

Influence of multiple factors on plant local adaptation: soil type and folivore effects in *Ruellia nudiflora* (Acanthaceae)

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Received: 27 December 2010/Accepted: 3 July 2011
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Abstract Different environmental factors can have contrasting effects on the extent of plant local adaptation (LA). Here we evaluate the influence of folivory and soil type on LA in *Ruellia nudiflora* by performing reciprocal transplants at two sites in Yucatan (Mexico) while controlling for soil source and folivory level. Soil samples were collected at each site and half of the plants of each source at each site were grown with one soil source and half with the other. After transplanting, we reduced folivory by using an insecticide applied to half of the plants of each population source grown on each soil at each site. This resulted in a fully-crossed design with site, population source, soil source and folivory as main effects. We evaluated LA by means of a significant site × origin interaction showing a home-site advantage of native plants. Additionally, to test for an effect of soil source and folivores on LA, we estimated the three-way interactions of site × origin × soil source and site × origin × folivory. We recorded fruit number and survival throughout an 8-month period. For survival, we found evidence of home-site advantage at one site, while for fecundity we found no evidence of LA and at one site even observed evidence of lower fecundity for local relative to foreign plants. Importantly, folivory had no influence on the degree of home-site advantage for either response variable, while soil source influenced the degree of home-site advantage in fecundity at one site (suggesting some degree of specialization to soil characteristics in *R. nudiflora*). Our results emphasize the need for simultaneously evaluating multiple factors of influence in tests of LA.

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Keywords Herbivory · Local adaptation · Soil conditions · Yucatan

Introduction

Local adaptation, defined as the condition whereby resident genotypes exhibit higher relative fitness in their local habitat compared to genotypes originating from other habitats (Kawecki and Ebert 2004), has been reported for a number of plant species over the last four decades (e.g., Antonovics and Bradshaw 1970; Schmidt and Levin 1985; Van Tienderen and Van der Toorn 1991; Geber and Eckhart 2005; Anderson and Geber 2009). Based on these results, it has been often assumed that plant local adaptation is a common condition across populations and species. Nevertheless, several studies have found no evidence for local adaptation in plants (e.g., Platenkamp 1990, Leiss and Muller-Scharer 2001; Abdala-Roberts and Marquis 2007; Ortegón-Campos et al. 2009), and a recent meta-analysis suggests that local adaptation is less common than previously thought (Leimu and Fischer 2008), thus questioning the ubiquity of the phenomenon.

Theory predicts that local adaptation will be favored by some combination of low gene flow between populations and divergence in the strength and/or sign of natural selection across sites (Kingsolver et al. 2002; but see Hoeksema and Forde 2008 for implications of relative gene flow levels in the context of coevolutionary dynamics). In the case of plants, traits such as mating system, longevity and clonality also have the potential to influence local adaptation given their effects on the degree and spatial distribution of genetic variation (Leimu and Fischer 2008). For example, it is predicted that short-lived self-compatible species will tend to have more differentiated populations which are prone to local adaptation at smaller spatial scales (Linhart and Grant 1996; but see Leimu and Fischer 2008; Hereford 2010). Finally, other factors such as large population sizes (Jakobsson and Dinnetz 2005; but see Becker et al. 2006, 2008), greater time since establishment of a population (Parker et al. 2003), and greater distance between populations (Galloway and Fenster 2000; Becker et al. 2006; but see Becker et al. 2008) will increase the likelihood of plant local adaptation. The degree to which some or all of these factors predominate will determine the strength of local adaptation.

Traditionally, plant studies have focused on the influence of abiotic factors (e.g., soil, precipitation) on local adaptation (e.g., Jain and Bradshaw 1966; Antonovics and Bradshaw 1970). However, during the last decade there has been an increase in the number of investigations which have addressed the degree to which plants are locally adapted to biotic factors such as insect herbivores (Sork et al. 1993; Abdala-Roberts and Marquis 2007; Ortegón-Campos et al. 2009), pathogens (e.g., Crémieux et al. 2008), mutualists (Abdala-Roberts and Marquis 2007; Gómez et al. 2009), as well as the influence competition has on local adaptation (e.g., Sambatti and Rice 2006). The most powerful approach to measure the contribution of biotic (or abiotic) factors to plant local adaptation is to perform reciprocal transplants while simultaneously manipulating the abundance or effect of the biotic counterpart (i.e., herbivore, pollinator, competitor; Nuismer and Gandon 2008). Unfortunately, very few studies have conducted experimental manipulations to test the effect of biotic interactions on plant local adaptation (Biere and Verhoeven 2008), and even fewer have simultaneously evaluated the influence of biotic and abiotic factors in order to evaluate the relative importance of each factor for plant local adaptation and specialization (but see Fine et al. 2004; Macel et al. 2007). This paper presents the results of an experimental study aimed at elucidating multi-factor influences on local adaptation in a perennial herb.

Ruellia nudiflora is a perennial herb with limited seed dispersal (Cervera and Parra-Tabla 2009). It is non-native to Mexico and has a broad distribution throughout the Yucatan Peninsula where it is found in open disturbed sites and is subject to a wide range of biotic and abiotic conditions. A previous study found that *R. nudiflora* was not locally adapted in terms of fitness-related traits, but that folivores have a significant effect on its reproductive output (Ortegón-Campos et al. 2009). In addition, although damage by other insect herbivore guilds has been shown to vary significantly between populations, it has shown to remain relatively constant between years within a given site (Abdala-Roberts et al. 2010). On the other hand, *R. nudiflora* populations are found on different soil types which vary in pH, fertility and depth (Bautista et al. 2007). Although we currently do not have any information on the amount of time *R. nudiflora* populations have been present in southeast Mexico, previous studies have reported evidence of rapid adaptation of non-native herbaceous plant species (Richards et al. 2006; Leger and Rice 2007) which could favor local adaptation. Based on these conditions, we hypothesized that herbivore and soil effects could act independently or in concert to promote local adaptation in this species.

