

MEASURING THE EFFECTS OF POLLINATORS AND HERBIVORES: EVIDENCE FOR NON-ADDITIVITY IN A PERENNIAL HERB

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Abstract. Using an experimental approach, this study addresses the following two questions for the perennial herb *Paeonia broteroi* (Paeoniaceae) at a location in southeastern Spain. (1) What are the relative magnitudes of the effects of pollinators and herbivores (invertebrates and vertebrates feeding on flowers and developing fruits) on maternal fecundity (total seed production per plant)? (2) Are the effects of pollinators and herbivores on fecundity additive, or is there some significant interaction between them? A two-factor (“Pollinators” and “Herbivores,” each with two levels, “present” and “excluded”) experimental design was used, with individual plants being treated as experimental units and maternal fecundity as the response variable. On average, the effects of pollinators and herbivores were of opposite sign and roughly similar absolute magnitude (~7.5 seeds per plant), thus approximately canceling each other. A significant interaction (non-additivity) between factors did exist, with plants exposed to pollinators experiencing a disproportionately higher incidence of herbivores than those from which pollinators had been excluded. This was mainly due to mammals browsing only on the larger fruits from flowers that had been exposed to pollinators. Only in the absence of herbivores did pollinators account for a significant amount of between-plant variance in maternal fecundity. It is concluded that the interaction between pollinators and herbivores will lead to variable, herbivore-dependent “opportunity for selection” (sensu Arnold and Wade 1984) on *P. broteroi* by its pollinators. Regional variation in herbivore incidence experienced by *P. broteroi* populations will presumably generate local variation in the degree of adaptedness of the plant to its pollinators.

Key words: fruit selection; herbivory; indirect interactions; mammalian herbivores; maternal fecundity; Mediterranean; non-additive effects; *Paeonia broteroi*; perennial herbs; pollination; seed production.

INTRODUCTION

Possibly because of their inherent biological complexities, plant–animal interactions traditionally have been studied after exercising some sort of conceptual abstraction based on functionality. With relatively few exceptions, the research program on plant–animal interactions has generally produced “single-interaction” studies focusing on just one kind of interaction (i.e., herbivory, pollination, or seed predation studies, some of the most commonly studied interaction categories). This compartmentalization, albeit convenient, leads to an artificial oversimplification, as most plant populations and individuals presumably “perceive,” over ecological and evolutionary time, the composite result of their interactions with a broad array of animal interactors. Whenever efforts at realism and completeness have been undertaken, evidence has been gathered showing that different sets of plant interactors (e.g., herbivores, pollinators, seed predators, seed dispersers) combine to exert an intricate influence on plants (e.g., Heithaus et al. 1982, Schemske and Horvitz 1988, Herrera 1989, 1993, Cunningham 1995). Considering the most popular cases of herbivores and pollinators, a

number of recent studies have illustrated that interaction between herbivory and pollination is not confined to the relatively infrequent case in which the same organisms play a dual role as both herbivores and pollinators (e.g., Wiebes 1979, Pellmyr 1992, Pettersson 1992, Powell 1992), and have documented the complex influence frequently exerted on plants by the combined action of these two sets of functionally contrasting interactors. These investigations have shown, for example, that herbivores have the capacity to indirectly modulate the nature, strength, and consequences of the interaction between plants and their animal pollinators (Karban and Strauss 1993, Quesada et al. 1995, Lohman et al. 1996, Mutikainen and Delph 1996, Strauss et al. 1996, Lehtilä and Strauss 1997, Strauss 1997). It has also been shown that plant reproductive traits may sometimes reflect a compromise between the conflicting selective pressures exerted by pollinators and herbivores (Brody 1992, Euler and Baldwin 1996, Brody and Mitchell 1997, Ehrlén 1997, Strauss 1997, Kudoh and Whigham 1998) and that the concurrent interaction of plants with their herbivores and pollinators may help to explain the macroevolutionary patterns of defense and reward systems in some plant lineages (Armbruster 1997, Armbruster et al. 1997).

