

FLORAL VARIATION IN THE GENERALIST PERENNIAL HERB *PAEONIA BROTEROI* (PAEONIACEAE): DIFFERENCES BETWEEN REGIONS WITH DIFFERENT POLLINATORS AND HERBIVORES¹

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This paper investigates the differences in floral phenotype in *Paeonia broteroi* (Paeoniaceae) in four populations at two distant mountainous regions in southern Spain. *Paeonia broteroi* flowers exhibit traits of a highly generalized pollination system, but previous studies have revealed that not all flower visitors are effective at pollen delivery. Plants differed between regions in the number of flowers per plant, petal size, number of stamens per flower, and ovules per carpel. Differences between regions could not be attributable to differences in the size structure of the plants. Flower visitors in the two regions differed in assemblage and body size at all the spatiotemporal scales. Larger visitors were more effective as pollinators in the region with the largest and more rewarding (as measured by the number of stamens) flowers, suggesting that pollinators may create opportunities for selection of certain floral traits. In contrast, the two regions did not differ in the probability of damage by herbivores, which did not select flowers based on any of the measured traits, nor affected maternal fecundity. Despite the differences in flower phenotype, potential maternal fecundity, and pollinator effectiveness, plants did not differ between regions in seed production. The role of pollinators as determinants of the differences between regions in floral phenotype, through male and female reproductive success, is discussed. Also, alternative explanations to divergence are addressed, with special reference to the patterns of resource allocation between sexual functions and genetic drift.

Key words: floral traits; floral variation; herbivores; *Paeonia*; pollinators; selection.

Evolutionary biologists have assessed the adaptive value of floral traits in relation to pollinating agents since the early 20th century, and numerous authors have presented evidence suggesting that the evolutionary responses of zoophylous flowering plants to their pollinators have yielded specialized flowers. Flower specialization, however, is not necessarily a universal outcome of the interaction between flowering plants and their pollinators (see Waser et al., 1996; Gómez and Zamora, 1999), but the information on the evolutionary dynamics of generalists species (i.e., those exhibiting unspecialized, unrestricted floral phenotypes, with exposed floral rewards) is still scarce (but see Gómez and Zamora, 1999, 2000).

Zoophylous plants with generalized pollination systems may also be subjected to selection by pollinators. If flower visitors preferentially select particular floral phenotypes within populations, then spatiotemporal variation in visitors assemblage may cause divergence in floral phenotype (Wilson and Thomson, 1996; Gómez and Zamora, 2000). Further, recent research has focused on how herbivores may interfere with evolutionary responses of flowers to pollinators. For example, herbivory on vegetative parts may alter the balance of resources available for the expression of sexual functions, while herbivory on flowers may affect their attractiveness or pollen presentation (Thomson et al., 2000). Finally, herbivory on developing or

matured fruits may cancel the results of selection by pollinators (e.g., Mutikainen and Delph, 1996; Strauss, Conner, and Rush, 1996; Brody, 1997; Strauss, 1997; Lethilä and Strauss, 1999; Herrera, 2000; Mothershead and Marquis, 2000).

This paper analyzes the variation in floral phenotype in *Paeonia broteroi* Boiss. & Reut., (Paeoniaceae), and its relation to fitness via maternal fecundity in two areas with different pollinators and herbivores. *Paeonia broteroi* is a hermaphroditic herb that produces only a few flowers per season (1–8, Sánchez-Lafuente et al., 1999). The flowers exhibit a highly generalized pollination system, with bowl-shaped, large, unrestrictive corollas and exposed pollen reward (Stern, 1946; see Fig. 1). Nevertheless, previous studies have demonstrated that not all flower visitors are equally efficient at pollen delivery (Sánchez-Lafuente et al., 1999), which is a precondition for specialization (Schemske and Horvitz, 1984; but see Aigner, 2001). I, therefore, hypothesize that selective pressures exerted by pollinators may have resulted in variation in floral traits in regions with different pollinator faunas.

Specifically, I address the following questions: Do floral traits, visitors assemblage, herbivory damage, and maternal fecundity differ seasonally and regionally? Do floral visitors define an “opportunity for selection” by preferentially visiting flowers of particular phenotypes? Are herbivores able to cancel the “opportunity for selection” created by efficient pollinators? Are the interactions among plants, pollinators and herbivores consistent seasonally and regionally? Are particular floral phenotypes more successful than others at seed production?

MATERIALS AND METHODS

Study sites—The study was conducted in two mountainous areas in southern Spain—Sierra de Cazorla and Sierra de Jaén—which are separated by ca. 150 km. Two populations were selected in Cazorla: Roblehondo (RH) and Fuente de la Umbra (FU). The latter is surrounded by a fence to exclude ungulates. These are the same populations used earlier by Sánchez-Lafuente

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Fig. 1. Flowering *Paeonia broteroi* plants in (A) Cazorla and (B) Jaén. Both photos presented at the same scale. Circles and arrows in (A) indicate the location of two flower visitors (*Halictus scabiosae*, [Hymenoptera: Halictidae]). Note the visitors' size in relation to the flowers' size.

et al. (1999) and Herrera (2000). The populations were studied during the flowering seasons of 1994 and 1995. Another two populations were selected in Jaén: Cañada de las Hazadillas (CHZ) and Llanos de Navalpo (NAV). These populations were studied during the flowering seasons of 1997 and 1998. The populations in Jaén are in a mixed pine and oak forest located between 1000–1200 m (above sea level) a.s.l. They are separated by ca. 3 km and each has between 120 and 160 plants.

Overall, 566 plants were tagged: 221 in Cazorla (1994: RH = 53, FU = 48; 1995: RH = 82, FU = 38) and 345 in Jaén (1997: CHZ = 100, NAV = 99; 1998: CHZ = 70, NAV = 76). To avoid later differences among plants in their ability to set fruit or to attract pollinators because of early herbivory events (e.g., Strauss, Conner, and Rush, 1996; Krupnick, Weis, and Campbell, 1999; Lethilä and Strauss, 1999), I initially disregarded plants with signs of herbivory on vegetative parts or flower buds.

Plant natural history—*Paeonia broteroi* (Fig. 1) is a perennial herb that usually grows in the understory of oak and pine woods. Plants consist of one to several leafy stems that sprout every season from tuberous roots. Usually, each stem bears a single, terminal flower bud, although some stems may not produce flowers at all. In the study populations the number of flowers per plant ranges up to eight, but most plants produce a single flower per season. Flowers are large (6–12 cm in diameter when open) and actinomorphic, with five to eight dark pink petals. Flowering extends from mid-April to early June, depending on the region and population. Extrafloral nectaries occur at the base of an elongated sepal outside the perianth. Flowers are protogynous and apocarpous and contain ca. 170 stamens that produce copious pollen (the main reward for flower visitors). Stigmas are wet and fleshy and are borne directly on the top of the carpels. They remain receptive for ca. 4 d if unpollinated. Flowers are self-compatible, but pollination services are required for substantial seed production (Sánchez-Lafuente et al., 1999). Bees are the main pollinators, although a very diverse insect assemblage, including beetles and ants, visit receptive flowers (Sánchez-Lafuente et al., 1999). Seed maturation takes ca. 10–16 wk.

Herbivory is the main risk for plant fecundity; however, its incidence can differ radically between regions (A. M. Sánchez-Lafuente, unpublished data). Plants are consistently subjected to severe herbivory by ungulates in Cazorla,

but not in Jaén. Herbivory by invertebrates (mainly Lepidoptera larvae and Orthoptera) occurs at the two study regions, but the effects are less than those caused by ungulates.

