Pollination success across an elevation and sex ratio gradient in gynodioecious *Daphne laureola*[1]

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Gynodioecy is a dimorphic breeding system in which hermaphrodite and female individuals coexist in populations. Sex ratio and gender-relative lifetime seed production determine the stability of gynodioecy, and both genetic and ecological factors may influence these parameters. I analyzed the consequences of variation in population sex ratio and site elevation for the relative pollination success of female and hermaphrodite individuals of *Daphne laureola* in southern Spain, where previous studies failed to detect female fecundity advantages at two mid-elevation sites. Pollination success, estimated as stigmatic pollen loads, number of pollen tubes per style, and percentage of fertilized flowers, was higher for hermaphrodites than females in populations with 20–56% females. Furthermore, female quantitative disadvantage in pollination success increased with elevation, suggesting that the higher availability of pollen due to the increased proportion of hermaphrodites could not mitigate the negative effect that other factors associated with elevation apparently had on pollination. Supplemental hand pollinations showed that female seed production was pollen limited in populations with a proportion of females >50%, although both pollination success and natural fruit set of females in these sites were the highest recorded.

Key words: altitudinal gradients; *Daphne laureola*; fruit set; gynodioecy; pollen competition; pollen limitation; pollen tube numbers; sex ratio.

Gynodioecy results when a male-sterile mutant (''female'' hereafter) invades and becomes established in a cosexual plant population (Webb, 1999). In order to spread and persist within populations, female plants must have some consistent fecundity advantage that compensates for their gametic disadvantage. Population genetics models point out that the magnitude of compensation required for gynodioecy to be stable varies, depending primarily on the mechanism of gender inheritance (Lewis, 1941; Gouyon and Couvet, 1987). The expected female frequency at equilibrium depends also on the degree of selfing and inbreeding depression (Charlesworth and Charlesworth, 1978; Jacobs and Wade, 2003). These models have been extensively tested and, as predicted by resource allocation theory, females of many gynodioecious species actually have quantitative fecundity advantages, although exceptions exist (see Shykoff et al., 2003, for a review).

Intraspecific variation in female frequency is a widespread phenomenon in gynodioecious species (Webb, 1999). Frequency dependence models highlight the existence of a negative feedback relationship between local frequency of females and female fecundity advantage generated by pollen limitation, that contributes to maintenance of gynodioecy when spatial distribution of the two genders is not uniform (McCauley and Taylor, 1997; Graff, 1999). Under these models, increased sex ratio variation would contribute to the persistence of gynodioecy even when females have lower fecundity advantage than they should according to the genetic models, being suitable thus to explain the persistence of gynodioecy in those species lacking female fecundity quantitative advantages. Intraspecific variation in sex ratio has been related to nuclear-

cytoplasmic gender inheritance (Bailey et al., 2003) and founder effects combined with different age of populations (Couvet et al., 1986; Belhassen et al., 1989). Additionally, when sex ratio variation is associated with environmental gradients (Delph, 1990; Ashman, 1999; Alonso and Herrera, 2001; Barrett et al., 2001; Delph and Carroll, 2001), ecological factors may determine different sex ratios at equilibrium in different populations and, thus, environment could affect the evolutionary dynamics of gynodioecy.

Temporal and spatial variation in gender-relative fecundity of gynodioecious species may also contribute to maintenance of gynodioecy (Eckhart, 1992a). However, there are only a few cases in which the effects of pollinators and environmental conditions on gender-relative seed fitness of gynodioecious species have been studied in detail (e.g., Eckhart, 1992b; Ashman, 2000; Delph and Carroll, 2001). More studies of intraspecific variation along geographical gradients in sex ratio and frequency-dependent pollination performance are needed to better understand the ecological mechanisms underlying breeding system evolution (Barrett et al., 2001).

