Noncorrelated evolution between herbivore- and pollinator-linked features in *Aristolochia chilensis* (Aristolochiaceae)

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The effect of mutualists (i.e. pollinators) and antagonists (i.e. herbivores) can have non-additive effects on plant fitness. This is often interpreted as evidence for correlated evolution on a suite of traits leading to an increase and decrease of the interaction of plants with mutualists and antagonists, respectively. This situation has been found to prevail in plants that have large floral and fruit displays but are not limited by pollinators for seed set. We suggest the alternative hypothesis, where plants limited by pollinators for seed set (e.g. deceit-pollinated plants) exhibit additive effects of pollinators and herbivores on fitness (i.e. noncorrelated evolution). Using a 2 × 2 factorial design, we tested this hypothesis by solely and simultaneously evaluating the effects of pollinators and the single herbivore, *Battus polydamas archidamas*, on female reproductive success of *Aristolochia chilensis*. Plants exposed to herbivores presented 2.6-fold greater herbivory than plants that excluded them. In addition, plants exposed to pollinators showed strong limitation by pollinators for seed set compared with other plants of the genus *Aristolochia*. However, only pollinators had a significant effect on fruit and seed set because plants that excluded them did not set fruits or seeds. Furthermore, herbivores and pollinators exerted additive effects on fruit and seed production. Collectively, these results indicate that herbivore- and pollinator-linked traits in *A. chilensis* exhibit noncorrelated evolution. © 2007 The Linnean Society of London, Biological Journal of the Linnean Society, 2007, 91, 239–245.

ADDITIONAL KEYWORDS: additivity – correlated evolution – fitness.

INTRODUCTION

Plants may face numerous selective pressures elicited by different kinds of animals, mostly mutualists (i.e. pollinators) and antagonists (i.e. herbivores), which may influence their ecological and evolutionary responses (Zamora, 2000; Herrera & Pellmyr, 2002). However, empirical evaluations assessing the combined and isolated effects of these suites of animals are rather scarce (but see Herrera, 2000; Ehrlén, Kåck & Ågren, 2002; Herrera *et al*., 2002; Gómez, 2005). Non-additivity of effects on plant fitness triggered by mutualists and antagonists can be interpreted as evidence for correlated evolution on a suite of traits tending to increase and decrease the interaction of plants with their mutualists and antagonists, respectively (sensu Herrera, 2000; Herrera *et al*., 2002; Gómez, 2005). This is particularly important because correlational selection may be a mechanism for the functional integration of one trait with another, most likely promoting genetic integration (Lande, 1980; Brodie, 1992).

Herrera (2000) and Herrera *et al*., (2002) proposed a herbivore-dependent selection pressure led by pollinators based on the non-additive effects elicited by mutualists and antagonists on seed production and seedling recruitment in the perennial herbs *Paeonia broteroi* and *Helleborus foetidus*, respectively. The same authors claimed that their findings should be particularly frequent if herbivores prey on plants bearing large floral and fruit displays (i.e. plants also preferred by pollinators). Similar conclusions were arrived at by Gómez (2005) for *Erysimum*...
**Material and Methods**

**Natural History and Study Site**

The pipevine, *A. chilensis*, is a creeping perennial herb with a foul-smelling purplish blossom, which grows in semiarid regions of Chile from 27°S to 34°S, and from sea level to 1500 m a.s.l. It is a deceit-pollinated, protogynous plant bearing nonrewarding flowers that are visited by several insect species during the pistillate and stamine phases. Flowering and fruiting occur from August to December. Fruits are dehiscent nuts, 3–4 cm long, bearing black seeds, 4–5 mm long. Its leaves are 3–10 cm in diameter (Ruiz, 2001). Although no information is available with respect to flower visitors and pollinators, the leaves are almost exclusively eaten by *B. p. archidamas* caterpillars, even though it is possible to find other insects, such as aphids, albeit very infrequently (C. Valdivia, pers. observ.). Caterpillars develop exclusively on *A. chilensis* and then pupate in suspension from nearby branches or rocks (Peña & Ugarte, 1996). The distribution range of *B. p. archidamas* is approximately the same as that of *A. chilensis* (Peña & Ugarte, 1996).

