

Interaction of pollinators and herbivores on plant fitness suggests a pathway for correlated evolution of mutualism- and antagonism-related traits

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Different kinds of plant–animal interactions are ordinarily studied in isolation, yet considering the combined fitness effects of mutualistic and antagonistic interactions is essential to understanding plant character evolution. Functional, structural, or phylogenetic associations between attractive and defensive traits may be nonadaptive or result from correlational selection on sets of herbivory- and pollination-linked traits. Nonadditivity of fitness effects of mutualists and antagonists, a requisite for correlational selection, was experimentally tested in the field. We created experimental populations of the insect-pollinated perennial herb, *Helleborus foetidus*, at 16 different locations distributed among three regions in the Iberian Peninsula. Plants experienced one of four possible selective regimes generated by independently weakening the effects of pollinators and herbivores (flower and fruit predators) according to a two-way fully factorial design. Effects were assessed in terms of number of next-generation offspring recruited per mother plant under natural field conditions. Differences among *H. foetidus* plants in the strength of their interactions with pollinators and herbivores translated into differential fitness, as measured in terms of recruited offspring, and subsequent changes in plant population densities. A strong, geographically consistent nonadditivity in the fitness consequences of pollinators and herbivores was found also. Plants possessing the particular combination of “traits” simultaneously enhancing pollination and escape from herbivores enjoyed a disproportionate fitness advantage over plants possessing any of the other three possible “trait” combinations. Results suggest a simple, possibly widespread ecological pathway favoring the adaptive correlated evolution of mutualism- and antagonism-related plant traits in pollinator-dependent plants suffering intense flower and fruit herbivory.

Investigations on the different sorts of plant–animal interactions (e.g., herbivory, pollination, and seed dispersal) have traditionally progressed along separate lanes, the vast majority of studies focusing on just one kind of interaction and ignoring the possible significance of the others. This compartmentalization leads to an artificial oversimplification that may hinder our understanding of the ecology and evolution of plant–animal interactions. It is the composite result of the distinct interactions with all their animal counterparts that plants “perceive” in ecological and evolutionary time, and the combined action of different sets of counterparts (e.g., herbivores, pollinators, seed predators, and seed dispersers) exerts intricate ecological and evolutionary influences on plants extending beyond simple additivity of effects (1–5). The combined action of herbivores and pollinators may influence the ecology of plant reproduction and the evolution of pollination-related traits. Through direct or indirect effects on floral displays, flower characteristics, pollen quantity and quality, and plant attractiveness to pollinators, herbivores may ultimately modulate the nature, strength, and fitness consequences of the interaction between plants and their pollinators (6–15). Furthermore, herbivores and pollinators may

exert conflicting selective pressures on particular plant traits, and certain characteristics of flowers and inflorescences may reflect compromise adaptations to mutualists and antagonists (5, 12, 16–20).

Another possible consequence of the concurrent interaction of plants with herbivores and pollinators that has begun to be recognized recently and the evolutionary origin of which is still poorly understood is the close functional, structural, or phylogenetic association sometimes existing between herbivory- and pollination-related traits. In *Hypericum* flowers, the same UV pigments play a defensive role in the stamens and ovaries and an attractive role in the petals (21). In tropical vines of the genus *Dalechampia*, anthocyanins and resins involved in chemical defense against herbivores have secondarily assumed a role in pollinator attraction and reward, respectively (22–24). *Hypericum* dual-role, floral UV pigments mentioned earlier also may have been secondarily derived from foliar defensive compounds (21). Correlated evolution of defensive foliar anthocyanins and anthocyanin-based floral pigmentation has been documented in trees of the genus *Acer* (24). These associations between mutualism-linked traits (floral rewards and attractive pigmentation) and antagonism-linked traits (defensive compounds in flowers or vegetative parts) may reflect pleiotropy or developmental associations between characters (24) or, alternatively, may be the adaptive outcome of correlational selection on sets of attractive and defensive characters taking place at the microevolutionary scale.

