

Ecological Context of Breeding System Variation: Sex, Size and Pollination in a (Predominantly) Gynodioecious Shrub

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- **Background and Aims** Species that exhibit among-population variation in breeding system are particularly suitable to study the importance of the ecological context for the stability and evolution of gender polymorphism. Geographical variation in breeding system and sex ratio of *Daphne laureola* (Thymelaeaceae) was examined and their association with environmental conditions, plant and floral display sizes, and pollination environment in a broad geographic scale was analysed.
- **Methods** The proportion of female and hermaphrodite individuals in 38 populations within the Iberian Peninsula was scored. Average local temperature and precipitation from these sites were obtained from interpolation models based on 30 years of data. Pollination success was estimated as stigmatic pollen loads, pollen tubes per ovule and the proportion of unfertilized flowers per individual in a sub-set of hermaphroditic and gynodioecious populations.
- **Key Results** *Daphne laureola* is predominantly gynodioecious, but hermaphroditic populations were found in northeastern and southwestern regions, characterized by higher temperatures and lower annual precipitation. In the gynodioecious populations, female plants were larger and bore more flowers than hermaphrodites. However, due to their lower pollination success, females did not consistently produce more seeds than hermaphrodites, which tends to negate a seed production advantage in *D. laureola* females. In the northeastern hermaphroditic populations, plants were smaller and produced 9–13 times fewer flowers than in the other Iberian regions, and thus presumably had a lower level of geitonogamous self-fertilization. However, in a few southern populations hermaphroditism was not associated with small plant size and low flower production.
- **Conclusions** The findings highlight that different mechanisms, including abiotic conditions and pollinator service, may account for breeding system variation within a species' distribution range and also suggest that geitonogamy may affect plant breeding system evolution.

Key words: *Daphne laureola*, environmental gradients, floral display, geographic variation, geitonogamy, gynodioecy, pollination success, sex ratio, Thymelaeaceae.

INTRODUCTION

Most angiosperms are hermaphroditic, which is the ancestral condition from which all other reproductive systems have apparently evolved (Barrett, 2002). The evolutionary pathway from hermaphroditism to dioecy via gynodioecy, a state where hermaphrodite and female individuals co-exist within populations, is theoretically and empirically well supported (Charlesworth, 1999; Webb, 1999; Weiblen *et al.*, 2000). In contrast, the ecological contexts favouring or hindering the two required transitions, namely the spreading of female mutants into co-sexual populations and selection for increased male function in hermaphrodites, are still poorly understood (Ashman, 2006). Indeed, gynodioecy can also be a stable polymorphic breeding system favoured by its most frequent inheritance mode that involves several cytoplasmic male sterility (CMS) genes and specific corresponding nuclear restorer alleles for male fertility (e.g. Bailey *et al.*, 2003), and metapopulation structure (Pannell, 1997). This study, based on an ample geographical survey encompassing contrasting ecological scenarios, was designed to evaluate the role of ecological factors in the evolution and maintenance of

gynodioecy. Both monomorphic (i.e. hermaphroditic) and gynodioecious populations of *Daphne laureola* occur within the Iberian Peninsula and, in gynodioecious populations, the proportion of females fluctuates widely, thus providing an exceptional model system.

To spread and persist within populations, female individuals of gynodioecious plants must have some consistent fecundity advantage that compensates for their gametic disadvantage, i.e. for the fact that they are not producing pollen. Such advantage may be achieved by resource compensation due to the reduced costs of their usually smaller and non-polleniferous flowers (Obeso, 2002; for a review, see Shykoff *et al.*, 2003) and/or decreased inbreeding when self-fertilization of hermaphrodites is frequent and inbreeding depression strong (Lewis, 1941; Charlesworth and Charlesworth, 1978). Previous studies on *D. laureola* have shown that spontaneous autogamy is extraordinarily infrequent among hermaphrodites (Alonso and Herrera, 2001). However, pollinator behaviour promotes geitonogamy in hermaphrodites, and consequently their seed progeny are characterized by extremely low outcrossing rates and considerable inbreeding depression in southern Spanish populations (Alonso, 2004; Medrano *et al.*, 2005).

Female frequency may also depend on environmental conditions and habitat quality (Delph and Carroll, 2001;