In a previous study conducted in a montane area in southern Spain, the frequency of females in populations of the gynodioecious *Daphne laureola* was highly variable and negatively related to site elevation (Alonso and Herrera, 2001). Pollen limitation in females was not evident and no female fecundity advantage was detected until seed germination at two populations with ~33% females. These previous results led to the present study on fitness frequency dependence in this species. Here, I analyze the relationship between female frequency and pollination success of females and hermaphrodites of *D. laureola* in several southern Spanish populations selected along the species sex ratio and altitudinal range. The inverse relationship between altitude and sex ratio led to opposite predictions in regards to pollination success. On one hand, differences between genders were expected to decrease at high elevations where females were less abundant due to increased pollen availability. However, the lower rate of pollinator visits likely occurring at high-altitude sites in a winter-flowering species (Herrera, 2002) might hinder this pattern. The study was

TABLE 1. Elevation and sex ratio of *Daphne laureola* populations studied, listed in order of increasing elevation. Number of plants sampled each year (Fem = females, Her = hermaphrodites) and codes used in text and figures to designate populations are also shown.

Population	Code	Elevation (m a.s.l.)	Sex ratio (% fem)	Sample size 2001 Fem	2001 Her	2002 Fem	2002 Her
Huerta Vieja	HV	700	35.5	6	6	—	—
Central Eléctrica[a]	CE	830	50.0	8	8	6	6
Coto del Valle[a]	CV	950	56.1	8	8	6	6
Cuevas Bermejas	CB	1210	32.2	7	7	7	6
Roblehondo[a]	RH	1235	34.5	9	12	6	6
Prados de Navahondona	PN	1490	33.3	—	—	6	6
Cañada de La Medianega	LM	1560	33.3	—	—	6	6
Cañada del Espino	CDE	1575	21.4	—	—	6	6
Nava de las Correhuelas	NC	1615	30.1	5	5	6	6
Nava de Cabeza del Tejo	CT	1640	28.1	6	6	6	6
Puerto Llano[a]	PLL	1800	27.4	5	5	6	6
Navilla de Cagasebo	NG	1830	20.6	5	5	6	6
Pico Cabañas	CAB	1920	20.0	6	6	—	—

[a] Populations in which supplemental hand pollinations were conducted in 2002.

conducted during two consecutive years because pollination success of winter-flowering species can vary yearly (e.g., Herrera, 2002), and, as mentioned before, temporal variation in the gender-relative female fecundity could contribute to maintenance of gynodioecy. In addition, during the second study year, pollen limitation of females was estimated by supplemental hand pollinations conducted at four populations differing in sex ratio. Differences in fruit set between control and hand-pollinated flowers were expected to be positively related to female frequency if pollen was limiting fruit production.

The following questions are addressed in this paper: (1) Does gender divergence in pollination success vary among populations? If so, (2) are the patterns consistent between years? (3) Does pollination success of females decrease with reduced hermaphrodite abundance? (4) Are *D. laureola* female plants more pollen-limited in populations with lower proportion of hermaphrodite individuals?

MATERIALS AND METHODS

Study species—*Daphne laureola* L. (Thymelaeaceae) is a winter-flowering evergreen shrub growing in the undergrowth of coniferous and mixed Mediterranean mountain forests. Southern Spanish populations consist of a mixture of hermaphrodite and female individuals, with the local frequency of females ranging between 10 and 56%. In the study area, *D. laureola* is in flower from January through April, a period characterized by low temperatures, frequent rains, and irregular snowfalls. Each plant produces a large number of small, tubular, green-yellowish flowers aggregated into several compact inflorescences per stem. The two sexes are similar in size, flower production, and fruit set (Alonso and Herrera, 2001). Individual flowers of both genders have a single ovule and remain open continuously for approximately 1 mo. Hermaphrodite plants are fully self-compatible but fruit production requires flower visitation by pollinators, and an excess of self-pollen may clog up the stigma of hermaphrodites, reducing their fruit set (Alonso and Herrera, 2001). Direct observations on *D. laureola* flowers indicated that nitidulid beetles *Meligethes elongatus* Rosenhauer, solitary bees, and noctuid moths are the ordinary pollinators in the area (Alonso, 2004). Black drupes ripen in June.

