

Test of local adaptation to biotic interactions and soil abiotic conditions in the ant-tended *Chamaecrista fasciculata* (Fabaceae)

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Abstract Few previous studies have assessed the role of herbivores and the third trophic level in the evolution of local adaptation in plants. The overall objectives of this study were to determine (1) whether local adaptation is present in the ant-defended plant, *Chamaecrista fasciculata*, and (2) the contribution of ant-plant-herbivore interactions and soil source to such adaptation. We used three *C. fasciculata* populations and performed both a field and a greenhouse experiment. The first involved reciprocally transplanting *C. fasciculata* seedlings from each population-source to each site, and subsequently applying one of three treatments to one-third of the seedlings of each population-source at each site: control, reduced ant density and reduced folivory. The greenhouse experiment involved reciprocal transplants of population-sources with soil sources to test for a soil-source effect on flower production and local adaptation to soil conditions. Field results showed that ant and herbivore treatments reduced ant density (increasing folivory) and herbivore damage relative to controls, respectively; however, these manipulations did not impact *C. fasciculata* reproduction or the likelihood of survival. In contrast, greenhouse results showed that soil

source significantly affected flower production. Overall, plants in both experiments, regardless of population-source, always had higher reproductive output at one specific site. Native populations did not outperform nonnative ones, causing us to reject the hypothesis of local adaptation. The absence of treatment effects on plant reproduction and the likelihood of survival suggest a limited effect of ants and folivores on *C. fasciculata* fitness and local adaptation during the study year. Temporally inconsistent effects of biotic forces across years, coupled with the young age of populations, relative proximity of populations and possible counter effects of seed predators may reduce the likelihood of local adaptation in the populations studied.

Keywords Ant defense · *Chamaecrista fasciculata* · Herbivory · Local adaptation · Spatial variation

Introduction

Spatial variation in environmental factors is universal in ecological systems. In response to such variation, plant populations can become genetically fixed for a phenotype at each location in which they occur, resulting in local adaptation (Van Tienderen 1990; Stearns 1992; Alpert and Simms 2002), or exhibit a range of phenotypes across sites against a homogeneous genetic background, i.e., phenotypic plasticity (Bradshaw 1965; Lortie and Aarssen 1996; Alpert and Simms 2002). Together, local adaptation and phenotypic plasticity across sites represent end points in a continuum of potential evolutionary responses. In general, low gene flow (Kingsolver et al. 2002; Sultan and Spencer 2002) and divergent natural selection across sites by biotic (e.g., herbivory; Sork et al. 1993) and abiotic factors (e.g., abiotic soil factors; McGraw and Chapin 1989) will

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promote local adaptation. Conversely, high gene flow and temporal variability in the strength and direction of selection are expected to favor phenotypic plasticity (Scheiner 1993; Sultan and Spencer 2002).

Local adaptation in plants is common (Antonovics and Bradshaw 1970; Schmidt and Levin 1985; Waser and Price 1985; Schmitt and Gamble 1990; Van Tienderen and Van der Toorn 1991; Miller and Fowler 1994; Nagy and Rice 1997; Joshi et al. 2001; Geber and Eckhart 2005), but not universal (Platenkamp 1990; Leiss and Muller-Scharer 2001). Most studies addressing plant local adaptation have focused on the relative importance of gene flow and natural selection (Slatkin 1973; Linhart and Grant 1996), and on the role of abiotic factors (e.g., wind, elevation, soil) as forces promoting local adaptation (Jain and Bradshaw 1966; Galen et al. 1991). Except for a few studies involving competition (McGraw and Chapin 1989; Prati and Schmid 2000) and leaf herbivores (Schemske 1984; Sork et al. 1993; Lau 2006), the influence of biotic interactions on plant local adaptation generally has been overlooked (although for animals this has not been the case; see Fauth 1998 and references therein for examples on competitive interactions and local adaptation in amphibians).

Herbivory and ant defense can act as important selective forces in plant populations (e.g., Marquis 1984, 1992a; Koptur 1984; Fonseca 1994; Rudgers 2004; Rutter and Rausher 2004). In addition, spatial variation in these selective forces can lead to geographic structuring of interactions (Barton 1986; Marquis 1992b; Rudgers and Strauss 2004; Thompson 2005), which may lead to local adaptation. Similarly, soil abiotic conditions can also have a strong impact on plant fitness components (Shaver et al. 1986; Burslem 1995; Hangelbroek et al. 2003), and soil effects have been shown to vary spatially, potentially leading to local adaptation (Schwaegerle and Bazzaz 1987). Furthermore, it is likely that tritrophic interactions are in many cases affected by abiotic soil conditions (e.g., via extrafloral nectar), such as nutrient and moisture levels (e.g., Frazee and Marquis 1994; Kersch and Fonseca 2005).

In *Chamaecrista fasciculata* Michx. (Fabaceae), ant visitation to extrafloral nectaries (EFNs) decreases herbivory, increasing seed production relative to ant-excluded plants (Barton 1986; Rios et al., in review). Leaf damage and ant abundance vary spatially among study sites in Missouri and Illinois, and appear to have selected for population differences in EFN characteristics (e.g., nectar production and concentration remained different between populations under greenhouse conditions suggesting a genetic basis for population differences; Rios et al., in review). In addition, *C. fasciculata* populations occur on different soil types in Missouri and Illinois, from sandy to very dense, clayey soils (Foote and Jackobs 1966); these soils vary in water-retention ability and fertility (LAR and RJM, unpublished

data), which can affect EFN nectar production (Chen 2003). Finally, given the observed low level of gene flow in this species (Fenster 1991), differential selection by biotic and/or abiotic factors across sites could promote local adaptation in *C. fasciculata*.