The present paper is an attempt at contributing to this recent line of inquiry, which focuses on the im-

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plications of the interaction between herbivores and pollinators. Using an experimental approach, I will evaluate the relative importance of pollinators and herbivores as determinants of maternal fecundity in the perennial herb *Paeonia broteroi*. As a way of illustrating some of the perils involved in studying plant–animal interactions as dissociated functional categories, particular attention will be paid to the issue of possible non-additive effects of these two particular sets of interactors. Specifically, I address two questions. (1) What are the respective magnitudes of the effects of pollinators and herbivores on the maternal fecundity of *Paeonia broteroi* individuals? (2) Do the effects of pollinators and herbivores on fecundity merely add up, or, alternatively, is there some significant interaction (non-additivity) between them?

METHODS

Plant natural history

Paeonia broteroi Boiss. & Reut. (Paeoniaceae) is a perennial herb frequently found in the understory of mid-elevation to montane oak forests in the Iberian Peninsula (Muñoz Garmendia and Navarro 1993). The leafy stems, bearing terminal preformed flower buds, sprout from the underground tuberous roots in early spring. Plants consist of one or a few leafy ramets, each of which produces a maximum of one flower. In the study population and year (see *Study site and field methods*), most plants consisted of single ramets and produced single flowers (1.8 ± 1.2 flowers per plant, mean \pm 1 SD; range = 1–7 flowers; $N = 121$ plants). The large (8–12 cm across), showy flowers are actinomorphic, with a bowl-shaped corolla made up of 5–8 pinkish-red petals. Pollination is accomplished by insects, mainly by solitary bees of the families Andrenidae and Halictidae, and several species of small beetles of the families Malachiidae, Oedemeridae, and Scarabaeidae (Sánchez-Lafuente et al. 1999). Flowers are self-compatible, but seed production is very low in the absence of pollinators. Among experimentally self-pollinated flowers, fruit set is lower and seeds are smaller than among cross-pollinated flowers (Sánchez-Lafuente et al. 1999). Flowers are apocarpous, bearing one or a few independent carpels (2.1 ± 0.9 carpels per flower, mean \pm 1 SD; range = 1–5; $N = 89$ flowers). Carpels develop as independent follicles from late spring to early autumn, and dehisce in September–October. Each follicle bears an average of 5.4 ± 4.6 arillate seeds ($N = 94$ follicles), with a range in fresh mass of 50–200 mg. In my study population and elsewhere, flower buds, open flowers, and developing fruits and seeds are quite often partially or totally consumed by both invertebrate (larvae of several lepidopteran species in the families Noctuidae and Geometridae, and adult orthopterans) and vertebrate (mainly deer) herbivores (Sánchez-Lafuente et al. 1999; C. M. Herrera, *personal observation*). The two groups of herbivores

leave characteristic signs after feeding on flowers and developing fruits. Deer remove the whole flower or the full complement of follicles plus the receptacle, leaving only the cut pedicel. Invertebrates, in contrast, generally fed on only a fraction of the follicles or enclosed seeds, and never completely remove the follicle walls or the receptacle. These differences were used in this study to assess the relative importance of these two major groups of herbivores. It must be noted, however, that my estimates of invertebrate and vertebrate fruit predation rates are only approximate. Unless vertebrates were to systematically reject invertebrate-damaged fruits, their consumption of whole fruits would remove any evidence of prior partial consumption by invertebrates.

Study site and field methods

This study was carried out during April–September 1980 with a large population of *P. broteroi* located near the Casa Forestal de Roblehondo, at 1330 m elevation, in the Sierra de Cazorla, Jaén province, southeastern Spain (this is the “Roblehondo” population of Sánchez-Lafuente et al. 1999). Plants grow in and around a large clearing in a dense mixed forest dominated by *Pinus nigra* and *Quercus faginea*.

The effects of pollinators and herbivores on the maternal fecundity of individual plants (assessed using total seed production per plant) were simultaneously studied by means of a factorial experiment. Individual plants, identified as either single, isolated ramets or groups of ramets that emerged from a common rootstock, were used as experimental units. During the flowering period, all flowers on each experimental plant were either exposed to natural pollinators or enclosed inside mesh bags that precluded insect pollinator access (“Pollinators” factor). The combined effect of invertebrate and vertebrate herbivores was evaluated by protecting flowers and developing fruits with mesh bags (similar to those used in the pollination treatment) on some plants, while leaving them unprotected in others (“Herbivores” factor). These two two-level treatments were combined factorially, leading to the following four combinations.

