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Spatially inconsistent direct and indirect effects of herbivory on floral traits and pollination success in a tropical shrub

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Investigations on plant–animal interactions have traditionally focused on single interactions at a time (e.g. herbivory, pollination), yet plant fitness is generally influenced in complex ways by several interactions operating concurrently, and very little is known on the degree of spatial consistency of the direct and indirect effects that link different interactions. This paper evaluates experimentally whether direct and indirect effects of herbivory on male and female flower size and pollination success of the monoecious tropical shrub *Cnidoscolus aconitifolius* (Euphorbiaceae) remain consistent at three distant regions in Yucatan (southeastern Mexico). Plants were subjected to different levels of leaf defoliation, and treatment effects on floral traits (corolla area, corolla tube length, pollen production), and male and female components of pollination success (percent pollen removal, number of pollen tubes) were subsequently measured to evaluate the indirect effect of herbivory on plant reproductive success via pollination. Defoliation had significant direct effects on floral traits, but its indirect effects differed between the male and female components of pollination success. Moreover, the relationships between defoliation, floral traits and male and female pollination success varied spatially (i.e. between regions), although they were frequently more spatially-consistent for male success than for female success. Results from this study stress the importance of explicitly testing for spatial variation in direct and indirect effects arising from plant-animal interactions.

Multiple biotic interactions acting in concert may have ecological and evolutionary consequences that cannot be predicted from the separate study of single interactions, and examination of a given plant–animal interaction without regard to the others may lead to erroneous or biased conclusions about its actual effects on plant fitness (Herrera 2000, Herrera et al. 2002, Strauss and Irwin 2004, Rey et al. 2006). For instance, when two interactions occur simultaneously and impinge upon the same structures (e.g. pollinators and flower-feeding herbivores), opposing selective pressures acting on the same trait may generate tradeoffs and favour compromise phenotypes (Galen and Cuba 2001, Cariveau et al. 2004, Irwin et al. 2004). Moreover, if interactions take place sequentially, the preceding interaction may indirectly influence the subsequent one. Folivores for example, may indirectly influence subsequent pollination events and plant reproductive success by modifying pollinator foraging behaviour via changes in floral traits (Strauss et al. 1996, Lehtilä and Strauss 1997, Mothershead and Marquis 2000). Pollinators, in turn, may also indirectly influence subsequent interactions between plants and seed predators. This will happen, for example, when fruit predators select larger fruits or those with more seeds because they originate from flowers which enjoyed improved pollinator service (Herrera 2000, Herrera et al. 2002, Cariveau et al. 2004).

Investigations on the interaction between pollinators on one side, and herbivores or fruit predators on the other, have so far furnished compelling evidence pointing at significant evolutionary effects (Strauss and Irwin 2004). Some studies have documented changes in plant fitness correlates of trait variation when multiple interactors are involved (Mothershead and Marquis 2000, Cariveau et al. 2004, Irwin 2006). Others have proven the non-additivity in effects of different sets of interactors on plant fecundity (Herrera 2000, Herrera et al. 2002, Gómez 2005, Pohl et al. 2006). There is still, however, one important aspect of the interaction between pollinators and herbivores that remains largely unexplored, namely that of its spatial consistency (Strauss and Irwin 2004, Rey et al. 2006). The evolution of species interactions can only be fully understood by considering their variation (i.e. intensity, presence–absence) across space (Thompson 1994, Travis 1996). Recent advances in coevolutionary theory have arisen from the recognition that the form of an interaction often varies across geographic landscapes (Thompson 1994), and examples now abound documenting selection mosaics in plant–animal interactions (Benkman 1999, Zangerl and Berenbaum 2003, Gómez et al. 2009).

Since the ecological and evolutionary consequences of single interactions have been so often proven to vary spatially (Thompson and Fernández 2006, Gómez et al. 2009), it would be also expected that the consequences of the effects between different interactions will also vary spatially. Therefore, properly understanding the ecological and evolutionary role of multiple interactions will also require placing these in a geographic context. This paper describes an experimental investigation designed precisely to evaluate whether the direct and indirect effects of herbivory on pollination success of the monoecius tropical shrub *Cnidoscolus aconitifolius* (Euphorbiaceae) remained consistent at three distant regions.

By experimentally removing different amounts of leaf tissue, and subsequently measuring treatment effects on floral traits and male and female components of pollination success, this study was aimed at answering the following questions: a) is there a direct effect of leaf defoliation on female and male floral traits? b) does defoliation have an indirect effect on male and female pollination success via changes in such floral traits; and c) does the direct and indirect effects of defoliation on plant pollination success vary spatially (i.e. across study regions)?

Material and methods

Study species

The tropical shrub *Cnidosculus aconitifolius* (Euphorbiaceae) has a wide distribution which ranges from south Mexico to Central America (Standley and Steyemark 1949). In Yucatan, this species is typically found at disturbed sites or edges of tropical dry forest patches throughout the state. It can reach up to 3–5 m in height and flowers from June to September. This species is monoecious and produces white male and female flowers which are arranged in inflorescences which have a dichotomous ramification pattern and measure between 15–40 cm in length (Standley and Steyemark 1949). Female flowers are located at the base of each ramification, while male flowers are found at the apex of each ramification. The average male-to-female flower sex ratio is 8:2 (Parra-Tabla et al. 2004). Male flowers have five anthers which vary in length and both male and female flowers last for only one day. Although *C. aconitifolius* is self-compatible, it requires pollinator visitation to form fruits. Female flowers contain four ovules, but fruits frequently produce only three seeds.

Flowers are visited by more than 10 bee species, of which the most abundant are: the honeybee *Apis mellifera* (Apidae), and the native species *Trigona nigra*, *T. fulviventris* (Apidae) and *Melipona* spp. (Apidae) (Arango et al. 2000, Rodríguez 2004). In addition, at least 30 butterfly species have been reported as common floral visitors of this species (Rodríguez 2004). Pollinator visitation is more intense early in the morning, and decreases towards noon. Bees typically visit male flowers to obtain pollen and nectar, while butterflies visit both male and female flowers indistinctly (Parra-Tabla et al. 2004). Preliminary observations suggest that the species composition of the pollinator guild associated to *C. acontifolius* remains spatially consistent across populations throughout the state of Yucatan. Leaves, stems, inflorescences and fruits are covered by glandular trichomes that serve as chemical and physical defense against herbivores (Abdala-Roberts and Parra-Tabla 2005). Extrafloral nectaries can be found at the base of each leaf blade, and are visited by at least five species of ants that actively defend the plant against natural enemies (Rico-Gray and Oliveira 2007). Despite all these defense mechanisms, *C. aconitifolius* frequently experiences high levels of folivory (up to 100% at some sites), and defoliation intensity has been shown to vary greatly between populations (Parra-Tabla et al. 2004, Abdala-Roberts and Parra-Tabla 2005, below). Leaf damage is caused mainly by larvae of Lepidoptera, the most important of which are *Chioides catillus albofasciatus* (Hesperiidae) and *Anteos maerula* (Pieridae) (Abdala-Roberts and Parra-Tabla 2005). As for other species belonging to the genus *Cnidosculus* (Dillon et al. 1983), larvae cut the latex flow to the leaf by bending the petiole and subsequently consuming rapidly the entire leaf blade. Folivory is most intense at the start of the rainy season, when leaf production is greatest (Parra-Tabla et al. 2004). Previous defoliation experiments have shown that artificial clipping increases trichome production on leaves and stems of *C. aconitifolius* (Abdala-Roberts and Parra-Tabla 2005), alters the number and proportion of male and female flowers, and reduces fruit production (Parra-Tabla et al. 2004, Arceo-Gómez et al. 2009).

