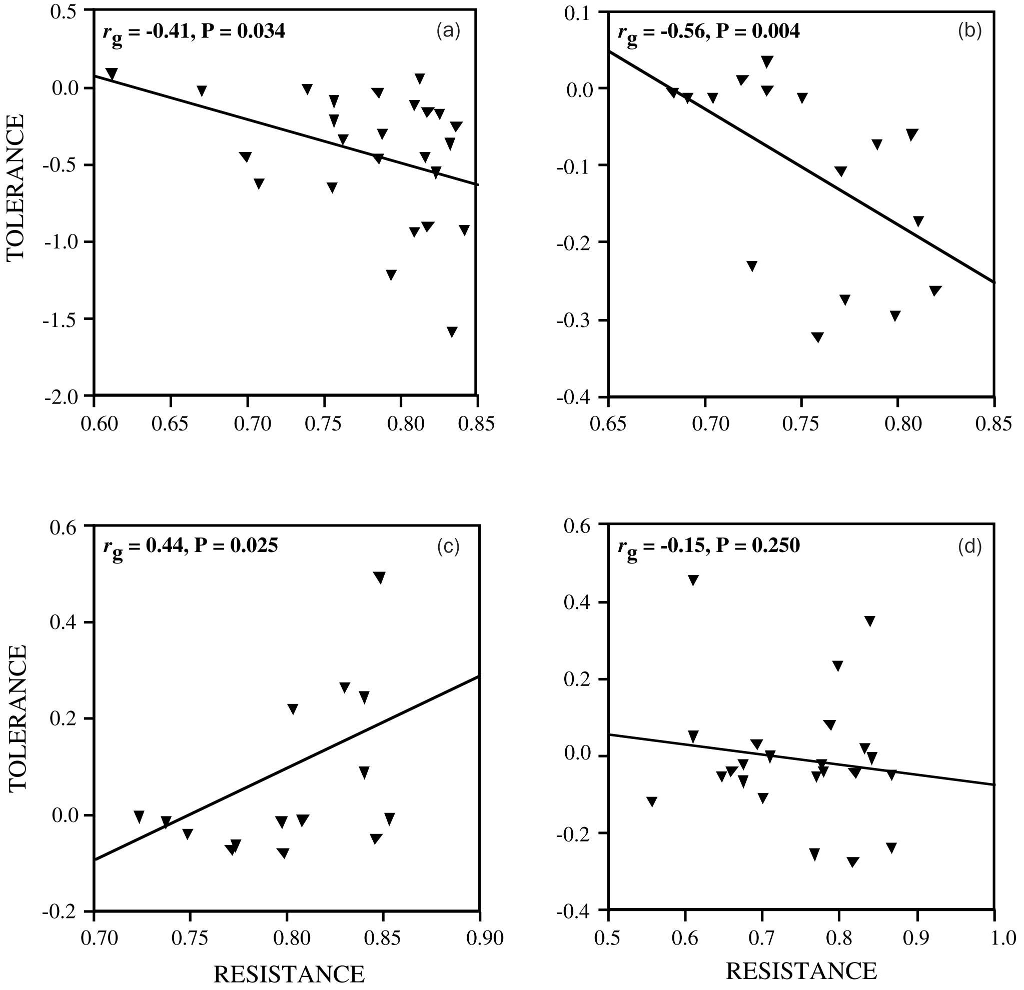

Fig. 1. Genetic correlation between tolerance and resistance for two populations of *Datura stramonium* grown in a reciprocal transplant experiment. (a) Population of Ticumán grown at its native site. (b) Population of Sto. Domingo grown at Ticumán. (c) Population of Ticumán grown at Sto. Domingo. (d) Population of Sto. Domingo grown at its native site.

produced 3% of the total number of seeds/plant produced on average at Ticumán (Fig. 2a). Across sites, plants from Sto. Domingo produced on average 47% fewer seeds than plants from Ticumán (Fig. 2a). At Ticumán, the native population produced 44% more seeds than the alien population. However, in Sto. Domingo, no difference in fitness was detected between the native and alien population (Fig. 2a). There was no significant site × population interaction (Table 4).

