Local adaptation of *Ruellia nudiflora* (Acanthaceae) to biotic counterparts: complex scenarios revealed when two herbivore guilds are considered

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Abstract

This study evaluated whether the herb *Ruellia nudiflora* is locally adapted to a specialist insect seed predator (SP) and insect folivores, and if plant local adaptation (LA) to the former is more likely. A reciprocal transplant experiment was conducted using three sites in Yucatan (Mexico) ($n = 864$ plants). A third of the plants of each origin were placed at each site, and we recorded the following during a 9-month period: fruit number, leaf damage, and fruits attacked by SP. Results indicated lack of plant LA for all the variables measured. Instead, seed predation was *c.* 100% greater for native plants at one study site, suggesting insect LA or plant maladaptation; folivory was homogeneous across sites/origins. Based on these results, we discuss differences in the potential each herbivore guild has to promote plant LA, as well as divergent evolutionary outcomes of plant–herbivore interactions across sites.

Introduction

Plant species are frequently exposed to different environmental conditions along their distribution range, and thus may exhibit variation across populations in terms of performance or specific traits. Spatial variation in plant traits may result from two contrasting evolutionary conditions: Phenotypic plasticity and local adaptation (LA) (Becker *et al.*, 2006). The former refers to the ability one genotype has to express different phenotypes across different environments (Bradshaw, 1965; Lortie & Aarssen, 1996; Alpert & Simms, 2002); this condition will be favoured by environmental variability, as well as moderate to high levels of gene flow (Kingsolver *et al.*, 2002; Sultan & Spencer, 2002). On the other hand, LA occurs when each phenotype corresponds to a different genotype as a result of specific environmental conditions present at each site (Stearns, 1992; Alpert & Simms, 2002); this condition will be favoured when natural

selection is of different magnitude and/or direction across sites or populations, temporally constant within sites, as well as by limited gene flow (Linhart & Grant, 1996; Kingsolver *et al.*, 2002; Sultan & Spencer, 2002), which will promote genetic isolation and differentiation between populations (Kawecki & Ebert, 2004). LA has been typically evaluated by reciprocally trasplanting plants from different sites, and looking for an origin by site interaction (i.e. genotype by environment) for which native plants outperform non-natives (e.g. Van Tienderen, 1992; Nuismer & Gandon, 2008; Hereford, 2009).

A number of studies have looked at plant LA to abiotic selective factors such as temperature, humidity, precipitation and soil nutrients (e.g. Schwaegerle & Bazzaz, 1987; Leger & Rice, 2007; Dechamps *et al.*, 2008). In contrast, much less attention has been paid to the role of biotic factors as promoters of LA in plant populations, specifically the role of plant–insect interactions (but see Abdala-Roberts & Marquis, 2007; Crémieux *et al.*, 2008). This gap in research remains even though there is an increasing body of knowledge pointing at an almost universal condition of spatial variation in the intensity and outcome of plant–insect interactions (Thompson,

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1994; Herrera *et al.*, 2002; Rey *et al.*, 2006). Such scenario suggests the potential for plant LA as a result of mutualistic or antagonistic interactions with insects (Thompson, 2002).

Herbivory is recognized as an important biotic interaction that influences plant growth, reproduction and overall performance (Marquis, 1992; Crawley, 1997). In addition, it has been documented that herbivore impacts on plants vary spatially, which may be a result of changes in herbivore species composition, abundance (e.g. Gómez & Zamora, 2000) and/or plant defensive mechanisms (Coley & Barone, 1996). Despite such evidence, very few studies have looked at plant LA to insect herbivores (except for: Sork *et al.*, 1993; Abdala-Roberts & Marquis, 2007; Crémieux *et al.*, 2008), while studies on LA of insect herbivores to their host plants are far more common (e.g. Mopper *et al.*, 1995; Amarillo-Suarez & Fox, 2006; Ruhnke *et al.*, 2006). Moreover, plants are subject to different types of herbivores such as suckers, chewers, miners and gallers which exhibit constrasting feeding modes, life history traits, degree of specialization (i.e. temporally, taxonomically and by type of tissue) and degree of impact on plant fitness measures (Marquis, 1992). Such condition suggests differences in the selective pressure exerted by each herbivore guild (Bernays & Chapman, 1994) and thus of the potential plants have to locally adapt to each one and the overall strength of plant–insect herbivore evolutionary responses.