1) Plants with both herbivores and pollinators excluded from both flowers and developing fruits. These individuals had mesh bags placed on flower buds just before flower opening. Flowers opened inside the bags, and any resulting fruits developed within the enclosures, thus being also protected from herbivores.

2) Plants whose developing fruits were exposed to herbivores, but that previously had insect pollinators excluded from flowers. In these plants, flowers opened inside mesh bags that were removed immediately after petals were shed, so that fruits were exposed to the action of herbivores during their whole development period.

3) Plants with flowers exposed to pollinators, but with herbivores subsequently excluded. In these plants,

mesh bags were placed on withered flowers immediately after petals were shed, so developing fruits were protected from the action of herbivores.

4) Control plants, whose flowers and developing fruits were exposed to the action of both pollinators and herbivores.

Most plants of the population were marked at the beginning of the study and randomly assigned to each of the four treatment combinations. Some of them had to be excluded from the study because they were trampled by large mammals or had their mesh bags broken or bitten off, which led to some differences in sample size between treatments. Data from a total of 221 flowers and 121 plants were ultimately included in the study. For all these, nearly ripe "fruits" (pedicel + receptacle + the set of expanded follicles from the same flower) were collected in late September and brought to the laboratory. Signs of herbivory by invertebrates or vertebrates were noted for each fruit. Follicles were dissected individually, and the number of undamaged, apparently viable seeds ("sound seeds," hereafter) was determined. The total number of sound seeds eventually produced by each plant (NSEEDS hereafter) was obtained by summing these figures for all follicles from the same plant. This latter variable was used as the response variable in all analyses.

Statistical analyses

I assessed the absolute magnitude and statistical significance of the effects of herbivores and pollinators on seed production by individual plants, as well as the significance of their interaction, by fitting the following linear model to the data:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk} \quad (1)$$

where y_{ijk} equals the k th observed total sound seed production for the (i, j)th cell, μ denotes the experimental population grand mean, α_i stands for the effect of the i th level of the Pollinators treatment, β_j is the effect of the j th level of the Herbivores treatment, $(\alpha\beta)_{ij}$ is the interaction effect of the i th level of Pollinators and the j th level of Herbivores, and ε_{ijk} is the random error associated with individual observations. Model 1 was fit using the SAS procedure MIXED and restricted maximum likelihood estimation (SAS Institute 1996). When fitting the model, I included the total number of carpels initially produced by the plant (summed over all its flowers) as a covariate to account for differences between plants in their inherent potential for seed production. Inclusion in the model of the two interaction terms between the covariate and Pollinators and Herbivores did not qualitatively change the results, so they were left out of the models presented here.

NSEEDS exhibited an extremely right-skewed distribution, with the vast majority of plants in the sample eventually producing none (67.8% of plants) or only 1–10 (19.0%) sound seeds, and the distribution could

TABLE 1. Summary of the ANOVA testing for the effects of pollinators (present or excluded) and herbivores (present or excluded) on the total number of sound seeds produced by whole plants of *Paeonia broteroi*.

Source	df	F	P†
Number of carpels	1, 116	50.02	<0.0001
Pollinators (P)	1, 116	4.90	0.045
Herbivores (H)	1, 116	5.36	0.042
P × H	1, 116	7.50	0.024

Notes: The total number of carpels per plant was included as a covariate to account for differences between plants in seed production potential. The model was fit using SAS procedure MIXED and restricted maximum likelihood estimation. Overall fit of the model (residual log-likelihood = -473.47) was highly significant ($P < 0.0001$; determined by randomization with 10 000 repetitions).

†Significance values of individual effects were determined using a randomization procedure and 10 000 repetitions (see *Methods, Statistical analyses*, for justification and further details).

not be normalized by logarithmic transformation. Failure of the response variable to meet the normality requirement presumably has little effect on the comparative magnitudes of treatment effect estimates, but might substantially affect the reliability of parametric statistical significance tests based either on F statistics or on asymptotic estimates of standard errors of effect sizes and model-adjusted cell means. To overcome this problem, the statistical significance of the two main effects (Pollinators and Herbivores) and their interaction was assessed using randomization tests (randomizing the response variable while holding fixed the predictor variables and the covariate). A similar method was used to assess the statistical significance of "simple main effects" (sensu Pedhazur 1982), or "interaction slices" (sensu SAS Institute 1996; SLICE option in the LSMEANS statement of the MIXED procedure). In factorial designs with significant interaction effects, "simple main effects" refer to the (different) effects of a given factor at different treatment levels of the other factor(s) (Pedhazur 1982: 362–365). Approximate standard errors for effect sizes and model-adjusted cell means were obtained by bootstrapping. I wrote all routines for randomization and bootstrapping using SAS Macro Processing Language (SAS Institute 1990) and algorithms in Manly (1991) or Efron and Tibshirani (1993).