In the present study we performed reciprocal transplants between two ecologically contrasting sites, and controlled soil origin and the level of damage on leaves of plants at each site, to evaluate if *R. nudiflora* is locally adapted, and whether soil characteristics and/or folivores influence the degree to which *R. nudiflora* is locally adapted. Specifically, we tested the following predictions arising from the local adaptation hypothesis: (a) folivory and soil conditions both have significant effects on plant fitness (i.e., pre-requisite in order for both factors to represent relevant selective forces on the plant), and (b) assuming local genotypes are adapted to local folivore populations and to local soil conditions, then a fitness advantage of resident genotypes should disappear or weaken when folivory is reduced or no longer prevalent, as well as when resident genotypes are grown with non-native soil.

Methods

Study species

Ruellia nudiflora is a self-compatible perennial herb, typically found in open or partially shaded disturbed areas (Cervera and Parra-Tabla 2009). It has a mixed reproductive system as it produces both cleistogamous and chasmogamous flowers, of which the former do not open but self-pollinate obligately; chasmogamous flowers also exhibit high rates of self-pollination (Abdala-Roberts et al. 2009). Although seeds fall within a 1 m-radius around the parent plant and this species does not have any known seed dispersers (Parra-Tabla unpublished data), preliminary data indicate fairly high levels of gene flow between populations of this species in Yucatan (Ortegón-Campos 2010). As a result, genetic differentiation has been observed between populations only at larger spatial scales (50–100 km; Ortegón-Campos 2010). In the Yucatan Peninsula, the flowering season of this species is from May to September and chasmogamous flowers are visited by several bee species such as *Apis mellifera* and *Trigona fulviventris*, as well as butterflies such as *Microtia elva* (Abdala-Roberts et al. 2009). Leaves are oval-shaped, arranged in pairs, and are mainly eaten by larvae of species of Lepidoptera such as *Anartia jatrophae* and *Siproeta stelenes* (Lepidoptera: Nymphalidae), with consumption levels typically ranging between 10 and 20% of total leaf area consumed per plant depending on the population (Ortegón-Campos et al. 2009). In addition, soil conditions vary considerably across *R. nudiflora* populations,

from cambisol and leptosol soils, to luvisols and nitisols, which vary widely in fertility and relevant physical properties (Bautista et al. 2007).

Study sites

The study was conducted at two sites located in the state of Yucatan, Mexico. The first site, Sinanche, is located on the northern portion of the state (ca. $21^{\circ} 12' 50.5''\text{N}$, $89^{\circ} 10' 58.9''\text{W}$), at 6 m a.s.l. Climate is warm with rains during summer, the mean annual temperature is 25.9°C and annual rainfall ranges from 450 to 650 mm (Chico-Ponce de León 1999). Soil texture is partly sandy, gray to black in color, and calcareous rock outcrops are common which make the organic horizon rich in calcium. Percent of organic matter is ca. 18%, and the depth of the organic horizon is <25 cm. Vegetation at this site is a tropical dry spiny forest 5–7 m tall and characterized by the presence of cacti species (Flores and Espejel 1994). Previous data for this site indicate that folivory levels associated to *R. nudiflora* are ca. 13% of leaf area consumed at this site (Ortegón-Campos et al. 2009).

The other site, Molas, is located ca. 55 km southwest of Sinanche, towards the central portion of the state of Yucatan (approx. $20^{\circ} 49' 51.1''\text{N}$, $89^{\circ} 36' 44.2''\text{W}$) at 10 m a.s.l. Climate at this site is warm subhumid with summer rains and an annual rainfall and temperature of 700–1,000 mm and 26.2°C , respectively (Chico-Ponce de León 1999). The soil at this site is red in color, and the organic horizon reaches greater depths than in Sinanche (between 25 and 50 cm). The percent of organic matter of this soil is ca. 12%. Vegetation is a tropical dry forest of 6–8 m in height on average (Flores and Espejel 1994). Folivory levels for *R. nudiflora* at this site are ca. 18% of total leaf area consumed per plant (Ortegón-Campos et al. 2009).

Experimental plots and reciprocal transplants

In January 2007, we collected 15 fruits (from chasmogamous flowers) of 10–15 plants found at each of two neighboring locations within the vicinity of Sinanche. Likewise, roughly the same number of plants and fruits were sampled from two locations found within the vicinity of Molas. Plants were sampled at two neighboring locations within each site (ca. ≤ 3 km away) in order to achieve a sufficiently large number of seeds to conduct the experiment. Biotic and abiotic conditions were homogeneous between locations at each site (Abdala-Roberts et al. 2010).

In March 2007, fruits were opened and seeds were planted in plastic growing trays which were watered 3–4 times a week. Once seedlings had at least four true leaves, they were transplanted to 2-l plastic pots and placed in a nursery at the Campus de Ciencias Biológicas y Agropecuarias of the Universidad Autónoma de Yucatan. Plants were grown under the same conditions of light, water and humidity throughout an 18-month period. This long acclimation period to which mother plants were subjected presumably controlled for maternal effects on the progeny used in the experiment. In November and December 2008, we collected all the fruits from cleistogamous flowers produced by each plant and pooled the seeds from the two sampling locations within each site, resulting in 566 seeds for Sinanche, and 518 for Molas. Seeds were then germinated in plastic growing trays in January 2009 using native soil in both cases. Seeds from cleistogamous flowers have shown no difference in the percent of germination or seedling survival compared to those from chasmogamous flowers (Gutiérrez, unpubl. data). Finally, in early February of 2009 we collected soil samples from each locality and pooled them by site. Soil samples were sieved to remove large-size debris, stored in plastic bags and used 1 month later for the

reciprocal transplant experiment. Soil samples were not sterilized, as soil microbiota may be an additional component which influences the effect of soil on local adaptation.