Study regions and experimental design

This study was conducted at three distant regions separated from each other by at least 50 km but no more than 170 km. Each region is characterized by presenting one of the following types of tropical dry forest: low-height thorny deciduous forest (LTD), medium-height subdeciduous forest (MSD) and low-height deciduous forest (LD). Regions differed in floristic composition and climate (Chico-Ponce et al. 1999); the LTD region was the driest (600–700 mm of annual rainfall), followed by LD and MSD (1000–1200 and 1000–1500 mm annual rainfall, respectively). Regions have shown significant variation in folivory intensity on *C. aconitifolius* (Abdala-Roberts and Parra-Tabla 2005, Parra-Tabla unpubl.). Average values across seasons range from 5 to 15% of leaf area removed at the LD region and 10–30% at the LTD region to 15–50% at the MSD region. At each region, two *C. aconitifolius* populations were selected, and separated by at least 300 m. Five blocks were established within each population. Each block consisted of a group of three to five plants, each of which was randomly chosen to receive one of the following defoliation treatment levels: ‘control’ (natural herbivory: ca 5% of total leaf area eaten per plant on average), 50% of leaf area artificially removed, and 100% of leaf area artificially removed. Artificial defoliation was conducted with scissors, and for the 50% defoliation treatment each leaf had half of its tissue removed starting at the base of the leaf blade, and following the midvein (which remained on the defoliatied leaf after clipping); 100% defoliated plants had all leaves clipped at the base of each leaf blade. Defoliation treatments were conducted once at the beginning of the rainy season, as this is the time of year when the intensity of natural herbivory is greatest (Parra-Tabla et al. 2004). Natural herbivory levels were monitored throughout the sampling season (July–September) by conducting monthly censuses of lost leaf area for all plants to assess the amount of natural damage which each experimental plant experienced throughout the experiment; control or 50%-defoliated plants exceeding 15% of total leaf area removed were discarded from the experiment. One drawback of this measure is that it can result in the exclusion of the most susceptible plants which may introduce a bias in the sample after specimen removal. However, throughout the experiment only five plants were excluded which in addition were not more frequently found in a region or of a particular defoliation treatment. One of the excluded specimens was a control plant which belonged to the LTD region, another two belonged to region MSD (one control and the other a 50% defoliation plant), and two belonged to the LT region (50% defoliation). We did not use any type of insect repellent to exclude herbivores as this might have affected pollinator visits and thus pollination success.

Male and female floral traits

Each region was visited three times during the peak of the flowering season (August). Flowers were collected from experimental plants around noon, and individually placed in microcentrifuge tubes containing FAA solution. A total of 370 male and 109 female flowers were collected for all regions combined throughout the study season. For both male and female flowers we measured corolla tube length (mm) and the area represented by the petals (corolla area: mm^2); the number of pollen grains produced for each male flower was also estimated. These variables were chosen because previous studies with other plant species have often shown that they are affected by herbivory and are related to pollination success (Strauss et al. 1996). Corolla tube length was measured with the use of a dissecting microscope (10×) and an ocular micrometer. To measure petal area, the contour of each flower was drawn on black paper and then carefully cut out and measured with a leaf area meter.

To estimate the number of pollen grains produced, a total of 180 closed male flowers were collected (30 flowers per defoliation level at each region). Flowers were then dissected with the use of an optic microscope, and three anthers were extracted from each one. Before counts were performed, pollen grains were dispersed using a ultrasonic cell disruptor for 30 s. Each anther yielded from two to four 0.5 μl subsamples, which were diluted using 50 ml salt solution. Pollen counts were conducted using a particle counter fitted with a 100 μm aperture tube, and set to a particle size counting range of 22–30 μm. The average pollen grain count from the three sampled anthers was then multiplied by five to have an estimation of pollen grain production per flower.

Pollination success

Female pollination success was defined as the number of pollen tubes penetrating the ovary per flower. Ovary and styles were carefully separated from the corolla using a dissecting microscope, and softened in a KOH 5 M solution for 2 min. They were then rinsed with distilled water and dyed with aniline blue for 20 min at 65°C. The number of pollen tubes penetrating the ovary was then counted with a fluorescence microscope. Male pollination success was estimated as the proportion of pollen grains removed per flower. Anthers were carefully separated with the use of a dissecting microscope, and remaining pollen grains were counted using the same methods described above for closed flowers. The number of pollen grains removed was estimated as the difference between the number of remaining pollen grains per flower and the average number of pollen grains produced per flower for each region and treatment, obtained from counts performed for closed flowers.

Floral trait measurements, as well as pollen tube and pollen grain counts were conducted by the same person. In all cases, the identity of defoliation treatments, region of origin and population were unknown to the person recording the observations and conducting measurements.

Statistical analyses

Data were analysed by means of a generalized mixed model using PROC GLIMMIX in SAS (Littell et al. 1996), which included the region, defoliation treatment, and their interaction (i.e. spatial variation in effects) as fixed effects (Herrera et al. 2002), and population (nested in region), and block (nested in population) as random effects. The plant (nested in block) was considered the experimental unit (subject; Verbeke and Molenberghs 2000). In addition, the number of degrees of freedom was corrected based on the Satterthwaite method (Littell et al. 1996). The models used to test effects on the number of pollen tubes and pollen grains assumed a Poisson error distribution and the log link function. Because we did not observe a phenotypic correlation between corolla area and tube length for none of the studied regions (female flowers: $r \leq 0.23$, $p > 0.05$; male flowers: $r \leq 0.46$, $p > 0.05$), we conducted separate analyses for these variables. The models used for corolla tube length and corolla area assumed a normal distribution and used the identity as link function, while that for the proportion of pollen grains removed assumed a binomial distribution and a logit link function (Littell et al. 1996). Whenever the region by treatment interaction resulted significant, the SLICE statement was included to perform pairwise comparisons between factor levels within each level of the other factor. When the interaction was not significant, differences between levels of each main effect were determined based on preplanned contrasts (Littell et al. 1996). Univariate linear regressions were used to describe the relationships between floral traits and female and male pollination success. These analyses were also used to indirectly link defoliation to pollination success via changes in floral traits. In all cases, we present average values ± standard deviation (SD), except when indicated otherwise.