Average resistance varied between sites. Plants in the locality of Ticumán were 1% less resistant to herbivory than in the locality of Sto. Domingo (Table 4, Fig. 2b). Also, significant differences in resistance between populations across sites were detected. Plants from Ticumán were on average 6% more resistant to herbivorous insects than plants from

Table 4. Results of two-way analyses of variance for fitness (seeds/plant), resistance and tolerance in two natural populations of *D. stramonium* grown according to a reciprocal transplant experiment

Character	Source	d.f.	MS	*F*	*P*
Fitness	Site	1	21.905	141.01	< 0.0001
	Population	1	0.960	6.18	0.0157
	Site × population	1	0.368	2.37	0.1289
	Error	60	0.155		
Resistance	Site	1	0.024	4.47	0.0386
	Population	1	0.025	4.66	0.0348
	Site × population	1	0.001	0.34	0.5612
	Error	60	0.005		
Tolerance	Site	1	0.013	23.31	< 0.0001
	Population	1	0.002	4.08	0.0476
	Site × population	1	0.005	9.40	0.0032
	Error	60	0.001		

Note: Values for tolerance correspond to the slope of the relationship between fitness and damage for each paternal family. Site and population were considered fixed factors. Type III sums of square were used.

Sto. Domingo (Fig. 2b). Population differences in resistance were similar at both transplant sites, as shown by the absence of a significant site × population interaction (Fig. 2b).

Another analysis of variance indicated differences in tolerance both between and within sites (Table 4). In the Ticumán site, plants were 89% less tolerant to foliar damage than at the Sto. Domingo site (Table 4, Fig. 2c). Across localities, the population of Ticumán was, on average, 63% less tolerant to foliar damage than the population of Sto. Domingo (Table 4, Fig. 2c). However, only within the site of Ticumán was there a significant difference in tolerance between populations. At this site, the population of Ticumán was 68% less tolerant than the population of Sto. Domingo (Fig. 2c). Differences in tolerance between populations at the Ticumán site accounted for the significant site × population interaction in the analysis of variance (Table 4, Fig. 2c).

DISCUSSION

The results of this study indicate that populations of *D. stramonium* are genetically differentiated in terms of tolerance and resistance. At their native site, each population expressed significant additive genetic variation in tolerance and resistance. The presence of additive genetic variation for tolerance and resistance in these populations suggests that genetic drift has not eliminated the variation in plant defences. The detection of similar heritability in resistance for the two populations suggests that further response to selection is not limited by heritable variation. The detection of a trade-off between tolerance and resistance only at the locality of Ticumán indicates the existence of a constraint to the simultaneous evolution of both defensive strategies. As long as the higher resistance at Ticumán reflects an adaptive response, the presence of a trade-off between tolerance and resistance may not represent a constraint to co-evolution, unless selection favours high levels of tolerance. For the population of Sto. Domingo, we found no evidence of constraints to the simultaneous