Study sites—The study was conducted in the Natural Park of Sierras de Cazorla, Segura y Las Villas (Jaén Province, southeast Spain). In 2001, 10 populations were selected between 700 and 1920 m a.s.l. In 2002, the study was repeated in eight of them, and another three sites located at intermediate altitudes (1490–1570 m) were added to better encompass the whole altitudinal range of the species (Table 1). A wide variation in population sex ratio was

therefore studied because of the inverse relationship between altitude and proportion of females in the region (Alonso and Herrera, 2001). Two of the mid-elevation sites, Roblehondo (RH) and Cuevas Bermejas (CB), are the two populations considered in the earlier study of fecundity components of female and hermaphrodite plants that failed to detect a female advantage (Alonso and Herrera, 2001).

Field methods—*Sex ratio and pollination success*—The proportion of female and hermaphrodite individuals were determined at flowering in all study populations. All plants examined for this purpose (55–145 plants per site) produced exclusively either hermaphrodite or female flowers and thus were unambiguously classified into one of the two sex morphs.

At the end of the flowering period 10–12 flowers per plant were collected from a subset of female and hermaphrodite individuals at each study site (see Table 1 for the number of individuals sampled). Flowers were preserved in FAA solution (2.5% formaldehyde, 2.5% acetic acid, 95% ethanol) until their dissection and measurement. Styles were softened in 1 N KOH at 65°C for 20 min, rinsed with distilled water, and stained for 20 min at 65°C in decolorized aniline blue. The number of pollen grains on the stigma and the number of pollen tubes developed into the style were counted under a fluorescence microscope. All the flowers (N = 1273 in 2001; N = 1607 in 2002) were analyzed by the same person to decrease measurement errors. Pollination success of individual plants was estimated with three different but complementary variables. Mean stigmatic pollen loads estimated the amount of floral visits (Engel and Irwin, 2003), mean number of pollen tubes developed into the style estimated the possibilities for gametophyte competition (Herrera, 2004), and the proportion of flowers within a plant without pollen tubes estimated the frequency of pollination failure. The relationship between pollen tubes and pollen grain load at the individual flower level was also studied as an estimate of pollination quality.

Supplemental hand pollination of females—In 2002, the magnitude of pollen limitation in females was evaluated after supplemental hand pollinations in four populations differing in sex ratio (Central Eléctrica [CE], Coto del Valle [CV], RH, Puerto Llano [PLL]; see Table 1). At each site, six female plants were haphazardly selected, and all the flowers in five inflorescences located on five different stems were hand-pollinated (number of treated flowers per plant ranged between 32 and 53, total = 1020 flowers). Another five inflorescences with similar number of flowers were marked as controls on the same stems (N = 1006), thus allowing for a paired design that controlled for possible differences between stems in the chance of setting fruits, although susceptible to resource competition within stems. Mature anthers from several hermaphrodite individuals in the same population were collected and the pollen from these anthers was thoroughly mixed into a small box and transferred

to stigmas with a brush. Counts of developing fruits 4 wk after pollination treatments were used to estimate fruit set.

Supplemental hand pollinations were not conducted in hermaphrodites for two reasons. Firstly, without emasculation the hand pollination would induce a reduction of fruit set (Alonso and Herrera, 2001) being, thus, useless to evaluate individual pollination deficit. Secondly, emasculation would greatly reduce natural self-fertilization, which in the study area is rather frequent at all elevations (Medrano et al., 2005), and, consequently, the treatment would be inadequate in the estimation of pollination deficit of hermaphrodites naturally receiving unknown amounts of cross pollen.

Data analyses—All statistical analyses were performed using the SAS statistical package (SAS Institute, 1996). Unless otherwise stated, means will be presented ±1 SD. Differences between populations in the proportion of the two sexes were analyzed by a χ^2 test. Correlations of population means with altitude and sex ratio were estimated by the Spearman rank coefficient.

Differences between sexes and populations for pollination success were analyzed by generalized linear mixed models (Glimmix macro in SAS; Littell et al., 1996) with Poisson error distribution for stigmatic pollen load and number of pollen tubes and binomial error distribution for the proportion of unfertilized flowers. Sex and population effects were treated as fixed. When replicated at this level, the effects due to differences between individual plants within populations were considered as random. Different years were firstly analyzed separately, and after that, the comparison between years was conducted only for data of the eight populations sampled in 2001 and 2002, including population and individual plants as random effects. Nonsignificant interactions at $P > 0.10$ were removed from models.