Although local adaptation of *C. fasciculata* populations has been reported in two other studies (Galloway and Fenster 2000; Etterson 2004), the potential contribution of biotic selective pressures to local adaptation has not yet been investigated in this system. Indeed, the role of the third trophic level as a selective force in the evolution of local adaptation appears not to have been studied previously in any plant species. The overall objective of the present study was to determine whether local adaptation is present in three populations of *C. fasciculata* and relate this result to ant-plant-herbivore interactions and abiotic soil characteristics as potential factors promoting local adaptation. We performed a field experiment in which three hypotheses were tested: (1) folivory, ant defense, and seed predation affect plant fitness, (2) local adaptation is present in *C. fasciculata*, and (3) folivory, ant defense, and seed predation contribute to such adaptation. In addition, a greenhouse experiment was performed to test the hypotheses that (4) soil source affects *C. fasciculata* reproduction, and (5) there is local adaptation to soil abiotic conditions in the populations studied.

Methods

Study system and sites

Chamaecrista fasciculata is a self-compatible, outcrossing annual (Fenster 1991) distributed throughout most of the eastern United States (Foote and Jacobs 1966). Seedlings emerge in late April and early May. Each leaf typically has a single EFN at the base of the petiole. A number of ant species (Formicidae) visit the EFNs. In the St. Louis region, the most abundant are *Monomorium minimum* (Buckley), *Paratrechina terricola* (Buckley), and *Creमतogaster lineolata* (Say) (Rios et al., in review; H. Wheeler, unpublished data). *Melanopus* spp. grasshoppers (Orthoptera) and *Eurema lisa* (Boisduval and Leconte) larvae (Lepidoptera) are the plant's major leaf herbivores, and a species of weevil (Coleoptera) feeds on seeds of developing fruits (Rios et al., in review).

Three *C. fasciculata* sites were used: Cuivre River (located at Northwood Prairie in Cuivre River State Park; near Troy, MO, USA), St. Charles Rock Road (located 100 m south of St. Charles Rock Road, on the east side embankment of Hwy I-170; St. Louis, MO, USA), and Poag Road Prairie (located between Poag Road and Hwy 255; Madison County, IL, USA). These sites were chosen

because they differ in leaf herbivory levels (lowest at St. Charles Rock Road, and highest at Cuivre River) and ant abundance (highest at Poag Road) on *C. fasciculata* plants (Rios et al., in review), as well as soil characteristics (see below). Each *C. fasciculata* native population consisted of at least 200 plants. All sites were at least 35 km apart, with few to no known intervening populations.

The Cuivre River site (CRSITE) was dominated by grasses (especially *Andropogon gerardii* Vitman) and some herbaceous species, which were, in many cases, more than 2 m tall; a matrix of deciduous *Quercus*-dominated forest surrounded the site. Deep (more than at the other two sites) and clayey soils occurred at this site. The Poag Road site (PRSITE) was abundant in xerophytic plant species (i.e., *Opuntia* sp.), several shrubs (*Cornus* spp., *Rubus* sp.), and the vine *Campsis radicans* Seem, as well as native grasses and invading *Q. stellata* Wangenh. Soil was sandy as this site was formerly a sand bank of the Mississippi River. Finally, the St. Charles Rock Road site (SCSITE) is within the St. Louis urban area, located on a highway embankment. The vegetation at this site was composed mainly of grasses, vines and shrubs of lower height and in lower densities compared to Cuivre River; soil at this site was compact and clayey.

Field experiment

Approximately 1 month after emergence (first week of June 2004), when seedlings were at least 10 cm tall, 135 seedlings were haphazardly collected from each site and placed in growing trays (No. 18, T.O. Plastics, MN, USA) to be transported to the University of Missouri, St. Louis, greenhouse (plants were kept in soil from their native site). Seedlings, provided daily with 30 ml water each, remained in the greenhouse for approximately 10 days to allow recovery from the physiological stress of initial collection. Forty-five randomly selected seedlings from each population were then transplanted to each of the three study sites, resulting in three experimental populations of 135 plants each: 45 native, and the remaining two-thirds nonnative (405 plants total for this experiment). One week after transplanting, all nonsurviving seedlings were replaced; mortality at this stage was considered to be mostly due to transplantation stress, and thus was not analyzed. Seedlings dying subsequent to this were not replaced. Once in the field, each seedling was given 150 ml of water per day on three nonconsecutive days during the first week following transplanting.

At each site, seedlings were planted 1 m apart in a plot consisting of 15 columns and 9 rows. The vegetation surrounding study plants within a radius of 25 cm was removed; vegetation was otherwise undisturbed. Each column was composed of plants of the same population-source, with population-source alternating by column.

During the last week of June 2004, one of three treatments was systematically applied to 15 plants of each population-source at each site: control (no manipulation), reduced EFN nectar production (RE), and herbivore exclusion (HE). Treatments were alternated within each row. The resulting design was three-factorial, with site, treatment, and population-source as the main (fixed) factors. Although the intended effect of both HE and RE treatment levels was to influence the amount of herbivore damage (and seed production of the plant), most of the variation in herbivory in the HE treatment compared to controls was expected to come from plant direct defenses (e.g., leaf chemistry), while that from RE compared to its control was expected to come from indirect defenses, i.e., ants (Rudgers et al. 2004). Including both treatment levels allowed us to determine which defense strategy or combination of strategies may be acting as a potential mechanism leading to local adaptation of the populations studied. Initial evidence (Rios et al., in review) suggested that plant population-level differences in nectar production are related to differences in herbivore abundance and the damage they cause.

To reduce ant visitation and its potential effects on plant fitness measures, EFN nectar production was blocked and thus made unavailable for ants by placing a drop of waterproof glue (Aleene's Ok to Wash It, Aleene's, Buelton, CA, USA) on each nectary (see Rudgers 2004). We predicted that plants in this treatment (RE) would suffer increased herbivory because of lower ant protection. Glue was applied weekly to all new leaves of plants subject to this treatment, and was done in such a way as to minimize petiole tissue necrosis (following recommendations by J. Rudgers). To control for the effect of the glue itself, a single drop was placed adjacent to the EFN on all leaf petioles of all other plants not subject to this treatment level.