Floral traits—Floral variation within and between regions was assessed for five traits measured from each plant: petal size (length of the longest petal, ± 0.1 mm), number of stamens per flower, number of carpels per flower, carpel size (length from the basal disc to the top of the longest carpel, ± 0.1 mm), and number of ovules per carpel (estimated after fruit maturation as the total number of ovules divided by the number of carpels). These measures integrate two different sets of variables. The first two measures may account for phenotypic variation in traits related to flower attractiveness (size and reward). The remaining three may account for variation in traits related to maternal fecundity.

Plant size (length of each flowering ramet ± 0.1 cm) and number of flowers per plant were also registered. Plant size may be an estimator of vigor or age and may be related to flower size. As for the number of flowers per plant, while most plants in the study regions produce a single flower each season (i.e., “plant = flower” in most cases), there may be differences between seasons in the number of flowers produced by particular plants (A. M. Sánchez-Lafuente, unpublished data). When a plant produced more than one flower per season, I averaged the plant and flower sizes for all the flowering ramets produced by that plant.

Composition and abundance of the visitor assemblage—Composition and abundance of the visitors assemblage were assessed by censuses on 20–40 single-flowered plants in each population and season (overall, Cazorla: $N = 61$; Jaén: $N = 58$, most plants used were the same in both seasons). Single-flowered plants were selected because they are the closest to the modal number of flowers per plant and because differences in allocation of resources for reproduction between flowers in multiflowered plants may preclude a comparison of the results obtained from plants with a different number of flowers.

Censuses lasted for 3 min per flower and were conducted from 0900 to 1600 (Greenwich mean time) from the day after anthesis until stigmas were no longer receptive. Each day a plant was randomly chosen for the first count, then the rest were sequentially monitored several times a day. During each

TABLE 1. Relative abundance (as percentage of total number of visits to *Paeonia broteroi* in each season, population, and region) of the different groups of flower visitors recorded during regular censuses.

Flower visitors	Cazorla				Jaén			
	FU		RH		CHZ		NAV	
	1994	1995	1994	1995	1997	1998	1997	1998
<i>Andrena</i> spp.	9.24	13.66	5.48	8.33	8.00	1.32	8.76	1.80
<i>Apis mellifera</i>	—	—	—	—	34.67	40.79	21.16	27.93
<i>Bombus terrestris</i>	—	—	0.68	0.93	22.67	27.63	25.29	36.04
Halictidae	39.10	32.24	36.99	40.47	30.66	30.26	18.68	21.62
Formicidae	33.65	46.99	49.32	47.22	—	—	—	—
Other Hymenoptera	0.71	—	—	—	—	—	7.93	6.31
Coleoptera	17.30	7.11	7.53	2.78	4.00	—	16.20	6.30
Other groups	—	—	—	0.27	—	—	1.98	—

Note: FU = Fuente de la Umbría, RH = Roblehondo, CHZ = Cañada de las Hazadillas, NAV = Llanos de Navalopo.

census, I noted the number and species (or genus, if the species was unknown) of the visitors to each focal flower. Overall, 2225 censuses were carried out. The plants that were censused were a subset of those measured morphologically. The only difference in the measurement of the censused plants was that the number of stamens per flower was recorded once the stigmas were no longer receptive, in order to avoid modifying the behavior of the flower visitors.

To assess the between-region differences in body size of the main visitors, a sample of them was measured (body length ± 0.01 mm). Insects from Cazorla ($N = 134$) were measured in 1997 at the Estación Biológica de Doñana, Sevilla, Spain, from collections maintained by Carlos M. Herrera. Insects from Jaén ($N = 119$) were collected in the field during the 1998 flowering season and measured in the laboratory at the University of Jaén, Jaén, Spain. Ants were not measured, because although they were the most abundant flower visitors in Cazorla, they have no effect on seed production (Sánchez-Lafuente et al., 1999).

Herbivory damage—*Paeonia broteroi* experiences herbivory by vertebrates and invertebrates. Plants may be consumed by wild ungulates in Cazorla and by domestic livestock in Jaén. Consumption by vertebrates usually occurs early in the season, when plants are sprouting, and results in complete removal of aboveground parts. Hence, herbivory by vertebrates precludes further development of vegetative or reproductive structures that season and, consequently, the estimation of floral phenotype, breeding success, or any interaction with biotic agents. Given the drastic damage vertebrates are capable of inflicting, I covered the study plants at RH population with large mosquito-net bags, which usually discourage ungulates, just after sprouting. The bags were removed when the plants had fully developed and the chance of complete destruction by vertebrates was reduced. Unlike vertebrates, invertebrates only partially consume aboveground parts. Noctuid larvae and grasshoppers are the most common invertebrate herbivores, consuming buds and flowers at different stages during the flowering season.

At the end of fruiting, fruit herbivory was scored for all tagged plants as a measure of the reduction in fitness (estimated from maternal fecundity; see next section). Damage was estimated as follows: none (fruit appears intact), moderate (<20% of the fruit consumed), intermediate (between 20 and 50% consumed), or severe (>50% consumed). It was not possible to determine the number of seeds consumed from those remaining intact in the damaged fruits, and therefore damage was estimated as a qualitative rather than a quantitative variable.

Maternal fecundity as a measure of plant fitness—For the purpose of this study, plant fitness resulting from the interactions of plants with biotic agents was evaluated from maternal fecundity (although incomplete, maternal fecundity is a measure that may be assumed to be correlated with total plant fitness; e.g., Schemske and Horvitz, 1989; Herrera, 1996). Maternal fecundity was estimated from two variables: the number of seeds initiated and the number of seeds matured. The number of seeds initiated is considered an early estimator of the outcome of pollination. Initiated seeds are fertilized ovules that

begin maturation but are eventually aborted. When carpels dehisce, these initiated seeds can be identified as large, flat ovules, ranging in color from red to brown. The second variable, the number of seeds matured, is an estimator of plant fitness gained through the female function. These seeds are identified inside the dehiscent fruit as spherical, plump, large, and black. The final number of seeds matured is what is left after the number of seeds initiated undergo losses owing to several causes (e.g., herbivory, seed abortion because of inbreeding depression, or resource limitation).

Data analyses—Unless otherwise stated, all statistical analyses reported here were performed with SAS 8.02 statistical package.

Differences in plant size and floral traits between seasons, populations, and regions were analyzed by generalized linear mixed models (e.g., Littell et al., 1996). Response variables were modeled according to their error distribution and default link function. Season was nested within population (random factors) and population within region (fixed factor).

Covariation among floral traits was examined by principal components analysis (PCA). I analyzed each population separately in order to detect if any patterns found differed between populations within regions.

At least 25 different insect species could be observed visiting *P. broteroi* flowers (see also Sánchez-Lafuente et al., 1999). Since many of the insect species were only rarely observed, I grouped flower visitors, for the purpose of the analyses, by pooling species in the same genera and of a similar body size into the lowest common taxon. Thus, genera *Halictus* and *Lasioglossum* were grouped as Fam. Halictidae, several *Andrena* bee and beetle species were grouped as *Andrena* spp. and Coleoptera, respectively, genera *Lasius* and *Camponotus* were grouped as Fam. Formicidae, and a number of bee species as Other Hymenoptera. Overall, the number of insect categories in analyses was reduced to seven (Table 1) accounting for more than 99% of all visitors recorded.