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Vaughton and Ramsey, 2004). In addition, frequency-dependent selection and pollen limitation of seed production may also act to limit the increase of female frequency beyond a certain threshold (McCauley and Taylor, 1997). In animal-pollinated gynodioecious plants, seed set, pollen limitation and selfing rate are largely determined by pollinator service (Eckhart, 1991; Sakai and Weller, 1999; Ashman, 2000, Williams *et al.*, 2000; Case and Barrett, 2004). Interestingly, at a local scale, the seed production of *D. laureola* was pollen limited only in populations with >50% of females. However, in these populations, pollination success was relatively high due to the characteristics of the habitats (i.e. lower altitude and more favourable conditions for pollinator service of this winter-flowering species; Alonso, 2005). Further studies of intraspecific variation in reproductive characteristics and the relative pollination success of the two sexual morphs along wide geographical and ecological gradients are expected to contribute significantly to understanding the role played by the environment and, more specifically, pollinators in the evolution and maintenance of gynodioecy. Such large-scale studies provide critical links between the micro- and macroevolutionary components of breeding system evolution (e.g. Barrett, 1995; Barrett *et al.*, 2001; Herrera *et al.*, 2006), yet this aspect remains relatively unexplored in gynodioecious plants (but see, for example, Delph, 1990; Wolfe and Shmida, 1997; Asikainen and Mutikainen, 2003; Case and Barrett, 2004; Nilsson and Ågren, 2006; Ramsey *et al.*, 2006).

One objective of the present investigation was to determine whether geographical variation in abiotic conditions is predictably related to variation in population breeding system and sex ratio. Such a relationship would be expected from hypotheses relating gynodioecy to environmental adversity (Ashman, 2006) and partially supported by previous studies on *D. laureola* conducted locally along an altitudinal gradient (Alonso and Herrera, 2001). Furthermore, breeding system variation is often correlated with variation in plant growth form and floral display size across species (Vamosi *et al.*, 2003). Thus, a second objective of this study was to test if there are significant correlations within a species between geographical variation in plant size, floral display size and pollination success on one side, and individual sex and population sex ratio on the other. Mean stigmatic pollen load, mean number of pollen tubes developed into the style, and the proportion of flowers without pollen tubes were used as surrogates for pollination success (Alonso, 2005). Finally, particular emphasis was given to the comparison between pollination success of hermaphrodite plants in monomorphic and dimorphic populations because the spread of male sterility mutations within populations would eventually depend on the mating system of hermaphrodites. The hermaphrodite individuals of monomorphic populations are expected to have higher maternal pollination success than hermaphrodites in gynodioecious populations, in which they would eventually reproduce mostly as males (e.g. Case and Barrett, 2004). If inbreeding avoidance is the main force maintaining gynodioecy, as suggested by recent studies on southern Spanish *D. laureola* populations (Medrano

et al., 2005) and by those on other species (Ramsey *et al.*, 2006), lower selfing rates and/or environmental conditions in which inbreeding is less detrimental should occur in monomorphic populations.

Specifically, the following questions were addressed. (a) Are population breeding system and female frequency related to abiotic conditions at the broad geographic scale considered? (b) In gynodioecious populations, do female individuals consistently outperform hermaphrodites in flower production and pollination success so as to obtain a higher maternal fitness? (c) Is the magnitude of the female seed production advantage contingent on population environmental conditions and/or female frequency? (d) Do hermaphrodite individuals experience higher maternal fitness in hermaphroditic than in gynodioecious populations?

MATERIALS AND METHODS

Study species

Daphne laureola L. (Thymelaeaceae) is an early-flowering, evergreen shrub growing in the undergrowth of shady mountain forests. It has a Palearctic distribution ranging from the Atlas Mountains, in Morocco, northwards to England and Hungary (Brickell and Mathew, 1976). In the Iberian Peninsula, the species is abundant in the central and western Cantabrian Range, the Pyrenean Mountains and the southern Betic Ranges. It is infrequent in the eastern Cantabrian Range, and absent from the central Iberian Ranges (Fig. 1). The southeastern Spanish populations of *D. laureola* consist of a mixture of hermaphrodite and female individuals, like other species in its genus (Kikuzawa, 1989). In this region, female frequency is highly variable (3.8–64.3%; 94 populations) and decreases with elevation (Alonso and Herrera, 2001). The genetic basis of gender inheritance in this species is currently unknown. *Daphne laureola* has a characteristic architecture, branches exhibit strongly monopodial growth and leaves occur only at the distal end of stems, where they form a well-defined rosette. The small, tubular, green-yellowish flowers are aggregated into compact axillary inflorescences, and open synchronously in late winter. Individual flowers of both sexes have a single ovule. Female flowers have vestigial stamens that do not produce pollen, and have shorter corolla tubes (mean \pm s.d.: 4.9 ± 0.45 mm, $n = 45$ flowers) than the perfect flowers of hermaphrodites (8.1 ± 0.92 mm, $n = 56$). Hermaphroditic plants are fully self-compatible. However, fruit production requires flower visitation by pollinators, and an excess of self-pollen may clog up the stigma and reduce fruit set (Alonso and Herrera, 2001). The pollen beetle *Meligethes elongatus* Rosenhauer (Coleoptera: Nitidulidae), small solitary bees and noctuid moths are the main pollinators in the southeastern Spanish populations (Alonso, 2004).