The herbivore-exclusion treatment (HE) was performed using diluted (0.1 l oil: 6.6 l water) neem oil (DYNA GRO, San Pablo, CA, USA) sprayed directly on leaves. Neem oil is obtained commercially from the Neem tree (*Azadirachta indica* Juss: Meliaceae) and acts as a broad-spectrum organic repellent, growth regulator, and insect poison against insects of the Homoptera, Lepidoptera, Orthoptera and Coleoptera (Olkowski et al. 1991; Ellis and Bradley 1996), with no known effect on hymenopteran pollinators (Ellis and Bradley 1996). Because neem-active compounds biodegrade within 5–7 days (Ellis and Bradley 1996), this insecticide was applied once a week throughout the field season to ensure exclusion of herbivores throughout the field season. In the greenhouse, neem insecticide did not affect flower production of *C. fasciculata* plants whose leaves were sprayed once a week for 3 months compared to nonsprayed controls: sprayed flower mean \pm SE = 175.3 \pm 12.14, nonsprayed = 137.8 \pm 9.43 (PROC GENMOD: $F_{1,28} = 2.40$, $P = 0.13$).

Dependent variables

The following dependent variables were measured for each plant at each site: flower number, fruit number, total seed number, proportion of leaf area lost (folivory), ant density, and the proportion of seeds lost to weevils (seed predation). In addition, the number of surviving experimental plants was recorded at each site the first week of September 2004. Leaf damage was measured during the second week of August 2004. Three ant censuses were performed the third weeks of July, August, and September 2004. Weekly flower counts (flowers last a single day) were conducted starting the second week of July 2004, and until the last week of September 2004. Finally, fruits were collected starting the third week of September 2004, and dissected in the laboratory to determine the total number of seeds produced per plant as an estimate of lifetime fitness. While dissecting fruits, it was possible to determine the number of seeds missing or damaged due to weevil predation. The number of seeds eaten was then divided by the total number of seeds produced by a plant (counted plus eaten) to obtain the proportion of seeds eaten. Values were averaged by plant.

Folivory was assessed by estimating missing area from each leaflet for each of five randomly chosen mature leaves per plant (the study species has pinnately divided compound leaves). We assigned, by visual inspection, each leaflet to one of five categories depending on percent area missing (0 = no damage, 1 = 1–25%, 2 = 25–50%, 3 = 50–75%, 4 = 75–100%; modified from Rios et al., in review). Damage for each leaflet was obtained by multiplying mean leaflet area for each leaf (dividing total leaf area by leaflet number) by the mid-value of the proportion of area missing assigned for that leaflet. The resulting values of missing area per leaflet were summed to estimate the area missing for each leaf, and then divided by total leaf area to estimate the proportion of leaf area missing. Values for each leaf were averaged by plant. Total leaf area for each leaf was estimated based on its length. Previously, we collected one mature leaf from 50 different plants of each population. The lengths of those leaves were measured, and the area of each leaf was estimated with a scanner (Hewlett Packard ScanJet 6300C) and a pixel-counting program (SigmaScan Pro 5.0). These values were used to calculate a regression equation for each population [$\text{area} = \lambda + \beta(\text{length})$] to predict total leaf area based on leaf length (R^2 values ranged from 0.94 to 0.97; $P < 0.001$ in all cases).

All ants present on a plant were counted without removal in the morning (0900 to 1200 hours) because this is the time of day when insect activity is greatest (both herbivores and ants) and interaction effects are assumed to be strongest. Sites were visited on consecutive days, and in a different order during each census; ants were not censused on rainy days. Ant density was expressed as the number of

ants divided by the plant's number of leaves (ant density responds to EFN number; each leaf has a single EFN) multiplied by 100. This response variable estimates the ability a plant has to attract ants based on its resource availability (Heil and McKey 2003). Finally, values were summed across the three censuses to represent the total number of ants a plant was able to attract throughout the sampling season, given that the average life span of *M. minimum* workers (by far the most abundant species found on *C. fasciculata* in this study) is approximately equal to the amount of time between each census. Results from an analysis using averages across censuses did not show qualitative differences from those using the cumulative value.

Treatment effects and local adaptation

A treatment effect on plant reproduction was considered evidence of the influence that biotic interactions (ant defense, folivory) have on the study species's fitness components. Evidence for local adaptation was taken to be a significant site \times population-source interaction for which native plants show greater reproductive output (or survival) than nonnative plants (Schluter 2000) for at least one study site (e.g., Schemske 1984; Van Tienderen 1992). We also looked for significant population-source \times site interactions in ant density, proportion of leaf area lost, and proportion of seeds eaten, as these variables are correlated to reproductive output (Rios et al., in review) and can be used to test for local adaptation. For such variables, however, a treatment effect would indicate that the treatments were effective at reducing folivore, ant and seed-predator numbers. Finally, treatment \times site \times population-source interactions for reproductive measures, correlated variables, and survival were of particular interest because they might indicate differences in the population-source \times site interaction depending on the treatment (HE, RE or controls), and thus provide clues as to the effects these insects (i.e., the biotic effect) have on local adaptation.

Statistics

All statistical analyses were conducted in SAS (SAS Institute 2002).