Results

Effect of defoliation on floral traits

Male flowers

For both corolla area and tube length significant differences were observed due to defoliation (Table 1, Fig. 1). In the case of corolla tube length, flowers from 100% defoliation plants had a significantly lower average value compared to flowers from 50% defoliation plants and controls ($F \geq 41.8$, $p < 0.0001$ in both cases, Fig. 1a). On average, 100% defoliation caused a 1 mm reduction in corolla tube length. On the other hand, corolla area was significantly smaller for 100% defoliation plants compared to control plants ($F_{1,\ 50.3} = 6.35$, $p = 0.01$, Fig. 1b); defoliation caused a reduction of 4 mm^2 on average. Corolla area for 50% defoliation plants did not differ significantly

Table 1. Mixed model results for region and defoliation treatment effects on male and female flower traits, and on male pollination success (proportion of pollen grains removed) and female pollination success (number of pollen tubes) in the monoecious tropical shrub *Cnidoscolus aconitifolius* (Euphorbiaceae). Significant effects are shown in bold.

Flower type/ pollination success	Male flowers						Male pollination success		Female flowers				Female pollination success	
Response variables	Number of pollen grains		Corolla length (mm)		Corolla area (mm^2)		Proportion of pollen grains removed		Corolla length (mm)		Corolla area (mm^2)		Number of pollen tubes	
Source	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Region	**3.5**	0.03	1.6	0.2	1.9	0.16	**16.9**	$<$0.001	3.0	0.06	1.9	0.16	**3.9**	0.02
Treatment	**3.4**	0.03	**25.6**	0.001	**3.3**	0.047	**3.8**	0.02	0.1	0.9	0.5	0.9	0.4	0.6
Region × Treatment	1.9	0.5	1.6	0.2	0.4	0.8	1.97	0.1	**4.1**	0.006	**22.8**	$<$0.001	**6.1**	$<$0.001

from control and 100% defoliation plants ($F \leq 1.16$, $p > 0.1$ in both cases). Within each region corolla tube length was significantly smaller for 100% defoliation plants compared to 50% defoliation and controls at all sites except MSD (Fig.1a). In contrast, corolla area did not vary significantly between treatment levels for any of the study regions (Fig. 1b).

Significant effects of region and defoliation treatment were observed on the number of pollen grains (Table 1, Fig.1c). Flowers from control and 50% defoliated plants did not differ ($F_{1,58.9} = 0.09$, $p = 0.75$), but both had significantly more pollen grains than flowers from 100% defoliation plants ($F \geq 4.54$, $p < 0.03$ in both cases). On average, 100% defoliated plants produced about 6000 fewer pollen grains than flowers of the other two treatment levels. Flowers from plants at LTD and LD showed a significantly greater average number of pollen grains compared to flowers from plants at the MSD region ($F \geq 20.74$, $p < 0.0001$ in both cases, Fig. 1c); the LTD and LD regions did not differ significantly ($F_{1,27} = 0.29$, $p = 0.59$). Male flowers of plants from LTD and LD produced about 5000 more pollen grains on average than male flowers from plants at MSD. Within regions, significant differences between defoliation levels were found for pollen grain number at LD and LTD; at both regions, control and 50% defoliated plants differed from 100% defoliated plants ($p < 0.05$ in all cases). Defoliation levels did not differ significantly at MSD (Fig. 1c).

Female flowers

For both corolla tube length and corolla area a significant defoliation × region interaction was observed (Table 1). Nonetheless, artificial defoliation did not always result in a reduction of flower size and in some cases even caused an increase depending on the region (Fig. 1). Within each region corolla tube length at MSD was significantly smaller for 50% defoliation plants compared to controls ($t_{88} = 2.41$, $p = 0.01$); defoliation caused a reduction of about 0.4 mm in length (Fig. 1d). However, corolla tube length did not differ between control and 100% defoliated plants ($t_{42} = 0.32$, $p = 0.7$). In contrast, 50% defoliation plants at LTD exhibited an increase of 0.6 mm on average relative to the other two treatment levels (Fig. 1a; $t \geq 2.4$, $p < 0.01$ in both cases). Finally, treatment levels did not differ at the LD region ($t \leq 0.27$, $p \geq 0.7$; Fig. 1d). Treatment differences in corolla area were only observed at MSD, where both 50% and 100% defoliation plants showed a significant reduction (about 3.5 mm^2 on average) relative to control plants ($t \geq 3.33$, $p \leq 0.002$; Fig. 1e).

Effect of defoliation on pollination success

Male success

Overall, the average proportion of pollen grains removed per flower was high (0.92 ± 0.04), significant effects of defoliation treatment and region were observed (Table 1). Flowers from 100% defoliation plants exhibited a significantly lower proportion of removed grains compared to 50% defoliation plants and controls ($F \leq 5.5$, $p < 0.03$ in both cases, Fig. 2a). Differences between regions indicated that the proportion of removed pollen grains was lower for flowers from plants at the MSD region compared to LTD and LD ($F \leq 14.38$, $p < 0.001$ in both cases, Fig. 2a). Within each region, only at LD were significant differences observed between defoliation treatment levels, as control plants showed a significantly greater average value compared to 50% and 100% defoliation plants ($t \geq 2.3$, $p < 0.03$ in both cases).

Female success

Significant region and defoliation × region effects were observed on the number of pollen tubes (Table 1). Nonetheless, similar to effects of defoliation on flower size, defoliation did not always cause a reduction in pollination success and even caused an increase in some cases (Fig. 2b). Significant differences between defoliation treatments in the number of pollen tubes per flower were observed at MSD and LTD ($F \geq 2.92$, $p \leq 0.055$; Fig. 2b). At LTD, flowers from 50% and 100% defoliated plants showed a marked decrease in the number of pollen tubes, flowers from control plants had on average 18 more pollen tubes than the other two treatment levels (Fig. 2b). In contrast, female flowers of 50% and 100% defoliated plants at MSD had on average more pollen tubes than flowers from control plants (Fig. 2b). At this region, female flowers from 50% defoliated plants had on average 10–15 more pollen tubes than those from control and 100% defoliated plants, although the difference between 50% and 100% defoliation plants was not significant (Fig. 2b). Finally, female flowers from 50% defoliated plants at LD showed a marginally greater