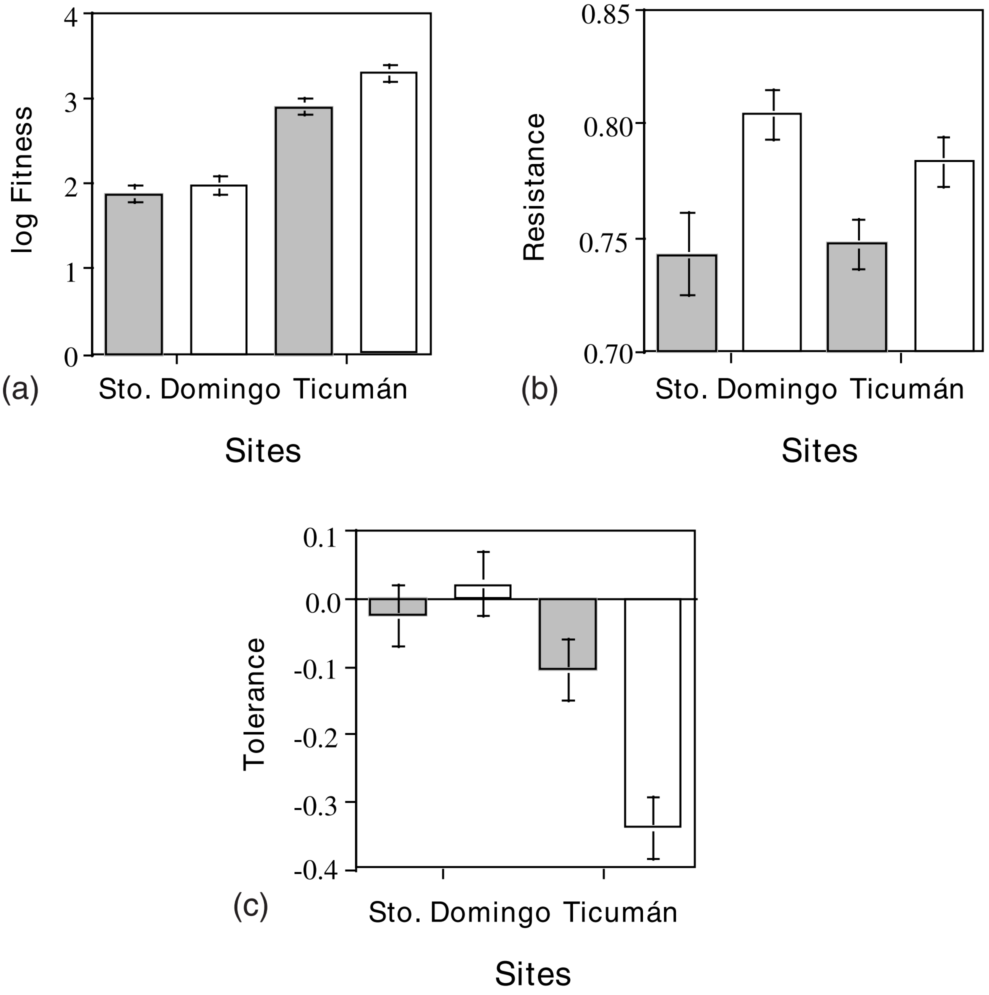

Fig. 2. (a) Log fitness (seeds/plant), (b) resistance and (c) tolerance for two natural populations of *Datura stramonium* grown in a reciprocal transplant experiment (mean ± standard error). ■, population of Sto. Domingo; □, population of Ticumán.

evolution of tolerance and resistance given the absence of a trade-off between them. The better performance of the population of Ticumán at its native site compared with plants from Sto. Domingo suggests that plants from Ticumán are locally adapted.

Local differentiation in plant defensive strategies

In marked contrast to the many studies that have analysed genetic variation for resistance to herbivores (Marquis, 1992), few have evaluated the existence of genetic differentiation in levels of tolerance among natural plant populations (Lennartsson *et al.*, 1997; Juenger *et al.*, 2000). In this study, we examined simultaneously the presence of genetic differentiation for tolerance and resistance among two natural populations of *Datura* *stramonium*. The pattern of differentiation indicated that the more resistant population (i.e. Ticumán) was, at the same time, the least tolerant. In contrast, the more tolerant population (i.e. Sto. Domingo) was the least resistant. This pattern supports previous results indicating that tolerance and resistance represent redundant strategies (van der Meijden *et al.*, 1988; Roy and Kirchner, 2000; Valverde *et al.*, 2003). Although there is some evidence to suggest that environmental effects on the expression of costs of defence may be responsible for across-population variation in tolerance and resistance, few studies have addressed this issue (but see Hochwender *et al.*, 2000; Koricheva, 2002). The difference in levels of damage between this and previous studies for the *D. stramonium* populations of Sto. Domingo and Ticumán (Valverde *et al.*, 2001) reveals the presence of an important environmental component related to the amount of damage suffered by plants.