Exploratory graphic analyses showed that linear regression was not appropriate to model the relationship between pollen tubes and pollen load at the individual flower level. Thus, a saturating negative exponential model [tubes = a(1 − exp$^{(-b \cdot \text{grains})}$)] was calculated for each sex and year separately, by using NLIN procedure (SAS Institute, 1996). Similar models have been used to predict the relationship between seed set and pollen loads (Mitchell, 1997; Cane and Schiffhauer, 2003). Differences between populations were tested by MIXED models on the residuals of the nonlinear regressions, modeling plant individual as a random effect.

In the supplemental pollination experiment, the effect of treatment, population, and their interaction on female fruit set was analyzed by generalized linear mixed models (Glimmix macro in SAS) with binomial error distribution and individual plant and stem as random effects. The SLICE option was used to determine at which populations the pollination treatment had a statistically significant effect on fruit set.

RESULTS

Sex ratio and pollination success—Variation between the study populations in the proportion of females was statistically significant ($\chi^2_{12} = 39.4$, $P < 0.0001$), and female proportion decreased with site elevation ($r_s = -0.91$, $N = 13$, $P < 0.0001$; Table 1).

On average, pollination success was higher for hermaphrodites independent of the parameter used to estimate it (Fig. 1). In both study years, stigmatic pollen loads and number of pollen tubes developed into the style were higher for hermaphrodites, whereas the proportion of unfertilized flowers (pollination failure) was significantly higher in female plants (Fig. 1; $P < 0.0001$ in all cases). Differences between populations were also statistically significant in all cases (Fig. 1; $P < 0.0001$). Contrary to expectations of sex ratio as the main determinant of pollination success, female *D. laureola* plants in populations with higher proportion of females recorded higher stigmatic pollen loads and consequently a larger number of pollen tubes. Those correlations were statistically significant in 2001 (r_s [pollen load] = 0.74, $P = 0.01$; r_s [pollen tubes] = 0.77, $P = 0.009$; $N = 10$ populations) but not in
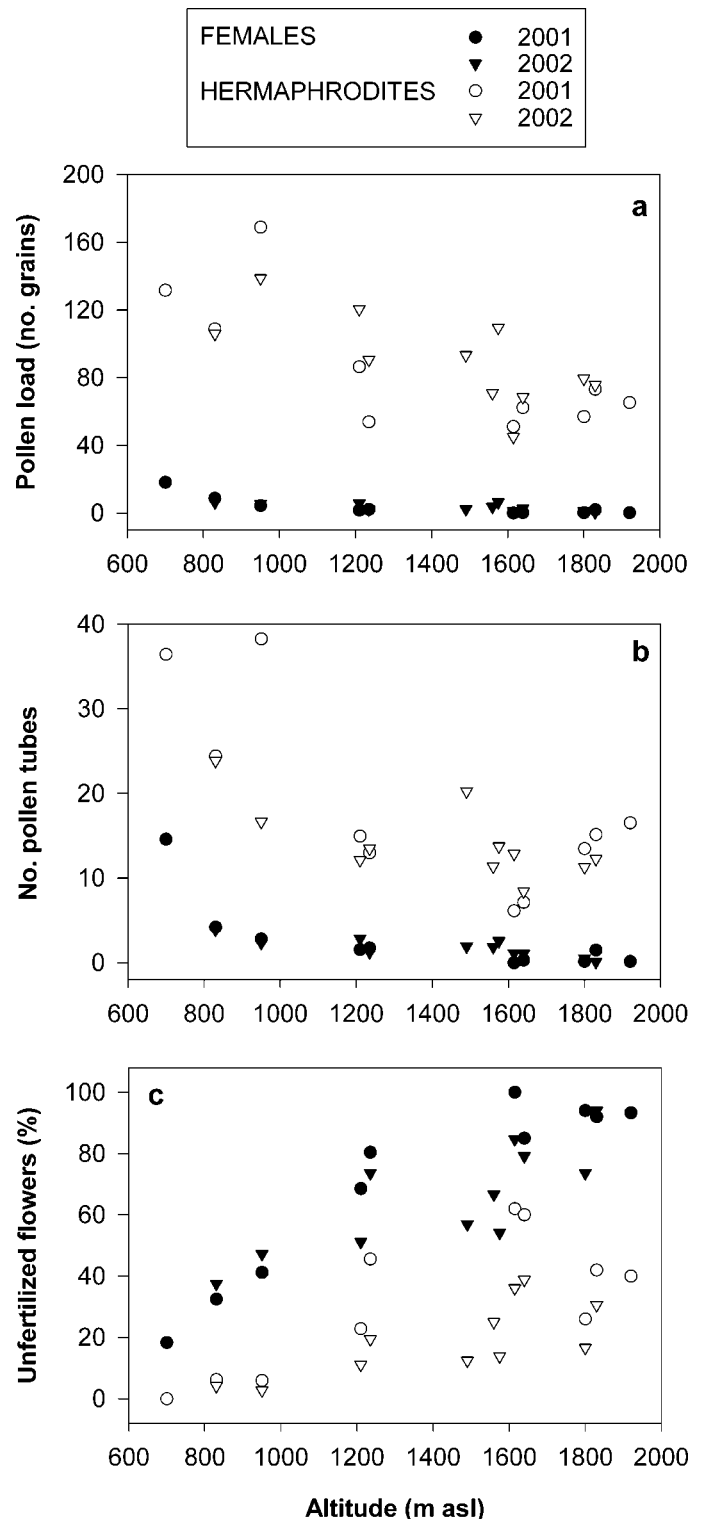