Correlational selection on two or more traits occurs when some particular combination of traits is selectively favored at the expense of other possible combinations (25). One necessary requirement is the existence of significant interactions (i.e., nonadditivity) between the fitness effects of different traits (26). Nonadditivity of the fitness effects of mutualists and antagonists on plants may be tested by field experiments where phenotypic variance in mutualism-related and antagonism-related traits is experimentally increased and distinct combinations of traits are generated according to a factorial design (3). This approach allows a dissection of the fitness effects of mutualists, antagonists, and their interaction by using the analytical tools inherent to analyses of variance and linear models (3). We present here the results of a field study adopting such an approach and designed to dissect the effects of pollinators, flower- and fruit-feeding herbivores (hereafter termed “herbivores” for simplicity), and their interaction on the reproductive success of the perennial herb, *Helleborus foetidus* L. (Ranunculaceae), in the Iberian Peninsula. By amplifying within-population variance in the strength of individual plants’ interaction with pollinators and herbivores, and adopting a two-way fully factorial design, we

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Fig. 1. Map of the Iberian Peninsula showing the location of the three study regions. The figures in brackets indicate the number of separate *H. foetidus* populations (treated as blocks in statistical analyses) used in each region. In Caurel, *H. foetidus* plants grew at 950- to 1,350-m elevations in pine (*Pinus sylvestris*) plantations, *Castanea sativa* groves, open successional scrublands, and *Brachypodium rupestre* meadows. In Cazorla and Mágina, plants were in pine (*Pinus nigra*)- or oak (*Quercus rotundifolia*)-dominated forests at 900- to 1,600-m elevations.

created experimental populations of *H. foetidus* in the field with individuals that experienced one of four possible selective regimes generated by selectively weakening the effects of pollinators and herbivores independently. The effects of experimental manipulations then were assessed in terms of number of next-generation offspring recruited per mother plant under field conditions, thus a direct index of maternal fitness. We found that differences among *H. foetidus* plants in the strength of their interactions with pollinators and herbivores translated into differential fitness as measured in terms of recruited offspring. Furthermore, strong nonadditivity in the fitness consequences of the interaction with pollinators and herbivores occurred consistently over the significant portion of the species' geographical range considered in this study. The latter finding suggests a simple ecological pathway favoring the correlated evolution of mutualism- and antagonism-related traits in pollinator-dependent plants suffering intense herbivory on flowers and/or fruits.

Methods

The study was conducted between February 1999 and October 2001 at three separate regions in the Iberian Peninsula (Fig. 1). The reproductive ecology of *H. foetidus* in these areas has been described (27–30). Plants consist of one or a few ramets, each of which produces a single terminal inflorescence after 3–5 seasons of vegetative growth and subsequently dies. Flowering takes place during January–March. Each inflorescence produces 25–75 flowers, which are hermaphroditic, self-compatible, and predominantly bumble bee-pollinated. The species is self-compatible, and up to 30% of flowers may set fruit in the absence of pollinators via spontaneous self-pollination, yet insect pollination is required for abundant seed production (natural fruit set 65–90%). Open flowers (in January–March) and then developing fruits (March–June) are frequently eaten by mice (mainly *Apodemus sylvaticus*) and generalist lepidopteran larvae (mainly *Trigonophora flammea*, Noctuidae), which may collectively de-

stroy or seriously damage up to 75–100% of flowers and/or developing fruits over the flowering and fruit-growth period. When feeding on flowers, lepidopteran larvae mainly eat the carpels and pieces of the large sepals, whereas mice generally remove whole flowers by chewing the pedicels. When feeding on developing fruits, larvae eat the soft, unripe seeds through a hole bitten through the follicle wall, whereas mice remove whole fruits. Foliage herbivory by either vertebrates or invertebrates on *H. foetidus* plants was virtually nonexistent at all our study sites. Fruit maturation and seed shedding take place in June through early July. As expected from their well developed elaiosome, seeds are often but not invariably dispersed by ants after falling to the ground (A.J.M., unpublished data). In our study localities most seeds either remain undispersed under the parent plant or are dispersed short distances by ants, and seedling recruitment mostly occurs within ≈ 2 m of maternal parents. Seeds have an extended dormancy, with the vast majority germinating during the second spring after entering the seed bank.