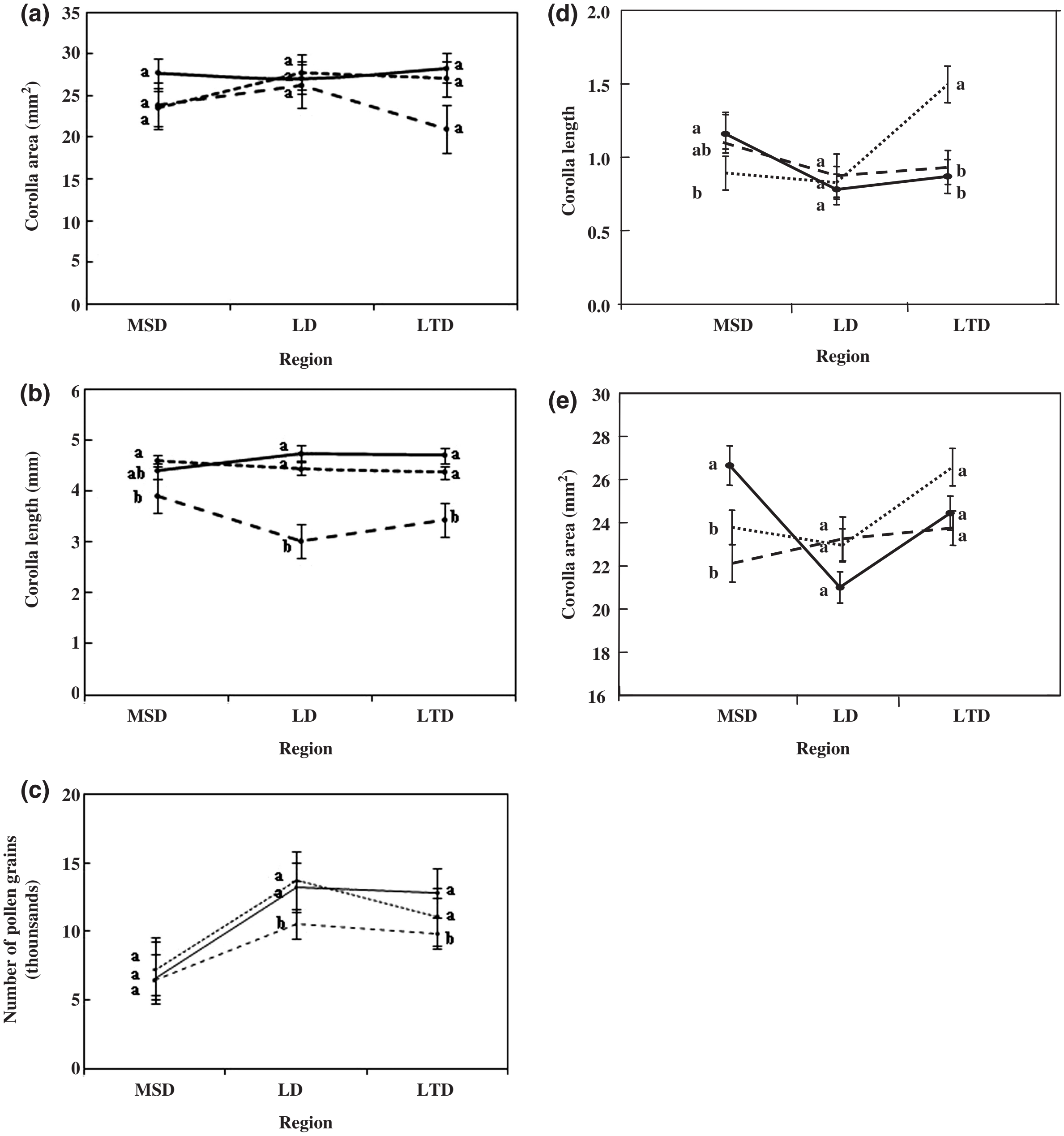

Figure 1. Interaction graphs for region and defoliation treatment effects on male flowers: (a) corolla tube length, (b) area, and (c) number of pollen grains produced; and female flowers: (d) corolla tube length, and (e) corolla area (means ± SE) of *Cnidoscolus aconitifolius*. Continuous line = control plants (natural herbivory), dotted line = 50% defoliation plants, and broken line = 100% defoliation plants. Different letters indicate significant differences between treatment levels within each region ($p < 0.05$).

number of pollen tubes relative to the other two treatment levels (Fig. 2b, $p = 0.09$).

Floral traits, pollination success and indirect effects of defoliation across regions

Male flowers

Although male reproductive success (i.e. proportion of pollen grains removed) and its relationship with floral traits were consistent across regions (Fig. 3), some differences were observed. For instance, at the MSD and LTD regions, where defoliation reduced the proportion of pollen removed, a significant negative relationship existed between corolla area and male reproductive success, while a positive relationship existed between corolla length and reproductive success, suggesting indirect effects of defoliation on male reproductive success, but of different sign depending on the floral trait considered (Fig. 3a, 3c). Thus, given that artificial defoliation reduced male flower corolla area and tube length of plants from these regions, then a negative indirect effect of defoliation on male success may be expected via reduced corolla length, while a positive indirect effect would be expected via corolla area for plants at MSD and LTD regions. At the LD region a significant relationship was observed between corolla tube

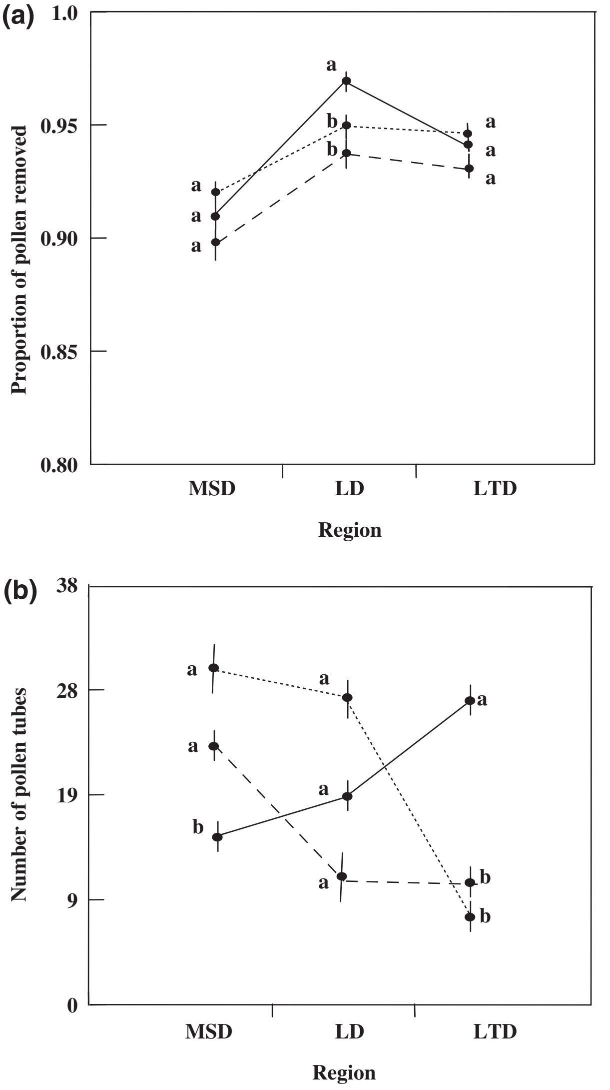

Figure 2. Interaction graphs for region and defoliation treatment effects of (a) number of pollen tubes, and (b) proportion of pollen removed (means ± SE) in female and male flowers of *Cnidosculus aconitifolius*. Continuous line = control plants (natural herbivory), dotted line = 50% defoliation plants, and broken line = 100% defoliation plants. Different letters indicate significant differences between treatment levels within each region ($p < 0.05$).