Plant reproduction and survival Log-linear model analyses (PROC GENMOD) were conducted separately for flowers, fruits, and seeds (observed plus those attacked by predators) as dependent variables; site, treatment, and population-source (three levels each) and their interactions were used as main effects. Initial total leaf area, measured as the average area per leaf ($n = 7$ leaves per plant) per plant multiplied by total leaf number was used as a covariate; interactions of the covariate with the main effects were

not significant for any reproductive measure in this experiment. These analyses only include plants that survived until the end of the reproductive season (late September). Using all plants (dead and alive by the end of September; dead plants were assigned a zero) showed no qualitative differences in the results. In addition, an analysis with blocks within sites was also performed in order to further characterize within-site variation that could mask the effects of the main factors; results from this analysis (not reported here) did not change qualitatively from those using plants still alive by late September. Finally, logistic regression (PROC PROBIT) was conducted using survival as a dichotomous response variable for each individual (0 = died, 1 = survived), with site, treatment, population-source, and their interactions as predictor variables. Nonsignificant effects and interactions were removed from the regression model in a stepwise-backward fashion (Floyd 2001; Gentry and Dyer 2002).

Folivory, ant density, and seed predation Three-way analyses of variance (PROC GLM; site, treatment and population-source) were performed separately for proportion of leaf area lost and ant density. The proportion of seeds lost to weevil predation at PRSITE and SCSITE was also analyzed with a three-way ANOVA (site, treatment and population-source); CRSITE was exempt from seed damage and thus not included in this analysis. Initial total leaf area was also used as covariate, and its interactions with the main effects were not significant for any of these response variables.

Other statistical considerations Tukey tests were performed as follow-up for comparison of means for ANOVA-type analyses (PROC GLM), and preplanned contrasts were conducted using the ESTIMATE option for log-linear model analyses (PROC GENMOD). Nonsignificant factors were removed from each model in a backward fashion, except when involved in a significant interaction. Data transformations were conducted for ANOVA-type analyses when a significant deviation from normality was observed based on Kolmogorov–Smirnov or Shapiro–Wilk tests ($P < 0.05$ to reject normality). Skewness and kurtosis values also were used to assess normality. When transformations were necessary, normality was achieved (normality tests $P > 0.05$ in all cases after transformation). Ant, folivory, and seed predation data were arcsine square-root transformed (Zar 1999). Power tests were conducted using GLMPOWER for linear models in SAS. All statistical analyses were performed using type III sums of squares for ANOVA-type analyses (GLM) and type 3 analyses for log-linear models (GENMOD). Given that flower, fruit, and seed data were overdispersed (values ≥ 5), a scaling factor was applied (SCALE = PEARSON) to control for such a condition (Allison 1999; Stokes et al. 2000).

Greenhouse experiment

Thirty seedlings and 30 soil samples were collected from each site the last week of May 2004, and taken to the University of Missouri, St. Louis, greenhouse to perform a population-source-soil source reciprocal transplant experiment. Seedlings and soil samples were haphazardly collected at different locations at each site from the area surrounding the experimental plots used for the field experiment. Seedlings were given 30 ml of water daily for a week while in growing trays, and then randomly transplanted to 1-l pots with soil from one of the three sources. After all transplants were conducted, each plant population had 10 plants growing under each soil source. Once in pots, seedlings were given 130 ml of water daily. The experimental setup was a crossed design ($n = 90$; 10 replicates per cell), using soil source (three levels) and plant population-source (three levels) as main effects to assess the effect of soil origin on reproductive output of the three population-sources. As a measure of fitness, flowers were counted daily for each plant from the start of July 2004 until the end of September 2004 (flower number is significantly positively correlated to seed number: $r_p = 0.77$, $P < 0.0001$; field data, this study).

Statistics

The effects of soil source (soil effect), population-source and their interaction (local adaptation to soil) on flower number were analyzed with a log-linear model (PROC GENMOD; type 3 analysis). Initial total leaf area per plant was used as covariate. The interaction of the covariate with the main effects was not significant. Preplanned contrasts (ESTIMATE option) were conducted to compare means of levels for significant factors. Nonsignificant factors were removed from the model in a backward fashion, except when involved in a significant interaction. Given that the data were overdispersed (value > 4), a scaling factor was applied (SCALE = PEARSON) to control for such a condition (Allison 1999; Stokes et al. 2000).

Results

Field experiment

Site and population-source effects

Ant density A significant site effect occurred (Table 1), with SCSITE having the highest ant density (32.38 ants per 100 leaves ± 4.53), while intermediate levels were observed at PRSITE (19.02 ± 1.97), and very few ants at CRSITE (0.65 ± 0.25) (all sites differed significantly;

Table 1 Field-experiment results from the three-way ANOVA (population-source, treatment, and site) for ant density and proportion of leaf area lost, as well as from three-way ANOVA for SCSITE and PRSITE for proportion of seeds eaten (arcsine square-root transformed)

Source of variation	Proportion of leaf area lost				Ant density				Proportion of seeds eaten			
	SS	df	F-value	P-value	SS	df	F-value	P-value	SS	df	F-value	P-value
	Population-source	0.02	2,306	0.47	0.622	63.46	2,218	8.60	0.0003	0.032	2,164	0.98
Treatment	0.76	2,306	15.77	<0.0001	52.51	2,218	7.12	0.001	0.004	2,164	4.10	0.018
Site	1.84	2,306	38.35	<0.0001	881.04	2,218	119.43	<0.0001	0.236	1,164	129.24	<0.0001
P. source × treatment	0.27	4,306	2.85	0.024	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R
P. source × site	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R	0.152	2,164	4.59	0.011
Treatment × site	T/R	T/R	T/R	T/R	55.51	4,218	3.76	0.005	T/R	T/R	T/R	T/R
P. source × treat × site	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R
Initial foliar area	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R

Values presented are for terms that remained significant after backward removal (i.e., final model). T/R Nonsignificant terms removed during backward selection