RESULTS

A summary of the ANOVA of the effects of pollinators, herbivores, and their interaction on NSEEDS, is shown in Table 1. Not unexpectedly, the number of carpels (included as a covariate) had a highly significant effect on NSEEDS. After statistically accounting for this relationship, both pollinators and herbivores had significant effects on NSEEDS, although the corresponding P values were, in both cases, barely less than the critical 0.05 level. The effect of pollinators on plant fecundity was 7.4 ± 2.8 seeds per plant (mean

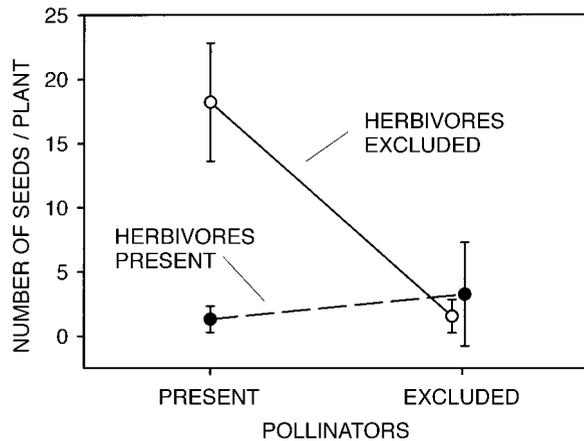


FIG. 1. Interaction graph for the effects of pollinators and herbivores on the total number of undamaged, apparently viable seeds produced by individual plants of *Paeonia broteroi*. Circles denote model-adjusted cell means with herbivores present (solid circles) or excluded (open circles), and vertical segments extend over ± 1 SE (standard errors were determined by bootstrapping with 10 000 repetitions). Results of tests of simple main effects are shown in Table 2.

± 1 SE, bootstrap estimated), whereas that of herbivores was -7.7 ± 2.6 seeds per plant. This means that, on average, *Paeonia broteroi* individuals exposed to pollinators produced 7.4 seeds more than those without pollinators, whereas those exposed to the action of herbivores experienced a fecundity reduction of 7.7 seeds relative to those that were protected.

These estimates of the magnitudes of main effects would meaningfully reflect the relative importance of pollinators and herbivores as determinants of maternal fecundity in *P. broteroi* plants only if the two main factors did not have any joint effect (interaction) on NSEEDS. This was not the case in the present instance. The interaction between pollinators and herbivores was statistically significant (Table 1), thus indicating that the sign and/or magnitude of the effect of either of these main factors on *P. broteroi* fecundity depended on the level of the other factor. The interaction between herbivores and pollinators is graphically illustrated in Fig. 1, and tests of simple main effects (or interaction slices) are summarized in Table 2. Pollinators had a highly significant effect on NSEEDS in the absence of herbivores. In the presence of herbivores, however, the presence of pollinators was inconsequential for NSEEDS (plants without pollinators but in the presence of herbivores even had a slight seed production advantage over plants with pollinators; Fig. 1). This was caused by the extreme impact of herbivores on seed production in the presence of pollinators. Herbivores had a highly significant effect on NSEEDS in the presence of pollinators, but had no effect when these were excluded. The marginal significance level of the main effects of pollinators and herbivores treatments (Table

1) should thus be attributed to their strong "disordinal" interaction (sensu Pedhazur 1982).