Plants of *H. foetidus* were either exposed to natural pollination or enclosed inside enclosures that precluded pollinator access (“Pollinators” factor; hereafter, factor names are referred to by using capitalized initials). The effect of flower and fruit herbivores was evaluated by protecting some plants while leaving unprotected others (“Herbivores” factor). These factors were combined in a fully factorial design leading to the following four combinations. (i) Both herbivores and pollinators excluded: Plants were surrounded with a 0.5-m-high open-topped wire-netting cylinder (1-cm mesh), which then was covered with fine black tulle. Protection from noctuid larvae, which climbed up *H. foetidus* plants at night to feed on flowers or fruits, was achieved by liberally sprinkling on the ground around the base of plants a malathion-based persistent powdery insecticide (Verdecion Mat 4P, 4% malathion) on several occasions through the flowering and fruit-growing period. (ii) Exposed to herbivores but with pollinators excluded: Plants were inside a tulle-covered 0.5-m-high open-topped wire-netting cylinder similar to those in i, but four 8×8 -cm openings were made at ground level in the cylinder to allow the access of herbivores. Insecticide was not applied. (iii) Exposed to pollinators but protected from herbivores: Plants were surrounded with a 0.25-m-high open-topped wire-netting cylinder (1-cm mesh) that precluded the access of rodents without interfering with the pollinators' access to the flowers, as the upper border of the cylinder stood below the lowermost flowers of the inflorescence. Insecticide was also periodically applied around the base of the plant for protection from noctuid larvae. (iv) Fully exposed to both pollinators and herbivores.

A randomized complete block design was used within each region. In January 1999, before the start of flowering, five areas with suitable *H. foetidus* populations were selected in Mágina and Cazorla and six areas in Caurel. The distances between areas in the same region ranged between 0.3 and 5.0 km. Each area was treated as a block in our experimental design. Eight 1.5×1.5 -m. square plots were set up in each block, each plot being centered on an isolated *H. foetidus* individual having a single or, rarely, two to three inflorescences. These plots were both the experimental and sampling units used for this study and were assigned randomly to one of the four combinations of the Pollinators and Herbivores treatment levels described earlier. Two plot replicates were used per combination in each block. Enclosures remained in place throughout the flowering and fruiting period and were checked frequently for signs of possible intrusions by herbivores or pollinators. Tulle coverings of wire cages were removed shortly after completion of the flowering season. Plants in experimental plots matured fruits and shed seeds naturally in June 1999, and wire-netting cylinders were removed soon afterward. Three plots were excluded from analyses because inflorescences were affected by a fungal pathogen and withered

Table 1. Summary of mixed-model ANOVA testing for the effects of Region (Caurel, Mágina, and Cazorla; Fig. 1), Pollinators (present or excluded), and Herbivores (present or excluded) on the total number of seedlings emerged and recruited (= postsummer survivors; both log-transformed) per experimental plot

Effect	df	1999 Seedlings				2000 Seedlings				2001 Seedlings			
		Emerged		Recruited		Emerged		Recruited		Emerged		Recruited	
		F	P	F	P	F	P	F	P	F	P	F	P
Region (R)	2,13	50.52	<0.0001	3.22	0.07	12.99	0.001	4.72	0.03	18.53	0.0002	5.51	0.02
Pollinators (P)	1,100	0.24	0.63	3.62	0.06	0.37	0.54	0.11	0.74	5.44	0.02	6.77	0.01
Herbivores (H)	1,100	0.26	0.61	0.35	0.56	1.19	0.28	0.01	0.97	0.41	0.53	0.56	0.46
R × P	2,100	2.81	0.07	0.82	0.44	2.27	0.11	0.63	0.53	2.06	0.13	2.60	0.08
R × H	2,100	0.16	0.85	0.08	0.93	0.04	0.96	0.43	0.65	0.46	0.63	0.02	0.98
P × H	1,100	0.66	0.42	0.01	0.96	1.15	0.26	0.06	0.81	12.52	0.0006	9.71	0.002
R × P × H	2,100	0.29	0.75	0.81	0.45	0.34	0.71	0.46	0.63	0.28	0.76	1.81	0.17

Manipulations were conducted during the reproductive season of 1999. As seeds of *H. foetidus* germinate in the second year after entering the seed bank, effects of experiments should show up in the spring germination season of 2001 but not in 1999 or 2000.

without producing fruits. In six of the plots with herbivores excluded we found evidence of intrusion by mice. For biological realism, these plots have been treated in the analyses as if they had been actually exposed to herbivores. Nevertheless, neither excluding them from the analyses nor keeping them in their original treatment-level category made any difference to results.