Fig. 1. Pollination success variation between genders, years, and populations of the gynodioecious shrub *Daphne laureola* along an altitudinal gradient in southern Spain. (a) The mean number of pollen grains deposited on the stigma was higher in hermaphrodites and at lower altitude populations. (b) A similar altitudinal trend for the mean number of pollen tubes per style, indicated that microgametophyte competition was stronger within hermaphrodite flowers and at lower sites. (c) A higher percentage of unfertilized flowers symbolized lower pollination success in female plants and in populations at higher altitudes.
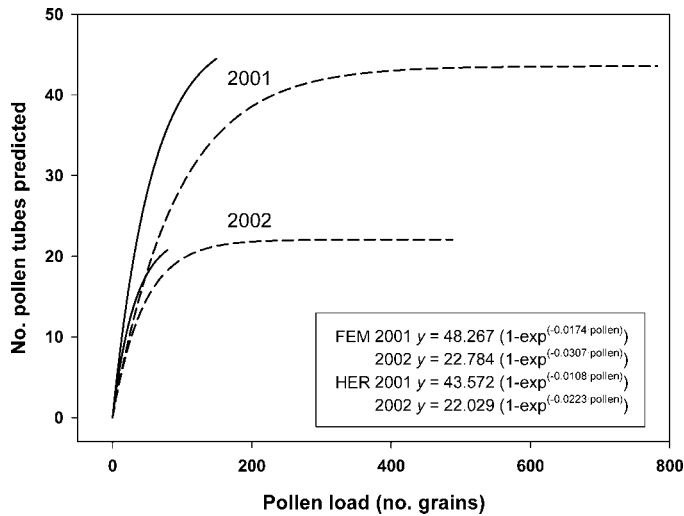
Fig. 2. Nonlinear saturating negative exponential equations fitted to model a dose-dependent relationship between the number of pollen tubes developed into the style and the pollen loads observed on the stigma. Lines are adjusted along the pollen load real data range; solid lines represent females and dashed lines represent hermaphrodites. The adjusted R^2 of the models were 0.67 and 0.76 for females in 2001 and 2002 and 0.58 and 0.42 for hermaphrodites in 2001 and 2002, respectively.
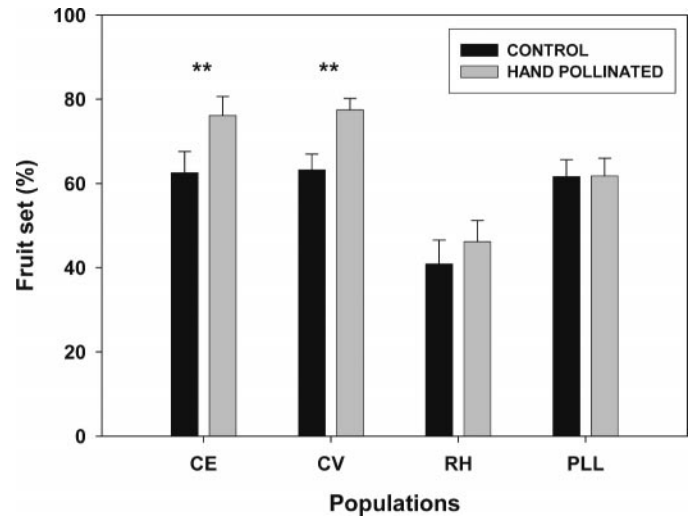