length and male reproductive success, while a marginally significant relationship was observed between corolla area and reproductive success (Fig. 3b). Using only flowers from control plants, a significant and positive relationship existed between corolla tube length and male reproductive success at MSD and LTD ($R = 0.6$, $p < 0.01$, and $R = 0.4$, $p < 0.01$, respectively; Fig. 3a, 3c). In contrast, a significant but negative relationship existed between corolla area and male reproductive success was observed at both these regions ($R = -0.4$, $p < 0.01$, and $R = -0.6$, $p < 0.01$, respectively; Fig. 3a, 3c). At LD, a non-significant relationship in control plants was observed for both floral traits and male pollination success ($R \leq 0.3$, $p > 0.1$ in both cases; Fig. 3b).

Female flowers

In contrast to results for male flowers, the relationship between changes in floral morphology and female flower pollination success were not clear (Fig. 4). For example, although defoliation resulted in a significant decrease in corolla area and an increase in female pollination success at the MSD region, female flower corolla area and length were not related to the number of pollen tubes (Fig. 4a), while at the LD region only marginally significant relationships were observed (Fig. 4b). Finally, a contrasting pattern was observed at the LTD region where a significant negative relationship existed between corolla tube length and female reproductive success (Fig. 4c); however, defoliation significantly increased corolla length at this site, thus indirectly and negatively linking defoliation with female reproduction success via increased tube length, agreeing with the negative effect of defoliation on number of pollen tubes at this site (Fig. 2b). Using only flowers from control plants, only a significant and positive relationship was observed at the MSD region between corolla tube length and corolla area with female reproductive success ($R = 0.7$, $p < 0.05$ and $R = 0.9$, $p < 0.03$, respectively; Fig. 3a).

Discussion

By using the monoecious tropical shrub *Cnidosculus aconitifolius* as study system, this work experimentally showed that defoliation has direct effects on male and female flower traits, as well as indirect effects on pollination success. Moreover, results from this study also showed that such effects may vary from one region to another. Nonetheless, results from our study should be taken with caution as the strongest differences for the traits measured were observed between control and 100% defoliation plants, and total defoliation is not a frequent condition at the *C. aconitifolius* natural populations. In spite of this, 50% defoliation also produced not only significant direct effects which, nonetheless, varied between male and female flowers, and between flower morphological traits, but also significant indirect effects on male and female pollination success which varied across sites.

Many studies have reported indirect effects of folivory on plant pollination, suggesting that indirect relationships of this sort are relatively common in nature (Strauss and Irwin 2004). The principal mechanism responsible for such indirect link is that folivory reduces available photosynthetic tissue, which cause tradeoffs between growth, reproduction and defense (Marquis 1992). However, most studies looking at direct and indirect effect of folivory have been conducted at one population, and have not evaluated the degree of spatial consistency of direct and indirect effects of herbivory on pollination success (but see Herrera et al. 2002, Gómez et al. 2009). Overall, our results suggest that the direct effects of folivory vary spatially for *C. aconitifolius*, and that the potential indirect effects of herbivory on plant reproductive success depend on flower sex and intensity of simulated herbivory. Such indirect effects are partly linked to changes in floral characteristics due to defoliation, as discussed below.

Direct effects of defoliation on floral characteristics

Overall, findings from all three study regions indicated that male flowers were generally smaller for defoliated plants and

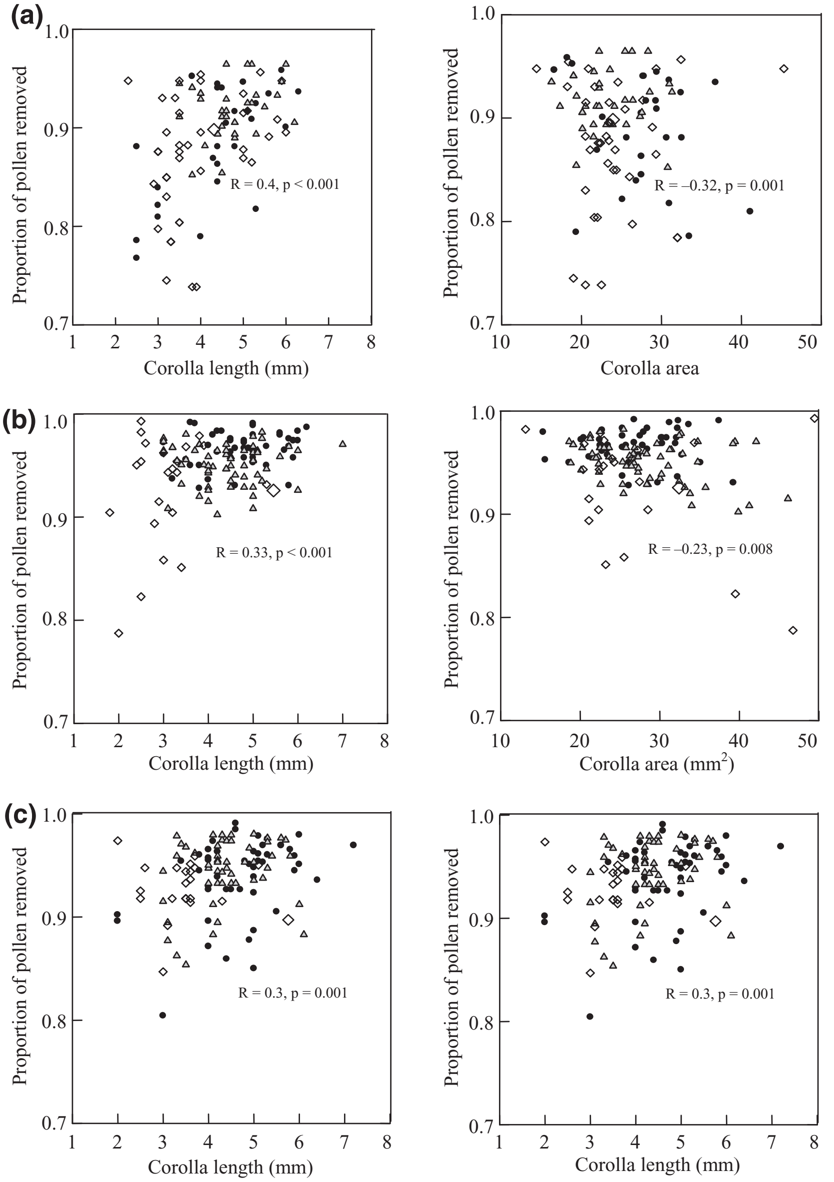

Figure 3. Relationship between corolla tube length and area and the proportion of pollen grains removed for male flowers of *Cnidosculus aconitifolius* subjected to different levels of artificial defoliation for each study region: (a) MSD, (b) LD, and (c) LTD. Filled circles = control plants (natural herbivory), grey triangles = 50% defoliation plants, and open diamonds = 100% defoliation plants. Regression values and significance are shown (ns = not significant).

that the production of pollen grains was also negatively affected, although the significance of these affects varied across regions. On the other hand, defoliation effects on female flower traits not only differed in strength across regions, but also in sign, since artificial defoliation caused an increase or a decrease in female flower size, depending on the region and damage level.