Fieldwork was carried out from July to December 2004 at Cuesta Lo Prado in the coastal range of central Chile (33°28′S, 70°55′W), in a Mediterranean region characterized by dry summers and rainfall mainly in the autumn and winter (Hajek & Di Castri, 1975).

**Correlated Evolution Assessment**

To determine a possible pathway for correlated evolution on herbivore- and pollinator-linked traits, we conducted an experiment with a 2 × 2 factorial design following Herrera (2000) by excluding solely and simultaneously herbivores (*B. p. archidamas* caterpillars) and pollinators. In brief, this methodological approach, rather than modifying a specific set of traits, allowed for a statistical dissection of plant fitness following the experimental exclusions. Thus, herbivore exclusions mimicked an ancestral situation in which plants presented a suite of traits that allowed them to escape from herbivores (or show resistance to them), which in turn produced an increased fitness of plants. On the other hand, pollinator exclusions mimicked an ancestral situation whereby plants exhibited a suite of traits that reduced the optimal mutualistic relationship between plants and pollinators, which in turn led to a decreased fitness of plants. Therefore, this artificial array allows the dissection of the isolated effects of herbivores and pollinators, as well as the combined actions of both. Furthermore, it allows for the detection of non-additive effects of herbivores and pollinators, which is the obligate condition for the occurrence of correlated evolution on traits related to them (for methodological details, see Herrera, 2000; Herrera et al., 2002).

Given that *B. p. archidamas* has a wing-span of 7.5–10.5 cm (Peña & Ugarte, 1996), herbivores were excluded by enclosing plants with wire-net cages, 0.3 m high (~1 cm mesh) anchored in the ground and covering a surface of 1.5 × 1.5 m, thus ensuring that no butterfly would oviposit on the plants. Plants in the population studied were not infested by caterpillars at the beginning of the experiment; furthermore, caterpillars were never found in plants that were excluded from butterfly visits. Given that all floral visitors presented body sizes below 1 cm, it is likely that no pollinator was excluded from plants excluded to herbivores. On the other hand, because plants produced flowers which opened sequentially, they were individually enclosed each month at the bud stage with a tulle-mesh bag for preventing pollinator access, albeit allowing access of butterflies to leaves. Both treatment levels were factorially combined to conform the following four combinations: (1) control plants (i.e. exposed to herbivores and pollinators); (2) herbivore treatment (i.e. only exposed to herbivores); (3) polli-
nator treatment (i.e. only exposed to herbivores); and (4) simultaneous herbivore and pollinator treatment (i.e. excluded to both herbivores and pollinators).

For each treatment, 15 plants were selected and monitored from July to December 2004. In these plants, herbivory was monthly recorded by using the herbivory index (HI) developed by Dirzo & Domínguez (1995). One-hundred randomly chosen leaves per plant were checked monthly by registering the degree of foliar surface disappearance triggered both by herbivores and by damage due to senescence. A distinction between these two factors could not be obtained. Each leaf was assigned to one of the following six categories (0, 1, 2, 3, 4, or 5) depending on whether 0%, 1–6%, 7–12%, 13–25%, 26–50%, or 51–100%, respectively, of foliar surface was absent. The HI per plant was determined by the equation:

$$HI = \frac{\sum_{i=0}^{5} x_i n_i}{N}$$

where $n$ is the frequency of observations, $x_i$ is the damage category, and $N$ is the total number of observations. A single per-plant estimate of HI was monthly obtained and assessed through a repeated measures analysis of variance (ANOVA).

When the reproductive season was over, fruits with their respective seeds were collected to determine the reproductive success of plants after experiencing the four treatments. Because fruit and seed production did not meet the normality assumption even after applying appropriate transformations (analyses not shown but assessed through a Kolmogorov–Smirnov test), correlational selection was evaluated by randomizing the original values 10 000 times (Manly, 1997; Herrera, 2000). Furthermore, to estimate the power ($1-\beta$) of the ANOVA used to detect correlational selection, a power test for the interaction effect was applied *sensu* Kirk (1982).