In February 2009, when seedlings were 1 month old and had at least four true leaves, 160 seedlings from each origin were randomly selected, half of which were randomly assigned a site for transplantation. Additionally, half of the seedlings of each origin at each site (i.e., 40) were randomly assigned a soil origin (Sinanche or Molas) to which they would be transplanted. In late February 2009, prior to transplantation, we established one 4.5×10.5 -m plot at each site and marked a grid made up of 8 columns and 20 rows, and removed the vegetation present within and around the plot. At each grid point, we dug a 10 cm (diameter) by 20 cm (deep) pit; distance between pits was 0.5 m (both within rows and between columns). Pits were filled with either Sinanche or Molas soil; soil origin was the same within rows and was alternated every two rows (i.e., two rows of the same soil origin, followed by two of the other soil origin). The first week March 2009, seedlings from both origins were transplanted to each plot; each column was composed of plants of the same origin, and origins were alternated between columns (i.e., a column of one plant origin followed by a column of the other plant origin).

Seedlings which died during the first week after transplantation were replaced with seedlings of the same origin. This was done to achieve large enough sample sizes and because mortality during this time is associated mainly with transplanting stress and not to the factors under evaluation in this study (Abdala-Roberts and Marquis 2007). Thus, mortality during this initial week was neither taken into consideration nor analyzed. Plants dying after the first week were not replaced and were included in data analyses. Seedlings were watered twice a week (300–400 ml) for 2 months until the onset of the rainy season in June.

Starting in May 2009 and until October 2009, we incorporated an additional treatment aimed at reducing folivory on experimental plants in order to test for an influence of folivores on *R. nudiflora* local adaptation. We chose this time of year because insect abundance is greater during the rainy season (June–October), and is thus when herbivory is greatest. This treatment consisted in the application of an insecticide composed of piperonyl butoxide (0.2%) and pyrethrum (0.02%) (Ortho® Plant Care, Marysville, OH, USA), which was sprayed on leaves at a distance of 20 cm. To avoid possible interference with pollinator activity, we were careful to avoid contact of the insecticide with chasmogamous flowers. This was feasible because flowers are found in the terminal portion of 20–30 cm-high inflorescences, while most leaves occur at the base of the plant, close to the ground. This insecticide is a synthetic pyrethroid with a short life span (7–14 days); for this reason, it was applied once a week on plants randomly assigned to receive this treatment (control plants were not sprayed). The insecticide treatment was applied to half of the plants of each plant origin on each soil source at each site (i.e., 20 plants), and was alternated between individuals within each row (one individual sprayed, followed by another that was not), with each row beginning with a different treatment relative to the previous so that vertically- or horizontally-adjacent plants did not have the same folivory treatment. The resulting design was a fully-crossed factorial experiment (site \times population origin \times soil origin \times folivory treatment).

Response variables

For each experimental plant we recorded survival throughout the duration of the experiment (whether living or dead on observation dates), number of fruits, and proportion of leaf area consumed by folivores. In addition, we also recorded the number of leaves for each plant at the beginning of the experiment as a proxy of initial plant size. Fruit number was

recorded every 2 weeks, survival every 3 weeks, and folivory once a month throughout the sampling season (March 2009 to October 2009).

In order to determine if the insecticide treatment was effective at reducing folivory on treated plants relative to controls, we measured leaf damage on each experimental plant once a month from August 2009 to October 2009. Damage was measured by selecting a pair of completely developed leaves and drawing the contour and holes (due to damage) of each leaf blade on a paper in the field. Drawings were then carefully cut out, and remaining area (not consumed) and consumed area (consumed portions) were estimated with a leaf area meter (Li-COR[®], Li-3100A, Lincoln, NE, USA). Different pairs of leaves were selected on each census date. The proportion of total leaf area consumed per plant was calculated by summing leaf area consumed for all leaves of a given plant and dividing this value by total leaf area (consumed and remaining). In <5% of the cases, total leaf area could not be estimated in the previously described manner because more than half of the leaf had been consumed and the leaf contour could not be inferred. In these cases, the opposite leaf (similar in size to damaged one) was taken as reference to draw the potential contour of the damaged leaf. A repeated-measures ANOVA (for all three folivory censuses) using leaf area consumed as response variable indicated that the insecticide treatment significantly reduced the proportion of total leaf area consumed of treated plants in comparison to the natural level exhibited by control plants (insecticide-treated plants mean = $3.89 \pm 0.34\%$ [SE]; controls: $11.68\% \pm 1.00\%$; $F_{1,212} = 83.9$, $P < 0.0001$).

Statistical analyses

Generalized linear models were fitted to the data using procedure GENMOD (Type 3 analyses) in SAS ver. 9.1 (Cary, NC, SAS 2002), for which we included site, population origin (origin hereafter), soil origin (soil hereafter) and folivory treatment as main effects on the number of fruits produced per plant (fecundity) and the number of censuses each plant remained alive throughout the study season (i.e., survival). Given that data for these two models showed some degree of overdispersion a scaling factor was applied (SCALE = PEARSON). Both models included the following interactions between main effects: site \times origin, site \times origin \times soil, and site \times origin \times folivory. The remaining interactions were generally not significant and were left out to optimize the models and focus on the relevant interactions aimed at testing the hypotheses. Additionally, these models assumed a Poisson distribution with a log link function and included initial plant size (leaf number) as covariate; the model for fruit number also included the number of censuses alive as covariate because fruit production is conditioned by how long a plant remained alive. Neither of the covariates interacted significantly with any of the main effects for the GENMOD models. A significant site \times origin interaction in which local plants outperformed foreign plants (i.e. higher survival, greater fruit production), was taken as main evidence of local adaptation given that home versus away comparisons confound the effects of divergent natural selection and between-habitat differences in habitat quality (Kawecki and Ebert 2004). In the results section, however, we also report home versus away comparisons to provide additional interpretation of the observed patterns for each population source (although this comparison is not as directly related to local adaptation as the first). Finally, the three-way interactions site \times population origin \times folivory treatment and site \times population origin \times soil, were used to assess the influence of folivores and soil, respectively, on local adaptation.