Differences in abundance and composition of flower visitors were then tested as the probability that each focal flower was visited during a standard census by each visitor group (binomial response: visited vs. not visited), weighted by the relative abundance of each group in each season, population, and region. Differences in body size of the main visitors were analyzed by two-way ANOVA (region and pollinator as fixed factors). Insects used for body measurement were grouped according to the same criteria described above. Similarly, differences between regions in herbivory damage were analyzed as the probability that each focal flower suffered different levels of damage. The dependent variable was modeled as binomial (number of flowers damaged in each level divided by the total number of flowers in each season, population, and region).

The role of floral traits as determinants of pollinator and herbivore attraction was analyzed by considering visitation rate (visits per flower per hour, normally distributed) and herbivory damage (multinomial distribution) as dependent variables and regressing these variables on each trait for each study region. Visitation rate was analyzed by multiple linear regression and herbivory damage by logistic multiple regression. Statistical significance of the regression coefficients (selection gradients; Lande and Arnold, 1983) was as-

TABLE 2. (A) Floral traits measured at all populations and seasons (means ± 1 SE and coefficients of variation [CV]). (B) Differences between seasons, populations, and regions in absolute and relative estimators of plant size and flower size and maternal fecundity of *Paeonia broteroi*. Given the consistent departure from normality of the number of carpels per flowers, *P* values were calculated by randomization with 5000 repetitions. (C) Summaries of simple regressions between plant size and each of the traits that differed between regions.

A) Traits	Cazorla			Jaén		
	<i>N</i>	Mean ± 1 SE	CV (%)	<i>N</i>	Mean ± 1 SE	CV (%)
PlantSiz	158	45.4 ± 0.9	23.44	345	41.8 ± 0.4	16.98
FlowPlant	221	1.4 ± 0.1	61.44	345	1.0 ± 0.1	7.09
PetalSiz	158	46.4 ± 0.7	18.28	345	52.4 ± 0.4	15.58
NStams	113	168.4 ± 3.7	23.30	265	180.2 ± 2.4	21.73
CarpSiz	120	12.8 ± 0.3	26.55	345	12.2 ± 0.2	26.88
NCarps	221	2.0 ± 0.1	35.12	345	1.2 ± 0.1	31.67
OvulCarp	221	19.5 ± 0.4	29.47	295	16.9 ± 0.3	28.06

B) Traits	Fixed factor		Random factors			
	Region		Population (Region)		Season (Population)	
	χ ²	<i>P</i>	Wald Z	<i>P</i>	Wald Z	<i>P</i>
PlantSiz ^a	0.55	0.46	0.06	0.95	1.35	0.18
FlowPlant ^b	17.64	0.001	0.09	0.85	1.41	0.16
PetalSiz ^a	6.57	0.02	0.09	0.70	1.52	0.13
NStams ^a	3.90	0.05	0.13	0.91	1.39	0.16
CarpSiz ^a	0.53	0.47	0.04	0.97	1.29	0.20
NCarps ^c	1.06	0.45	0.27	0.79	1.15	0.26
OvulCarp ^a	5.20	0.03	0.13	0.90	1.09	0.27

C) Traits	Cazorla				Jaén			
	1994 (<i>N</i> = 75)		1995 (<i>N</i> = 38)		1997 (<i>N</i> = 119)		1998 (<i>N</i> = 131)	
	<i>b</i>	<i>r</i> ²	<i>b</i>	<i>r</i> ²	<i>b</i>	<i>r</i> ²	<i>b</i>	<i>r</i> ²
PetalSiz	0.50***	0.35	0.46	0.20	1.01***	0.29	0.93***	0.21
NStams	0.49	0.01	0.57	0.06	2.01***	0.25	1.39**	0.24
OvulCarp	0.20*	0.05	0.11	0.03	0.13	0.03	0.14**	0.05

Note: PlantSiz = plant size, FlowPlant = number of flowers per plant, PetalSiz = petal size, NStams = number of stamens per flower, CarpSiz = carpel size, NCarps = number of carpels per flower, OvulCarp = number of ovules per flower. Error distribution: ^a normal, ^b Poisson, ^c random. * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001.

essed by an accelerated bootstrap (Efron and Tibshirani, 1993) with 1000 repetitions. The relationships between floral traits and maternal fecundity (seeds initiated and matured) were estimated in a similar way. I also included visitation rate and herbivory damage as independent variables, in order to account for their presumed beneficial and detrimental effects on plant fecundity. The quadratic terms of the floral traits were also included in the analyses, in order to detect nonlinear relationships. These analyses were carried out for each season in each study region and also on data pooled from all seasons and populations in each study region. The purpose of pooling was to detect if, despite the presumed spatiotemporal variation in the composition and abundance of pollinators and herbivores, any selection gradients were statistically significant, thus acting as feasible indicators of consistent selection. To pool both seasons from each region I calculated the mean values of floral traits, visitation rates, herbivory damage, and seeds initiated and produced for the same plants between seasons. I only used those plants flowering in both study seasons in each region. Analyses carried out with mean values that yielded similar results to those carried out on each season alone indicated consistency between seasons. If the significant effects disappeared when analyses were done with mean values, I assumed that there was no consistency between seasons.

I used path analysis and structural equation modeling (SEM) (Crespi and Bookstein, 1989; Mitchell, 1992, 1994), with values pooled for both seasons for each region (with the same criteria indicated above), to test the best hypothetical causal model that explained the patterns found in previous analyses. I constructed a basic model and a number of alternative ones (nested models; Mitchell, 1992, 1994) and compared them. The criteria I used to select the best-fit model were similar to those described by Gómez and Zamora (2000). Statistical significance of the path coefficients was assessed from 95% confidence intervals for the mean coefficients, obtained by an accelerated bootstrap (Efron and Tibshirani, 1993) with 5000 repetitions. Coefficients were considered significant if their confidence interval did not include zero.

RESULTS

Differences between regions in floral traits—Initially, the analysis of differences between regions in floral traits included plant size as a covariate. However, the interaction region × plant size was statistically significant in all cases, suggesting that the slope of the relationship between plant size and floral traits differed between regions. This significant interaction violates the parallelism assumption of the analysis of covariance (ANCOVA; e.g., Sokal and Rohlf, 1981), thus plant size was removed as a covariate and analyzed separately by simple regression. The results of the regression analyses indicate that plants did not differ between regions in size, but they did in the number of flowers per plant (plants produced more flowers in Cazorla; Table 2). Flowers were larger and developed more stamens in Jaén; however, they produced more ovules per carpel in Cazorla (Table 2). Considering that pollen is the main reward for flower visitors in *P. broteroi* and that flower size may affect visitation rate, these findings indicate that flowers in Jaén allocated more resources to structures related to advertising and reward. In Cazorla, however, the combination of more flowers per plant and more ovules per carpel indicated a higher potential female fecundity. No differences were found in plant size or floral traits between seasons within populations or between populations within regions.

Petal size was related to plant size in all seasons and regions (except Cazorla in 1995). The number of stamens was related to plant size only in Jaén. The rest of the relationships were either nonsignificant or the variance explained was negligible (Table 2).

TABLE 3. Results of the principal components analysis on flower measures of *Paeonia broteroi* in (A) Cazorla and (B) Jaén.