Plots were surveyed regularly for emerged *H. foetidus* seedlings during the germination periods of 1999–2001. All seedlings emerging were marked and monitored through October 2001. Because of the 2-yr dormancy of *H. foetidus* seeds, any seedlings emerging in the experimental plots in the springs of 1999 (the year when the experiment was conducted) and 2000 would come from seeds already present in the seed bank before our experiments, not from those produced by our experimental plants in 1999. Information of seedling emergence in experimental plots in 1999 and 2000 thus provided a “blank” against which to compare seedling emergence patterns in 2001. To be biologically meaningful, any effects on seedling numbers of the experimental manipulations of pollinators and herbivores conducted in 1999 should be restricted to the seedling cohort emerged during the spring of 2001, and no effects should be apparent on the cohorts emerged in the springs of either 1999 or 2000. We also tested whether the number of seedlings emerged per plot actually reflected the total progeny produced by the focal *H. foetidus* plants and hence its maternal fitness. Seed dispersal was precluded in half the plots for each Herbivores × Pollinators combination by excluding ants during the seed-dispersal period. Malathion-based insecticide was sprinkled once over the whole surface of ant-exclusion plots. The rest of the plots were left untreated such that foraging ants could enter and take seeds away. The difference between plots with and without ants in number of emerged seedlings will provide an indication of the extent to which our plot-scale measurements of seedling recruitment actually reflected total progeny production of focal plants.

Fitness consequences for *H. foetidus* plants of Pollinators, Herbivores, and their interaction were assessed by fitting linear mixed models separately to the seedling-emergence and seedling-recruitment data for 1999–2001. Two models were fit for each year’s data, having as the dependent variable either the cumulative number of seedlings emerged per plot (“emerged seedlings” hereafter) or the number of seedlings surviving in each plot past the first summer after emergence (“recruited seedlings” hereafter), both log-transformed to achieve normality. Region, Pollinators, Herbivores, and all two- and three-way interactions were included in the models as fixed effects. Blocks within region were incorporated as random effects. Computations were carried out by using SAS procedure MIXED and restricted maximum-likelihood estimation (31). When signifi-

cant interaction between main effects occurred, the SLICE option in the LSMEANS statement was used to assess the statistical significance of a given factor at different treatment levels of the other factor(s) (“simple main effects”; ref. 32).

Results

Emerged and recruited seedlings per plot were correlated in all years ($r = 0.677, 0.776, \text{ and } 0.952$ for 1999, 2000, and 2001, respectively; $n = 125$; $P < 0.0001$ in all cases), hence patterns of variation among years and regions were similar for emerged and recruited seedlings. The mean number of emerged seedlings per plot differed significantly among regions (Table 1), being largest in Mágina (mean \pm SD = $55 \pm 53, 91 \pm 100, \text{ and } 96 \pm 127$ seedlings per plot in 1999, 2000, and 2001, respectively), smallest in Cazorla ($5 \pm 5, 8 \pm 13, \text{ and } 7 \pm 10$ seedlings per plot), and intermediate in Caurel ($4 \pm 7, 17 \pm 41, \text{ and } 14 \pm 25$ seedlings per plot). Likewise, the mean number of recruited seedlings per plot was largest in Mágina ($5.1 \pm 7.9, 26.8 \pm 57.4, \text{ and } 61.6 \pm 121.9$ seedlings per plot for 1999, 2000, and 2001, respectively), smallest in Cazorla ($1.2 \pm 1.4, 0.7 \pm 1.7, \text{ and } 1.8 \pm 3.1$ seedlings per plot), and intermediate in Caurel ($1.6 \pm 3.9, 9.4 \pm 18.6, \text{ and } 8.7 \pm 20.9$ seedlings per plot).

Statistically significant effects of Pollinators or Herbivores on the number of seedlings emerged or recruited per plot occurred for the 2001 seedling cohort but not for either the 1999 or 2000 cohorts (Table 1). This result agrees with expectations based on the 2-yr-long dormancy period of *H. foetidus* seeds and provides clear support for relating patterns of seedling emergence and recruitment in our plots in 2001 with the experimental treatments conducted there in 1999. On average, the number of seedlings emerged in 2001 was slightly greater in plots that had ants excluded during the 1999 seed-dispersal period than in plots with ants (12.0 vs. 8.5 seedlings per plot), but the difference was not statistically significant [$F(1,106) = 1.80, P = 0.18$; mixed-model ANOVA on log-transformed data]. This result suggests that only a minor fraction of the seeds produced by individual *H. foetidus* plants in 1999 were dispersed by ants beyond the confines of individual plots, hence the numbers of seedlings appearing in plots can be used as reliable indicators of maternal fitness of focal plants.