Differences between levels of significant main effects were evaluated with the LSMEANS statement using corrected P values, and local versus foreign and home versus away comparisons were tested for using pre-planned contrasts (CONTRAST statement).

Results

Survival

The number of censuses plants remaining alive was significantly affected by site and soil effects, as well as by the site \times origin interaction (Table 1). Specifically, plants at Molas (center site) remained alive over significantly more time intervals (mean: 10.8 ± 0.3 [SE] intervals) compared to plants at Sinanche (8.5 ± 0.4 intervals). On the other hand, plants grown with Sinanche soil remained alive significantly longer compared to plants grown with Molas soil (Sinanche: $10.1 \text{ intervals} \pm 0.3$; Molas: 9.1 ± 0.4). Initial plant size did not have a significant effect on subsequent survival (Table 1). The significant site \times origin interaction was due to plants of Sinanche origin surviving a greater number of censuses on average at their native site relative to plants originating from Molas, thus indicating local adaptation for plants from Sinanche (i.e., local vs. foreign comparison: $\chi^2 = 8.83$, $P = 0.003$; Fig. 1A); plants originating from Sinanche performed equally well at both sites (home vs. away comparison: $\chi^2 = 2.27$, $P = 0.132$; Fig. 1A). On the other hand, at the Molas site, native and non-native plants performed equally well (local vs. foreign: $\chi^2 = 0.46$, $P = 0.50$; Fig. 1A). In addition, plants originating from Molas showed a significantly lower average value when transplanted outside their native site (home vs. away: $\chi^2 = 26.38$, $P < 0.001$; Fig. 1A).

Although the origin \times site interaction showed evidence of local adaptation in terms of survival for at least one of the population sources, none of the three-way interactions (origin \times site \times soil and origin \times site \times folivory) were significant (Table 1), thus indicating that the pattern described by the origin \times site interaction did not change substantially as a result of manipulating soil source or folivory level.

Table 1 Results from the generalized linear model analyses to evaluate the effects of site, plant origin, soil source, folivory treatment and their interactions on *Ruellia nudiflora* survival (measured as the number of censuses a plant remained alive) and number of fruits produced per plant (n number of plants)

| Source of variation | Survival (n = 320) | | | Fruit number (n = 320) | | |
|--|--------------------|-------|-------------------|------------------------|--------|-------------------|
| | df | F | P value | df | F | P value |
| Site | 1,307 | 22.77 | <0.0001 | 1,305 | 0.25 | 0.620 |
| Origin | 1,307 | 3.14 | 0.077 | 1,305 | 0.72 | 0.395 |
| Soil | 1,307 | 4.16 | 0.042 | 1,305 | 0.01 | 0.933 |
| Folivory treatment | 1,307 | 0.02 | 0.884 | 1,305 | 10.11 | 0.001 |
| Site \times Origin | 1,307 | 7.11 | 0.008 | 1,305 | 10.81 | 0.001 |
| Origin \times Soil | 1,307 | 0.02 | 0.893 | 1,305 | 0.68 | 0.409 |
| Site \times Soil | 1,307 | 0.01 | 0.971 | 1,305 | 10.03 | 0.001 |
| Site \times Origin \times Soil | 3,307 | 0.01 | 0.999 | 1,305 | 0.36 | 0.547 |
| Site \times Origin \times Folivory | 3,307 | 1.04 | 0.374 | 3,305 | 0.72 | 0.539 |
| Initial plant size | 1,307 | 1.43 | 0.233 | 1,305 | 0.76 | 0.385 |
| Survival | — | — | — | 1,305 | 330.08 | <0.0001 |
| Survival \times Site | — | — | — | 1,305 | 0.08 | 0.780 |

Values in bold represent significant P values

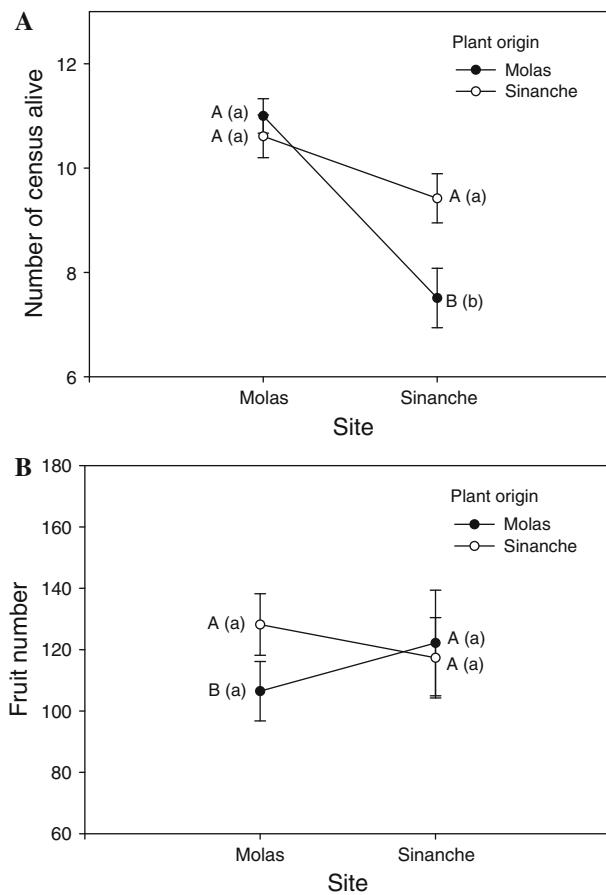


Fig. 1 Number of **A** censuses alive and **B** fruits produced for *Ruellia nudiflora* plants of each origin across sites (values are the mean \pm SE). Different *upper case letters* indicate significant differences between plant origins within a given site (local vs. foreign comparison), while *lower case letters* indicate differences across sites for a given plant origin (home vs. away comparison). Significance value set at 0.05 for comparison of levels of significant effects