Variation in the expression of the trade-off between tolerance and resistance

The presence of a trade-off between tolerance and resistance for both the native and alien population in one locality (i.e. Ticumán site) indicates that the expression of the negative genetic correlation can vary depending on the environmental conditions. In addition, the detection of a significant genetic correlation for the populations of Sto. Domingo and Ticumán grown at their alien site suggests that the undetected genetic variation may be related to an increase in the within-sire variance.

Since damage was used for the estimation of both tolerance (i.e. fitness change per unit of damage) and resistance (1 − relative damage), the genetic correlation between them could be biased by a statistical artifact. In any biological scenario the artifact, if present, would be expected to affect the correlation in a similar way. Thus, the pattern of differences found between sites in the expression of the correlation strongly suggests that the artifact had a relatively small effect on the magnitude and direction of the correlation. Inversely, if the artifact had a strong effect upon the correlation, a similar trend should have been observed in every combination of site and population. Although we cannot conclude that the artifact does not exist, it does appear that its effect was rather minor. Hence, we limit our discussion about the environmental effect on the trade-off to the comparison of relative rather than quantitative values.

Theoretical work on the distribution of genetic correlations has indicated that the mechanism behind the variability in the expression of genetic correlations may be related to variation in resource acquisition relative to resource allocation (Houle, 1991; Mole, 1994). When variation in acquisition is greater than variation in allocation, a positive correlation is expected and vice versa (Houle, 1991; Roff, 1997). At the locality of Ticumán, the presence of a negative genetic correlation suggests that variation in patterns of allocation to defence rather than acquisition of resources may explain the presence of a negative genetic correlation. Conversely, at Sto. Domingo, the presence of a positive and a non-significant correlation for the alien and native population, respectively, suggests that variation associated with acquisition rather than allocation to defence could have precluded the detection of a trade-off (Houle, 1991).

Provided both tolerance and resistance are costly, the second main assumption behind the existence of a trade-off is the presence of redundancy between both strategies (Simms and Triplett, 1994). Hence, another explanation for the absence of a negative genetic correlation is that redundancy may be reduced or absent at the site of Sto. Domingo, if fitness benefits of tolerance and resistance were not equivalent. This may occur if (1) different defensive strategies are selected by different species of natural enemies, or (2) if the costs of tolerance and resistance differ. The first scenario may occur when a plant species is eaten by different species of natural enemies (Fritz and Simms, 1992; Thompson, 1994), and tolerance or resistance function as species-specific responses against natural enemies such that both become complementary defences (de Jong and van der Meijden, 2000). The second possibility may occur if, for example, costs of tolerance are comparatively low relative to those of resistance. Hence, a highly resistant genotype will not pay a fitness cost for being tolerant, weakening or obscuring the negative genetic correlation (Fineblum, 1991; Simms and Triplett, 1994). Conversely, when both resistance and tolerance have similar costs, selection will increase the strength of the negative association between tolerance and resistance (Fineblum, 1991). The scatterplot between tolerance and resistance for the population of Ticumán at its native site suggests that the costs of tolerance and resistance may differ (Simms and Triplett, 1994). The absence of a negative correlation at the site of Sto. Domingo suggests that either the difference in costs is higher than at Ticumán or the benefits of tolerance and resistance are not redundant.