A) Traits	RH (N = 65)			FU (N = 48)	
	PC1	PC2	PC3	PC1	PC2
Cazorla					
PetalSiz	0.90	0.03	-0.12	0.71	0.36
NStams	0.70	-0.53	0.25	0.80	-0.18
CarpSiz	0.01	0.03	0.98	0.47	-0.57
NCarps	-0.07	0.86	0.01	-0.01	0.77
OvulCarp	0.27	0.78	0.21	0.06	0.76
Cumulative variance	33.80	61.03	81.46	29.68	52.08
B) Traits	CHZ (N = 175)		NAV (N = 90)		
	PC1		PC1		
Jaén					
PetalSiz		0.77		0.73	
NStams		0.72		0.85	
CarpSiz		0.82		0.77	
NCarps		0.56		0.62	
OvulCarp		0.60		0.44	
Cumulative variance		48.15		58.10	

Note: Fu = Fuente de la Umbría, RH = Roblehondo, CHZ = Cañada de las Hazadillas, NAV = Llanos de Navalopo. Seasons at each population pooled by averaging measured traits between seasons (see text). Only eigenvalues ≥ 1 were retained. Heaviest factor loadings are presented in bold. See Table 2 for abbreviations of floral traits.

Coefficients of variation of the measured traits were moderate to high (Table 2; see Cresswell, 1998), and there were no differences between regions ($t = 1.57$, $P = 0.14$, $df = 12$). These findings suggest that (1) the variability in traits in each region occurred within a similar proportional range and (2) a spatiotemporal consistency in floral phenotype within regions.

Covariation among floral traits—Principal components analysis for Cazorla extracted three factors for RH and two

TABLE 4. Results of the generalized linear mixed model on differences in pollinator abundance between seasons, populations, and regions for *Paeonia broteroi*.

Effects	df	χ^2	P
A) Random			
Season (Population)	4, 4692	3947.43	$\ll 0.001$
Population (Region)	2, 4692	1196.44	$\ll 0.001$
Fixed			
Region	1, 4692	1345.52	$\ll 0.001$
Pollinator	6, 4692	5859.56	$\ll 0.001$
Region \times pollinator	4, 4692	659.85	$\ll 0.001$
B) Random			
Season (Population)	4, 18	0.06	0.99
Population (Region)	2, 18	0.06	0.94
Fixed			
Region	1, 18	0.08	0.78
Level of damage	3, 18	29.17	$\ll 0.001$
Region \times level of damage	3, 18	0.71	0.54

Note: In part A, tests of region, population, and season refer to the probability that a plant received at least one visit during a 3-min census by any visitor group. Test of pollinator refers to the probability that a visit was made by a particular visitor group. The response variable was modeled as a binomial (visited vs. not visited). The relative abundance of each visitor group was used as a weighting variable. In part B, the results of a similar analysis on differences in the probability that developing or matured fruits received different levels of damage by herbivores. The response variable was modeled as a binomial (flowers damaged in each level divided by the total number of flowers).

for FU (Table 3). The first two factors for each population were similar. The first one loaded on traits related to flower advertising and reward (petal size and number of stamens per flower). The second one accounted for variation in traits related to maternal fecundity (number of carpels per flower and number of ovules per carpel). The third factor for RH accounted for carpel size (larger carpels may contain more ovules or larger ovules that may eventually produce larger seeds; A. M. Sánchez-Lafuente, unpublished data). For Jaén, only one factor was extracted for each population, loading mainly on traits accounting for flower advertising and carpel size (Table 3B). Thus, variation in floral phenotype in Cazorla fluctuated along two independent lines. At Jaén all floral traits seemed to covary along the same line of variation. These results suggest a greater phenotypic integration of flowers in Jaén.

Composition and abundance of the visitor assemblage

Overall visitation rates differed between regions and were higher in Cazorla (Cazorla: mean ± 1 SE = 0.122 ± 0.004 , $N = 859$ visits; Jaén: mean ± 1 SE = 0.089 ± 0.004 , $N = 383$; Table 4). Differences were also found between seasons in each population and between populations in each region. There were also differences in abundance of the visitors groups (measured as the probability that a visit was made by members of each group); furthermore, the relative abundance of the different visitors groups was not the same across regions (significant region \times pollinator interaction; Table 4A, Fig. 2A). Tests of simple main effects (SLICE option in LSMEANS in Proc MIXED; SAS, 1996), revealed that the most abundant visitors in Cazorla were ants (mainly *Lasius niger* L.) followed by solitary bees (Halictidae [*Halictus* sp. and *Lasioglossum* sp.], and *Andrena* spp.) (Fig. 2A, Table 1). Other hymenopterans were rarely observed (e.g., *Xylocopa violacea* L. and *Scolias* sp.). In Jaén, the most abundant visitors were bees (both solitary and social), including *Apis mellifera* L., and *Bombus terrestris* L. (the latter was almost absent in Cazorla; Fig. 2A). A number of species were also occasionally recorded in Jaén (e.g., *Anthophora* sp., *Eucera* sp.,

TABLE 5. Results of the mixed ANOVA on differences in plant fecundity of *Paeonia broteroi* between seasons (nested in population) and populations in each region.

Variables	Fixed factor		Random factors			
	Region		Population (Region)		Season (Population)	
	χ^2	<i>P</i>	Wald Z	<i>P</i>	Wald Z	<i>P</i>
Seeds initiated	0.01	0.98	0.21	0.84	1.58	0.06
Seeds matured	0.02	0.91	0.35	0.73	1.35	0.08

Colletes sp. [included in Other Hymenoptera], and *Forficula auricularia* L. [Dermaptera].

There were significant, although subtle, differences between regions in body size of visitors (Cazorla: 12.25 ± 0.34 mm; Jaén: 12.78 ± 0.24 mm; $F_{1,252} = 5.83, P < 0.02$). *Bombus terrestris* was the largest visitor ($F_{3,252} = 88.29, P < 0.001$), followed by *Andrena* spp., *A. mellifera* (absent in Cazorla), and halictid bees. The region × pollinator interaction was significant ($F_{2,252} = 31.90, P < 0.001$), and tests of simple main effects revealed that mean body length of *B. terrestris* and halictid bees differed between regions (Fig. 2B), while this was not the case for *Andrena* spp.

In summary, these results indicate that the most abundant flower visitors in Jaén were larger than the most abundant ones in Cazorla.

Differences in herbivory damage—Plants were not consumed by ungulates or livestock in either season or region, and only herbivory by invertebrates was evident. Plants did not differ in the probability of being damaged on any of the spatiotemporal scales considered (Table 4B). Further, the probability of receiving different intensities of damage did not differ between regions (region × damage interaction; Table 4). Thus, 84.84% of plants in both regions escaped from herbivory, and only a small percentage suffered any damage (6.16%, 4.26%, and 4.74% with moderate, intermediate, and severe damage, respectively).

Differences in plant fecundity—There were no spatiotemporal differences in seeds initiated or seeds matured per flower (Table 5). Despite the differences in potential plant fecundity and visitors assemblage, the number of seeds initiated (SI) and matured (SM) were roughly the same in both regions (SI_{Cazorla}: 17.83 ± 1.68; SI_{Jaén}: 20.68 ± 1.67; SM_{Cazorla}: 10.37 ± 0.87; SM_{Jaén}: 11.05 ± 0.86; $N_{Cazorla} = 108, N_{Jaén} = 103$). Available data (not shown) indicate that seed production was not pollen

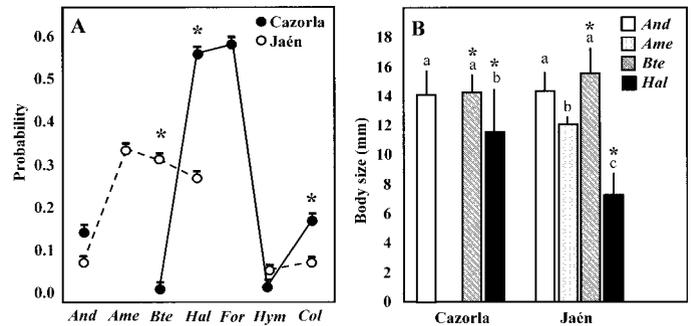


Fig. 2. (A) Probability (mean + 1 SD) that a *Paeonia broteroi* flower was visited, during a standard census, by the different insect groups considered. Asterisks indicate the groups that differed significantly in abundance between regions. (B) Differences (mean + 1 SD) in body size among the main visitors species or groups considered at each study region. Asterisks indicate the groups that differed significantly in size between regions. Groups with a different letter differ significantly in size within regions. Visitors: *And* = *Andrena* spp., *Ame* = *Apis mellifera*, *Bte* = *Bombus terrestris*, *Hal* = *Halictidae*, *For* = Formicidae, *Hym* = Other Hymenoptera, *Col* = Coleoptera.

limited in any population and season (Sánchez-Lafuente et al., 1999; A. M. Sánchez-Lafuente, unpublished data).