Most studies that have evaluated the effect of herbivory on floral display characteristics for plants with hermaphrodite flowers have generally found negative effects (Strauss and Irwin 2004). However, the few studies that have examined such effects for monoecious species have found contrasting results. For instance, chronic levels of herbivory caused an increase in the investment in the male function through a greater production of male reproductive structures in *Pinus edulis* (Cobb et al. 2002), while previous studies with *C. aconitifolius* showed that artificial defoliation caused an increase in the proportion of female flowers (Parra-Tabla et al. 2004, Arceo-Gómez et al. 2009), which is similar to what was found for *Cucumis sativus* (Thomson et al. 2004). Results for *C. aconitifolius* in this study also showed contrasting effects of defoliation depending on the flower type and size variable measured. For instance, although we found that defoliation consistently reduce pollen grain production (in agreement with previous studies; Quesada et al. 1995), its effect on flower size changed depending on the flower sex. In contrast to our results, previous studies have shown that defoliation can reduce female flower size but not male flower size in *Cucumis sativus* (Thomson et al. 2004), or not affect any of the floral types as in the case of *Cucurbita moschata* (Hladun and Adler 2009).

The observed response to defoliation by *C. aconitifolius* in terms of both male and female flower size was interesting

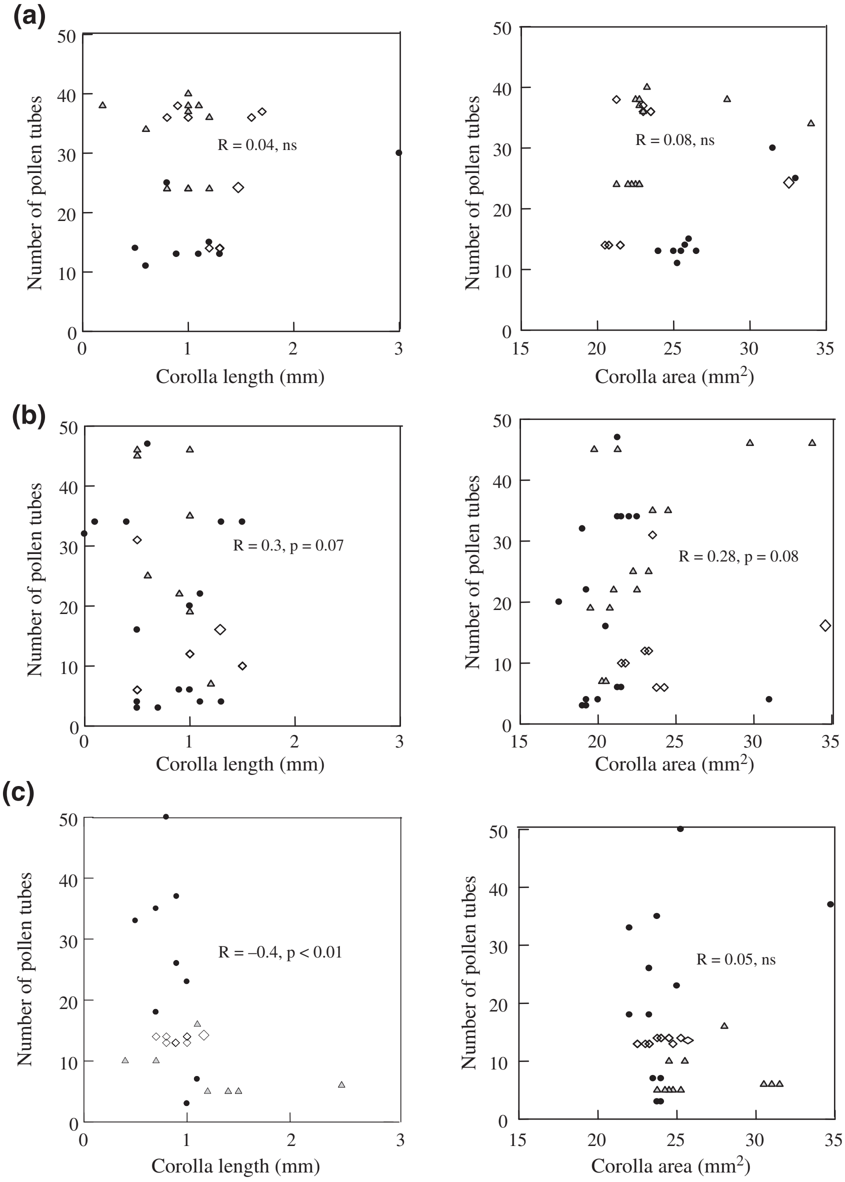

Figure 4. Relationship between corolla tube length and area and the number of pollen tubes for female flowers of *Cnidosculus aconitifolius* subjected to different levels of artificial defoliation for each study region: (a) MSD, (b) LD, and (c) LTD. Filled circles = control plants (natural herbivory), grey triangles = 50% defoliation plants, and open diamonds = 100% defoliation plants. Regression values and significance are shown (ns = not significant).

because it indicated that defoliation can reduce flower size in some cases, while in others it may cause an increase. Such differences in results may respond to the plant's ability to compensate for herbivore damage based on nutrient availability and tradeoffs between the male and female function (Wise and Cummins 2006). Such limits to compensation may help explain the observed pattern for male and female flower size of *C. aconitifolius* because the degree of investment in one function or the other may depend on water and nutrient availability (Parra-Tabla et al. 2004). Moreover, a previous study with this species showed that its capacity to increase the proportion of female flowers in response to defoliation was consistent across regions but variable in magnitude (Arceo-Gómez et al. 2009). Such evidence suggests a tradeoff between male and female flower production for this species which is mediated by nutrient availability and folivory.

However, based on the studies published so far, it is still unclear for monoecious species if defoliation affects male and female functions in the same way, or if tradeoffs occur between these two fitness components. In *C. aconitifolius*, as well as in other monoecious species, further studies are needed which experimentally control resource availability, as well as perform reciprocal transplant experiments to understand the ecological and genetic implications of such tradeoffs. Future research should also consider long-term measurements in order to characterize changes in sexual expression across time, as well as identify the factors which underlie such variation (Cobb et al. 2002, Lázaro and Méndez 2007).