Although variation in the expression of genetic correlations among populations in ecological and life-history traits has been documented previously (Clausen and Hiesey, 1958, p. 150: Table 46; Biere, 1995; Roff, 1997; Pigluicci and Schimtt, 1999; Schapper and Shore, 2000), we still know little about the causes of this variation (Lynch and Walsh, 1998). The environment-dependent expression of a cost of resistance in terms of reduced tolerance suggests the possible existence of an environmental effect upon the genetic interaction between resistance and tolerance loci. This, in turn, would influence the pattern of adaptive variation in plant defences among populations and would be one of the causal factors shaping the selection mosaic of the interaction between plants and their natural enemies. The presence of a trade-off between tolerance and resistance in some interacting populations validates previous theoretical foundations (Fineblum and Rausher, 1995; Mauricio *et al.*, 1997; Tiffin, 2000). A consideration of the potential factors related to variation in the expression of the trade-off would help to understand the generation of geographic variation in the outcome of plant–natural enemy interactions. Future theoretical work should incorporate plant tolerance as an important parameter to understand the co-evolutionary dynamic of plants and their natural enemies at a geographic scale, as well as variation in the adaptive value of tolerance and resistance related to physic and biotic environmental variation.

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REFERENCES

Abrahamson, W.G. and Weis, A.E. 1997. *Evolutionary Ecology Across Three Trophic Levels: Goldenrods, Gall-makers and Natural Enemies*. Monographs in Population Biology No. 29. Princeton, NJ: Princeton University Press.

Agrawal, A.A. 2001. Phenotypic plasticity in the interactions and evolution of species. *Science*, **294**: 321–326.

Agrawal, A.A., Strauss, S.Y. and Stout, M.J. 1999. Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. *Evolution*, **53**: 1093–1104.

Benkman, C.W., Holimon, W.C. and Smith, J.W. 1999. The influence of a competitor on the geographic mosaic of coevolution between crossbills and lodgepole pine. *Evolution*, **55**: 282–294.

Biere, A. 1995. Genotypic and plastic variation in plant size: effects on fecundity and allocation patterns in *Lychnis flo-cuculi* along a gradient of natural soil fertility. *J. Ecol.*, **83**: 629–642.

Buckling, A. and Rainey, P.B. 2002. Antagonistic coevolution between a bacterium and a bacteriophage. *Proc. R. Soc. Lond. B*, **269**: 931–936.

Clausen, J. and Hiesey, W.M. 1958. *Experimental Studies on the Nature of Species. IV. Genetic Structure of Ecological Races*. Publication No. 615. Washigton, DC: Carnegie Institution of Washington.

de Jong, T.J. and van der Meijden, E. 2000. On the correlation between allocation to defence and regrowth in plants. *Oikos*, **88**: 503–508.

Ehrlich, P.R. and Raven, P.H. 1964. Butterflies and plants: a study in coevolution. *Evolution*, **18**: 586–608.

Falconer, D.S. and Mackay, T.F.C. 1996. *Introduction to Quantitative Genetics*, 4th edn. London: Longman.

Fay, P.A., Hartnett, D.C. and Knapp, A.K. 1996. Plant tolerance of gall-insect attack and gall-insect performance. *Ecology*, **77**: 521–534.

Fineblum, W.L. 1991. Genetic constraints on the evolution of resistance to host plant enemies. Doctoral dissertation, Duke University, Durham, NC.

Fineblum, W.L. and Rausher, M.D. 1995. Tradeoff between resistance and tolerance to herbivore damage in a morning glory. *Nature*, **377**: 517–520.

Fornoni, J. and Núñez-Farfán, J. 2000. Evolutionary ecology of *Datura stramonium*: genetic variation and costs for tolerance to defoliation. *Evolution*, **54**: 789–797.

Fritz, R.S. and Simms, E.L. 1992. *Plant Resistance to Herbivores: Ecology, Evolution, and Genetics*. Chicago, IL: University of Chicago Press.

Fry, J.D. 1992. The mixed-model analysis of variance applied to quantitative genetics: biological meaning of the parameters. *Evolution*, **46**: 540–550.