Selection on floral traits and effects on plant fecundity—I approached the evaluation of selection on floral traits in two steps: (1) determining the selection by pollinators and herbivores as a function of the traits measured; (2) determining the outcome, in terms of plant fecundity, of such selection.

The first step revealed a positive and significant effect of variables related to flower advertising and reward on visitation rates in the two regions (Table 6). For herbivory, none of the measured traits was significant in either region, for each season alone or for both seasons pooled (Cazorla: $\chi^2_{1994} = 6.32, \chi^2_{1995} = 7.83, \chi^2_{\text{pooled}} = 8.19$; Jaén: $\chi^2_{1997} = 8.32, \chi^2_{1998} = 11.90, \chi^2_{\text{pooled}} = 11.36$; see Table 6 for sample sizes). Thus, flower visitors seemed to be attracted preferentially by larger and more rewarding flowers, while according to the traits measured, no selection was exerted by herbivores. Some quadratic terms were also positive and significant, particularly the number of stamens per flower, suggesting disruptive selection. For each significant quadratic term, I analyzed its relationship with the residuals of a regression analyses that did not include the significant quadratic term. In all cases the relationship was not significant (results not shown) suggesting that the relationship

TABLE 6. Floral traits of *Paeonia broteroi* as determinants of visitation rates in Cazorla and Jaén.

Traits	Cazorla			Jaén		
	1994 (N = 61) β	1995 (N = 47) β	Pooled (N = 45) β	1997 (N = 45) β	1998 (N = 58) β	Pooled (N = 45) β
Petalsiz	0.38**	—	—	—	0.34***	—
NStams	—	0.72***	—	—	—	—
NStams ²	—	—	0.30**	0.34*	0.74***	0.46**
NCarps ²	0.33*	—	—	—	—	—
<i>F</i>	5.89**	30.80***	5.95*	6.05**	24.08***	18.77***
<i>r</i> ²	0.23	0.50	0.20	0.26	0.62	0.26

Note: Model fitted by linear multiple regression. Visitation rate (visits per flower per hour) was the dependent variable. Original variables and their quadratic terms were included as independent. The statistical significance of the standardized coefficients (β) was assessed by bootstrapping with 5000 repetitions. Only significant coefficients are presented, to shorten the output. See Table 2 for abbreviation of floral traits.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

TABLE 7. Floral traits and biotic agents as determinants of maternal fecundity of *Paeonia broteroi* in (A) Cazorla and (B) Jaén.

A) Cazorla Traits	1994 (N = 61)		1995 (N = 47)		Pooled (N = 45)	
	Seeds initiated β	Seeds matured β	Seeds initiated β	Seeds matured β	Seeds initiated β	Seeds matured β
CarpFlow	0.53***	0.39**	0.37***	0.38**	0.48**	0.29*
OvulCarp	—	—	—	0.44**	—	—
OvulCarp ²	—	—	0.58***	—	0.25*	0.21*
F	3.71*	3.90*	12.22***	7.24***	13.29***	6.71**
r ²	0.18	0.11	0.53	0.38	0.31	0.13
B) Jaén Traits	1997 (N = 45)		1998 (N = 58)		Pooled (N = 45)	
	Seeds initiated β	Seeds matured β	Seeds initiated β	Seeds matured β	Seeds initiated β	Seeds matured β
CarpSiz	0.24*	—	—	—	—	—
CarpFlow	—	—	0.55***	0.46***	—	—
OvulCarp	0.39***	0.36***	—	0.42***	0.39***	—
CarpSiz ²	—	0.21*	—	—	—	0.22*
CarpFlow ²	0.44***	0.33**	—	—	0.54***	0.41***
OvulCarp ²	—	—	0.39***	—	—	0.40***
Visit. rate	0.21*	—	0.22*	—	0.15*	—
F	22.94***	24.11***	30.79***	22.77***	54.62***	35.95***
r ²	0.60	0.68	0.51	0.43	0.52	0.51

Note: Models were fitted by linear multiple regression. Seeds initiated and matured were square-root transformed to meet normality. Original variables and their quadratic terms were included as independent. The statistical significance of the standardized coefficients (β) was calculated by bootstrapping with 5000 repetitions. Only significant coefficients are presented, to shorten the output. See Table 2 for abbreviations of floral traits.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

between the dependent variables and the traits was actually directional but nonlinear.

The second step revealed that only traits related to maternal fecundity significantly affected seed production (Table 7). Seed initiation was positively, albeit weakly, related to visitation rate only in Jaén, while the effect of herbivores on maternal fecundity was not significant in any season or population. As in the previous analyses (see above), none of the quadratic terms contributed to explain the dependent variables (seeds initiated and seeds matured), suggesting a directional, nonlinear relationship between the dependent variables and the traits. Overall, the significance level and the proportion of the variance explained by the models in Cazorla were much lower than in Jaén.

Path analyses—Figure 3A depicts the basic unparameterized path model proposed for the causal relationships among variables. According to the results from previous analyses, this model assumes that traits associated with flower advertising and reward may affect visitation rates, while those associated with maternal fecundity may affect seed initiation. Visitation rates were assumed to affect seed initiation and the latter to affect seeds matured (Fig. 3A). No herbivory effects were included, because they did not appear to be related to floral traits or to affect seed production. The alternative models considered were all possible combinations of flower-advertising traits, related to visitation rates, and all possible combinations of fecundity traits, related to seed initiation. The relationships between visitation rate, seeds initiated, and seeds matured were maintained in all models. Overall, 21 models were tested for each region (not shown).

The most parsimonious models are depicted in Fig. 3B, C (Cazorla: $\chi^2 = 3.62$, $df = 5$, $P = 0.60$; Jaén: $\chi^2 = 5.20$, $df = 5$, $P = 0.39$). Although similar, they differ in the selected component of maternal fecundity that best explained seed initiation: number of ovules per carpel in Cazorla and number of carpels per flower in Jaén. These variables may be considered

as the two components of the overall female reproductive potential (i.e., total number of ovules per flower; see Sánchez-Lafuente et al., 1999). Visitation rates were related to the number of stamens per flower in both regions, but the effect of visits on seed initiation was significant only in Jaén. Thus, while both models explained a similar proportion of the variance in seed production (Cazorla: 56%; Jaén: 60%), the model for Jaén explained almost twice the variance in seed initiation than did the model for Cazorla (Cazorla: 20%, Jaén: 38%).