Fig. 3. Mean (SE) percentage of flowers setting fruits in control naturally pollinated, and hand-pollinated female flowers within individual *Daphne laureola* plants located at four populations ($N = 6$ plants/site) in 2002. Treatment induced a statistically significant percentage increase ($P < 0.01$) in the fruit set of females at the two populations with more than 50% females, CE and CV, but not in the other two populations, RH and PLL, having less than 35% females.

2002 (r_s [pollen load] $= 0.40$, $P = 0.22$; r_s [pollen tubes] $= 0.56$, $P = 0.07$; $N = 11$ populations). Likewise, the proportion of unfertilized female flowers (pollination failure) decreased at sites with higher proportion of females in 2001 ($r_s = -0.78$, $N = 10$, $P = 0.007$) and 2002 ($r_s = -0.63$, $N = 11$, $P = 0.04$).

As regards altitudinal patterns, mean female (fem) and hermaphrodite (her) stigmatic pollen loads were both negatively related to site elevation (Fig. 1a), and the correlation coefficients obtained for females in 2001 (r_s [fem] $= -0.77$; r_s [her] $= -0.58$; $N = 10$ populations) and those for both sexes in 2002 (r_s [fem] $= -0.70$; r_s [her] $= -0.69$; $N = 11$ populations) were statistically significant (all $P < 0.02$). Similar results in sign and magnitude were obtained for the number of pollen tubes (2001, r_s [fem] $= -0.81$, r_s [her] $= -0.44$ NS; 2002, r_s [fem] $= -0.86$, r_s [her] $= -0.64$; Fig. 1b). The proportion of unfertilized flowers increased with site elevation in both females and hermaphrodites (Fig. 1c) with correlation coefficients being always statistically significant ($r_s > 0.63$, $P < 0.05$ in all cases). Gender divergence, measured as the difference between hermaphrodites and females in the proportion of unfertilized flowers, was positively correlated to site elevation in 2001 ($r_s = 0.72$, $N = 10$, $P = 0.019$) and 2002 ($r_s = 0.56$, $N = 11$, $P = 0.07$), indicating that female pollination disadvantage consistently increased with elevation.

Nonlinear regressions adjusted to model the relationship between pollen loads and pollen tubes for each sex and year are shown in Fig. 2. Saturation of the relationship was rather frequent in hermaphrodite flowers, but it rarely occurred in female flowers. Sexes did not differ in the predicted asymptote value, although in 2001 the values were double than those in 2002. The rate of pollen tubes to grains, represented by the exponential parameter, was higher in female than in hermaphrodite flowers (Fig. 2) suggesting that selfing in hermaphrodites was substantial and, based on confidence limits (not shown), the difference between sexes was statistically signif-

icant in 2001, when pollination was more successful. Analyses of the residuals indicated that populations differed in the dose-response relationship of hermaphrodites in 2001 ($F_{9,58} = 4.77$, $P < 0.0001$) and 2002 ($F_{10,55} = 5.98$, $P < 0.0001$) and in dose-response of females in 2001 ($F_{9,55} = 8.56$, $P < 0.0001$) but not in 2002 ($F_{10,57} = 1.71$, $P = 0.10$). Differences between populations were not related to altitude or sex ratio ($P > 0.20$).