Study area and sex ratio

The Iberian Peninsula is extraordinarily heterogeneous in terms of climate, topography and biogeographical

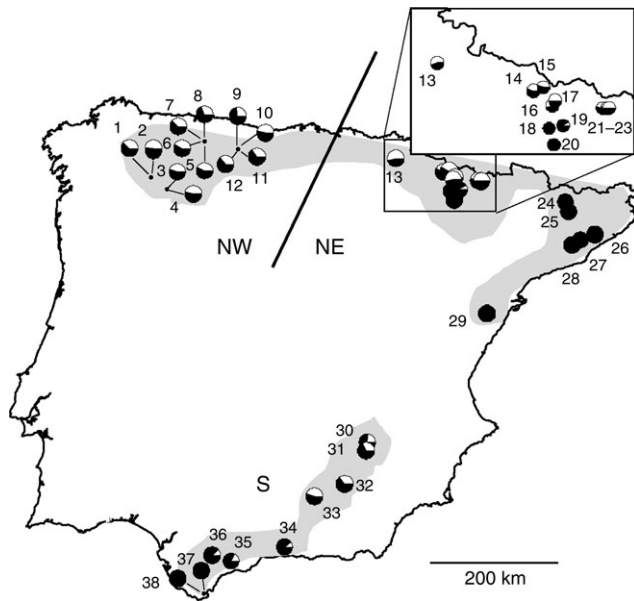


FIG. 1. The locations of the 38 *Daphne laureola* populations surveyed for sex ratio at the Iberian Peninsula. Population #32 (Robledo) is the only one represented here that is located within the Natural Park of Sierras de Cazorla, Segura y Las Villas, where previous studies of altitudinal variation in sex ratio referred to in the text were conducted. White areas of the pie chart graphs indicate the percentage of females in every study population. Fully hermaphroditic populations were not found in the NW region. For reference, the grey shading shows the species distribution range in the Iberian Peninsula.

background, which leads to a considerable diversity of environmental conditions, habitat types and plant communities (e.g. Capel Molina, 1981; Peinado Lorca and Rivas-Martínez, 1987). The southern and central, Mediterranean-climate regions are characterized by evergreen sclerophyllous and sub-sclerophyllous woodlands, dominated by holm or cork oaks (*Quercus ilex*, *Q. suber*), frequently mixed with pines (*Pinus halepensis*, *P. nigra*, *P. pinaster*, *P. sylvestris*), and occasionally with pinsapo fir (*Abies pinsapo*). In contrast, natural vegetation of the northern Cantabrian and Pyrenean regions is characterized by Atlantic forests dominated by beech (*Fagus sylvatica*), pines, silver fir (*A. alba*) and deciduous oaks (*Quercus robur*, *Q. petraea*, *Q. pyrenaica*) [see, for example, Bellot (1978) for a thorough description of the Iberian vegetation mosaic].

During 2003–2005 a total of 38 Iberian populations of *D. laureola* were visited during the flowering season. The proportion of female and hermaphrodite individuals was estimated at each site by checking the sexual expression of all, or at least 100, individuals per site (Fig. 1). Because the main objective of this study was to assess the possible ecological correlates of breeding system variation, particular effort was made to sample localities throughout the whole range of habitat types, ecological conditions and geographical regions where the species is known to occur in the Iberian Peninsula (Fig. 1). Sampling gaps in Fig. 1 mainly denote zones where the species is absent or very rare. In this study, three wide geographical regions were recognized for the analyses based on major

differences in latitude and longitude and sampling discontinuities (northeast, northwest and south, hereafter NE, NW and S, respectively). The abundance of *D. laureola* is very low between the NE and NW, which provides justification for considering these separately for the analyses.

Pollination success

At the end of the 2003 flowering period, 12 flowers were collected per plant from a sub-set of individuals at 18 study sites widely distributed and differing markedly in female frequency, even though all totally hermaphroditic populations were located in the NE region because the S ones were discovered later on (see Table 1 for locations and number of individuals sampled). Flowers were preserved in FAA solution (2.5 % formaldehyde, 2.5 % acetic acid, 95 % ethanol) until 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 an epifluorescence microscope for a total of $n = 2282$ flowers. The pollination success of individual plants was estimated using three different but complementary variables (Alonso, 2005): mean stigmatic pollen loads, which estimated cumulative flower visitation; mean number of pollen tubes per ovule, which reflected the likelihood of microgametophyte competition; and the proportion of flowers within a plant without pollen tubes, which provided an index of the frequency of pollination failure.

Plant and floral display sizes

A more detailed study was conducted in ten northern populations during the 2004 flowering season. Particular effort was invested in the NE region where the highest variation in plant architecture and sex ratio is found. At each site, 40 individuals (20 females and 20 hermaphrodites when available) were randomly selected and plant size was estimated as the total number of leaf whorls. Three partial measures of floral display were also obtained for each plant: proportion of stems bearing flowers; number of inflorescences/stem (based on counts of three stems per plant); and number of flowers/inflorescence (counted in one inflorescence per sampled stem). Total flower production per plant was then estimated by multiplying the number of inflorescences/stem \times average number of flowers/inflorescence \times number of flowering stems. Similar data for the southern populations are reported in Alonso and Herrera (2001).