DISCUSSION

Several floral traits of the pollination generalist *P. broteroi* differed between two regions in southern Spain which, in turn, varied in composition and abundance of flower visitors on several spatiotemporal scales. The regions differed in the relationships between plant size and some floral traits. Larger plants produced larger flowers, but only within the range of flower sizes found in the particular region. This suggests that differences in floral traits do not depend exclusively on differences in plant size. In particular, they fail to explain the different covariation patterns among floral traits found at each region, and other alternatives (e.g., the role of biotic agents) need to be considered to assess variation in floral traits between regions.

Known limitations and assumptions—This study has been carried out for two consecutive seasons in each population and region. Considering the fluctuations and changes that long-lived plant species, such as *P. broteroi*, can undergo in their relationships with biotic agents, any inferences based on such a short study period should be made, and taken, with caution. There is evidence, however that the relationships found in this study are consistent at least in the mid-term. For example, the interaction between pollinators and herbivores was previously found by Herrera (2000) in a 1980 study carried out in one of the study populations (RH).

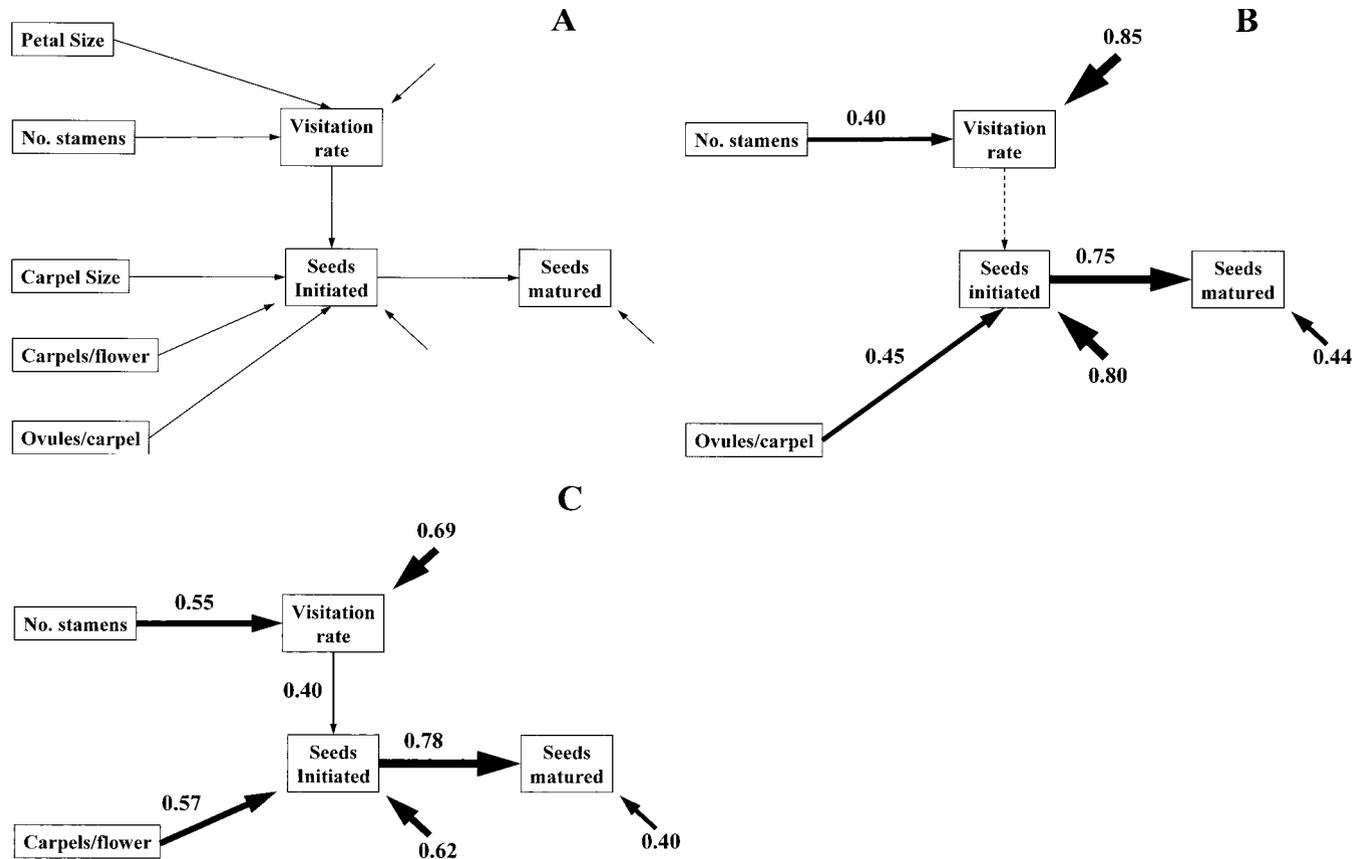


Fig. 3. (A) Common basic path model tested at each region of causal relationships among floral traits, visitation rate, and plant fecundity of *Paeonia broteroi*. See text for a detailed explanation. (B) Best-fit model obtained for Cazorla. (C) Best-fit model obtained for Jaén. The numbers by the arrows are the path coefficients. Only significant path coefficients are detailed. The solid lines indicate positive effects; the dashed line indicates a negative effect.

The study presented here relies on the assumption that phenotypic correlations among traits can be used as a surrogate for genetic correlations (Cheverud, 1988; Waitt and Levin, 1998). Although the application of this assumption to field studies have been criticized (e.g., Willis, Coyne, and Kirkpatrick, 1991), it is not yet clear how much the results may be compromised (Roff, 1995). The phenotypic surrogate has been accepted and used in several other field studies relating phenotypic variation to the evolution of floral traits (e.g., Conner and Via, 1993; Armbruster et al., 1999; Herrera et al., 2002).

The pollinator \times herbivore interaction as a determinant of floral variation—Traits related to flower size and amount of reward covaried in both regions and appeared to influence visitation rates: larger and more rewarding flowers received significantly more visits (see also Young and Stanton, 1990; Eckhart, 1991; Conner and Rush, 1996; Totland et al., 1998). Although abundance and composition of the visitor assemblage varied in the two regions on all the spatiotemporal scales (see also Herrera, 1988; Eckhart, 1992), visitor preferences were consistent across the study populations (but see Schemske and Horvitz, 1989; Inoue, Maki, and Masuda, 1996).

Even though visitation was higher in Cazorla, visitation rates affected seed initiation and subsequently seed set (i.e., the female reproductive success) only in Jaén. Although the morphology of *P. broteroi* flowers does not restrict access to a wide variety of insects (that actually visit the flowers), the

“size ratio” between flowers and visitors may be relevant in the explanation that the relationship between pollinator visits and seed initiation may be found only in Jaén (see also Totland et al., 1998). Thus, the most abundant visitors in Jaén (*A. mellifera* and *B. terrestris*) were significantly larger than the most abundant ones in Cazorla (halictid bees; ants are not considered pollinators; see Sánchez-Lafuente et al., 1999), and larger visitors may be more efficient pollinators in larger flowers because the likelihood of contacting the stigmas is higher (Sánchez-Lafuente et al., 1999). Furthermore, *A. mellifera* and *B. terrestris* seem to be much more effective at pollen delivery to *P. broteroi* flowers (A. M. Sánchez-Lafuente, unpublished data; see also Fishbein and Venable, 1996; but see Thomson and Thomson, 1992; Totland et al., 1998) than halictid bees (e.g., Inoue, Maki, and Masuda, 1996). However, the role of the male reproductive success as a determinant of floral differentiation via pollen export is not clear yet. Currently, available data suggest that plants undergo inbreeding depression and that crossed pollen is a better competitor than selfed pollen at seed siring. Thus, a successful pollen export would translate into a higher likelihood of seed siring, and pollen export may be particularly high in plants that are visited more because they produce more stamens per flower. Nevertheless, no data are available on how efficient the different flower visitors are at promoting pollen export. Consequently a differential male reproductive success cannot be excluded as a force driving floral divergence in *P. broteroi*.