In particular, seed predators (SP) have a strong impact on plant fitness by killing potential new recruits, and in this way may be more strongly and negatively linked to plant fitness and population dynamics than other herbivore guilds such as folivores (Leimu & Lehtilä, 2006; Kolb *et al.*, 2007). In addition, concealed insect herbivore guilds such as pre-dispersal SP have life cycles that are usually well-synchronized with respect to plant flowering and fruiting phenology (Diniz & Morais, 2002) and tend to be highly specialized in terms of their host plant species (Novotny & Basset, 2005), suggesting a more intense relationship with the host plant compared with external feeders such as leaf-chewers. Thus, given some degree of spatial variation in damage intensity, combined with other factors such as limited gene flow between plant populations and limited insect dispersal ability, we hypothesize that plant LA to concealed insect SP (and vice versa) is more likely than plant LA to leaf-chewing insects, and may even lead to coevolutionary dynamics.

Ruellia nudiflora (Acanthaceae) is a perennial herb attacked by larvae of a moth species which feed on seeds prior to their dispersal. Leaves are also attacked by larvae of several generalist species of Lepidoptera and previous research conducted at different sites in the state of Yucatan (Mexico) indicates that *R. nudiflora* presents spatial variation in seed predation intensity (Abdala-Roberts *et al.*, 2009b). In addition, *R. nudiflora* populations form discrete units and typically present high rates of self-pollination and limited seed dispersal (Abdala-

Roberts *et al.*, 2009a) all of which are conditions that promote genetic divergence between populations. These characteristics, together with varying intensity of herbivore selection across sites, make of *R. nudiflora* a suitable model to test hypothesis on LA to herbivores, as well as differences in the potential folivores and SP have to promote such evolutionary condition.

The goal of this study was to evaluate if *R. nudiflora* exhibits LA across three distant sites in the state of Yucatan (Mexico) in terms of the amount of damage inflicted by a pre-dispersal SP and by folivores. Specifically, we addressed the following: (a) is *R. nudiflora* locally adapted in terms of reproductive output (i.e. greater fruit production for natives vs. non-native plants)? (b) is *R. nudiflora* locally adapted to a pre-dispersal SP and folivores (i.e. lower damage for native vs. non-native plants)? and (c) based on the results, we discuss if plant LA to the SP is more likely than plant LA to the folivores.

Methods

Study system

Ruellia nudiflora (Acanthaceae) is a self-compatible perennial weed which measures between 20 and 30 cm in height, and grows in disturbed open or partially shaded areas (Standley *et al.*, 1974; Tripp, 2007). It occurs from Texas to southeast Mexico, and is widely distributed across the state of Yucatan (Mexico). It has a mixed reproductive system, that is, it produces both flowers that open (chasmogamous, CH) and flowers that do not (cleistogamous, CL) and obligately self-pollinate; CH flowers also present high levels of automatic self-pollination which takes place during corolla dehiscence, as anthers come into contact with the stigma when the corolla falls off the plant (up to 70% of flowers set fruit automatically at some sites; Abdala-Roberts *et al.*, 2009a). The CH flowering season starts in May and ends in late September; CL flower production extends for a longer period of time. Fruits are dry and dehiscent, each one normally bearing between seven and 12 seeds which typically fall within a metre of the parent plant after the fruit explodes (V. Parra-Tabla, personal observation). Fruits are attacked by larvae of an unidentified moth species (Lepidoptera: Noctuidae) that feed on seeds prior to their dispersal and are the only known SP of *R. nudiflora* in the Yucatan Peninsula. Larvae attack from 15% to 60% of the fruits produced by each plant depending on the site (Abdala-Roberts *et al.*, 2009b). Adult female moths oviposit on recently formed fruits, and the larva grows inside the developing fruit and typically consumes all the seeds. Before pupating, each larva excavates a tunnel which the adult uses to exit the fruit. On the other hand, leaves, which are oval-shaped and arranged in pairs, are attacked by larvae of several species of Lepidoptera. The most important are *Anartia*

jatrophae luteipicta and *Siproeta stelenes biplagiata* (Lepidoptera: Nymphalidae), both of which are considered generalist species which feed on a number of species of Acanthaceae, as well as Scrophulariaceae and Verbenaceae (DeVries, 1987; Lederhouse *et al.*, 1992; Chávez, 1995). Together, these two species typically consume between 10% and 20% of total leaf area per plant depending on the site (I. Ortegón-Campos, unpublished data) and are present at all *R. nudiflora* populations sampled so far.

Phenotypic variation has been observed both within and between sampled populations in terms floral traits, reproductive output and plant size. For example, greater values of herkogamy (anther-stigma separation) have been observed for *R. nudiflora* populations found in the south of the Yucatan compared with populations in the north and centre of state (mean ± SE north: 0.90 ± 0.157 mm, centre: 1.485 ± 0.112, south: 2.534 ± 0.239; $F_{2,77} = 69.01$, $P = 0.0001$; D. Marrufo, unpublished data). Moreover, differences between populations in plant size (total leaf area) have shown to remain under common garden conditions (population effect: $F_{3,301} = 7.97$, $P = 0.0001$), suggesting genetic differentiation between populations (G. Rivera-Solís, unpublished data).