Data analyses

All statistical analyses were performed using the SAS statistical package (SAS Institute, 2002). Due to strong heterogeneity of variances among the regions, regional differences in female frequency were analysed by means of an exact-probability median test. Differences between

TABLE 1. Average estimates of pollination success in the 18 *Daphne laureola* Iberian populations sampled in spring 2003 (s.d. in parentheses; n = 12 flowers per plant). For population number and location see Fig. 1.

Region	Population number (% females)	Sex*	No. of plants	No. of pollen grains	No. of pollen tubes	% unfertilized flowers
NE	29	Herm	6	145.0 (200.2)	16.5 (11.8)	15.3 (33.7)
	28	Herm	10	106.5 (256.2)	20.1 (44.3)	25.0 (75.8)
	27	Herm	10	85.6 (115.3)	14.5 (27.6)	30.8 (49.2)
	26	Herm	10	143.7 (122.2)	19.1 (23.4)	16.7 (47.1)
	25	Herm	6	155.0 (222.5)	20.1 (19.3)	15.1 (38.3)
	24	Herm	10	202.2 (192.0)	25.7 (27.0)	9.2 (37.1)
	20	Herm	6	186.9 (132.7)	25.9 (14.8)	8.6 (31.7)
	18	Herm	9	112.7 (133.4)	21.0 (22.8)	16.5 (48.3)
	19 (7.8 %)	Fem	4	9.5 (48.3)	1.1 (6.5)	83.3 (66.7)
		Herm	9	103.7 (123.1)	20.2 (31.5)	25.9 (71.4)
	14 (44.0 %)	Fem	6	3.7 (11.1)	1.8 (6.5)	73.6 (46.2)
		Herm	6	84.6 (117.8)	7.2 (12.0)	20.8 (59.9)
	16 (54.0 %)	Fem	7	6.2 (13.9)	1.1 (5.4)	77.4 (77.7)
		Herm	5	76.3 (51.3)	3.4 (10.5)	42.4 (86.9)
	NW	1 (39.4 %)	Fem	6	3.5 (6.9)	2.5 (3.9)
		Herm	6	81.6 (158.1)	8.0 (14.7)	34.7 (84.5)
6 (41.76 %)		Fem	6	4.2 (18.9)	1.1 (4.8)	78.1 (77.6)
		Herm	6	70.9 (73.1)	1.9 (6.6)	65.3 (71.7)
		Fem	6	11.0 (22.2)	4.4 (6.5)	35.6 (79.5)
S	36 (13.29 %)	Herm	6	93.3 (97.9)	14.1 (18.7)	8.3 (25.8)
		Fem	6	15.4 (36.3)	5.8 (15.2)	50.0 (104.8)
	35 (17.19 %)	Herm	6	115.1 (230.2)	12.1 (15.4)	20.8 (83.2)
		Fem	6	3.7 (9.9)	2.2 (5.2)	62.5 (101.2)
	33 (45.0 %)	Herm	6	89.0 (148.2)	13.2 (21.2)	20.5 (84.7)
		Fem	6	1.8 (5.0)	0.8 (2.6)	62.5 (65.2)
	32 (34.5 %)	Herm	6	89.3 (147.5)	16.2 (15.9)	9.7 (21.7)
		Fem	6	5.8 (10.4)	4.4 (9.9)	31.9 (82.5)
	31 (32.32 %)	Herm	6	89.4 (212.3)	13.9 (19.1)	11.1 (35.0)

* Herm, hermaphrodite individuals; Fem, female individuals.

sexes and among regions in pollination success, plant size and floral display size were analysed by generalized linear mixed models that allowed the effects of fixed and random factors on response variables that do not fit to normal distribution of errors to be analysed simultaneously (Procedure Glimmix in SAS 9.13; Littell *et al.*, 1996). Negative binomial error distribution was used for count data (stigmatic pollen load, number of pollen tubes,

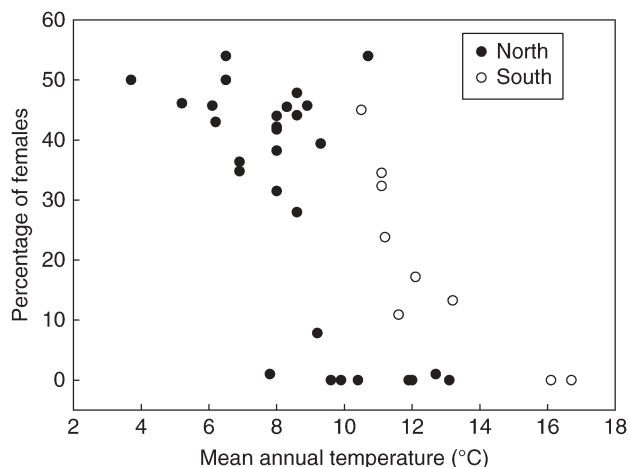


FIG. 2. Relationship between the mean annual local temperature and sex ratio of the studied Iberian *Daphne laureola* populations. Northern and southern locations are as indicated.