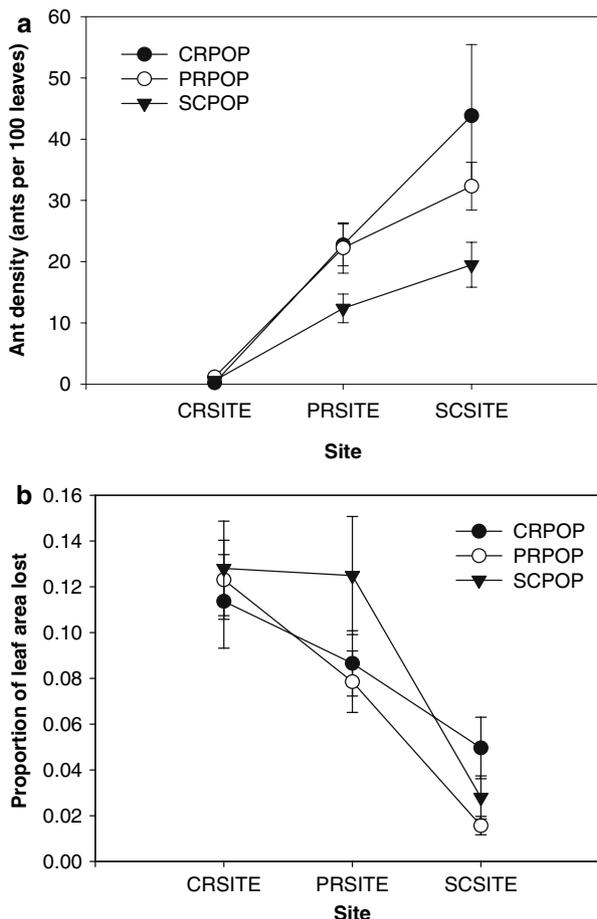


Fig. 1 Ant density values (a) and proportion of leaf area lost to herbivores (b) for *C. fasciculata* population-sources at each site. All treatment levels included. Values shown are means \pm SE

$P < 0.05$, Tukey; Fig. 1a). In addition, a significant population-source effect was observed (Table 1): Cuivre River native plants (CRPOP) had the highest ant densities

(20.51 ± 4.00), more than twice that of SCPOP plants (9.64 ± 1.45) ($P < 0.05$, Tukey), and almost 1.5 times that of PRPOP plants (16.88 ± 2.14) (Fig. 1a).

Folivory There was a significant site effect on leaf damage (Table 1), with plants at SCSITE having the lowest average damage level ($3 \pm 0.5\%$ of total leaf area lost), and differing significantly from those at CRSITE and PRSITE (12 ± 1.1 and $9.7 \pm 1.1\%$, respectively), which themselves did not differ ($P > 0.05$, Tukey; Fig. 1b). Population-sources did not show significant differences in leaf damage (Table 1).

Reproductive output Log-linear analyses for flower, fruits, and seeds all showed a significant site effect, but no population-source effect (marginal population-source effect on seed number: $F_{2,282} = 2.71$, $P = 0.06$); the final models after backward elimination all showed adequate fits compared to the saturated models. The SCSITE (408.1 ± 50.6) had on average more than 10 times the seed production of plants at CRSITE (30.8 ± 3.6), and more than 5 times that of PRSITE (76.49 ± 12.89) (Fig. 2a), and differed significantly from the other sites for all reproductive measures ($\chi^2 \geq 40$, $P < 0.0001$). Finally, the site effect on seed predation was also significant (Table 1), with SCSITE and PRSITE differing significantly from each other ($P < 0.05$, Tukey). Seed predation at PRSITE ($10 \pm 1.6\%$ of seeds eaten) was more than 15-fold that of SCSITE ($0.6 \pm 0.21\%$) (Fig. 2b). Seed predators were absent at CRSITE.

Survival Logistic regression showed a strong site effect ($\chi^2 = 20.23$, $df = 2$, $P < 0.0001$; overall model likelihood ratio = 22.0, $df = 2$, $P < 0.0001$). The population-source effect was not significant ($\chi^2 = 2.57$, $df = 2$, $P = 0.27$) and was removed from the model along with all other factors and interactions that were not significant. The overall