The main effect of Herbivores on the number of emerged and recruited seedlings per plot in 2001 was not statistically significant (Table 1), but this result cannot be interpreted separately because of the significant interaction between Herbivores and Pollinators described below. The main effect of Pollinators on emerged and recruited seedlings was statistically significant (Table 1). Excluding pollinators from plants resulted in an average differential of -5.51 seedlings emerged and -2.47

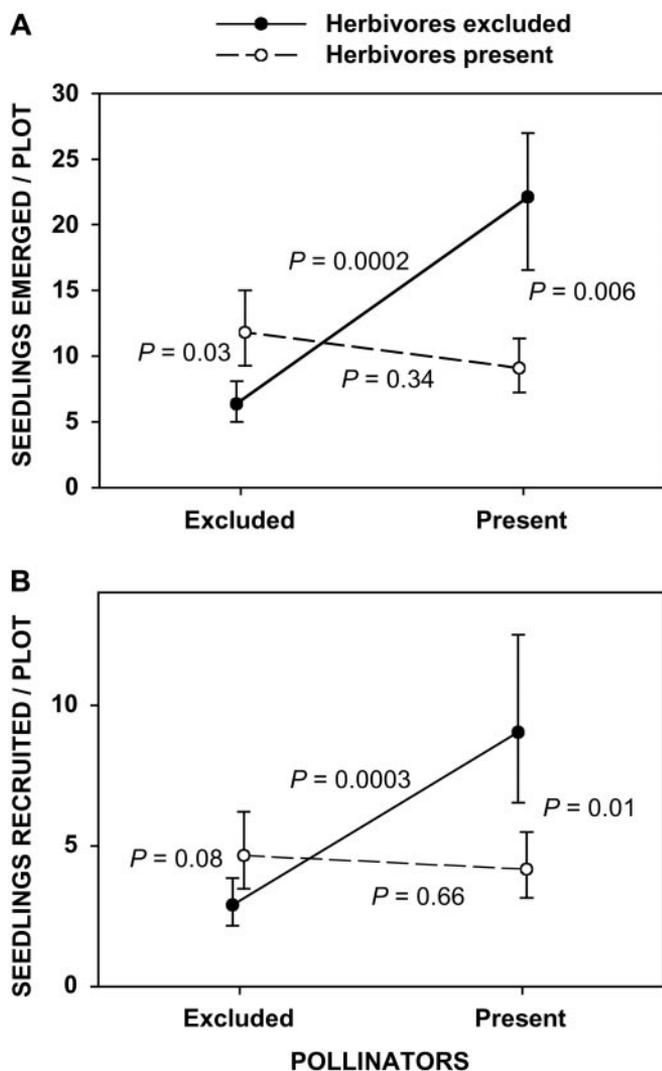


Fig. 2. Interaction graphs for the effects of Pollinators and Herbivores on the mean number of emerged (A) and recruited (B) seedlings of *H. foetidus* per experimental plot in 2001. Circles denote model-adjusted cell means with herbivores present (open circles, dashed line) or excluded (filled circles, continuous line), and vertical segments extend over ± 1 SE. The values plotted are back-transforms of the results obtained from the analyses of log-transformed data (hence the asymmetry of vertical segments with respect to the mean). In each graph, *P* values correspond to tests of significance of the four simple main effects involved in each interaction (see Table 1 for significance tests of main effects and interactions).

seedlings recruited per plot (effect sizes obtained as the difference of back-transformed model-adjusted cell means on the log scale, obtained by using the LSMEANS statement in the MIXED procedure). The nearly significant Pollinators \times Region interaction effect on the number of emerged and recruited seedlings in 2001 (Table 1) might suggest a weak trend for the fitness effects of pollinators to vary among regions. Nevertheless, this result is attributable most likely to some spurious correlation rather than reflecting a biological consequence of our experiments, because the Pollinators \times Region interaction effect was also close to statistical significance for emerged seedlings in 1999 and 2000.