Study area

The study was conducted from May 2005 to March 2007 using three distant experimental sites distributed along a latitudinal gradient in the state of Yucatan (SE Mexico). Each site was located within a different region of the State (namely: north, centre and south), and regions were defined based on marked differences in biotic (herbivore attack rates, surrounding vegetation type) and abiotic (rainfall) conditions. For example, SP attack intensity has shown to vary strongly across regions ($F_{3,17} = 9.33$, $P = 0.0007$; Abdala-Roberts *et al.*, 2009b),

and additional data collected over 2 years at different *R. nudiflora* populations within each region suggests temporal consistency in attack rates [n.s. effects of year ($F_{1,709} = 0.24$, $P = 0.62$) and of year × region ($F_{2,709} = 0.19$, $P = 0.83$); V. Parra-Tabla, unpublished data]. Importantly, most abiotic and biotic conditions are relatively consistent across locations within a given region (Chico-Ponce de León, 1999), and each experimental site presented biotic and abiotic conditions which were representative of the entire region (e.g. precipitation, type of vegetation and herbivore attack levels); distance between study sites was at least 50 km, but no > 200 km (Fig. 1).

The first experimental site was located in the northern region of Yucatan (21°31′N, 21°17′W) at 7 m a.s.l., and was the driest site with a mean annual rainfall and temperature of 450–650 mm and 25.9 °C respectively. The site used was dominated by herb species such as *Sanvitalia procumbens*, *Amaranthus* sp., *Borreria verticillata* and *Porophyllum punctatum*, all typical of secondary succession vegetation of low-height deciduous forest of this region; the arboreal legume *Lisiloma latisiliquum* was also frequent at the study site (Arellano-Rodríguez *et al.*, 2003). The second site was located in the central region of the Yucatan (20°48′N, 89°42′W), at 10 m a.s.l. with a mean annual rainfall and temperature of 750–1200 mm and 27.6 °C respectively. Vegetation found at this site was succesional low-height deciduous forest (Arellano-Rodríguez *et al.*, 2003) of 10–15 years of age, which was dominated by the tree *Gymnopodium floribundum* (Arellano-Rodríguez *et al.*, 2003). Lastly, the third and more moist site was located in the southern region of the State (20°00′N, 89°01′W), at 36 m a.s.l. with a mean annual rainfall and temperature of 1300–1500 mm and 26.6 °C respectively (Chico-Ponce de León, 1999). Vegetation at this site was medium-height subdeciduous forest (Arellano-Rodríguez *et al.*, 2003), and the
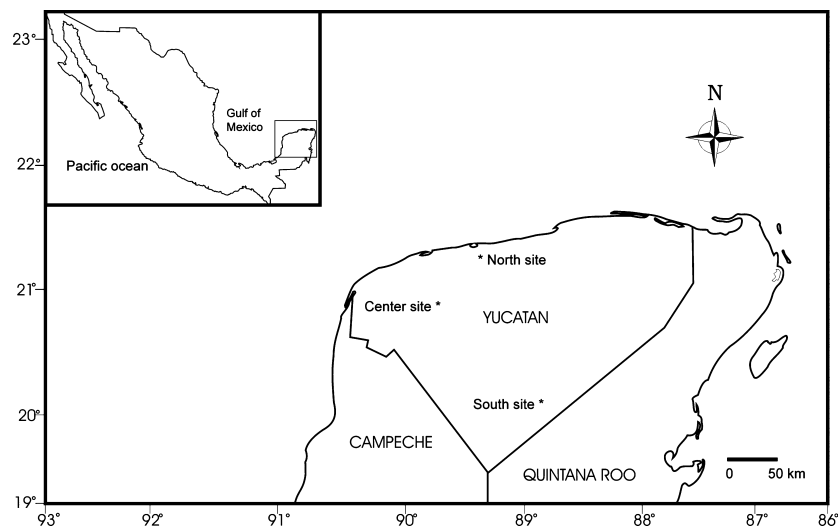

Fig. 1 Map of the Yucatan Peninsula (Mexico) showing the location of the *Ruellia nudiflora* study sites used for the reciprocal transplant experiment.

dominant tree species found were *Piscidia piscipula*, *Guazuma ulmifolia* and *Bauhinia divaricata* (Arellano-Rodríguez *et al.*, 2003).