Futuyma, D.J. and Peterson, S.C. 1985. Genetic variation in the use of resources by insects. *Annu. Rev. Entomol.*, **30**: 217–238.

Gómez, J.M. and Zamora, R. 2000. Spatial variation in selection. *Am. Nat.*, **155**: 657–668.

Gomulkiewicz, R., Thompson, J.N., Holt, R.D., Nuismer, S.L. and Hochberg, M.E. 2000. Hot spots, cold spots, and the geographic mosaic theory of coevolution. *Am. Nat.*, **156**: 156–174.

Han, K. and Lincoln, D.E. 1994. The evolution of carbon allocation to plant secondary metabolites: a genetic analysis of cost in *Diplacus aurantiacus*. *Evolution*, **48**: 1550–1563.

Hochwender, C.G., Marquis, R.J. and Stowe, K.A. 2000. The potential for and constraints on the evolution of compensatory ability in *Asclepias syriaca*. *Oecologia*, **122**: 361–370.

Hougen-Eitzman, D. and Rausher, M.D. 1994. Interaction between herbivorous insects and plant–insect coevolution. *Am. Nat.*, **143**: 677–697.

Houle, D. 1991. Genetic covariances of fitness correlates: what genetic correlations are made of and why it matters. *Evolution*, **45**: 630–648.

Jaenike, J. 1990. Host specialization in phytophagous insects. *Annu. Rev. Ecol. Syst.*, **21**: 243–271.

Juenger, T., Lennartsson, T. and Tuomi, J. 2000. The evolution of tolerance to damage in *Gentianella campestris*: natural selection and the quantitative genetics of damage. *Evol. Ecol.*, **14**: 393–419.

Koricheva, J. 2002. Meta-analysis of sources of variation in fitness costs of plant antiherbivore defenses. *Ecology*, **83**: 176–190.

Koskela, T., Salonen, V. and Mutikainen, P. 2000. Local adaptation of a holoparasitic plant, *Cuscuta europaea*: variation among populations. *J. Evol. Biol.*, **13**: 749–755.

Koskela, T.S., Puustinen, S., Salonen, V. and Mutikainen, P. 2002. Resistance and tolerance in a host–holoparasitic plant interaction: genetic variation and costs. *Evolution*, **56**: 899–908.

Lawrence, M.J. 1984. The genetical analysis of ecological traits. In *Evolutionary Ecology* (B. Shorrocks, ed.), pp. 27–63. Oxford: Blackwell Scientific.

Lennartsson, T., Tuomi, J. and Nilsson, P. 1997. Evidence for an evolutionary history of overcompensation in the grassland biennial *Gentianella campestris* (Gentianaceae). *Am. Nat.*, **149**: 1147–1155.

Lynch, M. and Walsh, B. 1998. *Genetics and Analysis of Quantitative Traits*. Sunderland, MA: Sinauer Associates.

Marquis, R.J. 1992. The selective impact of herbivores. In *Plant Resistance to Herbivores and Pathogens: Ecology, Evolution and Genetics* (R.S. Fritz and E.L. Simms, eds), pp. 301–325. Chicago, IL: University of Chicago Press.

Mauricio, R., Rausher, M.D. and Burdick, D.S. 1997. Variation in the defense strategies of plants: are resistance and tolerance mutually exclusive? *Ecology*, **78**: 1301–1311.

Medel, R. 2001. Assessment of correlational selection on tolerance and resistance traits in a host plant–parasitic plant interaction. *Evol. Ecol.*, **15**: 37–52.

Mitchell-Olds, T. and Shaw, R.G. 1987. Regression analysis of natural selection: statistical inferences and biological interpretation. *Evolution*, **41**: 1149–1161.

Mole, S. 1994. Trade-offs and constraints in plant–herbivore defence theory: a life history perspective. *Oikos*, **71**: 3–12.