Despite annual variation in pollination success estimates, these patterns of among-population variation were consistent between years, and population $\times$ year interaction was never statistically significant in tested models ($P > 0.10$). The eight populations that were sampled in 2001 and 2002 did not have statistically significant differences between years in mean stigmatic pollen loads (44.9 ± 60 and 45.9 ± 53 in 2001 and 2002, respectively; $F_{1,2080} = 0.21$, $P = 0.64$; Fig. 1b), and no sex $\times$ year interaction suggested different trends between sexes. The mean number of pollen tubes per style in hermaphrodite flowers was remarkably high and tended to be lower in 2002 (13.9 ± 6.3 pollen tubes/style) than in 2001 (17.5 ± 14.2 pollen tubes/style; $F_{1,1020} = 25.72$, $P < 0.0001$), whereas in females variation between years was not statistically significant (1.7 ± 1.9 and 1.6 ± 1.8 pollen tubes/style for 2001 and 2002, respectively; $F_{1,1040} = 0.001$, $P = 0.97$). Furthermore, the proportion of unfertilized flowers was lower in 2002 (67.8 ± 25.3 and 20.0 ± 18.2 for females and hermaphrodites, respectively; Fig. 1c) than in 2001 (70.4 ± 24.3 and 32.4 ± 29.8; $F_{1,56} = 8.79$, $P = 0.004$) and the sex $\times$ year interaction was not statistically significant. As expected, the population random effect was always highly statistically significant ($P < 0.0001$).

Supplemental hand pollinations and natural fruit set—In 2002, the effect of hand pollination varied between populations (Fig. 3). Slicing of the nearly significant treatment $\times$ population interaction ($F_{3,114} = 2.43$, $P = 0.069$) indicated that fruit set of female plants was pollen limited at CE ($F_{1,114} = $

9.09, $P = 0.0032$) and CV ($F_{1,114} = 9.79$, $P = 0.0022$), the two populations studied with a proportion of females equal to or greater than 50%, but not in RH and PLL ($F_{1,114} < 1.42$, $P > 0.23$), where females represent 34.5% and 27.4%, respectively. However, populations with pollen limitation were not those with the lowest natural fruit set.

Natural fruit set was studied for other purposes at CV and RH populations in 2001 (C. Alonso, unpublished data). The mean percentages of flowers naturally setting fruits in females and hermaphrodites were 55.4% and 77.7% at the CV site and 16.0% and 32.6% at the RH site, respectively. Fruit set was significantly lower in females at both sites ($F_{1,49} = 10.48$, $P = 0.002$), and in both sex morphs at the RH site ($F_{1,49} = 34.44$, $P < 0.0001$). Natural fruit set of individual plants was expected to be inversely related to the proportion of unfertilized flowers. This relationship could be explored for a subset of 14 female and 18 hermaphrodite individuals. The slope of the relationship was negative in females and marginally statistically significant ($F_{1,11} = 4.29$, $P = 0.06$), but it did not differ from zero in hermaphrodites ($F_{1,15} = 0.19$, $P = 0.67$), suggesting that other phenomena in addition to frequency of pollination are affecting fruit production more strongly in hermaphrodites than females. The effect of population was treated as random and in no case accounted for a statistically significant proportion of variance.

DISCUSSION

In *Daphne laureola* pollination was quantitatively less successful in females than in hermaphrodites along the whole species altitudinal and sex ratio gradient in southern Spain and during two consecutive years, confirming previous results in which no female fecundity advantage was found in this species (Alonso and Herrera, 2001). Female quantitative disadvantages were not constant between sites, suggesting that sex ratio could affect pollinators and consequently determine gender-relative seed fitness (Ashman and Diefenderfer, 2001). However, disadvantages decreased with female frequency within populations, in contrast to the expected negative frequency dependence in female success (McCauley and Taylor, 1997). When pollinators are scarce, as in early blooming species like *D. laureola* (Alonso, 2004), direct estimates of pollinator abundance and behavior are too time consuming and inaccurate. Indirect measures of pollination abundance (e.g., pollen load, Engel and Irwin, 2003; proportion of unfertilized flowers) and quality (e.g., pollen tubes, Herrera, 2004; pollen tubes to grains relationship) can be used instead to analyze variation in pollination success and its consequences for individual plant fitness.