Reciprocal transplants and experimental design

In May 2005, five 'parent' plants were collected from each of four previously selected populations per region. The criteria used to select these populations was that they were all located at disturbed open areas next to tropical dry forest fragments, and that within each region, they were no more than 20 km away from the study site. In addition, all sampled populations had similar plant densities (although their size varied from 40 to more than 100 *R. nudiflora* individuals), and the sites where they were found at presented similar biotic (surrounding vegetation, herbivore attack levels) and abiotic (rainfall) characteristics compared with those found at the experimental site of the region each one belonged to.

Within each population, 'parent' plants were separated by a distance of at least 5 m and were carefully selected based on size (number of leaves, height) and location (open areas) to minimize maternal effects. All 60 parent plants were transported to nurseries of the Ecology Department of the Autonomous University of Yucatan. During July and August of 2005, once plants had adapted to nursery conditions and while they were still flowering, CH flowers were manually self-pollinated to additionally purge maternal effects. The resulting fruits were collected and seeds from plants of populations belonging to the same region were mixed, resulting in a pool of 450 seeds per region (total of 1350 seeds). In November 2005, seeds were then germinated in plastic growing trays and seedlings were watered every 3 days for 2 weeks. Once seedlings had 3–4 real leaves they were transplanted to individual 3–l plastic containers filled with commercial substrate.

Once in plastic containers, plants remained inside the nurseries for a 4-month period. In June of 2006, once the rainy season had started, a total of 324 plants from the north region, 324 from the centre and 216 from the south (total $n = 864$ plants) were used to conduct the reciprocal transplant experiment; a third of the plants of each origin where transported to each experimental study site and left in the plastic containers. We used less number of plants from the south because the percentage of germinated seeds was lower for these populations. Within each site, plants were distributed in the following way: Three experimental plots were established at each site (96 plants per plot), and each plot was subdivided into four 5-m^2 blocks with 24 plants each (nine plants of north origin, nine of centre origin and six of south origin). Plants were randomly selected at the plot and block levels. Within each site, plots were separated by an average distance of 1 km while blocks within each plot were separated by 10–15 m. Herbaceous vegetation present inside each block had been previously removed, and experimental plants were placed at a density of one plant per square metre. This experimental design was statistically robust because it took into account the effects of block and plot, thus controlling for within-site variation while testing for main effects.

The experimental design used here differed from that of a classic reciprocal transplant experiment which uses as transplant sites those where the populations were originally sampled at. This approach, however, combined with using a 'pool' of seeds from populations of each region, allowed us to evaluate LA at a 'regional' scale while achieving a better representation of genotypes from each region (see Santamaría *et al.*, 2003; Becker *et al.*, 2006); thus, hereafter we refer to a 'region' effect instead of a site effect. Using a regional scale approach to test for LA is justified for three reasons. First, previous studies have pointed out that plant LA can be tested for as long as experimental plants are sampled from natural populations found within a range of conditions relevant to the environmental variability that exists in the field (e.g. Antonovics & Bradshaw, 1970; Schemske, 1984; Sultan, 1995; Becker *et al.*, 2006). Based on this, we consider that differences in biotic and abiotic factors between regions in Yucatan justify testing for plant LA to insects at a regional level. Second, many studies on plant population genetics have shown that native populations may be locally adapted over a variety of spatial scales (Endler, 1986; Linhart & Grant, 1996), and not only at the site or local level. Finally, the adult stages of the herbivores which attack *R. nudiflora* present greater mobility compared with other more sedentary insect herbivores (e.g. scale insects; see Hanks & Denno, 1994), which could result in neighbouring *R. nudiflora* populations being adapted to the same or at least a similar pool of insect populations.

Response variables

Starting in July 2006 and until March 2007 we visited each site monthly to measure leaf area consumed (folivory), and perform fruit counts. Fruits were then opened in the laboratory to record SP presence. Initial plant size was also estimated during the first census.

Folivory

During each census two lower (basal) and two upper leaves were randomly chosen along the vertical axis of each experimental plant (different pairs of leaves were drawn during each census). Leaf contours were drawn, cut out, and then taken to the laboratory to measure leaf area with a leaf area metre (Li-Cor®; Li-3000A, Lincoln, NE, USA). For each leaf drawing, the damaged portions (i.e. holes) were cut out and measured separately from the remaining (intact) leaf portion. Leaves that were completely eaten (petiole remaining), as well as those which were not damaged were not drawn (directly assigned 100% or 0% damage in the field; the former case was rare). For each plant, values for consumed leaf

area from all folivory censuses were summed and then divided by the total leaf area (consumed + remaining) of the corresponding leaves to estimate the proportion of leaf area consumed per plant throughout the study period.