Fecundity

A total of 38,077 fruits were counted for experimental plants throughout the sampling season at the two sites. There were significant effects of the folivory treatment, the site \times origin interaction, and the site \times soil interaction on fruit number (Table 1). Survival, measured as the number of time intervals plants remained alive, was used as covariate in this model and yielded a highly significant result as expected because fruit production throughout the study season was constrained by the amount of time each plant remained alive (Table 1).

The significance of the folivory treatment showed that plants subjected to insecticide application exhibited a 27% increase in fruit production (control plants: 105.6 ± 8.2 fruits; insecticide-treated: 131.4 ± 9.8 fruits), which was presumably due to reduced leaf consumption. However, against expectations, significance of the site \times origin interaction did

not result from a home site advantage of native plants at one or both sites, but rather from an inverse pattern: native plants at Molas exhibited lower average fruit production than foreign plants (local vs. foreign: $\chi^2 = 7.28, P = 0.007$; Fig. 1B); no pattern was observed at Sinanche native (local vs. foreign: $\chi^2 = 2.97, P = 0.09$; Fig. 1B). It is important to note, however, that the low fruit production of Molas plants at the Molas site was largely due to the low reproductive output of plants from this population when they were grown on non-native soil at this (native) site (see following paragraph). Finally, plants of Molas and Sinanche origin did not differ in their mean fruit number between sites (home vs. away comparisons: $\chi^2 \leq 1.81, P \geq 0.18$; Fig. 1B).

Interestingly, we found a significant site \times soil interaction (Table 1) and this was because fruit production at both sites was on average lower for plants grown on soils that were non-native to that site, than on soils originating from that site (Fig. 2A, B; comparison of soils that are native to the site versus soils that are non-native to the site: $\chi^2 > 4.85, P < 0.03$ in both sites). Specifically, at the Sinanche site (Fig. 2B), both Molas plants and Sinanche plants had ca. 25% lower fruit production on Molas soil than on Sinanche soil, even though the Molas soil was the home soil for Molas plants. Likewise, at the Molas site (Fig. 2A), fruit production was on average lower in Sinanche soil than in (local) Molas soil, a pattern that was mainly due to the low fruit production of Molas plants on Sinanche soils at this site (Fig. 2A): whereas Molas plants had significantly lower fruit production in Sinanche than in Molas soils at this site ($\chi^2 = 6.13, P = 0.013$), Sinanche plants performed equally well on both soils at this site (Fig. 2A). This also indicates that the low fruit production of Molas plants at their home site (compared to Sinanche plants), that was mentioned above (Fig. 1B), is solely due to the low fruit production of these Molas plants in Sinanche soil (Fig. 2A); in the Molas soil, the Molas plants exhibited similar fruit production as Sinanche plants at this site (Fig. 2A).

Discussion

Results from the present field experiment showed contrasting patterns for survival and fruit production in *R. nudiflora*. With respect to the former, local adaptation was detected at one site (Sinanche), while for fruit production local adaptation was not observed at either site. Moreover, the three-way interactions were not significant which indicated that the site \times origin patterns were not dependent on folivory or soil source. We did, however, observe that the soil effects were contingent upon the site and that a significant change in reproductive output was present for native plants at one site which was due to soil source. This latter finding suggests that the relative performance of local versus foreign genotypes of *R. nudiflora* can be influenced by soil type.

Survival

Local adaptation was observed at Sinanche in terms of the number of censuses alive, as denoted by the significant site \times origin interaction. It is important to note, however, that a strong site effect on survival was also observed, as the overall number of censuses a plant remained alive was ca. 30% higher at Molas compared to Sinanche. Interestingly, local adaptation was not observed at Molas. We hypothesize that the lack of local adaptation at Molas and its occurrence at Sinanche could be explained by the contrasting environmental conditions prevailing at the two sites, which may alter the likelihood of plant local adaptation, as discussed below.