number of stems, number of inflorescences/stem, total flower production), binomial error distribution for the proportion of unfertilized flowers and normal error distribution for the average number of flowers/inflorescence. A single analysis for each response variable tested simultaneously for the effect of region and sex, considering both effects as fixed and defining populations as random. In particular, differences between sexes were analysed as *a priori* contrasts testing for differences between female and hermaphrodite plants in gynodioecious populations, and between hermaphrodite plants growing in gynodioecious and hermaphrodite populations. Data obtained from multiple flowers per plant were averaged, and subsequent analyses were based on the individual means. Unless otherwise stated, least-squares means (\pm s.e.) that estimate the marginal means corresponding to the fixed effects over a balanced population will be shown. Correlations of population means with sex ratio and local temperature and precipitation predicted by interpolation models based on 30 years of meteorological data (Bustamante, 2003) were calculated by the Spearman rank coefficient.

RESULTS

Geographical variation in sex ratio

Not all *D. laureola* populations in the Iberian Peninsula are gynodioecious. Female frequency ranged from zero, i.e. totally hermaphroditic populations, to >45% in some

gynodioecious populations (Fig. 1). The observed variation in female frequency was not homogeneously distributed among regions ($\chi^2_2 = 10.55$, $P = 0.005$; exact-probability median test). NW populations have consistently more females (mean \pm s.d. = $39.6 \pm 6.1\%$; median = 40.6% ; $n = 12$ populations) than those in the NE ($23.3 \pm 24.5\%$; median = 7.8% ; $n = 17$) and S ($19.67 \pm 15.6\%$; median = 17.2% ; $n = 9$).

In the two northern regions, female proportion tended to decrease from west to east (Fig. 1; $r_s = -0.37$, $n = 29$, $P = 0.045$); the longitudinal location was not significantly correlated to mean annual temperature ($r_s = -0.15$, $n = 29$, $P = 0.45$) but correlated negatively to precipitation ($r_s = -0.44$, $n = 29$, $P = 0.02$). Female proportion correlated negatively with mean annual temperature ($r_s = -0.60$, $n = 29$, $P = 0.0006$; Fig. 2), and positively with the total annual precipitation ($r_s = 0.62$, $n = 29$, $P = 0.0003$) that ranged between 635.6 and 2093 mm. Congruently, the hermaphroditic populations were characterized by significantly higher temperatures ($10.9 \pm 0.6^\circ\text{C}$ vs. $7.6 \pm 0.4^\circ\text{C}$; $F_{1,27} = 23.46$, $P < 0.0001$) and lower precipitation levels (726.5 ± 106.7 mm vs. 1325.0 ± 65.9 mm; $F_{1,27} = 22.77$, $P < 0.0001$) than the gynodioecious ones. Within the data on the NE region, both the sign and magnitude of differences between hermaphroditic and gynodioecious

populations in temperature ($10.9 \pm 0.7^\circ\text{C}$ vs. $6.9 \pm 0.7^\circ\text{C}$; $F_{1,15} = 17.64$, $P = 0.0008$) and precipitation (726.5 ± 59.5 mm vs. 1320.0 ± 56.1 mm; $F_{1,15} = 52.6$, $P < 0.0001$) were similar to those observed in the whole data. Since the NE region is a geographically more restricted area, where both monomorphic and dimorphic populations were equally represented in the sample (Fig. 1), these results suggest that the differences between the hermaphroditic and gynodioecious populations were not likely to be artefacts due to spatial autocorrelation.

In the southern region, the percentage of females decreased from east to west ($r_s = 0.77$, $n = 9$, $P = 0.013$). Similarly to northern populations, the percentage of females was inversely related to mean local annual temperature ($r_s = -0.94$, $n = 9$, $P = 0.0002$; Fig. 2), and hermaphroditic populations were associated with higher temperatures than gynodioecious ones ($16.4 \pm 0.6^\circ\text{C}$ vs. $11.5 \pm 0.3^\circ\text{C}$; $F_{1,7} = 53.11$, $P = 0.0002$). In contrast, female frequency was not significantly related to local precipitation level ($r_s = -0.34$, $n = 9$, $P = 0.37$), that was much lower than in northern sites and ranged between 470.5 and 1270 mm.