When a large percent of the leaf had been consumed, making it impossible to calculate the total leaf area (<5% of cases), consumed leaf area was calculated by subtracting the remaining area of those leaves from an overall average of leaf area of 150 undamaged leaves (19.84 ± 0.5 cm^2) previously selected from experimental plants in nurseries before their placement at each site (leaf area was also measured with a leaf area metre). These values of consumed leaf area and total leaf area (average from the undamaged leaves) were summed to those calculated as described in the previous paragraph when necessary to estimate the proportion of leaf area consumed.

Seed predator attack

Fruits collected in the field were opened in the laboratory to record the presence of seed-eating larvae. Attacked fruits were easily identified because of direct (i.e. larvae) or indirect (i.e. frass and escape tunnel) evidence of predator presence. For each plant we estimated the proportion of attacked fruits as the total number of fruits attacked (throughout the entire sampling period) divided by the total number of fruits collected. Because experimental plants seldomly produced CH flowers, the data analysed are only from fruits of CL flowers. Results from a field experiment conducted in 2008 at one of the centre populations reported that the proportion of attack did not vary significantly between fruits of CH and CL flowers (CH mean ± SE: 0.16 ± 0.02; CL: 0.15 ± 0.04; paired t-test: $t_{49} = 0.7$, $P = 0.5$; P. Carbajal-Borges, unpublished data).

Plant size

We estimated initial plant size by measuring the area of an ellipse represented by each individual using the formula: $\pi * D_1 * D_2 / 4$, where D1 was the plant's largest diameter (from tip to tip of the largest pair of leaves) and D2 was the smaller diameter, perpendicular to the former.

Statistical analyses

We used PROC GLIMMIX in sas (ver. 9.1; SAS, 2002) to evaluate the effects of region (fixed, three levels), origin (fixed, three levels), plot (random, nested within region; nine levels) and block (random, nested within plot; 36 levels) on the total number of fruits produced per plant, the proportion of leaf area consumed and the proportion of fruits attacked by seed-eating larvae. Evidence for LA was taken to be a significant region × origin interaction for which native plants showed greater reproductive output or lower herbivore damage than non-native plants at any given site ('local vs. foreign' comparison;

Kawecki & Ebert, 2004). This interpretation was also complemented by comparing the performance of each plant origin across sites, to gain perspective on the relative performance of each plant origin outside of its native site ('home vs. away' comparison; Kawecki & Ebert, 2004).

The analysis for total number of fruits per plant considered a Poisson distribution with the log link function, and included as covariates initial plant size and proportion of total leaf area lost to folivores (average value per plant across censuses); covariate effects did not interact significantly with main effects. On the other hand, analyses for proportion of leaf area eaten and proportion of attacked fruits assumed a binomial distribution with logit link function. Plant size and fruit number were initially used as covariates for the folivory and seed predation models, respectively, but were later removed because of their non-significance. Pre-planned contrasts were conducted using the LSMEANS statement to test for differences between levels of significant main effects ($P \le 0.05$). When a significant region × origin interaction was observed, the SLICE option was used to test for differences between origins within a given site (local vs. foreign plants), or differences for a given origin across sites (home vs. away comparison) (Littell et al., 1996). P-values from multiple comparisons were corrected based on the Bonferroni adjustment. Data for all three response variables showed not to be overdispersed.

Results

Fruit number

A total of 2818 fruits were collected throughout the sampling period at the three study sites. Results showed significant effects of origin, region × origin, initial plant size and proportion of leaf area consumed (Table 1); the effect of region was only marginally significant. Pre-planned contrasts showed that plants of north origin produced significantly more fruits (mean ± SE: 7.48 ± 0.56) than plants from the south and the centre ($P < 0.01$ in both cases); the last two did not differ significantly (south origin: 5.64 ± 0.57; centre origin: 5.45 ± 0.52; $P = 0.23$). The significant region × origin interaction did not reveal a pattern of LA; instead it was probably partly because of plants of north origin nearly doubling their fruit production at the south region relative to their reproductive output at their native region (Fig. 2); plants of north origin produced at least 40% more fruits than plants from the centre and south origins at the south region (pre-planned contrasts: $t_{396} = -3.64$, $P < 0.001$ and $t_{396} = 2.14$, $P = 0.03$ respectively; Fig. 2). None of the plant origins performed significantly better at their native site compared with non-native sites (see Fig. 2 for multiple comparison results). There was only weak evidence of a 'home advantage' at the north site where native plants

Table 1 Results from the mixed model analyses to evaluate the effects of region, origin and their interaction on the number of fruits produced per plant, the proportion of leaf area consumed and the proportion of attacked fruits by a seed predator in *Ruellia nudiflora* (*n* values are sample sizes used for each model).