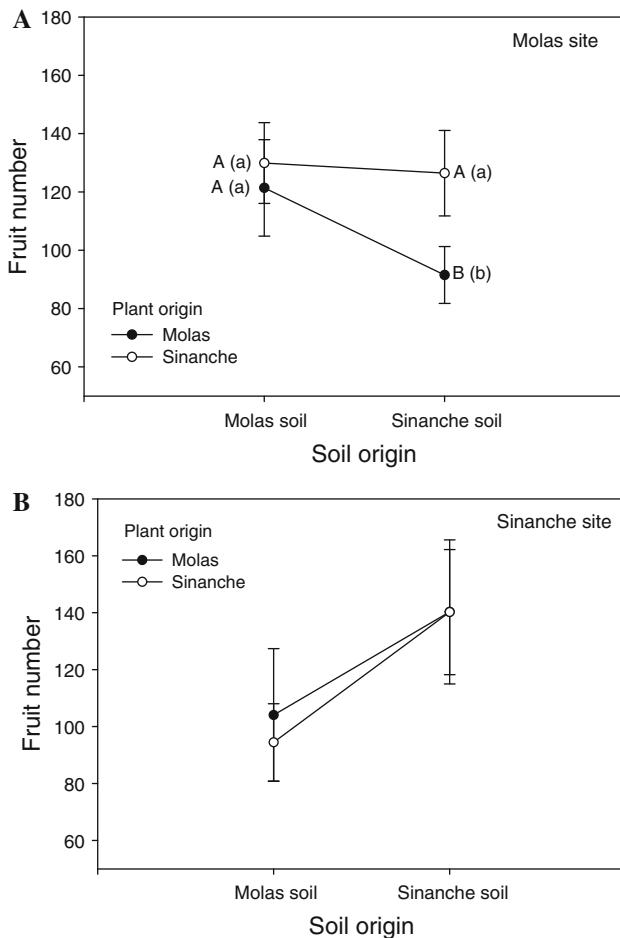


Fig. 2 Number of fruits produced by *Ruellia nudiflora* plants of each origin across soil sources at each site (dots are means \pm SE). Different upper case letters indicate significant differences between plant origins within a given soil source, while lower case letters indicate differences between soil sources for a given plant origin; comparisons were performed for each site separately (**A** Molas site, **B** Sinanche site)

Climate conditions are different between Molas and Sinanche. For instance, total precipitation throughout the study period was 524 mm at Molas and only 155 mm at Sinanche. Such difference in the amount of precipitation probably had a strong influence on the observed patterns of survival for each site. Therefore, we could speculate that at the Molas site, Sinanche plants had higher survival than at their native site (Fig. 1A) as a result of a plastic response to higher water availability, homogenizing performance of both plant origins, which may have limited the detection of local adaptation at this site. By contrast, the harsher environmental conditions at the Sinanche site could have reduced phenotypic plasticity (Lortie and Aarssen 1996), which would favour the detection of local adaptation at this site, mediated by genotypes adapted to drier conditions (drought tolerance has been shown to be a relevant condition for *R. nudiflora* establishment; Cervera and Parra-Tabla 2009).

Soil from Sinanche was more favorable for survival than soil from Molas, and this could be explained by the former soil type having greater electrical conductivity, greater % of

organic matter and coarser grain which facilitates water diffusion to the plant (Nobel 1999). More importantly, however, when addressing the potential effect of soil source on local adaptation we did not detect a significant soil source \times origin \times site interaction, suggesting that soil type did not have an effect on the degree of local adaptation. Previous studies reporting plant local adaptation to soil conditions have often used particularly stressful soil types for their comparisons (e.g., serpentine soils; Wright et al. 2006; Sambatti and Rice 2006). Thus, it is possible that differences in soil types used in this study were not large enough to detect an influence of soil source on the degree of local adaptation in *R. nudiflora*, at least not in terms of survival.

The insecticide treatment, despite being effective at reducing leaf damage, did not have a significant effect on survival. This could presumably be due to high tolerance to leaf damage by *R. nudiflora* mediated by high investment in roots and resource storage during the first year of life (G. Rivera, unpublished data). Moreover, the level of folivory did not influence the degree of home-site advantage, as corroborated by a non-significant folivory \times origin \times site interaction. Although a previous study found evidence of an effect of folivory on reproductive output of *R. nudiflora*, no evidence was found of plant local adaptation to folivores or vice versa (Ortegón-Campos et al. 2009). This finding, together with results from the present work, so far suggests limited evolutionary potential for folivores to promote local adaptation in this species.

Fecundity

In contrast with the results for survival, analyses for fruit number showed that local adaptation did not occur. The observed pattern could even be interpreted as maladaptation (Hereford and Winn 2008) of Molas plants at the Molas site, as foreign plants outperformed native plants. However, this finding was driven mainly by low performance of Molas plants at their native site when grown with non-native soil, hence suggesting an influence of soil source on the relative performance of local versus foreign genotypes at this site. It is interesting to note that the comparison of results for survival and fecundity reveals that local adaptation may arise for one fitness component (i.e., survival, in the present instance) but not for another (fecundity), suggesting that different life history strategies could be selected for in order for plants to adapt to local conditions at each site (Sambatti and Rice 2006). Accordingly, differences in results may be related to contrasting evolutionary strategies used across plant ontogenetic stages (Juravcic et al. 2002), which may lead to local adaptation at one stage (i.e., in terms of survival during early stages), but not in another (reproduction as adults). Based on results from this study, it appears that survival plays an important role for plant fitness during early life stages for which local adaptation is expressed. However, subsequent performance of individuals beyond that initial period, measured as reproductive output, shows no evidence of local adaptation (see Ortegón-Campos et al. 2009).

The insecticide treatment resulted in a significant effect on fruit production, since control plants produced ca. 25% fewer fruits than insecticide-treated plants. Despite this result, however, and similarly to what was observed for survival, the folivory \times site \times origin interaction was not significant. This result is not surprising and agrees with the absence of an effect of folivory on local adaptation based on survival, thus suggesting that selection imposed by folivores on *R. nudiflora* does not lead to locally adapted plant genotypes (Ortegón-Campos et al. 2009). In addition, this finding agrees with a previous study by Abdala-Roberts and Marquis (2007) which did not find evidence of an effect of folivory level on local adaptation of the annual legume *Chamaecrista fasciculata*. It is possible that

herbivore selection pressures are not divergent or strong enough to promote local specialization of *R. nudiflora* populations, which would also contribute to explain the observed findings.

The significant soil source \times site interaction effect on fecundity resulted from lower plant reproductive output on soils that were non-native to that site. This finding could be suggestive of a methodological effect of reduced pit size and heterogeneous soil conditions between transplanted foreign soil and the surrounding soil matrix possibly influencing root growth (and not so much having to do with soil specialization). Although this issue remains speculative, its potential influence on the effects of soil source on the relative performance of local and foreign plants cannot be discarded. However, independently of this methodological effect, by analyzing the soil \times origin interaction at each site separately we were able to identify a significant difference in fecundity for Molas plants when grown with native versus non-native soil at the Molas site; specifically, we observed lower reproductive output when grown with non-native soil, which in turn influenced the difference in fitness between native and non-native plants across soil sources (Fig. 2A). No such effects were observed for Sinanche plants. We take this finding as evidence of a change in the relative performance of local versus foreign plants depending on soil source which was largely responsible for the overall lower reproductive output of Molas plants at their native site (Fig. 1B).