**RESULTS**

**HERBIVORY AND POLLINATION**

Overall, only herbivores had significant effects on foliar removal of *A. chilensis* throughout the time assessed (Table 1). At the end of the reproductive season, plants with pollinators excluded presented 2.6-fold more foliar surface removal in the presence of herbivores than in their absence ($P < 0.001$). Similarly, plants with pollinators included presented 2.7-fold more foliar surface removal in the presence of herbivores than in their absence ($P < 0.001$). The highest herbivore abundance, in terms of the number of larvae per plant, occurred in November (Fig. 1A). However, plants presented the highest foliar surface removal in December, most likely due to the senescence of leaves (Fig. 1B).

Flowers of *A. chilensis* were visited by five orders of insects as well as by some species of acari. Sexually mature flowers presented more than 90% of all floral visitors, with the main floral visitors being coleopterans, which were mainly found at the staminate stage of flowers (Table 2). The only floral visitor observed carrying pollen from *A. chilensis* was the Asilidae *Tillobroma* sp., which represents all dipterans found inside the flowers.

| Table 1. Summary of repeated measures analysis of variance testing the effects of herbivores and pollinators on foliar removal of *Aristolochia chilensis* |
|---|---|---|
| **Source** | **d.f.** | **MS** | **F** |
| Within-subjects | | | |
| Herbivores | 1 | 57.631 | 61.447* |
| Pollinators | 1 | 0.006 | 0.007 |
| Herbivores × Pollinators | 1 | 0.004 | 0.004 |
| Error | 56 | 52.521 | |
| Between-subjects | | | |
| Time | 3 | 98.108 | 161.886* |
| Time × Herbivores | 3 | 24.093 | 39.750* |
| Time × Pollinators | 3 | 0.111 | 0.184 |
| Time × Herbivores × Pollinators | 3 | 0.013 | 0.022 |
| Error | 168 | 33.942 | |

Pollinators were maintained in the analyses to discard the eventual effects of pollinator exclusion on the feeding behaviour of *Battus polydamas archidamas*, and hence on foliar surface removal.

*P < 0.001.

**d.f.**, degrees of freedom.
From the herbivore perspective, no significant difference in seed and fruit set was observed when pollinators were either absent or present in plants exposed to herbivores (Tukey’s HSD test: \( P = 0.80 \) for fruit set; \( P = 0.83 \) for seed set; Fig. 2). Similarly, when herbivores were excluded, seed and fruit set were not reduced by the absence of pollinators (\( P = 0.12 \) for fruit set; \( P = 0.20 \) for seed set; Fig. 2).
**DISCUSSION**

According to Herrera *et al*. (2002) and Gómez (2005), non-additivity in the effect of pollinators and herbivores is expected to be frequent in systems where herbivores cause copious flower and fruit losses due in part to large floral and fruit crops attracting more herbivores. However, although our results showed that *B. p. archidamas* herbivory had a significant effect on foliar removal on *A. chilensis*, there was no effect on fruit and seed production. This result is surprising because previous evidence highlights the importance of herbivores of the *Battus* genus in modulating the reproductive success of *Aristolochia* plants. Rausher & Feeny (1980) reported a negative effect of *Battus philen- nor* on the reproductive success of *Aristolochia reticulata* regardless of whether it grows at low or high density. This negative effect was even more intense when interspecific competition for limiting resources occurred (Fowler & Rausher, 1985).