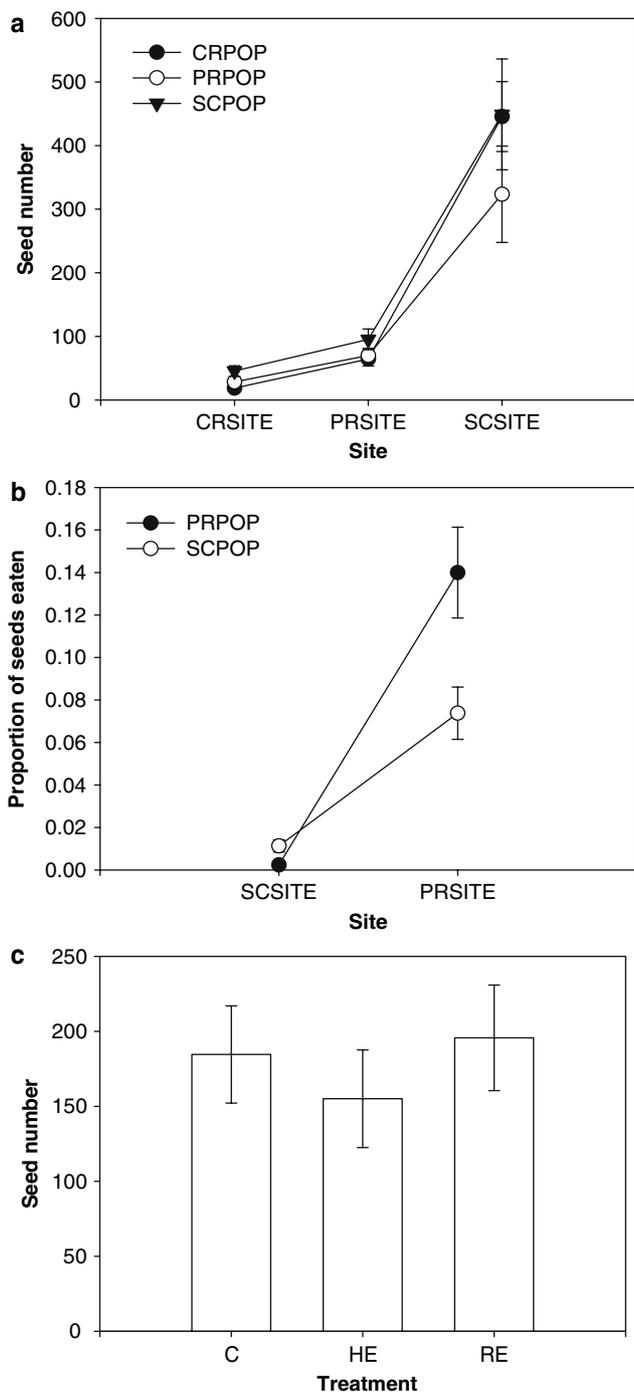


Fig. 2 *Chamaecrista fasciculata* seed production for each population-source by site (a), proportion of seeds lost to predation for PRPOP and SCPOP plants by site (b), and seed production by insect treatment level (c). Values are means ± SE

model with site had an adequate fit given the observed data (Pearson $\chi^2 = 17.20$, $df = 20$, $P = 0.63$). Of the three sites, only PRSITE had a significant parameter estimate, which was positive (estimate = 0.98, $P = 0.0002$), indicating that the likelihood of a plant surviving was higher at PRSITE.

Overall, 77% of the plants at PRSITE had survived by early September while only 56 and 52% remained alive at SCSITE and CRSITE, respectively.

Treatment effects

Ant density The treatment effect was significant (Table 1): ant-reduced (RE) plants had the lowest densities (10.14 ± 1.43) and differed significantly from the control treatment (21.93 ± 4.11) ($P < 0.05$, Tukey), which shows that gluing of EFNs reduced ant numbers. The HE treatment (15.30 ± 2.01) did not differ significantly from control plants, which shows that the insecticide did not have an important effect on ant abundance. The treatment × site interaction was significant (Table 1), but this result was due to the virtual absence of ants on plants at the CRSITE, which resulted in no treatment effect at this site.

Folivory There was a significant treatment effect on folivory (Table 1), for which reduced-EFN plants (RE) were the most heavily damaged ($12 \pm 1.2\%$) and differed significantly ($P < 0.05$, Tukey) from control ($8 \pm 1\%$) and herbivore-exclusion (HE) plants ($5 \pm 0.6\%$) (the latter had less damage than controls, although not significantly so). Finally, the treatment × population-source effect was significant (Table 1), because SCPOP controls had, on average, more damage than control plants from the other population-sources.

Reproductive output Log-linear model analyses for flower, fruits, and seeds all showed a nonsignificant treatment effect. The absence of a treatment effect suggests that, despite significantly affecting the amount of leaf area lost, folivory and ant defense had a limited impact on *C. fasciculata* reproductive measures during the study season. Although power test results indicated low values (<0.2), graph examination showed no trace of either HE or RE treatments having positive or negative effects, respectively, on plant fitness components compared to controls (e.g., see Fig. 2c).

Survival There was no significant treatment effect on the likelihood of survival (original model $\chi^2 = 0.7047$, $df = 2$, $P = 0.70$). The same held true for treatment × site and treatment × population-source terms (original model $\chi^2 = 1.75$, $df = 4$, $P = 0.78$, and $\chi^2 = 5.22$, $df = 4$, $P = 0.26$, respectively).

Evidence for local adaptation

Ant density The population-source × site interaction was not significant, although this result can be considered marginal ($F_{4,214} = 2.13$, $P = 0.07$; power = 0.6); however, there was an overall trend towards higher ant densities at

SCSITE regardless of population-source (Fig. 1a), and none of the native populations showed higher ant abundance than nonnative ones. The three-way interaction was not significant ($F_{8,202} = 1.29$, $P = 0.24$; power = 0.6).

Folivory The population-source \times site interaction was not significant ($F_{4,297} = 1.48$, $P = 0.20$; power = 0.3) because all plants, regardless of source, experienced lower damage at the SCSITE (Fig. 1b). Native populations did not exhibit significantly lower leaf damage than nonnative ones (Fig. 1b). The treatment \times population-source \times site interaction was not significant ($F_{8,290} = 1.48$, $P = 0.16$; power = 0.5).

Reproductive output The log-linear model analyses for flowers and seeds did not show significant population-source \times site interactions (flowers $F_{4,321} = 1.55$, $P = 0.18$; seeds $F_{4,268} = 0.91$, $P = 0.45$; power values $0.2 \geq 0.5$; Fig. 2a); however, fruit number did (Table 2). This latter result was not due to local adaptation, but rather because CRPOP plants produced more fruits than SCPOP plants at the SCSITE (SCPOP = 51.9 ± 9.75 , CRPOP = 71.0 ± 16.49). A similar analysis using relative seed production (see Lau 2006) showed that there was no population-source effect or population-source \times site interaction: none of the native populations outperformed nonnative ones (Fig. 2a). Finally, the three-way interaction was never significant (flowers $F_{8,312} = 0.58$, $P = 0.79$; fruits $F_{8,313} = 0.34$, $P = 0.94$; seeds $F_{8,260} = 0.55$, $P = 0.81$; power values > 0.5 in all cases). In contrast, there was a significant population-source \times site interaction for seed predation (Table 1); however, this result arose due to native plants having the greatest proportion of seeds eaten at their site of origin, an opposite pattern to that expected if plants were locally adapted to their seed predators (Fig. 2b).

Survival Population-source \times site and treatment \times population-source \times site interactions were not significant

($\chi^2 = 2.40$, $df = 4$, $P = 0.66$, and $\chi^2 = 7.93$, $df = 8$, $P = 0.44$, respectively), thus they were eliminated during backward selection as mentioned previously. However, although not a significant result, the proportion of native plants surviving at one site was higher than that of nonnative plants: more SCPOP plants remained alive (68%) than those of CRPOP (56%) and PRPOP (49%) at the SCSITE.