The results above suggest that plants in Jaén may have the potential to modify floral traits to fit their local pollinators. Since the trait that best explained visitation rates in Jaén was the number of stamens per flower (that, therefore, may be under strong selection), this trait may have increased in this region as a response to selection. If petal size and number of stamens are genetically correlated (see Stanton and Preston, 1988) and if flower size is able to respond quickly to selection (e.g., Campbell, 1996), flowers in Jaén may have evolved their larger size indirectly through selection for more rewarding ones.

In contrast, none of the measured floral traits influenced herbivore consumption of developing fruits during the study period. Furthermore, maternal fecundity was not negatively affected by the herbivory levels observed in the study. Thus, herbivores did not appear to influence plant fitness, nor did they seem to be responsible for canceling out eventual selective pressures exerted by pollinators (but see Herrera, 2000). This conclusion, however, should be viewed with caution because (1) it is based on a very limited period of observation (see above) and (2) I intentionally prevented early herbivory episodes by ungulates in Cazorla to avoid plant destruction before flowering. In other words, in Cazorla I artificially forced the same situation found by Herrera (2000) in 1980. At that time, ungulates predominantly consumed plants during fruit maturation, while today they usually consume plants while sprouting. In either case, and if ungulates are accounted for, herbivory may strongly affect opportunities for selection eventually created by pollinators in exposed populations in Cazorla. In Jaén, however, herbivory was only by livestock and it was very limited or completely absent for years (A. M. Sánchez-Lafuente, unpublished data). Therefore, plants in Jaén may have more chances to escape drastic herbivory and to interact with pollinators.

Plant fecundity and alternative explanations for floral variation—Traits related to maternal fecundity covaried within regions and were significantly related to seed initiation and seed production. However, none of the traits related to visitation rates were in turn related to seed production. Although seed initiation was related to visits in Jaén, differences in the number of seeds initiated between regions were not significant. In addition, there were no overall differences between regions in seed production. The best-fit SEMs suggested that the traits that best explained seed initiation were the number of ovules per carpel in Cazorla and the number of carpels per flower in Jaén. The number of carpels per flower did not differ between regions, whereas plants in Cazorla had significantly more ovules per carpel (Table 2). However, a larger number of ovules per carpel in Cazorla did not result in differences between regions in maternal fecundity, at least when single-flowered plants were compared. In fact, if one assumes that all flowers in a given plant may be similarly successful (A. M. Sánchez-Lafuente, unpublished data), plants in Cazorla might be more successful at seed production than those in Jaén (in a hypothetical scenario without ungulates), because they produce, on average, more flowers per season.

Opportunity for selection exists when differences in phenotypic characters result in consistent differences in rates of survival or reproduction (Futuyma, 1986). Although the causes for the similarity between regions in maternal fecundity may be diverse, a consistent similarity over time might, thus, suggest that the differences between regions in flower size and

reward cannot be solely attributable to the role of pollinators, and alternative hypotheses must be discussed.

One plausible explanation of the similarity between seasons in female fecundity may be that there is a within-species upper limit to seed production imposed by the abundance of resources available to plants and the pattern of resource allocation to floral structures related to sexual functions. Thus, in a previous experiment in Cazorla populations, Sánchez-Lafuente et al. (1999) found that, while self-pollinated flowers suffered inbreeding depression at seed production, plants pollinated with crossed pollen did not produce more seeds than control flowers (in which seed production was not pollen-limited) and did not reach a full seed set in any case. Hermaphroditic species are subjected to high nutrient stress (e.g., Eckhart and Chapin, 1997), and it is unlikely that the few-flowered *P. broteroi* could allocate flowers to act solely through one of the sexual functions, as may happen in hermaphroditic species that produce many flowers (see Sutherland and Delph, 1984). Thus, both sexual functions must be shared within a flower and, if resources are limited, there must be a trade-off in the allocation of resources between male- and female-related structures and functions (see Wilson et al., 1994; Campbell, 1998). In plants with larger and more rewarding flowers, such as those in Jaén, the allocation of resources to seed production may be reduced even though pollinators may be more effective at pollen delivery. In contrast, in Cazorla more resources may be available for seed production. Therefore, I hypothesize that flower divergence may be also mediated by the patterns of resource allocation to sexual functions (see also Haig and Westoby, 1988).

Alternatively, it may be argued that differences in floral phenotype may be caused by processes in which selection is not involved (e.g., genetic drift). Genetic drift can promote genetic divergence particularly in small populations, such as those of *P. broteroi* in the study regions. Further, genetic drift cannot be ruled out as an explanation for the observed differences in floral traits and integration even among populations of species exhibiting more specialized flowers (Herrera et al., 2002). However, genetic drift can be overwhelmed by even weak selection (e.g., Futuyma, 1986), and the optimal allocation explanation presented above runs counter to the importance of genetic drift creating differences.

In summary, the null hypothesis presented here suggests that pollinator-mediated selection may be an important force promoting floral divergence in the generalist *P. broteroi*. Alternatively, floral differentiation may have been originated by patterns of optimal allocation of the available resources to flower formation and seed production. If the null hypothesis is true, then changes in the abundance and composition of efficient pollinators may still promote further divergence among populations. However, if opportunities for selection in *P. broteroi* depend more on the availability of resources than on pollination services, I suggest that there should not be many chances for biotic agents to modify the relationships among floral traits in this species. Further research is still needed to ascertain these issues.

LITERATURE CITED

- AIGNER, P. A. 2001. Optimality modeling and fitness trade-offs: when should plants become pollinator specialists? *Oikos* 95: 177–184.
- ARMBRUSTER, W. S., V. DI STILIO, J. D. TUXILL, T. C. FLORES, AND J. L. VELASQUEZ RUNK. 1999. Covariance and decoupling of floral and veg-