Plant and floral display sizes

Adult plants of *D. laureola* vary widely in size and architecture across the Iberian Peninsula, ranging from slim single-stemmed individuals to large shrubs consisting of >400 stems. Data collected in the ten northern populations studied more intensively (see Supplementary Information available online for study sites and sample sizes) reveal that plants in the NE were smaller ($F_{1,388} = 10.38$, $P = 0.0014$; Fig. 3A), and had fewer flowering stems ($F_{1,356} = 123.75$, $P < 0.0001$; one population excluded, see Supplementary Information for details) than in NW populations. However, the mean numbers of inflorescences/stem and flowers/inflorescence were similar between these two regions ($P > 0.20$ in both cases). Regional differences in floral display size are confirmed when all the reproductive parameters measured are combined multiplicatively to estimate total flower production per individual ($F_{1,388} = 6.23$, $P = 0.013$). Total flower production per plant was considerably lower in the NE (Fig. 3B; median = 54 flowers/plant) than in the NW populations (median = 967 flowers/plant).

As regards differences between sexes in gynodioecious populations, it was found that female plants were significantly larger than hermaphrodites (21.0 ± 6.9 vs. 13.4 ± 4.3 stems; $F_{1,388} = 10.38$, $P = 0.0014$), and they also tended to have more flowering stems, although the difference was only marginally significant (15.5 ± 2.2 vs. 10.9 ± 1.4 stems; $F_{1,356} = 3.45$, $P = 0.06$). Mean number of inflorescences/stem and the average number of flowers/inflorescence did not differ between the sexes ($P > 0.20$; see Supplementary Information for details). Altogether, female individuals produced more flowers than hermaphrodites in the gynodioecious populations (638.9 ± 286.2 vs. 414.8 ± 182.8 flowers; $F_{1,388} = 3.85$, $P = 0.05$).

Finally, the size of hermaphrodite plants growing in hermaphroditic and gynodioecious populations did not differ significantly (8.6 ± 4.3 vs. 13.4 ± 4.3 stems;

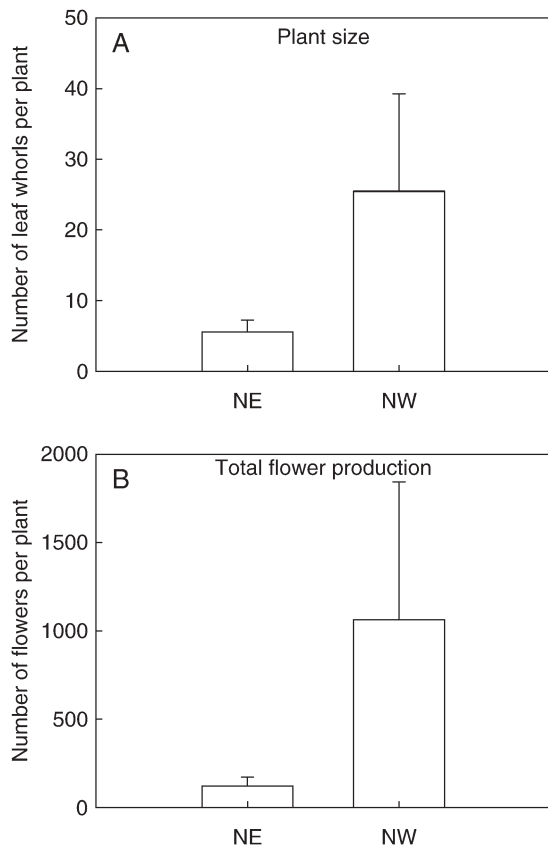


FIG. 3. Regional differences in average plant size (A) and total flower production (B) of *Daphne laureola* based on data of seven NE populations and three NW populations, and 40 individuals per site. Bars represent the least-square means (\pm s.e.) after accounting for sex (fixed effect) and population (random effect) variation.

$F_{1,388} = 0.54$, $P = 0.46$), neither did the total flower production per plant (258.1 ± 175.6 vs. 414.8 ± 182.8 flowers; $F_{1,388} = 0.34$, $P = 0.56$). Similar results were found when analyses were restricted to the NE region where both monomorphic and dimorphic populations are relatively common.

Pollination success and sex ratio

Maternal pollination success, estimated by the size of the pollen loads, number of pollen tubes per ovule and the frequency of unfertilized flowers (pollination failure), varied regionally (Table 1; note that all of the studied NW and S populations were gynodioecious). Pollen loads differed significantly between regions ($F_{2,183} = 3.01$, $P = 0.05$). The lowest number of pollen grains per pistil was observed in the NW region (43.3 ± 6.6), the highest in the S region (64.4 ± 6.9 pollen grains) and NE populations were intermediate (57.4 ± 3.8 pollen grains). The number of pollen tubes per ovule also differed between regions ($F_{2,183} = 12.87$, $P < 0.0001$), with NW populations showing the lowest number of pollen tubes per ovule (5.6 ± 1.0), S populations averaging more than twice this figure (14.0 ± 1.5 pollen tubes per ovule) and NE populations being intermediate (9.9 ± 0.7). In accordance with these results, pollination failure estimated as the proportion of unfertilized flowers per plant was lower in the S populations (0.14 ± 0.04 pollen tubes per ovule) than in either of the two northern regions (NE, 0.33 ± 0.04 ; and NW, 0.35 ± 0.09 ; $F_{2,169} = 5.3$, $P = 0.006$).