Stigmatic pollen loads and microgametophyte competition were extremely high in *D. laureola* hermaphrodites (Fig. 1). Even in 2002, the least favorable study season, 61–96% of hermaphrodite flowers had more than a single pollen tube per ovule depending on population, and the mean number of pollen tubes per ovule ranged from 8.4 to 23.8 among populations (for comparison see Erbar, 2003; Németh and Smith-Huerta, 2003). Simultaneously, obligate outcrossed females received much less pollen, and the mean number of pollen tubes per ovule ranged between 0.1 and 3.9, with *only* between 5% and 62% of flowers having more than a single pollen tube per ovule. Among sites, stigmatic pollen loads and the proportion of unfertilized flowers were indicators that pollination visits were less frequent at higher elevation sites, where weather is

less favorable for pollinators, and females were more strongly affected than hermaphrodites by shortage of pollination. The patterns were consistent between years despite the lower pollination success estimates during the second one. As regards pollination quality, the rates of pollen tubes to grains in hermaphrodite flowers were lower than in females (Fig. 2), indicating that the quality of pollen received by hermaphrodites was lower, likely because of a high deposition of self-pollen (see also Medrano et al., 2005). The extensive opportunities for microgametophyte competition could in principle palliate the lower quality of pollen received by hermaphrodites (Armbruster and Rogers, 2004), thus explaining the absence of a difference in germination rates between female and hermaphrodite offspring (Alonso and Herrera, 2001). Furthermore, differences in the rate of pollen tubes to grains recorded were not correlated with the population site elevation, supporting a lack of elevational effects in the quality component of pollination (but see, e.g., Utelli and Roy, 2000).

In 2001, estimates of natural fruit set in females were lower than in hermaphrodites in the two populations studied, thus confirming that female pollination disadvantages indirectly observed were consequential in terms of seed production. Hand pollinations conducted in 2002 showed *D. laureola* female fruit set was limited by pollen supply only at the lowest elevation sites (see also Alonso and Herrera, 2001), where females constitute more than 50% of the population, supporting the existence of a negative dependence between pollen limitation and female frequency (McCauley and Taylor, 1997). Female pollen limitation surprisingly was found in populations with the highest natural fruit set of females and the lowest divergence between genders in pollination success. Hence, in addition to the negative density dependence discernible only at extremely high female frequencies, differences between populations in resources (Campbell and Halama, 1993) or temperature (Totland, 2001) might be more limiting in determining the total and gender-relative fruit set of *D. laureola* than differences in female frequency and abundance of pollinators.

Under this scenario, gynodioecy would be maintained in *Daphne laureola* mainly by the increased outbreeding that results from it (Lewis, 1941) and not by resource compensation. Female advantage is usually enhanced in harsher environments, and changes in sex ratio associated with environmental factors such as moisture, latitude, or elevation would support that outbreeding benefits are context dependent (Delph, 1990; De Haan et al., 1997; Delph and Carroll, 2001; Asikainen and Mutikainen, 2003). In *D. laureola*, population sex ratio was not directly related to gender differential pollination success, suggesting that selection for outbred progenies should operate at later stages of the plant reproductive cycle (Alonso and Herrera, 2001; Medrano et al., 2005). Hence, fruit set and seed production, although correctly accounting for pollination success, would be poor estimators of individual fitness via female function in *D. laureola*.

In summary, this study demonstrates that environment can greatly modify intraspecific success of plant–pollinators interaction in space and time (Herrera, 1988; Utelli and Roy, 2000), and more interestingly, environment can also affect the relative pollination success between genders of dimorphic species (Delph and Carroll, 2001). In the case of *D. laureola*, density dependence of pollen limitation in females was overwhelmed by other environmental factors, and contrary to expectations, divergence in total and gender differential pollination success was not directly related to the relative frequency of genders

within populations. Apparently, pollination quality was more important than quantity in determining relative fitness of genders, and both variables were unrelated to each other. In addition, the fitness outbreeding advantage of females was not manifested at seed production but probably at later stages of the reproductive cycle. More studies of intraspecific variation in sex ratio and frequency-dependent pollination performance along geographical gradients are needed to better understand how the environment modify the evolutionary dynamics of gynodioecy.

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