Although herbivores did not affect the fitness of *A. chilensis*, pollinators did have a significant effect on reproductive success by increasing seed set. This result is in agreement with other species of the *Aristolochia* genus, which have been shown to be pollinator-limited for seed set (Sakai, 2002). Furthermore, the number of floral visitors was very low in *A. chilensis* compared with the tropical plant *Aristolochia grandiflora* because the latter presented as many as hundreds of insects inside the flowers at different stages of maturity (Burgess *et al.*, 2004). In *A. grandiflora*, 445 insects excluding larvae per flower were found at the pistillate stage (Burgess *et al.*, 2004), in contrast with 0.4 insects per flower found at the same stage of *A. chilensis*. Certainly, the very low number of floral visitors to *A. chilensis*, particularly of the Asillidae *Tillobroma* sp., which was the only insect observed carrying pollen, accounted for the very low amounts of fruits and seeds produced by plants exposed to pollinators.

Perhaps, the most revealing result of our study was that *A. chilensis* showed an additive effect of herbivores and pollinators on seed production, indicating that the selection pressures exerted by herbivores and pollinators are not correlated. Although this result might be considered a statistical artefact due to the low power of the interaction in the ANOVA, it is worthwhile highlighting that we worked with nearly all plants growing in the study site, and therefore a lack of statistical significance can be attributable to weak selection rather than an insufficient sample size (for a general example of natural selection, and sample and population sizes, see Medel, Botto-Mahan & Kalin-Arroyo, 2003). Consequently, it may be confidently proposed that plant features linked to herbivores and pollinators were not selected correlatedly by both types of plant animal-interactors.

The additive effect of herbivores and pollinators on seed production is contrary to other reports showing that pollinators and herbivores usually exert their selective pressures in a more complex ecological scenario, where herbivores may modulate or even cancel the effects of pollinators (Herrera, 2000; Ehrln *et al*., 2002; Herrera *et al*., 2002; Gómez, 2005; Valdivia & Niemeyer, 2005). The results of the present study may be explained by the fact that plants producing very few seeds, as a result of a decreased pollination service, may reinvest resources from vegetative tissues towards effectively pollinated flowers to insure reproductive success (Zimmerman & Pyke, 1988). Because deceit-pollinated plants such as *A. chilensis* are

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**Table 3. Summary of analysis of variance testing the effects of herbivores and pollinators on fruits and seeds per plant on *Aristolochia chilensis***

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fruits per plant:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herbivores</td>
<td>1</td>
<td>0.150</td>
<td>0.926</td>
</tr>
<tr>
<td>Pollinators</td>
<td>1</td>
<td>0.817</td>
<td>5.044*</td>
</tr>
<tr>
<td>Herbivores × Pollinators</td>
<td>1</td>
<td>0.150</td>
<td>0.926</td>
</tr>
<tr>
<td>Error</td>
<td>56</td>
<td>0.162</td>
<td></td>
</tr>
<tr>
<td><strong>Seeds per plant:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herbivores</td>
<td>1</td>
<td>756.2</td>
<td>0.657</td>
</tr>
<tr>
<td>Pollinators</td>
<td>1</td>
<td>4628.8</td>
<td>4.023*</td>
</tr>
<tr>
<td>Herbivores × Pollinators</td>
<td>1</td>
<td>756.2</td>
<td>0.657</td>
</tr>
<tr>
<td>Error</td>
<td>56</td>
<td>1150.5</td>
<td></td>
</tr>
</tbody>
</table>

Analyses were performed by randomizing the original data set 10,000 times.

*P < 0.05.

d.f., degrees of freedom.
restricted for seed production by pollinator activity rather than by environmental resources (Primack & Stacy, 1998; Johnson et al., 2003), there may be very few physiological restrictions that impair reallocations of resources within plants under herbivory.

The present study indicates that plants limited by pollinators for seed production may face the additive effects of mutualists and antagonists on reproductive success. This finding is in accordance with studies by Herrera et al. (2002) and Gómez (2005) who predicted the generality of non-additive effects (i.e. correlated evolution) exerted by different suites of animals on plants having large flower and fruit displays, but under high pollinator presence. Therefore, it is worthwhile noting that the evolution of herbivore- and pollinator-linked traits exhibited by A. chilensis may be representative of other plants exhibiting a high pollinator-limitation for seed set, even though they may be under selection by herbivores.

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