Greenhouse experiment

Soil- and population-source effect

Log-linear model analysis for flower number showed a significant soil-source effect (see Table 3), for which plants grown on St. Charles Rock Road soil (SCSOIL) had the highest flower production (241.43 ± 44.07), differing significantly from the other two soil sources ($P < 0.001$; pre-planned contrast). Plants grown on Poag Road soil (PRSOIL) (197.3 ± 36.02) and Cuivre River soil (CRSOIL) (169.5 ± 32.03) did not differ significantly ($P = 0.12$). In addition, we also found a significant population-source effect (Table 3), for which CRPOP plants had the highest flower production (231.43 ± 11.27 ; significantly different from SCPOP and PROP, $P < 0.0001$) and did best on nonnative soils (Fig. 3), whereas SCPOP plants (211.21 ± 12.03) did best on their soil of origin. Finally, PRPOP plants (168.36 ± 9.36) produced equally low numbers of flowers on all soil sources (Fig. 3) and did not differ significantly from SCPOP. The covariate (initial leaf area) was significant (Table 3).

Evidence for local adaptation

The population-source \times soil source interaction was significant for flower production (Table 3), however, no evidence of local adaptation was found as plants native to a given soil source never outperformed nonnatives (Fig. 3).

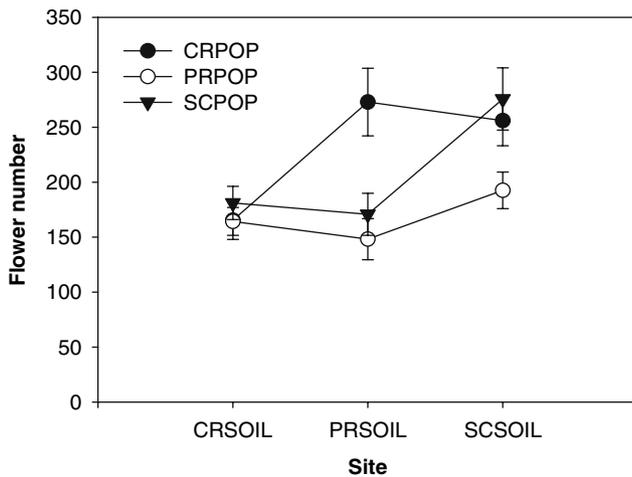
Table 2 Field-experiment results from the log-linear model analyses (population-source, treatment, and site) for flower, fruit, and seed number

Source of variation	Flower number			Fruit number			Seed number		
	df	F-value	P-value	df	F-value	P-value	df	F-value	P-value
Population-source	T/R	T/R	T/R	T/R	0.55	0.57	T/R	T/R	T/R
Treatment	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R
Site	2,337	89.20	<0.0001	2,331	36.91	<0.0001	2,284	70.08	<0.0001
P. source \times treatment	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R
P. source \times site	T/R	T/R	T/R	4,331	3.18	0.01	T/R	T/R	T/R
Treatment \times site	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R
P. source \times treat \times site	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R
Initial foliar area	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R	T/R

Values presented are for terms that remained significant after backward removal (final model). T/R Nonsignificant terms removed during backward removal

Table 3 Greenhouse results for log-linear model analysis for flower number

Source of variation	Flower number		
	df	F-value	P-value
Population-source	2, 78	12.54	<0.0001
Soil source	2, 78	9.18	0.0003
Population-source \times soil source	4, 78	4.30	0.003
Initial foliar area	1, 78	10.55	0.001

**Fig. 3** *Chamaecrista fasciculata* flower production for plant population-sources on each soil source (greenhouse experiment). Values are means \pm SE

Discussion

Local adaptation status of *C. fasciculata* study populations

Two previous studies have demonstrated local adaptation in *C. fasciculata* based on reproduction and/or survival (Galloway and Fenster 2000; Etterson 2004), and both generally found such a condition only at large spatial scales (e.g., ≥ 400 km). In contrast, the populations studied here were no more than 70 km apart, and most evidence from this study argues against the existence of local adaptation in the studied populations, as well as any possibility that ants and herbivores contributed to such adaptation during the study year. Population-source \times site interactions were usually not significant, and native plants did not outperform nonnative plants at any site in terms of flower, fruit, and seed number. Although power tests indicated low power for some of these interactions, graph examination (Figs. 1–3) confirmed a lack of home advantage of plant populations. In addition, other response variables measured (e.g., folivory, ant den-

sity) that could contribute indirectly to local adaptation did not appear to do so. For example, all population-sources had their highest ant density values at SCSITE, and native plants never showed higher ant densities than nonnative ones. Likewise, folivory results did not support local adaptation. Furthermore, seed predation results exhibited a pattern opposite to that expected, as native populations suffered the highest levels of seed predation. Finally, greenhouse results did not indicate local adaptation to soil conditions, as plants native to a given soil origin never outperformed nonnatives. In spite of all of this, some weak signal of local adaptation could be argued for SCPOP plants. First, these plants had 10–20% higher survival than other population-sources at SCSITE; however, this result was not significant. Secondly, this population had consistently lower seed production (Fig. 2a) and higher folivory (Fig. 1b) at nonnative sites. Nonetheless, SCPOP did not outperform (e.g., seed number) CRPOP at the former's native site (SCSITE) (Fig. 2a) or under native soil conditions (SCSOIL) in the greenhouse (flower number; Fig. 3).