The two major groups of herbivores recognized in this study responded differently to the levels of the Pollinators treatment, with mammals feeding exclusively on fruits from flowers that had been exposed to pollinators. Among plants whose developing fruits had been exposed to herbivores, 63.6% of damaged fruits ($N = 118$) exhibited signs of invertebrate damage, whereas the remaining 36.4% had been eaten by browsing mammals. These percentages, however, did not remain consistent across levels of the Pollinators treatment. Among plants whose flowers had been exposed to pollinators, vertebrates and invertebrates accounted for 39.5% and 60.5% of damaged fruits, respectively. The corresponding figures for plants with pollinators excluded were 0% and 100% ($P = 0.025$, Fisher's exact test).

DISCUSSION

By simultaneously accounting for more than one set of interactors, this study has been able to evaluate the relative importance of pollinators and herbivores as determinants of the maternal fecundity in *Paeonia broteroi*. On average, the effects of pollinators and herbivores, measured in terms of number of seeds produced per plant, were of opposite sign and roughly similar magnitude. In the study year and population, therefore, the detrimental effect of herbivores and the beneficial effect of pollinators roughly canceled each other. In addition to illustrating the use of a possible experimental protocol for measuring the effects of pollinators and herbivores on maternal fecundity, this

TABLE 2. Summary of tests of simple main effects (or "interaction slices"; SAS Institute 1996) for the effects of herbivores and pollinators on the total number of sound seeds produced by individual plants of *Paeonia broteroi*.

Source	df [†]	F	P [‡]
Pollinators, with herbivores excluded	1, 116	18.99	<0.0001
Pollinators, with herbivores present	1, 116	0.11	0.68
Herbivores, with pollinators excluded	1, 116	0.06	0.73
Herbivores, with pollinators present	1, 116	29.80	<0.0001

Note: See Table 1 for tests of main effects and their interaction in two-way ANOVA, and Fig. 1 for a graphical representation of the significant Herbivores \times Pollinators interaction.

[†]Because all simple main effects are tested in a single analysis, without separating the data into strata, they share a common denominator degrees of freedom, corresponding to the error degrees of freedom for the overall analysis of the factorial design (for justification and further details, see Pedhazur 1982:362–365).

[‡]Significance values of individual effects were determined using a randomization procedure and 10 000 repetitions (see *Methods, Statistical analyses* for justification and further details).

study has also documented a significant interaction between these two sets of interactors. Mammalian herbivores contributed decisively to the interaction by browsing exclusively on the fruits of those plants that had been exposed to pollinators, while neglecting fruits developing from flowers that had not been exposed to pollinators. Among plants protected from herbivores, the number of sound seeds per follicle was significantly greater in fruits from flowers exposed to pollinators (5.4 ± 4.6 seeds per follicle, mean ± 1 SD; $N = 94$ follicles) than in fruits from flowers with pollinators excluded (0.8 ± 1.5 seeds per follicle; $N = 90$ follicles) ($\chi^2 = 48.3$, $df = 1$, $P < 0.001$; Kruskal-Wallis ANOVA). Because follicle size and number of enclosed seeds are closely correlated, the strong preference of browsing mammals for the fruits from open-pollinated plants may be explained by size-based discrimination and a marked preference for the larger, and presumably more rewarding, fruits.

The experimental design used in this study has some inherent limitations that must be explicitly acknowledged. Although the Pollinators factor effectively assesses the influence of the whole set of pollinators on maternal fecundity, similar completeness is lacking in the case of the Herbivores factor. As measured in this study, the effect of herbivores on maternal fecundity accounts only for the fraction of herbivory that affects developing fruits and seeds, and it is in this limited context that results of the study can be properly interpreted. At the study site, leaves and flower buds of *P. broteroi* are also damaged by herbivores, often by the same invertebrate and vertebrate species that later damage the developing fruits. The possible direct and indirect effects of this fraction of herbivory on maternal fecundity, as well as its possible interaction with pollinators, was not addressed here because of practical limitations. Leaf damage by herbivores may modify flower characteristics and decrease overall plant attractiveness to pollinators (Strauss et al. 1996, Lehtilä and Strauss 1997). If this also occurs in *P. broteroi*, then the design used in this study might have missed one further potential source of interaction between herbivores and pollinators. In addition, maternal fecundity has been the only measure of reproductive success considered here, and the one used as the response variable in the ANOVA. In a hermaphroditic plant like *P. broteroi*, herbivory on flowers and leaves may also affect the reproductive success of individual plants via its influence on male reproductive function (Mutikainen and Delph 1996, Strauss et al. 1996).