A comparison of pollination success between female and hermaphrodite plants growing in gynodioecious populations reveals that female flowers received considerably smaller pollen loads ($F_{1,183} = 467.2$, $P < 0.0001$; Fig. 4A), developed fewer pollen tubes per ovule ($F_{1,183} = 129.96$, $P < 0.0001$; Fig. 4B) and displayed a much higher proportion of unfertilized flowers than hermaphrodite individuals ($F_{1,169} = 175.5$, $P < 0.0001$; Fig. 4C). Pollination success was higher for hermaphrodite plants growing in hermaphroditic populations than for hermaphrodite plants in gynodioecious populations. Only around 12% of hermaphrodite flowers remained unfertilized in the hermaphroditic populations, and pollen load was 1.6 times and the number of pollen tubes more than twice that in the gynodioecious populations ($P < 0.01$ in all cases; Fig. 4).

Pollination success at the population level was characterized by averaging plant mean values for each of the three parameters considered for each population and sex (Table 1). Across the gynodioecious populations, size of pollen loads and number of pollen tubes of hermaphrodite flowers were negatively correlated to the population proportion of females (Table 1; $r_s = -0.82$, $n = 10$, $P = 0.0038$; $r_s = -0.72$, $n = 10$, $P = 0.019$; for pollen load and pollen tubes, respectively) and positively correlated to mean annual temperature ($r_s = 0.74$, $n = 10$, $P = 0.014$; $r_s = 0.61$, $n = 10$, $P = 0.06$; for pollen load and pollen tubes, respectively). Similar patterns were also observed for pollination success of female flowers, although correlations with female frequency were not statistically significant (Table 1; $r_s = -0.49$, $n = 10$, $P = 0.15$; $r_s = -0.41$,

$n = 10$, $P = 0.24$; for pollen load and pollen tubes, respectively) and only mean annual temperature and the number of pollen tubes were significantly correlated

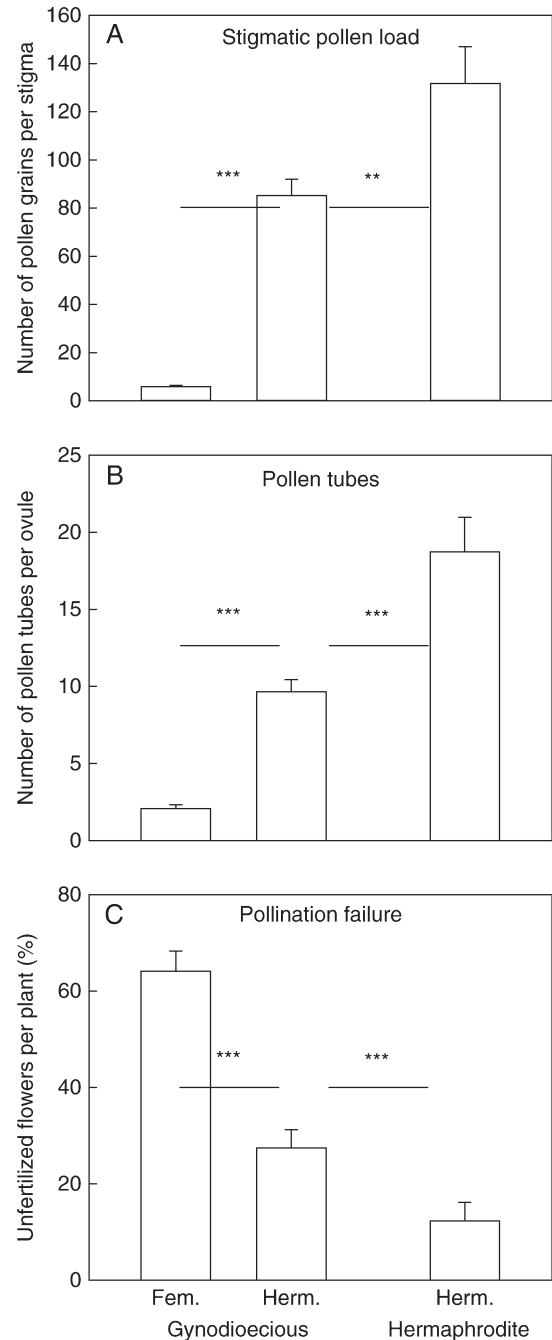


FIG. 4. Differences between sexes in three measures of quantitative pollination success of *Daphne laureola*. (A) Mean stigmatic pollen load, (B) mean number of pollen tubes per ovule and (C) the percentage of flowers without pollen tubes. Bars represent the least-square means (\pm s.e.) after accounting for region (fixed effect) and population (random effect) variation, and are based on ten gynodioecious and eight hermaphrodite populations sampled in 2003. *A priori* contrasts were used to test specifically for differences between female and hermaphrodite plants in gynodioecious populations, and also between hermaphrodite plants growing in gynodioecious and hermaphroditic populations, *** $P < 0.0001$ and ** $P = 0.002$.