- etative traits in nine Neotropical plants: a re-evaluation of Berg's correlation-pleiades concept. *American Journal of Botany* 86: 39–55.
- BRODY, A. K. 1997. Effects of pollinators, herbivores, and seed predators on flowering phenology. *Ecology* 78: 1624–1631.
- CAMPBELL, D. R. 1996. Evolution of floral traits in a hermaphroditic plant: field measurements of heritabilities and genetic correlations. *Evolution* 50: 1442–1453.
- CAMPBELL, D. R. 1998. Variation in lifetime male fitness in *Ipomopsis aggregata*: tests of sex allocation theory. *American Naturalist* 152: 338–353.
- CHEVERUD, J. M. 1988. A comparison of genetic and phenotypic correlations. *Evolution* 42: 958–968.
- CONNER, J. K., AND S. RUSH. 1996. Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*. *Oecologia* 105: 509–516.
- CONNER, J. K., AND S. VIA. 1993. Patterns of phenotypic and genetic correlations among morphological and life-history traits in wild radish, *Raphanus raphanistrum*. *Evolution* 47: 704–711.
- CRESPI, B. J., AND F. L. BOOKSTEIN. 1989. A path-analytic model for the measurement of selection on morphology. *Evolution* 43: 18–28.
- CRESSWELL, J. E. 1998. Stabilizing selection and the structural variability of flowers within species. *Annals of Botany* 81: 463–473.
- ECKHART, V. M. 1991. The effects of floral display on pollinator visitation vary among populations of *Phacelia linearis* (Hydrophyllaceae). *Evolutionary Ecology* 5: 370–384.
- ECKHART, V. M. 1992. Spatio-temporal variation in abundance and variation in foraging behavior of the pollinators of gynodioecious *Phacelia linearis* (Hydrophyllaceae). *Oikos* 64: 573–586.
- ECKHART, V. M., AND F. S. CHAPIN, III. 1997. Nutrient sensitivity and the cost of male function in gynodioecious *Phacelia linearis* (Hydrophyllaceae). *American Journal of Botany* 84: 1092–1098.
- EFRON, B., AND R. J. TIBSHIRANI. 1993. An introduction to the bootstrap. Chapman & Hall, New York, New York, USA.
- FISHBEIN, M., AND D. L. VENABLE. 1996. Diversity and temporal change in the effective pollinators of *Asclepias tuberosa*. *Ecology* 77: 1061–1073.
- FUTUYMA, D. J. 1986. Evolutionary biology, 2nd ed. Sinauer Associates Sunderland, Massachusetts, USA.
- GÓMEZ, J. M., AND R. ZAMORA. 1999. Generalization vs. specialization in the pollination system of *Hormathophylla spinosa* (Cruciferae). *Ecology* 80: 796–805.
- GÓMEZ, J. M., AND R. ZAMORA. 2000. Spatial variation in the selective scenarios of *Hormathophylla spinosa* (Cruciferae). *American Naturalist* 155: 657–668.
- HAIG, D., AND M. WESTOBY. 1988. On limits to seed production. *American Naturalist* 131: 757–759.
- HERRERA, C. M. 1988. Variation in mutualisms: the spatio-temporal mosaic of a pollinator assemblage. *Biological Journal of the Linnean Society* 35: 95–125.
- HERRERA, C. M. 1996. Floral traits and plant adaptation to insect pollinators: a devil's advocate approach. In D. G. Lloyd and S. C. H. Barrett (eds.), *Floral biology—studies on floral evolution in animal-pollinated plants*, 65–87. Chapman & Hall, New York, New York, USA.
- 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., X. CERDÁ, M. B. GARCÍA, J. GUITIÁN, M. MEDRANO, P. J. REY, AND A. M. SÁNCHEZ-LAFUENTE. 2002. Floral integration, phenotypic covariance structure and pollinator variation in bumble bee-pollinated *Helleborus foetidus*. *Journal of Evolutionary Biology* 5: 108–121.
- INOUE, K., M. MAKI, AND M. MASUDA. 1996. Evolution of *Campanula* flowers in relation to insect pollinators on islands. In D. G. Lloyd and S. C. H. Barrett (eds.), *Floral biology—studies on floral evolution in animal-pollinated plants*, 377–400. Chapman & Hall, New York, New York, USA.
- KRUPNICK, G. A., A. E. WEIS, AND D. R. CAMPBELL. 1999. The consequences of floral herbivory for pollinator service to *Isomeris arborea*. *Ecology* 80: 125–134.
- LANDE, R. L., AND S. J. ARNOLD. 1983. The measurement of selection on correlated characters. *Evolution* 37: 1210–1226.
- LETHILÁ, K., AND S. Y. STRAUSS. 1999. Effects of foliar herbivory on male and female reproductive traits of wild radish, *Raphanus raphanistrum*. *Ecology* 80: 116–124.
- LITTELL, R. C., G. A. MILLIKEN, W. W. STROUP, AND R. D. WOLFINGER. 1996. SAS system for mixed models. SAS Institute, Cary, North Carolina, USA.
- MITCHELL, R. J. 1992. Testing evolutionary and ecological hypotheses using path analysis and structural equation modelling. *Functional Ecology* 6: 123–129.
- MITCHELL, R. J. 1994. Effects of floral traits, pollinator visitation, and plant size on *Ipomopsis aggregata* fruit production. *American Naturalist* 143: 870–889.
- MOTHERSHEAD, K., AND R. J. MARQUIS. 2000. Fitness impacts of herbivory through indirect effects on plant-pollinator interactions in *Oenothera macrocarpa*. *Ecology* 81: 30–40.
- MUTIKAINEN, P., AND L. F. DELPH. 1996. Effects of herbivory on male reproductive success in plants. *Oikos* 75: 353–358.
- ROFF, D. A. 1995. The estimation of genetic correlations from phenotypic correlations: a test of Cheverud's conjecture. *Evolution* 49: 481–490.
- SÁNCHEZ-LAFUENTE, A. M., 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, *Paonia broteroii* Boiss. & Reut. (Paeoniaceae). *Ecoscience* 6: 163–172.
- SAS. 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. 1989. Temporal variation in selection on a floral character. *Evolution* 3: 461–465.
- SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry. Second edition. Freeman, New York, New York, USA.
- STANTON, M. L., AND R. E. PRESTON. 1988. Ecological consequences and phenotypic correlates of petal size variation in wild radish, *Raphanus raphanistrum*. *American Journal of Botany* 75: 528–539.
- STERN, F. C. 1946. A study of the genus *Paonia*. Royal Horticultural Society, London, UK.
- 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. Floral herbivory affects floral characters and plant attractiveness to pollinators: implications for male and female plant fitness. *American Naturalist* 147: 1098–1107.
- SUTHERLAND, S., AND L. F. DELPH. 1984. On the importance of male-fitness in plants: patterns of fruit set. *Ecology* 65: 1093–1104.
- THOMSON, J. D., AND B. A. THOMSON. 1992. Pollen presentation and viability schedules in animal-pollinated plants: consequences of reproductive success. In R. Wyatt [ed.], *Ecology and evolution of plant reproduction*, 1–25. Chapman & Hall, New York, New York, USA.
- THOMSON, J. D., P. WILSON, M. VALENZUELA, AND M. MALZONE. 2000. Pollen presentation and pollination syndromes, with special reference to *Penstemon*. *Plant Species Biology* 15: 11–29.
- TOTLAND, Ø., H. L. ANDERSEN, T. BJELLAND, V. DAHL, W. EIDE, S. HOUGE, T. R. PEDERSEN, AND E. U. VIE. 1998. Variation in pollen limitation among plants and phenotypic selection on floral traits in an early-spring flowering herb. *Oikos* 82: 491–501.
- WAITT, D. E., AND D. A. LEVIN. 1998. Genetic and phenotypic correlations in plants: a botanical test of Cheverud's conjecture. *Evolution* 80: 310–319.
- WASER, N. M., K. CHITTKA, M. V. PRICE, N. M. WILLIAMS, AND J. OLLERTON. 1996. Generalization in pollination systems, and why it matters. *Ecology* 77: 1043–1060.
- WILLIS, J. H., J. A. COYNE, AND M. KIRKPATRICK. 1991. Can one predict the evolution of quantitative characters without genetics? *Evolution* 45: 441–444.
- WILSON, P., AND J. D. THOMSON. 1996. How do flowers diverge? In D. G. Lloyd and S. C. H. Barrett (eds.), *Floral biology—studies on floral evolution in animal-pollinated plants*, 88–111. Chapman & Hall, New York, New York, USA.
- WILSON, P., J. D. THOMSON, M. L. STANTON, AND L. P. RIGNEY. 1994. Beyond floral Batemanian: gender biases in selection for pollination success. *American Naturalist* 143: 283–296.
- YOUNG, H. J., AND M. L. STANTON. 1990. Influences of floral variation on pollen removal and seed production in wild radish. *Ecology* 71: 536–547.