Spatial variation in effects linking defoliation, floral traits and pollination success

The indirect effect of herbivory on pollination success in *C. aconitifolius* varied across regions, as well as between male and female flowers. Male reproductive success was affected by defoliation, as leaf removal resulted in an overall decrease in pollen production, and/or in the proportion of pollen removed per flower. However, because the overall proportion of pollen removed was always high perhaps the impact of defoliation on male success may be quantitatively unimportant. Despite this, some evidence suggests that indirect effects of herbivory on male success in *C. aconitifolius* may be significant in some cases. For example, results showed that the number of pollen tubes in female flowers was generally low (5–30 tubes per flower), suggesting that pollen loads on stigmas were limited (low male success), which would then translate into a lower number of fertilized ovules. Thus, even small decreases in pollen removal may be of importance because an important relationship often exists between the number of pollen grains deposited on the stigma and ovule fertilization (Flanagan et al. 2009). On the other hand, the number of pollen tubes (female success) in *C. aconitifolius* flowers varied across regions, and results suggest that defoliation had a negative effect on this variable in some cases (e.g. at LTD), while in others it was positive (at MSD).

The importance of indirect effects of defoliation on plant–pollinator dynamics depends on herbivores producing significant changes on plant traits which serve for pollinator attraction; such trait changes may not only influence pollinator visitation rates, but also pollination efficiency. Even small changes in floral traits may have potentially large impacts on pollinator behaviour and thus plant reproductive success (Mothershead and Marquis 2000). The observed differences in pollination success across regions and treatments for *C. aconitifolius* may be explained at least partially by changes in floral traits, which in turn impact pollinator visit rates and behavior. It has been shown that pollinators visit damaged plants less frequently, and when they do, visit duration is often shorter (Lehtilä and Strauss 1996, Mothershead and Marquis 2000). However, these responses may change depending on pollinator species as each group uses different attraction signals (Strauss et al. 1996, Lehtilä and Strauss 1997, Young et al. 2007).

A common indirect effect of herbivory on pollination success can occur through a reduction in flower production because floral display size is one of the most important signals which plants use to attract pollinators (Strauss and Irwin 2004). However, a recent study by Arceo-Gómez et al. (2009) with *C. aconitifolius* that was conducted at the same study sites used here, showed that a decrease in flower number and changes in the female-to-male flower ratio resulting from defoliation did not affect pollinator visit rates at any region, suggesting that defoliation does not have an indirect effect on pollination success through these variables. However, results presented here suggest that changes in pollination success may have been indirectly mediated by the effect of defoliation on floral traits (i.e. flower size), although this effect varied between regions and flower type (male or female). For instance, our results showed that male reproductive success decreased as a result of artificial defoliation, and that for all three regions a decrease in corolla tube length and an increase in corolla area will cause a reduction in the proportion of pollen removed. In contrast, female pollination success was less clearly related to changes in flower size.

Flowers of *C. aconitifolius* are visited by at least 40 species of pollinators, of which the most common are bees and butterflies (Arango et al. 2000, Arceo-Gómez et al. 2009). Plants with specialized pollination systems often exhibit a clear relationship between floral morphology and efficiency of pollen removal and deposition (Johnson and Steiner 1997, Galen and Cuba 2001), as well as changes in floral traits due to herbivory and the indirect impact this has on seed production (Mothershead and Marquis 2000). In contrast, for species with a generalist pollination system such as *C. aconitifolius*, which are pollinated by many species with different foraging behaviours, it is difficult to identify specific changes in pollinator attraction signals due to herbivory, as well as the consequences these changes have on pollinator behaviour, and consequently on plant pollination success. Despite this, plants with generalist pollination systems have been shown to be affected not only by variation in pollinator efficiencies (Herrera 1986, Gómez et al. 2007), but also be subject to selection on specific floral traits (Gómez et al. 2009).

For *C. aconitifolius*, the observed inconsistency in the relationship between pollination success and male and female flower size may have been due to differences in pollinator preferences (e.g. between floral types), behavior and efficiency. For example, field observations have shown that bees visit *C. aconitifolius* male flowers more frequently to obtain pollen, while butterflies do not show any preference (Rodriguez 2004). In addition, many studies have shown a significant relationship between floral traits and pollen removal by bees, but not with measures of female pollination success (Young and Stanton 1990, Thomson et al. 2004).

Together, these results suggest that male and female pollination success in *C. aconitifolius* may not depend so much on flower number or pollinator abundance, but on the type of floral visitor and its behavior as suggested in previous studies (Gómez et al. 2007, Young et al. 2007). This condition may be particularly relevant in monoecious species for which investment on each sexual function is different and pollinator rewards change depending on the type of flower. In the case of *C. aconitifolius* it is clear that the direct effect of defoliation on flower size can indirectly influence male reproductive success through its interaction with different bee species. In contrast, female pollination success appears not to be related to folivory, agreeing with what has been found previously for other monoecious species (Thomson et al. 2004, Hladun and Adler 2009). Finally, it is important to keep in mind that defoliation may also cause changes in other floral traits such as nectar and scent production, which may directly affect pollinator behavior and thus plant pollination success.

Although our results showed that defoliation in *C. aconitifolius* may cause significant changes in floral traits which might in turn indirectly affect plant-pollinator interactions, and that such effects vary across space, further work is needed in order to have a rigorous quantitative assessment of pollinator diversity across regions, as well as to understand the relationship between floral traits and pollination efficiencies of contrasting pollinators' guilds (e.g. bees vs butterflies). In this sense an interesting note worth mentioning is that our results suggested a close link between flower traits and pollination success in *C. aconitifolius* control plants, because a significant relationship existed between floral traits and male success at two regions, and female success at one region. These observations suggest that natural variation (within and between populations) in flower traits is related to pollination efficiency and that these relationships could be potentially affected by herbivory.

Finally, one aspect which deserves to be addressed is that of temporal variation in indirect and direct effects of herbivores and pollinators mediated *C. aconitifolius*. A growing number of studies have shown significant temporal changes in the strength and sign of multiple interactions (Rey et al. 2006) and this underlines the need for studies which look at interaction effects across several years. By considering both a multiple species approach, as well as spatial and temporal variation in interaction outcomes it will be possible to fully understand the evolutionary implications of such interactions.

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References

Abdala-Roberts, L. and Parra-Tabla, V. 2005. Artificial defoliation induces trichome production in the tropical shrub *Cnidoscolus aconitifolius* (Euphorbiaceae). – Biotropica 37: 251–257.

Arango, A. M. et al. 2000. Population structure, seasonality, and habitat use by the green lynx spider *Peucetia viridans* (Oxyopidae) inhabiting *Cnidoscolus aconitifolius* (Euphorbiaceae). – J. Arachnol. 28: 185–194.

Arceo-Gómez, G. et al. 2009. Changes in sexual expression as a result of defoliation and environment in a monoecious shrub in México: implications for pollination. – Biotropica 4: 435–441.

Benkman, C. G. 1999. The selection mosaic and diversifying coevolution between crossbills and lodgepole pine. – Am. Nat. 153 Suppl.: S75–S91.