Source of variation	No. fruits ($n = 440$)			Proportion of leaf area eaten ($n = 616$)			Proportion of attacked fruits ($n = 440$)		
	df	F	P-value	df	F	P-value	df	F	P-value
Region (R)	2,6	3.83	0.08	2,6	0.31	0.75	2,6	2.03	0.21
Origin (O)	2,396	8.99	0.0002	2,574	4.94	0.01	2,398	8.64	0.0002
R × O	4,396	4.29	0.002	4,574	1.88	0.08	4,398	7.75	<0.0001
Plant size	1,396	58.76	<0.0001	1,574	–	–	1,398	–	–
Proportion leaf area consumed	1,396	22.36	<0.0001	1,574	–	–	1,398	–	–

Initial plant size and proportion of leaf area consumed were used as covariates for the analysis of number of fruits.
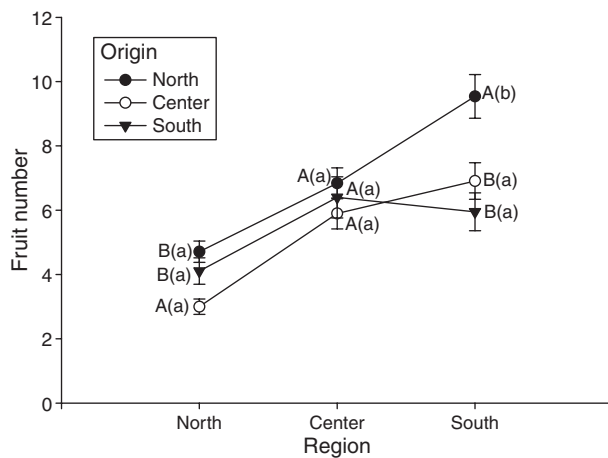

Fig. 2 Number of fruits produced by *Ruellia nudiflora* plants of each origin across regions (values are mean ± SE). Different upper case letters indicate significant differences between plant origins within a given region, while lower case letters indicate differences across regions for a given plant origin (significance value set at 0.05 for pre-planned contrasts; *P*-values adjusted for).

produced significantly more fruits compared with plants from the centre ($t_{396} = -2.82$, $P < 0.005$), but not significantly more than those from the south ($t_{396} = -0.05$, $P = 0.96$; Fig. 2). Finally, both covariates (initial plant size and proportion of leaf area eaten) had a significant effect on the number of fruits produced per plant indicating that larger plants and with less folivory produced more fruits (Table 1).

Proportion of leaf area eaten

On average, folivores consumed $12.70 \pm 0.45\%$ of the leaf area sampled per plant. Results showed only a significant effect of origin on the proportion of leaf area consumed by folivores, for which plants of centre origin were significantly more consumed ($17.15 \pm 0.9\%$), compared with those of north ($16.44 \pm 0.8\%$; $F_{2,574} = 7.43$,

$P = 0.006$) and south ($16.34 \pm 1.2\%$; $F_{2,574} = 5.18$, $P = 0.02$) origins; the last two origins did not differ significantly ($F_{2,574} = 0.05$, $P = 0.82$). Importantly, the region × origin interaction was not significant (although this result may be considered marginal; Table 1), and native plants did not show a significantly lower amount of damage than non-natives (Fig. 3a). In addition, never did any plant origin present less damage at its native site compared with non-native sites (Fig. 3a).