Nuismer, S.L., Thompson, J.N. and Gomulkiewicz, R. 1999. Gene flow and geographically structured coevolution. *Proc. R. Soc. Lond. B*, **266**: 605–609.

Núñez-Farfán, J. and Dirzo, R. 1994. Evolutionary ecology of *Datura stramonium* L. in Central Mexico: natural selection for resistance to herbivorous insects. *Evolution*, **48**: 423–436.

Núñez-Farfán, J., Cabrales-Vargas, R.A. and Dirzo, R. 1996. Mating system consequences on resistance to herbivory and life history traits in *Datura stramonium*. *Am. J. Bot.*, **83**: 1041–1049.

Pigliucci, M. and Schmitt, J. 1999. Genes affecting phenotypic plasticity in *Arabidopsis*: pleiotropic effects and reproductive fitness of photomorphogenic mutants. *J. Evol. Biol.*, **12**: 551–562.

Pilson, D. 2000. The evolution of plant response to herbivory: simultaneously considering resistance and tolerance in *Brassica rapa*. *Evol. Ecol.*, **14**: 457–489.

Rausher, M.D. 1996. Genetic analysis of coevolution between plants and their natural enemies. *TIG*, **12**: 212–217.

Rausher, M.D. 2001. Co-evolution and plant resistance to natural enemies. *Nature*, **411**: 857–864.

Roff, D.A. 1997. *Evolutionary Quantitative Genetics*. New York: Chapman & Hall.

Roff, D.A. and Gélinas, M.B. 2003. Phenotypic plasticity and the evolution of trade-offs: the quantitative genetics of resource allocation in the wing dimorphic cricket, *Gryllus firmus*. *J. Evol. Biol.*, **16**: 55–63.

Roff, D.A. and Preziosi, R. 1994. The estimation of the genetic correlation: the use of the jackknife. *Heredity*, **73**: 544–548.

Rosenthal, G.A. and Berenbaum, M.R. 1991. *Herbivores: Their Interactions with Secondary Plant Metabolites*, Vol. I. San Diego, CA: Academic Press.

Rosenthal, J.P. and Kotanen, P.M. 1994. Terrestrial plant tolerance to herbivory. *Trends Ecol. Evol.*, **9**: 145–148.

Roy, B.A. 1998. Differentiating the effects of origin and frequency in reciprocal transplant experiments used to test negative frequency-dependent selection hypotheses. *Oecologia*, **115**: 73–83.

Roy, B.A. and Kirchner, J.W. 2000. Evolutionary dynamics of pathogen resistance and tolerance. *Evolution*, **54**: 51–63.

SAS Institute, Inc. 1999. *SAS/STAT User's Guide*, v. 8.2. Cary, NC: SAS Institute.

Schapper, P.J. and Shore, J.S. 2000. Cyanogenesis in *Turnera ulmifolia* L. (Turneraceae). II. Developmental expression, heritability and cost of cyanogenesis. *Evol. Ecol. Res.*, **2**: 337–352.

Schluter, D. 2001. *The Ecology of Adaptive Radiation*. Oxford Series in Ecology and Evolution. Oxford: Oxford University Press.

Seger, J. 1992. Evolution of exploiter–victim relationships. In *Natural Enemies: The Population Biology of Predators, Parasites and Diseases* (M.J. Crawely, ed.), pp. 3–25. Oxford: Blackwell Scientific.

Shonle, I. and Bergelson, J. 2000. Evolutionary ecology of the trophane alkaloids of *Datura stramonium* L. (Solanaceae). *Evolution*, **54**: 778–788.

Simms, E.L. and Triplett, J. 1994. Costs and benefits of plant responses to disease. *Evolution*, **48**: 1973–1985.

Sokal, R.R. and Rohlf, F.J. 1995. *Biometry: The Principles and Practice of Statistics in Biological Research*, 3rd edn. New York: W.H. Freeman.