A highly significant interaction existed between Pollinators and Herbivores in their effects on both emerged and recruited seedlings per plot in 2001 (Table 1). The Pollinators effect on emerged (Fig. 2A) and recruited (Fig. 2B) seedlings per plot

heavily depended on the level of the Herbivores factor, i.e., whether plants had been exposed to herbivores. Pollinators had a statistically significant, positive effect on the number of seedlings emerged and recruited only under exclusion from herbivores. In the presence of herbivores, the Pollinators effect was not significantly different from zero. Both the sign and the magnitude of the Herbivores effect depended on the level of the Pollinators factor. Herbivores, in turn, had a statistically significant detrimental effect on the number of seedlings emerged and recruited only in presence of pollinators. A reversal in the sign of the Herbivores effect occurred under conditions of pollinator exclusion. In the absence of pollinators there was a significant (seedlings emerged, Fig. 2A) or nearly significant (seedlings recruited, Fig. 2B) trend for the presence of Herbivores to increase seedling numbers. The disordinal nature of the Pollinators \times Herbivores interaction from the perspective of the Herbivores factor accounts for the nonsignificance of the Herbivores main effect noted earlier.

The Region \times Pollinators \times Herbivores three-way interaction on both emerged and recruited seedlings per plot in 2001 was statistically nonsignificant (Table 1), thus denoting that the Pollinators \times Herbivores interaction should be considered homogeneous across regions. Separate interaction graphs for the three regions reveal a remarkable geographical constancy in the disordinal nature of this interaction on the number of both seedlings recruited (Fig. 3) and emerged (graphs not shown) per plot.

Discussion

Pollination studies have often compared seed production of plants exposed to natural pollination with those subject to pollinator exclusion or augmented pollination (33–36). Herbivory studies, on their part, have frequently revealed extensive herbivory on flowers or developing fruits, a situation particularly frequent among perennial herbs such as *H. foetidus* (37–41). Nevertheless, reductions in seed production with respect to some maximum potential value due to herbivores or insufficient pollination do not necessarily have to influence plant population dynamics, because seed production may exceed the habitat-specific requirements for maintaining average plant density (42–44). From an ecological viewpoint the critical question is whether reduced seed production caused by insufficient pollination or flower/fruit losses to herbivores actually translate into reduced seedling recruitment. This question can only be answered definitively by combining herbivore and pollinator exclusion experiments in the field with measurements of the effect in terms of recruited progeny. To date, this approach has been adopted by only a few investigations focusing on herbivores (reviewed in ref. 45) and, to our knowledge, by no study focusing on pollinators. Our study provides a simultaneous evaluation of the population effects of pollinators, herbivores, and their interaction in terms of recruited seedlings. Our results are especially strong, because the experiments were replicated both across separate regions and across populations within regions. Monitoring recruited seedlings until adulthood would have provided more precise estimates of the demographic effects of our experimental treatments (45). It is unlikely, however, that results of such longer-term study would have differed substantially from those reported here, because (i) mortality of *H. foetidus* seedlings tended to become negligible after the first summer past emergence in most of our study localities, and (ii) variation among plots in longer-term survivorship of the 1999 and 2000 seedling cohorts was random with respect to the plots' assignments to the Herbivores and Pollinators treatments.

Flower and fruit herbivory had detrimental population consequences for *H. foetidus*, as found also by some of the few previous investigations combining herbivore exclusion with measurements of subsequent seedling recruitment (refs. 46 and 47;