General overview of local adaptation in *R. nudiflora*

Ruellia nudiflora is non-native to Mexico, and has shown a vast potential for colonization of new environments. It is distributed throughout the Gulf of Mexico and most of the Yucatan Peninsula where it has successfully established in sites with varying biotic and abiotic conditions. *Ruellia nudiflora* could owe its colonization success to rapid adaptation in response to strong selective pressures in novel environments mediated by phenotypic plasticity (Richards et al. 2006; Leger and Rice 2007; Davidson et al. 2011), which could, in some cases, give rise to locally-adapted genotypes if enough time has passed for genetic differentiation to occur after initial establishment. Results from this study show that although local adaptation may occur during early life stages in terms of survival, beyond the initial stage of establishment genotypes do not appear locally adaptive in terms of reproductive output.

It has been previously shown that *R. nudiflora* typically exhibits high rates of autonomous selfing of chasmogamous flowers (ca. 70% of flowers self-pollinate automatically in absence of pollinators), as well as via cleistogamous obligate self-fertilization (Abdala-Roberts et al. 2009). Such conditions could potentially result in detrimental effects on fitness via reduced genetic variation (but see Leimu and Fischer 2008; Hereford 2010) and inbreeding which can limit local adaptation or even result in maladaptation (Núñez-Farfán et al. 2007). On the other hand, preliminary data have shown high levels of gene flow between *R. nudiflora* populations which would instead reduce population genetic differentiation (Slatkin 1987), limit local adaptation due to a decrease in frequency of locally-adapted genotypes, and even promote maladaptation as a result of the introduction of maladapted alleles (Anderson and Geber 2009 and references therein). Finally, the presence of frequent human disturbance (mowing, burning) at sites where *R. nudiflora* is found may reduce population sizes and thus call for attention to the effect of stochastic processes (e.g., genetic drift) influencing local adaptation in this species.

Concluding remarks

Results from this study highlight several important considerations for studies dealing with plant local adaptation. First, the detection of local adaptation can depend on the fitness component measured as well as the plant ontogenetic stage (e.g., seedling survival versus adult reproduction). Second, findings from this study agree with previous results showing that this species is not locally adapted when measured in terms of reproductive output, but that expression of local adaptation during early life stages of the plant should be taken as an important evolutionary condition. Third, more studies are needed which simultaneously evaluate the influence of multiple (biotic and abiotic) conditions in order to achieve a more robust evaluation of the conditions which influence plant local adaptation.

Acknowledgments The authors thank G. Pacheco, P. McManus, R. Moo, N. Celaya, P. Telléz and V. Hernández for field assistance, as well as L. Loria, D. Loria, D. Loria, and L. Aldana who kindly provided logistic support and accommodations at the study sites. Special thanks to R. J. Marquis for comments on a previous version of the manuscript, as well to Arjen Biere and two anonymous reviewers who improved the quality of this paper. This study was financially supported by the Consejo Nacional de Ciencia y Tecnología (CONACyT) through a doctoral scholarship to IOC and as part of a grant given to VPT (SEP 2004-CO1-4658A/A1).

References

- Abdala-Roberts L, Marquis RJ (2007) Test of local adaptation to biotic interactions and soil abiotic conditions in the ant-tended *Chamaecrista fasciculata* (Fabaceae). *Oecologia* 154:315–326
- Abdala-Roberts L, Parra-Tabla V, Salinas-Peña L et al (2009) Noncorrelated effects of seed predation and pollination on the perennial herb *Ruellia nudiflora* remain spatially consistent. *Biol J Linn Soc* 96:800–807
- Abdala-Roberts L, Parra-Tabla V, Salinas-Peña L et al (2010) Spatial variation in the strength of a trophic cascade involving *Ruellia nudiflora* (Acanthaceae), an insect seed predator and associated parasitoid fauna in Mexico. *Biotropica* 42:180–187
- Anderson JT, Geber MA (2009) Demographic source-sink dynamics restrict local adaptation in Elliott's blueberry (*Vaccinium elliottii*). *Evolution* 64:370–384
- Antonovics J, Bradshaw AD (1970) Evolution of closely related adjacent plant populations. VIII. Clinal patterns at a mine boundary. *Heredity* 25:349–362
- Bautista F, Aguilar Y, Rivas H et al. (2007) Los suelos del estado de Yucatán. Importancia del binomio suelo-materia orgánica en el desarrollo sostenible. Agencia Española de Cooperación Internacional y Centro de Edafología y Biología Aplicada del Segura
- Becker U, Colling G, Dostal P et al (2006) Local adaptation in the monocarpic perennial *Carlina vulgaris* at different spatial scales across Europe. *Oecologia* 150:506–518
- Becker U, Dostal P, Jorritsma-Wienk D et al (2008) The spatial scale of adaptive population differentiation in a wide-spread, well-dispersed plant species. *Oikos* 117:1865–1873
- Biere A, Verhoeven KJF (2008) Local adaptation and the consequences of being dislocated from coevolved enemies. *New Phytol* 180:265–268
- Cervera C, Parra-Tabla V (2009) Seed germination and seedling survival traits of invasive and non-invasive congeneric *Ruellia* species (Acanthaceae) in Yucatan, Mexico. *Plant Ecol* 250:285–293
- Chico-Ponce de León PA (1999) Atlas de procesos territoriales de Yucatán. Universidad Autónoma de Yucatán
- Crémieux L, Bischoff A, Smilauerová M et al (2008) Potential contribution of natural enemies to patterns of local adaptation in plants. *New Phytol* 180:524–533
- Davidson AM, Jennions M, Nicotra AB (2011) Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecol Lett*. doi:10.1111/j.1461-0248.2011.01596.x
- Fine PV, Mesones I, Coley PD (2004) Herbivores promote habitat specialization by trees in Amazonian forests. *Science* 305:663–665
- Flores S, Espejel I (1994) Tipos de vegetación de la Península de Yucatán. In: Flores S (ed) *Etnoflora Yucatanense*. Universidad Autónoma de Yucatán, Yucatán, pp 63–70