Proportion of attacked fruits

Seed-eating larvae attacked on average $15.47 \pm 1.27\%$ of fruits sampled per plant. Results showed a significant effect of origin and of region × origin (Table 1). Pre-planned contrasts showed that plants from the north and south origins were overall the most attacked and did not differ significantly in the proportion of attacked fruits by SP larvae ($18.46 \pm 1.9\%$ and $16.21 \pm 2.8\%$ respectively; $t_{398} = 0.11$, $P = 0.90$); both these origins differed significantly from plants of centre origin which suffered from the lowest attack rate (north vs. centre: $t_{398} = -4.01$, $P < 0.0001$; south vs. centre: $t_{398} = -3.35$, $P = 0.0009$). With respect to the significant region × origin interaction, and contrary to expectations, plants from the north showed by far the greatest proportion of attacked fruits at their native site (almost double the proportion of attacked fruits compared with non-native plants at the north; Fig. 3b), which resulted in a significantly greater proportion of damage than plants from the centre ($t_{398} = -3.67$, $P = 0.0003$) and south ($t_{398} = 3.07$, $P = 0.002$) at the north region (Fig. 3b). Moreover, plants of north origin were also significantly more attacked at the native site compared with non-native sites (Fig. 3b). On the other hand, plant origins did not differ in the proportion of attacked fruits at the south site, while only weak evidence of plant home site advantage was observed at the centre region as native plants showed a significantly lower proportion of attacked fruits compared with plants from the south ($t_{398} = -4.7$, $P < 0.0001$) but did not differ significantly from those
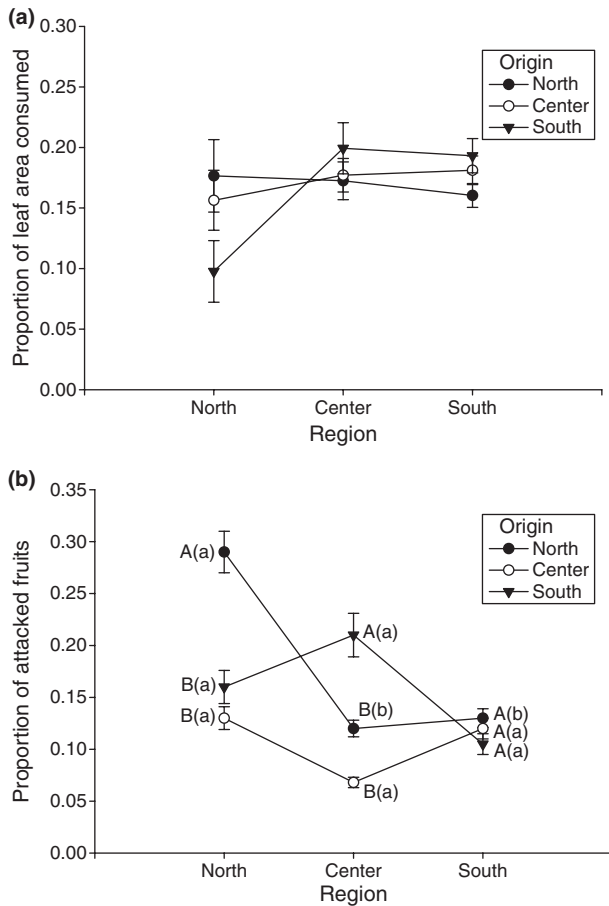
Fig. 3 Proportion of (a) leaf area consumed by folivores and (b) attacked fruits by the noctuid seed predator for *Ruellia nudiflora* plants from each origin across regions (values are mean ± SE). In the case of attacked fruits, different upper case letters indicate significant differences between plant origins within a given region, while lower case letters indicate differences across regions for a given plant origin (significance value set at 0.05 for pre-planned contrasts; *P*-values adjusted for).

of north origin (t_{398} = −0.92, *P* = 0.35; Fig. 3b). Finally, plant origins never showed significantly less damage at their native site compared with non-native sites, except perhaps for a tendency observed for plants of centre origin (Fig. 3b).

Discussion

Although a significant origin × region effect was found for fruit production, native plants generally did not outperform non-natives. Only at the north site native plants performed equally-well or better than non-native plants, but such pattern cannot be taken as evidence of home site advantage to argue LA. It is important to note, however, that lack of evidence of plant LA at the regional scale does not preclude the possibility of this condition

occurring at a finer spatial scale (although our experimental design did not allow us to test for it at this level). Instead of plant LA, the result which was most likely responsible for the significant interaction term was that plants of north origin nearly doubled their fruit production at the south site relative to their native site. This finding suggests a plastic response of northern plants to specific conditions in the south, which may be explained by changes in reproductive investment because of greater resource availability (Bazzaz, 1996). For instance, rainfall is greatest in the south and lowest in the north (Chico-Ponce de León, 1999), and greater water availability in the south may have triggered the observed response of plants of north origin at this site.

Folivory results indicated lack of plant LA to this herbivore guild. A significant effect was only observed in the case of plant origin, although the magnitude of differences was very small (1–2% difference in damage between origins). We note however, that folivory had a significant effect as a covariate for the analysis of fruit production, suggesting an effect of this herbivore guild on plant fitness. Nonetheless, this effect was consistent across plant origins, regions and their interaction (n.s. interaction of folivory with main effects; see Methods) which agrees with folivory results. In terms of LA, we found that regardless of the site, all plant origins exhibited practically the same amount of leaf damage within each site and no 'home advantage' was observed. Such homogeneous distribution of folivore damage across regions and plant origins suggests similar abundances and/or species composition for this guild across sites (e.g. Gómez & Zamora, 2000), and this was partially supported by preliminary field observations at each site which indicated that the abundance of the most common folivore species (belonging to Nymphalidae) was spatially consistent (I. Ortegón-Campos, data from this study). These results indicate that external leaf-chewing insects will probably have a limited potential to promote LA in *R. nudiflora*, which agrees with a previous study by Abdala-Roberts & Marquis (2007) who did not find evidence of LA to insect leaf-chewers in the annual herb *Chamaecrista fasciculata*. In contrast, a study by Sork *et al.* (1993) reported that native *Quercus rubra* saplings suffered less insect folivore damage than non-natives even at a fine spatial scale (< 4 ha). The same was true for *Plantago lanceolata* in terms of foliar damage by a Chrysomelid beetle according to a study by Crémieux *et al.* (2008). Based on the limited number of studies available and the inconsistency in their results, we argue that further research is needed which evaluates the influence of folivore degree of specialization, as well as the effect of spatial scale of analysis (local vs. regional). Such factors will contribute to the understanding of the potential insect folivores have to promote plant LA.