Sork, V.L., Stowe, K.A. and Hochwender, C. 1993. Evidence for local adaptation in closely adjacent subpopulations of northern red pine-oak (*Quercus rubra* L.) expressed as resistance to herbivores. *Am. Nat.*, **142**: 928–936.

Stinchcombe, J.R. 2002. Environmental dependency in the expression of costs of tolerance to deer herbivory. *Evolution*, **56**: 1063–1067.

Stinchcombe, J.R. and Rausher, M.D. 2001. Diffuse selection on resistance to deer herbivory in the ivyleaf morning glory, *Ipomoea purpurea*. *Am. Nat.*, **158**: 376–388.

Stinchcombe, J.R. and Rausher, M.D. 2002. The evolution of tolerance to deer herbivory: modifications caused by the abundance of insect herbivores. *Proc. R. Soc. Lond. B*, **269**: 1241–1246.

Stowe, K.A. 1998. Experimental evolution of resistance in *Brassica rapa*: correlated response of tolerance in lines selected for glucosinolate content. *Evolution*, **52**: 703–712.

Strauss, S.Y. and Agrawal, A.A. 1999. The ecology and evolution of plant tolerance to herbivory. *Trends Ecol. Evol.*, **14**: 179–185.

Strauss, S.Y., Rudgers, J.A., Lau, J.A. and Irwin, R.E. 2002. Direct and ecological costs of resistance to herbivory. *Trends Ecol. Evol.*, **17**: 278–285.

Thompson, J.N. 1994. *The Coevolutionary Process*. Chicago, IL: University of Chicago Press.

Thompson, J.N. 1999. What we know about and do not know about coevolution: insect herbivores and plants as a test case. In *Herbivores: Between Plants and Predators* (H. Olff, V.K. Brown and R.H. Drent, eds), pp. 7–30. Oxford: Blackwell Science.

Thompson, J.N. and Cunningham, B.M. 2002. Geographic structure and dynamics of coevolutionary selection. *Nature*, **417**: 735–738.

Tiffin, P. 2000. Are tolerance, avoidance and antibiosis evolutionary and ecologically equivalent responses of plants to herbivores? *Am. Nat.*, **155**: 128–138.

Tiffin, P. and Rausher, M.D. 1999. Genetic constraints and selection acting on tolerance to herbivory in the common morning glory *Ipomoea purpurea*. *Am. Nat.*, **154**: 700–716.

Underwood, N. and Rausher, M.D. 2000. The effects of host-plant genotype on herbivore population dynamics in a model system. *Ecology*, **81**: 1565–1576.

Valverde, P.L. 2001. Dinámica evolutiva de la resistencia a insectos herbívoros en *Datura stramonium*. Doctoral dissertation, Instituto de Ecología, Universidad Nacional Autónoma de México, México, D.F.

Valverde, P.L., Fornoni, J. and Núñez-Farfán, J. 2001. Defensive role of leaf trichomes in resistance to herbivorous insects in *Datura stramonium*. *J. Evol. Biol.*, **14**: 424–432.

Valverde, P.L., Fornoni, J. and Núñez-Farfán, J. 2003. Evolutionary ecology of *Datura stramonium*: equal plant fitness benefits of growth and resistance against herbivory. *J. Evol. Biol.*, **16**: 127–137.

van der Meijden, E., Wijn, H. and Verkaar, J. 1988. Defence and regrowth: alternative plant strategies in the struggle against herbivores. *Oikos*, **51**: 355–363.

van Valen, L. 1973. A new evolutionary law. *Evol. Theory*, **1**: 1–30.

Via, S. 1984. The quantitative genetics of polyphagy in an insect herbivore. II. Genetic correlations in larval performance within and among host plants. *Evolution*, **38**: 896–905.

Weaver, S.E. and Warwick, S.I. 1984. The biology of Canadian weeds. 64. *Datura stramonium* L. *Can. J. Plant Sci.*, **64**: 979–991.