In contrast, the most consistent result across all analyses was the presence of a strong site effect for which all population-sources had higher reproductive output at SCSITE. Possible reasons for this result might be (1) that this site had the lowest levels of folivory and the highest ant densities; this point would favor ants and herbivores as factors explaining a large portion of the variation in plant traits and overall success despite results found here (sensu Barton 1986; Rutter and Rausher 2004; Rios et al., in review), (2) high soil fertility at SCSITE (LA and RJM, unpublished data), which is supported also by greenhouse results as plants grown on SCSOIL had the greatest flower production, and (3) although a condition not exclusive to this site, vegetation density was low, which reduced competition for light and soil resources (L. Abdala, personal observation). Although plants at SCSITE did not show the highest proportion of surviving plants at the end of the reproductive season (September), the reason for this was probably additional mortality due to vertebrates (a gopher, *Geomys* sp., was observed several times adjacent to the study plot). Overall, these results suggest, instead of local adaptation (e.g., for SCPOP plants), a strong plastic response of all population-sources to SCSITE's resource-rich environment (e.g., increased output in high nutrient-level soils; Sultan and Bazzaz 1986; Hangelbroek et al. 2003).

According to Forde et al. (2004), gene flow can increase the chance of local adaptation occurring, presumably by elevating the average fitness of a population due to greater genetic variation and arrival of "appropriate" genes. However, in this study it seems unlikely that gene flow could promote local adaptation because, just as native populations did not outperform nonnatives (i.e., no significant population-source \times site term), a significant

population-source \times site interaction for which *nonnatives* were outperforming *natives* did not occur either. The only exception for this took place for the greenhouse experiment as CRPOP plants had a much higher flower production on PRSOIL than native plants (PRPOP), resulting in a significant soil source \times population-source interaction (Fig. 3). Thus, it could be argued that CRPOP genes arriving at PRSITE could increase the average fitness of the native population, thus increasing the extent to which local adaptation could presumably take place there in the future. However, this result pertained only to the flower production response to soil abiotic conditions in the greenhouse; the field experiment did not show this pattern for reproductive measures (Fig. 2a). Thus, it seems unlikely that gene flow between CRSITE and PRSITE would increase plant local adaptation at the latter site, and even less so between any other pair of populations.

Effect of ants, herbivores, and soil source on *C. fasciculata* fitness and local adaptation

Although the reduced-nectar treatment lowered ant density (significantly increasing folivory) and herbivore-exclusion treatment decreased folivory, these manipulations did not affect plant reproduction or the likelihood of survival (despite power level being low, graph examination did not reveal a tendency in favor of predictions). These results agree with a previous study with *C. fasciculata* by Kelly (1986), who found no effect of ant exclusion on plant fitness components; however, Barton (1986) and Rios et al. (in review) did find an ant-exclusion effect via increased herbivory. Finally, the three-way interaction (treatment \times population-source \times site) was never significant for reproductive measures, survival, or variables correlated to the former (e.g., folivory, ant density). All together, these results are evidence of a temporal inconsistency in the effect of ants and herbivores on plant fitness components, which brings into question their role as relevant evolutionary forces influencing local adaptation of *C. fasciculata* at the study sites. Possible causes of temporal variation in the effect these interactions have on plant fitness measures may include changing ant communities (e.g., DiGiusto et al. 2001), changing herbivore communities (Forkner et al. 2006), and changing resource availability to the plants so that they vary in traits that influence herbivory from year to year (Gratton and Denno 2003).

In contrast to the observed impact of ants and herbivores, there was a significant effect of soil source on *C. fasciculata* reproduction (flower production) in the greenhouse. Soil conditions from one site (SCSOIL) significantly increased flower production compared to soil from other sites. Of the three sites, SCSOIL had the highest percent organic matter, and the highest concentration of K,

Ca, Mg, and nitrates, as well as the least acid pH (LAR and RJM, unpublished data). Nevertheless, although soil conditions can be considered a temporally less variable selective force than biotic factors (given that environmental temporal variability favors plasticity; Scheiner 1993), plants growing on their native soil did not outperform nonnatives. It is important to mention that we did not consider the role soil biotic conditions might play in this study (e.g., levels of nitrogen-fixing bacteria; Slattery et al. 2001), and these also could influence plant fitness measures and local adaptation. Finally, although not included in this study, we also measured EFN nectar volume production in the greenhouse for the same plants used in the soil source \times population-source experiment, and results showed no home advantage (i.e., plants growing on their native soil did not produce more nectar than nonnatives). This result indicates that, for the populations studied, soil abiotic factors are not influencing potential local adaptation to the ant-herbivore system via EFN nectar production (LAR and RJM, unpublished data).

Finally, in the case of weevil seed predation, native plant populations suffered the highest damage levels, a pattern opposite to that expected for plant populations locally adapted to their seed predators. This result suggests another reason (besides resource-rich conditions at SCSITE) why local adaptation was not observed in this system: weevils could be locally adapted to each *C. fasciculata* population and may therefore disrupt other possible mechanisms leading to local adaptation of their host plants. Further studies are necessary to investigate fitness responses of weevil populations feeding on native and nonnative plants to properly assess local adaptation of these insects to host plant populations (e.g., Strauss 1997).

The two previous studies that found local adaptation in *C. fasciculata* involved large spatial scales: Galloway and Fenster (2000) tested distances from 0.1 to 2,000 km and found local adaptation only at the largest distance, and Etterson (2004) tested climate as a factor determining local adaptation using sites that were on average 400 km apart from each other. In the current study, distance between sites was ≤ 70 km, and at least one of the populations may be of relatively recent establishment (e.g., SCSITE may be no more than 30 years old; G. Luttrell, personal communication). Thus, distance between populations (i.e., spatial scale resolution) and age (and therefore limited genetic divergence and potential founder effects) (Kingsolver et al. 2002; Sultan and Spencer 2002) might be additional factors that have decreased the likelihood of finding local adaptation in this study. Nevertheless, by using a smaller spatial scale, our study implicitly contributes to a better understanding of the geographic and temporal scale at which local adaptation is occurring in *C. fasciculata*. Finally, although we did not find an influence of herbivores, ants, and abiotic soil conditions on local adaptation, and despite

temporal variation in the selective impact of the former two on plant fitness, we suggest that more attention should be paid to biotic interactions as they might promote local adaptation in plant populations.

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