- Galloway LF, Fenster CB (2000) Population differentiation in an annual legume: local adaptation. *Evolution* 54:1173–1181
- Geber MA, Eckhart VM (2005) Experimental studies of adaptation in *Clarkia xantiana*. II Fitness variation across a subspecies border. *Evolution* 59:521–531
- Gómez JM, Abdelaziz M, Camacho JPM et al (2009) Local adaptation and maladaptation to pollinators in a generalist geographic mosaic. *Ecol Lett* 12:672–682
- Hereford J (2010) Does selfing or outcrossing promote local adaptation? *Am J Bot* 97:298–302
- Hereford J, Winn AA (2008) Limits to local adaptation in six populations of the annual plant *Diodia teres*. *New Phytol* 178:888–896
- Hoeksema JD, Forde SE (2008) A meta-analysis of factors affecting local adaptation between interacting species. *Am Nat* 171:275–290
- Jain SK, Bradshaw AD (1966) Evolution in closely adjacent plant populations. I. The evidence and its theoretical analysis. *Heredity* 21:407–441
- Jakobsson A, Dinnetz P (2005) Local adaptation and the effects of isolation and population size—the semelparous perennial *Carlina vulgaris* as a study case. *Evol Ecol* 19:449–466
- Jurjavcic NL, Harrison S, Wolf A (2002) Abiotic stress, competition, and the distribution of the native annual grass *Vulpia microstachys* in a mosaic environment. *Oecologia* 130:555–562
- Kawecki T, Ebert D (2004) Conceptual issues in local adaptation. *Ecol Lett* 7:1225–1241
- Kingsolver JG, Pfennig DW, Servedio MR (2002) Migration, local adaptation and the evolution of plasticity. *Trends Ecol Evol* 17:540–541
- Leger EA, Rice KJ (2007) Assessing the speed and predictability of local adaptation in invasive California poppies (*Eschscholzia californica*). *J Evol Biol* 20:1090–1103
- Leimu R, Fischer M (2008) A meta-analysis of local adaptation in plants. *PLoS ONE* 3:e4010
- Leiss KA, Muller-Scharer H (2001) Performance of reciprocally sown populations of *Senecio vulgaris* from ruderal and agricultural habitats. *Oecologia* 128:210–216
- Linhart Y, Grant M (1996) Evolutionary significance of local genetic differentiation in plants. *Annu Rev Ecol Syst* 27:237–277
- Lortie CJ, Aarsen LW (1996) The specialization hypothesis for phenotypic plasticity in plants. *Int J Plant Sci* 157:484–487
- Mace M, Lawson C, Mortimer SR et al (2007) Climate vs. soil factors in local adaptation of two common plant species. *Ecology* 88:424–433
- Nobel PS (1999) Physiochemical and environmental plant physiology. Academic Press, Waltham
- Nuismer S, Gandon S (2008) Moving beyond common-garden and transplant designs: insight into the causes of local adaptation in species interactions. *Am Nat* 171:658–668
- Núñez-Farfán J, Fornoni J, Valverde PL (2007) The evolution of resistance and tolerance to herbivores. *Annu Rev Ecol Evol Syst* 38:541–566
- Ortegón-Campos I (2010) Adaptación local y diversidad genética de *Ruellia nudiflora* (Acanthaceae) en el estado de Yucatán. Ph.D. Dissertation. Universidad Autónoma de Yucatán
- Ortegón-Campos I, Parra-Tabla V, Abdala-Roberts L et al (2009) Local adaptation of *Ruellia nudiflora* (Acanthaceae) to biotic counterparts: complex scenarios revealed when two herbivore guilds are considered. *J Evol Biol* 22:2288–2297
- Parker I, Rodriguez J, Loik ME (2003) An evolutionary approach to understanding the biology of invasions: local adaptation and general purpose-genotypes in the weed *Verbascum thapsus*. *Conserv Biol* 17:59–72
- Platenkamp GA (1990) Phenotypic plasticity and genetic differentiation in the demography of the grass *Anthoxanthum odoratum*. *J Ecol* 78:772–788
- Richards CL, Bossdorf O, Muth NZ et al (2006) Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecol Lett* 9:981–993
- Sambatti JB, Rice KJ (2006) Local adaptation, patterns of selection, and gene flow in the Californian serpentine sunflower (*Helianthus exilis*). *Evolution* 60:696–710
- SAS (2002) Version 9.1. SAS Institute, Cary
- Schmidt KP, Levin DA (1985) The comparative demography of reciprocally sown populations of *Phlox drummondii* Hook. I. Survivorships, fecundities, and finite rates of increase. *Evolution* 39:396–404
- Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science* 236:787–792
- Sork V, Stowe KA, Hochwender C (1993) Evidence for local adaptation in closely adjacent subpopulations of northern red oak (*Quercus rubra L.*) expressed as resistance to leaf herbivores. *Am Nat* 142:928–936
- Van Tienderen PH, Van Der Toorn J (1991) Genetic differentiation between populations of *Plantago lanceolata* I. Local adaptation in three contrasting habitats. *J Ecol* 79:27–42
- Wright JW, Stanton ML, Scherson R (2006) Local adaptation to serpentine and non-serpentine soils in *Collomia sparsiflora*. *Evol Ecol Res* 8:1–21