Cariveau, D. et al. 2004. Direct and indirect effects of pollinators and seed predators to selection on plant and floral traits. – Oikos 104: 15–26.

Chico-Ponce de León, P. A. et al. 1999. Atlas de procesos territoriales de Yucatán. – Univ. Autónoma de Yucatán, Mérida.

Cobb, N. S. et al. 2002. Long-term sexual allocation in herbivore resistant and susceptible pinyon pine (*Pinus edulis*). – Oecologia 130: 78–87.

Dillon, P. M. et al. 1983. Disarming the "Evil Woman": petiole constriction by a sphingid larva circumvents mechanical defenses of its host plant, *Cnidosculus urens* (Euphorbiaceae). – Biotropica 15: 112–116.

Flanagan, R. J. et al. 2009. Interspecific pollinator movements reduce pollen deposition and seed production in *Mimulus ringens* (Phrymaceae). – Am. J. Bot. 96: 809–815.

Galen, C. and Cuba, J. 2001. Down the tube: pollinators, predators and the evolution of flower shape in the alpine skypilot, *Polemonium viscosum*. – Evolution 55: 1963–1971.

Gómez, J. M. 2005. Non-additive effects of herbivores and pollinators on *Erysimum mediohispanicum* (Cruciferae) fitness. – Oecologia 143: 412–418.

Gómez, J. M. et al. 2007. Pollinator diversity affects plant reproduction and recruitment: the tradeoffs of generalization. – Oecologia 153: 597–605.

Gómez, J. M. et al. 2009. A geographic selection mosaic in a generalized plant–pollinator–herbivore system. – Ecol. Monogr. 79: 245–263.

Herrera, C. M. 1986. Components of pollinator quality: comparative analysis of a diverse insect assemblage. – Oikos 50: 79–90.

Herrera, C. M. 2000. Measuring the effects of pollinators and herbivores: evidence for non-additivity in a perennial herb. – Ecology 81: 2170–2176.

Herrera, C. M. et al. 2002. Interaction of pollinators and herbivores on plant fitness suggests a pathway for correlated evolution of mutualism- and antagonism-related traits. – Proc. Natl Acad. Sci. USA 99: 16823–16828.

Hladun, K. R. and Adler, L. S. 2009. Influence of leaf herbivory, root herbivory, and pollination on plant performance in *Cucurbita moschata*. – Ecol. Entomol. 34: 144–152.

Irwin, R. E. 2006. The consequences of direct versus indirect species interactions to selection on traits: pollination and nectar robbing in *Ipomopsis aggregata*. – Am. Nat. 167: 315–328.

Irwin, R. E. et al. 2004. The dual role of floral traits: pollinator attraction and plant defense. – Ecology 85: 1503–1511.

Johnson, S. D. and Steiner, K. E. 1997. Long-tongued fly pollination and evolution of floral spur length in the *Disa draconis* complex (Orchidaceae). – Evolution 51: 45–53.

Lázaro, A. and Méndez, M. 2007. Variation in the sexual expression in the monoecious shrub *Baxus balearica* at different scales. – Plant Biol. 9: 736–744.

Lehtilä, K. and Strauss, S. Y. 1997. Leaf damage by herbivores affects attractiveness to pollinators in wild radish, *Raphanus raphanistrum*. – Oecologia 111: 396–403.

Littell, R. C. et al. 1996. SAS system for mixed models. – SAS Inst.

Marquis, R. 1992. The selective impact of herbivores. – In: Fritz, S. R. and Simms, E. L. (eds), Plant resistance to herbivores and pathogens, ecology, evolution and genetics. Univ. Chicago Press. pp. 301–325.

Mothershead, K. and Marquis, R. J. 2000. Fitness impacts of herbivory through indirect effects on plant–pollinator interactions in *Oenothera macrocarpa*. – Ecology 81: 30–40.

Parra-Tabla, V. et al. 2004. Effect of defoliation on leaf growth, sexual expression and reproductive success of *Cnidoscolus aconitifolius* (Euphorbiaceae). – Plant Ecol. 173: 153–160.

Pohl, N. et al. 2006. Nonadditive effects of flower damage and hummingbird pollination on the fecundity of *Mimulus luteus*. – Oecologia 149: 648–655.

Quesada, M. et al. 1995. Leaf damage decreases pollen production and hinders pollen performance in *Cucurbita texana*. – Ecology 76: 437–443.

Rey, P. J. et al. 2006. The geographic mosaic in pre-dispersal interactions and selection on *Helleborus foetidus* (Ranunculaceae). – J. Evol. Biol. 19: 21–34.

Rico-Gray V. and Oliveria, P. S. 2007. The ecology and evolution of ant–plant interactions. – Univ. of Chicago Press.

Rodríguez, R. F. P. 2004. Biología floral y diversidad genética en dos poblaciones contrastantes de *Cnidoscolus aconitifolius* (Mill) I. M. John (Euphorbiaceae). Bs thesis Licenciatura en Biología. – Univ. Autónoma de Yucatán, Mérida, Yucatán, México.

Standley, P. C. and Steyemark, J. A. 1949. Flora of Guatemala. Vol. 24, part VI. – Univ. of Chicago Press.

Strauss, S. Y. and Irwin, R. E. 2004. Ecological and evolutionary consequences of multispecies plant–animal interactions. – Annu. Rev. Ecol. Syst. 35: 435–466.

Strauss, S. Y. et al. 1996. Foliar herbivory affects floral characters and plant attractiveness to pollinators: implications for male and female plant fitness. – Am. Nat. 147: 1098–1107.

Thomson, V. P. et al. 2004. Herbivory differentially affects male and female reproductive traits of *Cucumis sativus*. – Plant Biol. 6: 621–628.

Thompson, J. N. 1994. The coevolutionary process. – Univ. of Chicago Press.

Thompson, J. N. and Fernandez, C. C. 2006. Temporal dynamics of antagonism and mutualism in a geographically variable plant–insect interaction. – Ecology 87: 103–112.

Travis, J. 1996. The significance of geographical variation in species interactions. – Am. Nat. 148 Suppl.: S1–S8.

Verbeke, G. and G. Molenberghs. 2000. Linear mixed models for longitudinal data. – Springer.

Wise, M. J. and Cummins, J. 2006. Strategies of *Solanum carolinense* for regulating maternal investment in response to foliar and floral herbivory. – J. Ecol. 4: 629–636.

Young, J. H. and Stanton, M. L. 1990. Influence of floral variation on pollen removal and seed production in wild radish. – Ecology 71: 536–547.

Young, J. H. et al. 2007. Foraging behavior affects pollen removal and deposition in *Impatiens capensis* (Balsaminaceae). – Am. J. Bot. 94: 1267–1271.

Zangerl, A. R. and Berenbaum, M. R. 2003. Phenotype matching in wild parsnip and parsnip webworms: causes and consequences. – Evolution 57: 806–815.