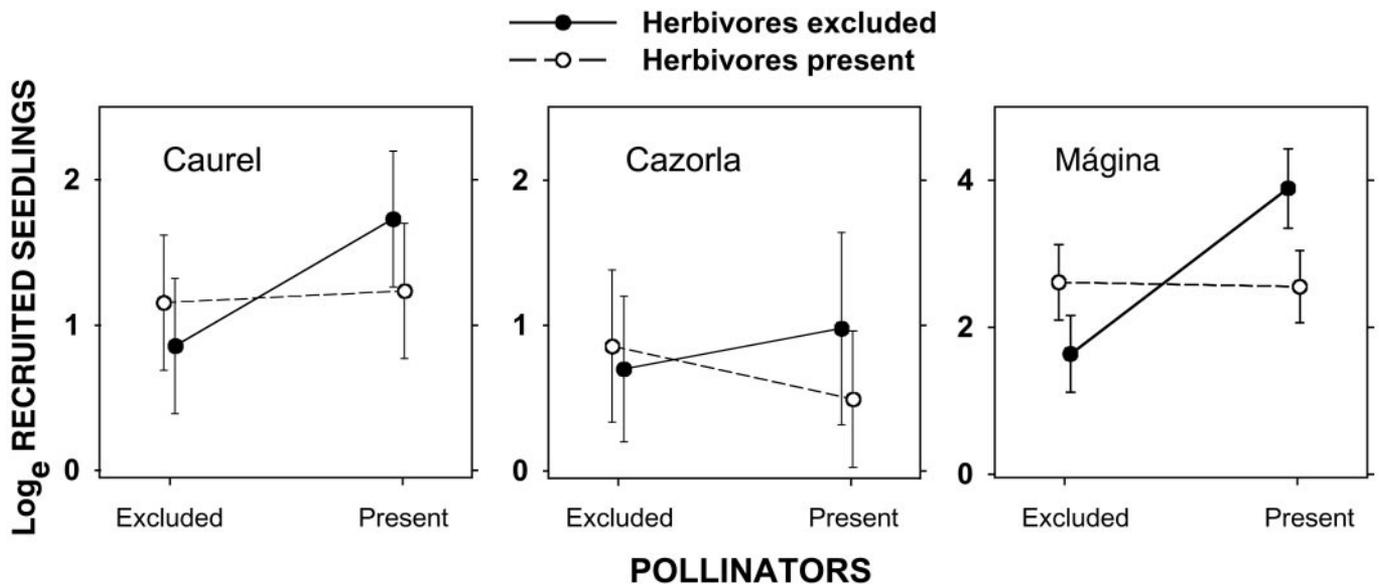


Fig. 3. Interaction graphs for the effects of Pollinators and Herbivores on the mean number of recruited seedlings of *H. foetidus* per experimental plot in 2001 for the three study regions (Fig. 1). Circles denote model-adjusted cell means with herbivores present (open circles, dashed line) or excluded (filled circles, continuous line), and vertical segments extend over ± 1 SE. Note the differences between regions in vertical scale.

review in ref. 45). Our results, however, differ in that the sign and magnitude of the Herbivores effect depended on the strength of the interaction with pollinators. Herbivory had detrimental population effects only in the presence of pollinators. When pollinators were excluded, the sign of the Herbivores effect was even reversed, and they had a small but significant positive effect on the number of emerged and recruited seedlings. (This reversal may have been caused by accidental pollination of some flowers by mice or lepidopteran larvae foraging on *H. foetidus* inflorescences inside pollinator exclosures, but this interpretation must remain speculative at present.) Pollinators, in turn, had a significant positive effect on number of seedlings only in the absence of herbivores and no effect when herbivores were present. Such reciprocal context dependence of the demographic effects of herbivores and pollinators has not been documented before for any plant species (but see ref. 3). The disordinal Pollinators \times Herbivores interaction remained remarkably constant in the three study regions, which supports its generality over a significant portion of the western European distribution range of *H. foetidus*.

A marked preference of herbivores for plants bearing abundant open flowers and/or developing fruits, eventually leading to a reduction in the number of seeds produced by these plants down to levels equivalent to those without pollinators, is the most parsimonious explanation for the disproportionate reduction in the number of seedlings in plots exposed to both pollinators and herbivores and for the lack of effect of pollinators on recruitment when herbivores were present. This interpretation is clearly supported by additional information on the differential impact of mice on developing fruits in our experimental Cazorla plots exposed to herbivores (equivalent data are not available for the other two regions). In these plots, the proportion of developing fruits eaten by mice was significantly higher in those exposed to pollinators (mean \pm SD = $65.4 \pm 28.3\%$, $n = 13$) than in those without pollinators ($22.7 \pm 31.3\%$, $n = 10$) [$F(1,20) = 11.1$, $P = 0.003$]. Furthermore, across plots the proportion of developing fruits eaten by mice was related directly to the proportion of carpels setting fruit [$F(1,21) = 58.5$, adjusted $R^2 = 0.72$, $P < 0.0001$]. These data show that, among plants exposed to herbivores, increases in pollination success predictably resulted in

greater proportional fruit losses to mice. A similar proximate mechanism accounted also for nonadditivity of effects of pollinators and herbivores on seed production in *Paeonia broteri* (Paeoniaceae), another perennial herb of southern Spain (3). In that species, browsing mammals fed selectively on the larger, many-seeded fruits of plants that had been exposed to pollinators and ignored the smaller, few-seeded fruits of plants with pollinators excluded, which led to seed production in presence of herbivores being eventually similar for plants with and without pollinators (3). Taken together, these results suggest the intriguing possibility of a complex link between naturally occurring spatio-temporal mosaics in pollinator abundance and concomitant variations in the population effects of flower and fruit herbivores.