The strong non-additivity of the effects of pollinators and herbivores found in this study implies that the effect on plants of one set of interactors was closely dependent on the treatment level of the other set of interactors. Pollinators did "matter" to *P. broteroi* plants (i.e., did account for a significant amount of between-plant variance in fecundity) only in the absence of herbivores, but became irrelevant when plants

were also exposed to the action of herbivores, which virtually wiped out the whole production of seeds of control plants during the study season. If analogous non-additivity between the effects of different interactors occurs frequently in nature, then interpretations of single-interaction studies will necessarily be strongly context dependent. Under natural, non-experimental conditions, an interaction similar to that observed in the present study will take place, for example, whenever herbivores are preferentially attracted to plants that have larger crops of developing fruits because they had been favored by pollinators (e.g., for their large floral displays), but tend to avoid those plants with smaller fruit crops that have been relatively avoided by pollinators. In *Lathyrus vernus*, flowering individuals are much more likely to be grazed than nonflowering ones of similar size, grazing risk increases with flower number, and individuals with larger inflorescences also suffer from higher invertebrate seed predation (Ehrlén 1996, 1997). Likewise, increased floral displays in *Ipomopsis aggregata* lead not only to increased flower visitation rate by hummingbird pollinators, but also to greater incidence of pre-dispersal seed predators, both in absolute numbers and in percentage of fruits damaged (Brody and Mitchell 1997).

The extreme herbivory on the developing fruits and seeds of *P. broteroi* documented in this study has also been observed in other years for the same species (C. M. Herrera, unpublished observations; A. M. Sánchez-Lafuente, personal communication), and similarly high levels are also common among other insect-pollinated perennial herbs of the forest understory in the study region. Extensive destruction of developing fruits by the combined action of ungulates and lepidopteran caterpillars has been also found in the taxonomically unrelated *Narcissus longispathus* (Amaryllidaceae), *Heliborus foetidus* (Ranunculaceae), and *Primula vulgaris* (Primulaceae) (C. M. Herrera, unpublished data). Similarly high intensities of vertebrate and/or invertebrate herbivory on the flowers and fruits of annual or perennial herbs have also been reported from other habitat types (e.g., Arnold 1982, Edwards 1985, Gómez 1996, Ehrlén 1997). Insofar as a disproportionate incidence of herbivores on the naturally pollinated, developing *P. broteroi* fruits was ultimately responsible for the non-additivity of the effects of herbivores and pollinators, the frequent occurrence of high herbivory levels on the reproductive structures of other insect-pollinated herbs suggests that such interactions may occur in other species as well.

The use of individual plants as experimental units, the evaluation of the effect of interactors in terms of individual fecundity, and the adoption of an ANOVA approach to measure effects and their interaction, as done here, all combine favorably to simplify the biological interpretations of results and to suggest some testable predictions. Assuming that individual variance in maternal fecundity of *P. broteroi* plants is roughly

proportional to their variance in fitness, the results of this study suggest that herbivores will greatly reduce the “opportunity for selection” (sensu Arnold and Wade 1984) of pollinators on plants. By wiping out virtually all possible variance in maternal fitness that could have been generated in the population by the interaction between the plants and their pollinators, herbivores were constraining the opportunity for selection of pollinators on plants. Through this mechanism, the interaction between pollinators and herbivores may thus become, depending on herbivore incidence, a critical constraint on the likelihood of adaptation of this plant to its pollinators. Sánchez-Lafuente et al. (1999), working on a *P. broteroi* population growing inside a large mammalian enclosure in the same region, found that the different insect pollinators differed widely in qualitative and quantitative aspects of pollinating effectiveness. Pollinators thus had the ability to generate a pollination-dependent component of population variance in maternal fitness (Sánchez-Lafuente et al. 1999), hence providing an essential prerequisite for plant adaptation to pollinators (e.g., Stebbins 1970, Schemske and Horvitz 1984, Herrera 1987). My results, however, point to the biological paradox that, even if differential interaction with pollinators of different effectiveness actually accounts for some individual variance in fecundity, the likelihood of adaptation to pollinators will, depending on herbivore incidence, eventually depend more on the action of herbivores than on the pollinators themselves. A similar conclusion was reached by Gómez (1996) from an observational study of the interactions among pollinators, herbivores, and the annual herb *Moricandia moricandioides* in an arid shrubsteppe of southeastern Spain. These results thus provide support for Ehrlén’s (1997) contention (see also Brody 1992) that the evolution of reproductive traits may sometimes be better understood if the effects of selective factors other than pollinators are taken into consideration. In southern Spain, the incidence of herbivores on *P. broteroi* varies widely among populations, with the population studied here probably falling on or near the upper extreme of herbivory intensity (A. M. Sánchez-Lafuente, *personal communication*). If the interaction between herbivores and pollinators actually limits the opportunities for the adaptation of *P. broteroi* to pollinators, then one testable prediction emerging from this study would be that the degree of local adaptability of flowers and flowering traits to pollinators in this species would be inversely correlated across regions with the mean local incidence of herbivores.