In contrast to folivory results, a more complex pattern arose for proportion of *R. nudiflora* fruits attacked by the SP. First, we found that native plants to the north region

experienced half the amount of damage when placed outside the north region, which suggests that plants from the north region appear to have escaped their SP populations when placed outside their native site thus supporting the enemy-release hypothesis (Keane & Crawley, 2002). In addition, plants of north origin experienced nearly double the amount of damage compared with non-native plants at the north site. These results may be because of (a) SP LA to native *R. nudiflora* populations at the north region, and/or (b) plants from the north region being maladapted to native SP populations (see Abdala-Roberts & Marquis, 2007; Crémieux *et al.*, 2008).

With respect to the first condition, Núñez-Farfán *et al.* (2007) suggest that insect herbivore specialization or LA to host plant populations may be more likely than the opposite condition because insect adaptive responses occur during shorter time scales (i.e. shorter generation times), and end up overruling plant LA in most cases. Such a scenario would probably be more likely for specialized concealed or endophytic feeders (see also Greischar & Koskella, 2007 in the case of pathogen LA). This idea is supported by a number of studies showing insect LA to host plants (e.g. Hanks & Denno, 1994; Mopper *et al.*, 1995; Mopper & Strauss, 1998), while only a few have showed plant LA to insects (Schemske, 1984; Sork *et al.*, 1993; Crémieux *et al.*, 2008) and with inconsistent results in some cases (although as mentioned before, more studies are still needed). In this study, SP did appear to be locally adapted to *R. nudiflora* plants at the north site, but this pattern was not observed at the south site or at the centre site. Such spatial inconsistency in results suggests that different stages of the adaptive response continuum between plants and SP may be occurring across regions (see discussion in Biere & Verhoeven, 2008), which may lead to coevolutionary dynamics (Ehrlich & Raven, 1964; Janzen, 1980; Berenbaum & Zangerl, 1998). Nonetheless, data recorded over multiple years in this system would be necessary to properly test this argument.

On the other hand, a second mechanism explaining greater SP attack rates on native plants in the north region is that these plants are maladapted to their SP. Such condition may be observed if, as mentioned earlier, the insect is able to evolve faster than its host plant, resulting in decreased fitness of native plant genotypes which appear maladapted (Greischar & Koskella, 2007; Crémieux *et al.*, 2008). However, from a phytocentric point of view plant local maladaptation may arise via inbreeding which may affect plant reproductive output and defensive responses because of reduced genetic variation (Núñez-Farfán *et al.*, 2007). High levels of inbreeding are probably common in *R. nudiflora* given that it produces cleistogamous flowers that obligately self-pollinate. Moreover, lower values of herkogamy (anther-stigma separation) have been observed for northern *R. nudiflora* populations (see Methods) and this

may result in limited outcrossing (Barrett, 2003), greater selfing rates and inbreeding levels, and finally suboptimal adaptation to SP (e.g. lower plant resistance; see Schmid, 1994). However, this argument would have predicted greater attack on plants of north origin at all three sites, and this was only true at the north site making the hypothesis of SP LA more suitable to explain the observed pattern.

In conclusion, although results from this study indicated lack of *R. nudiflora* LA for the response variables measured, some interesting but complex patterns emerged when comparing results for folivory and fruit attack. Damage by folivores was similar for native relative to non-native plants at all sites, while SP attack rates indicated SP LA and/or plant maladaptation in the north. Although more years of study are needed, this finding initially suggests that the interaction between plants and specialist pre-dispersal SP will more likely promote plant or insect LA (the latter being the case in this study). We argue that further plant LA studies are needed which contrast the influence of different insect herbivore guilds to understand the role of plant–herbivore interactions and degree of specialization on such evolutionary condition. One recommendation would be to manipulate the presence of each guild by means of experimenal exclusions and test for the contribution of each group to a specific plant fitness measure (Biere & Verhoeven, 2008; Nuismer & Gandon, 2008).

Acknowledgements

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