The consistent disordinal interaction between Pollinators and Herbivores revealed by this study suggests a novel pathway for the adaptive correlated evolution of defensive (antagonism-related) and attractive (mutualism-related) characters in insect-pollinated plants that are subject to the impact of flower and fruit herbivores. Our experimental *H. foetidus* populations were made up of a mixture of individuals differing widely in their interaction with mutualists and antagonists. Rather than attempting to manipulate particular phenotypic traits of plants that would putatively influence their interaction with pollinators and herbivores, we directly manipulated the strength of the mutualistic and antagonistic interactions themselves. We simulated a situation where conspecific individuals exhibited all possible combinations of two independently varying (i.e., statistically uncorrelated across individuals by virtue of our factorial experimental design), discrete phenotypic “traits,” each of them influencing the individual’s success in its interaction with antagonists and mutualists. Herbivore exclusion mimicked some hypothetical highly efficient (i.e., minimal or no metabolic cost to plants and high effectiveness against herbivores) defensive trait, whereas pollinator exclusion mimicked some trait causing a considerable decline in pollinator attraction. This hypothetical scenario of independent variation of defensive and attractive traits across individuals is admittedly unrealistic in biological terms, because such decoupling is highly unlikely to occur frequently in nature (e.g., herbivore defense and pollinator

attraction may compete for plant resources and generate defense–attraction trade-offs). This circumstance, however, does not invalidate our interpretation of results in relation to the correlated evolution of herbivory- and pollination-related traits. In fact, the experiments were aimed precisely at recreating a hypothetically ancestral, artificial situation where attractive and defensive traits still covaried independently across plants as a way of assessing the likelihood of correlated evolution between mutualism- and antagonist-related characters. Interpreted in this particular context, our results indicate that individual plants possessing the particular combination of traits that simultaneously performed well in pollination and escape from herbivores enjoyed a disproportionate fitness advantage over individuals possessing any of the other three possible combinations of traits. This well defined, steep fitness gradient in the space defined by combinations of character states sets a distinct directionality toward the joint evolution of mutualism- and antagonism-related traits. Spurious associations among actual pollination- and herbivory-related traits of our study plants (e.g., inverse correlations derived from differential resource allocation to floral attractive structures and defensive secondary compounds) are unlikely to be found so consistently across many separate populations and regions, which further strengthens our conclusion. Our results therefore suggest that correlated evolution of traits enhancing the interaction with pollinators and traits improving defense from flower- and fruit-feeding herbivores may be the most likely shortcut available to solve the evolutionary dilemma between enhancing the interaction with mutualists while at the same time escaping from antagonists in insect-pollinated plants that are subjected to intense herbivory on reproductive structures. From this we infer that instances of

association between pollination- and herbivory-related traits mentioned in the Introduction (21–22, 23, 24) may frequently be explained as the outcome of adaptive correlated evolution of defensive and attractive traits driven by nonadditivity of fitness effects of pollinators and herbivores of the kind reported here for *H. foetidus* (also ref. 3 for *P. broteri*).

Pollinator × Herbivores interaction effects similar to those found here for *H. foetidus* should be particularly frequent whenever (i) herbivores cause extensive flower and fruit losses on attacked plants and (ii) herbivores are preferentially attracted to individual plants that have larger floral displays or crops of developing fruits because they had been favored by pollinators (e.g., because of their larger floral displays) and tend to avoid those with smaller fruit crops that have been relatively neglected by pollinators. Both circumstances seem to be particularly common among herbaceous plants, which frequently experience devastating levels of flower and fruit herbivory (3, 37–41, 48, 49) by insect and mammalian herbivores that tend to be quite selective and concentrate on individuals with the larger floral displays and/or fruit crops (17, 18, 41, 50). We hypothesize that adaptive correlated evolution of mutualism- and antagonism-related driven by correlational selection should be particularly frequent among herbaceous plants.

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