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LITERATURE CITED

- Armbruster, W. S. 1997. Exaptations link evolution of plant–herbivore and plant–pollinator interactions: a phylogenetic inquiry. *Ecology* **78**:1661–1672.
- Armbruster, W. S., J. J. Howard, T. P. Clausen, E. M. Debevec, J. C. Loquvam, M. Matsuki, B. Cerendolo, and F. Anel. 1997. Do biochemical exaptations link evolution of plant defense and pollination systems? Historical hypotheses and experimental tests with *Dalechampia* vines. *American Naturalist* **149**:461–484.
- Arnold, R. M. 1982. Pollination, predation and seed set in *Linaria vulgaris* (Scrophulariaceae). *American Midland Naturalist* **107**:360–369.
- Arnold, S. J., and M. J. Wade. 1984. On the measurement of natural and sexual selection: theory. *Evolution* **38**:709–719.
- Brody, A. K. 1992. Oviposition choices by a pre-dispersal seed predator (*Hylemya* sp.). I. Correspondence with hummingbird pollinators, and the role of plant size, density and floral morphology. *Oecologia* **91**:56–62.
- Brody, A. K., and R. J. Mitchell. 1997. Effects of experimental manipulation of inflorescence size on pollination and pre-dispersal seed predation in the hummingbird-pollinated plant *Ipomopsis aggregata*. *Oecologia* **110**:86–93.
- Cunningham, S. A. 1995. Ecological constraints on fruit initiation by *Calyptrogyne ghiesbreghtiana* (Arecaceae): floral herbivory, pollen availability, and visitation by pollinating bats. *American Journal of Botany* **82**:1527–1536.
- Edwards, J. 1985. Effects of herbivory by moose on flower and fruit production of *Aralia nudicaulis*. *Journal of Ecology* **73**:861–868.
- Efron, B., and R. J. Tibshirani. 1993. An introduction to the bootstrap. Chapman and Hall, New York, New York, USA.
- Ehrlén, J. 1996. Spatiotemporal variation in predispersal seed predation intensity. *Oecologia* **108**:708–713.
- Ehrlén, J. 1997. Risk of grazing and flower number in a perennial plant. *Oikos* **80**:428–434.
- Euler, M., and I. T. Baldwin. 1996. The chemistry of defense and apparency in the corollas of *Nicotiana attenuata*. *Oecologia* **107**:102–112.
- Gómez, J. M. 1996. Predispersal reproductive ecology of an arid land crucifer, *Moricandia moricandioides*: effect of mammal herbivory on seed production. *Journal of Arid Environments* **33**:425–437.
- Heithaus, E. R., E. Stashko, and P. K. Anderson. 1982. Cumulative effects of plant–animal interactions on seed production by *Bauhinia unguolata*, a neotropical legume. *Ecology* **63**:1294–1302.
- Herrera, C. M. 1987. Components of pollinator “quality”: comparative analysis of a diverse insect assemblage. *Oikos* **50**:79–90.
- Herrera, C. M. 1989. Vertebrate frugivores and their interaction with invertebrate fruit predators: supporting evidence from a Costa Rican dry forest. *Oikos* **54**:185–188.
- Herrera, C. M. 1993. Selection on floral morphology and environmental determinants of fecundity in a hawk moth-pollinated violet. *Ecological Monographs* **63**:251–275.
- Karban, R., and S. Y. Strauss. 1993. Effects of herbivores on growth and reproduction of their perennial host, *Eriogon glaucus*. *Ecology* **74**:39–46.
- Kudoh, H., and D. F. Whigham. 1998. The effect of petal size manipulation on pollinator/seed-predator mediated female reproductive success of *Hibiscus moscheutos*. *Oecologia* **117**:70–79.

- Lehtilä, K., and S. Y. Strauss. 1997. Leaf damage by herbivores affects attractiveness to pollinators in wild radish, *Raphanus raphanistrum*. *Oecologia* **111**:396–403.
- Lohman, D. J., A. R. Zangerl, and M. R. Berenbaum. 1996. Impact of floral herbivory by parsnip webworm (Oecophoridae: *Depressaria pastinacella* Duponchel) on pollination and fitness of wild parsnip (Apiaceae: *Pastinaca sativa* L.). *American Midland Naturalist* **136**:407–412.
- Manly, B. F. J. 1991. Randomization and Monte Carlo methods in biology. Chapman and Hall, London, UK.
- Muñoz Garmendia, F., and C. Navarro. 1993. *Paeonia* L. Pages 144–150 in S. Castroviejo, C. Aedo, S. Cirujano, M. Lainz, P. Montserrat, R. Morales, F. Muñoz Garmendia, C. Navarro, J. Paiva, and C. Soriano, editors. Flora Iberica. Volume III. Plumbaginaceae (partim)–Capparaceae. Real Jardín Botánico, Consejo Superior de Investigaciones Científicas, Madrid, Spain.
- Mutikainen, P., and L. F. Delph. 1996. Effects of herbivory on male reproductive success in plants. *Oikos* **75**:353–358.
- Pedhazur, E. J. 1982. Multiple regression in behavioral research. Second edition. Holt, Rinehart and Winston, Fort Worth, Texas, USA.
- Pellmyr, O. 1992. The phylogeny of a mutualism: evolution and coadaptation between *Trollius* and its seed-parasitic pollinators. *Biological Journal of the Linnean Society* **47**:337–365.
- Pettersson, M. W. 1992. Pollination, oviposition and seed predation by flower visiting insects in bladder champions (*Silene vulgaris* s.l., Caryophyllaceae). *Acta Universitatis Upsaliensis* **399**:1–32.
- Powell, J. A. 1992. Interrelationships of yuccas and yucca moths. *Trends in Ecology and Evolution* **7**:10–15.
- Quesada, M., K. Bollman, and A. G. Stephenson. 1995. Leaf damage decreases pollen production and hinders pollen performance in *Cucurbita texana*. *Ecology* **76**:437–443.
- Sánchez-Lafuente, A., P. J. Rey, J. M. Alcántara, and F. Valera. 1999. Breeding system and the role of floral visitors in seed production of a 'few-flowered' perennial herb, *Paeonia broteroi* Boiss. & Reut. (Paeoniaceae). *Ecoscience* **6**:163–172.
- SAS Institute. 1990. SAS guide to macro processing. Version 6. Second edition. SAS Institute, Cary, North Carolina, USA.
- SAS Institute. 1996. SAS/STAT software: changes and enhancements through Release 6.11. SAS Institute, Cary, North Carolina, USA.
- Schemske, D. W., and C. C. Horvitz. 1984. Variation among floral visitors in pollination ability: a precondition for mutualism specialization. *Science* **225**:519–521.
- Schemske, D. W., and C. C. Horvitz. 1988. Plant–animal interactions and fruit production in a neotropical herb: a path analysis. *Ecology* **69**:1128–1137.
- Stebbins, G. L. 1970. Adaptive radiation of reproductive characteristics in angiosperms. I: Pollination mechanisms. *Annual Review of Ecology and Systematics* **1**:307–326.
- Strauss, S. Y. 1997. Floral characters link herbivores, pollinators, and plant fitness. *Ecology* **78**:1640–1645.
- Strauss, S. Y., J. K. Conner, and S. L. Rush. 1996. Foliar herbivory affects floral characters and plant attractiveness to pollinators: implications for male and female plant fitness. *American Naturalist* **147**:1098–1107.
- Wiebes, J. T. 1979. Co-evolution of figs and their insect pollinators. *Annual Review of Ecology and Systematics* **10**:1–12.