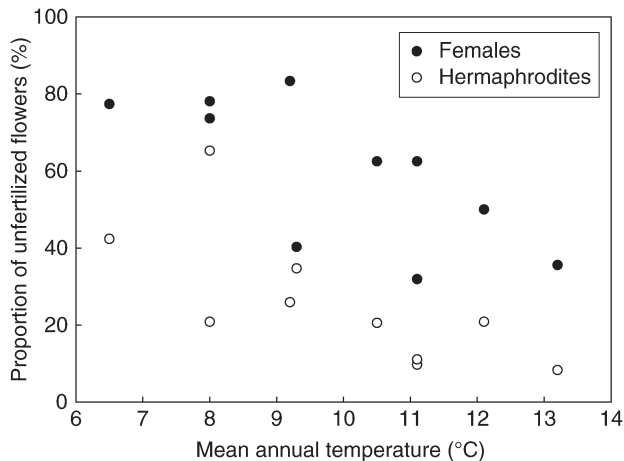


FIG. 5. Relationship between the mean annual local temperature and population pollination failure estimated as the average proportion of unfertilized flowers per plant. Female and hermaphrodite scores in gynodioecious populations are as indicated.

($r_s = -0.64$, $n = 10$, $P = 0.05$). The proportion of unfertilized flowers per plant tended to increase with the percentage of females, but the pattern was not statistically significant for either sex ($P > 0.25$). Temperature was apparently more influential (Fig. 5) because the correlation between proportion of unfertilized flowers per plant and mean annual temperature was highly significant in hermaphrodites ($r_s = -0.80$, $n = 10$, $P = 0.005$) and females ($r_s = -0.75$, $n = 10$, $P = 0.01$). No correlation was found between any measure of pollination success and mean annual precipitation at the population ($P > 0.11$).

DISCUSSION

Daphne laureola is an early-flowering shrub widely distributed in Europe. Previous field observations suggested that floral dimorphism in this species was restricted to high altitude forms (Brickell and Mathew, 1976). Nevertheless, more recent studies conducted at a regional scale and a broad range of elevations in southeastern Spain revealed that the frequency of females is highly variable and decreases with elevation (Alonso and Herrera, 2001). By broadening the spatial scale, the present study shows that gynodioecy has a patchy distribution, prevailing in the northwest and southeast of the Iberian Peninsula and, most probably, also in other areas of the species range (gynodioecious populations also occur in Morocco and Slovenia; C. Alonso, unpubl. data). In addition, there are at least two disjunct, distant areas in the northeast and southwest of the Iberian Peninsula in which purely hermaphroditic populations are frequent (Fig. 1).

Intraspecific variation in female frequency is a widespread phenomenon in gynodioecious species (Webb, 1999). In species with both monomorphic and dimorphic populations, gynodioecy seems to be associated with harsh dry environments (Vaughton and Ramsey, 2004; Ashman, 2006 and references therein). However,

gynodioecious populations of *D. laureola* were found at sites characterized by higher precipitation and lower temperatures than purely hermaphroditic populations; this pattern occurred consistently both at the scale of the whole Iberian Peninsula and within the NE region, where both monomorphic and dimorphic populations were relatively common. Indeed, hermaphroditic populations in this region were found in relatively isolated coastal and pre-Pyrenean mountains with Mediterranean climate, whereas gynodioecious populations were predominantly located in Atlantic-climate environments. Furthermore, female frequency was inversely related to local temperature (Fig. 2), and in the two northern regions it increased with annual precipitation. These results contrast with the negative relationship between female frequency and site elevation observed in a southeastern Mediterranean mountainous region (Alonso and Herrera, 2001), that was consistent with the expectation of a higher female frequency in harsher environments. The contrasting results obtained by these two investigations conducted at different spatial scales suggest that different selective and stochastic processes might prevail at different geographic scales (see also Nilsson and Ågren, 2006). In the following paragraphs there follows a discussion on how the selective pollination context might influence intraspecific geographic variation in the breeding system of *D. laureola*.

Plant and floral display sizes

Plants of *D. laureola* growing in different regions of the Iberian Peninsula differed mainly in their size. Plants growing in the NE region had fewer than six leaf whorls on average, with just 3–5 stems bearing flowers, and were thus considerably smaller than plants growing in the NW (Fig. 3A) and also in comparison with S populations in which average individual size ranged between 34 and 480 leaf whorls per plant ($n = 8$ populations; C. Alonso, unpubl. data).

The main consequence of plant size variation in terms of sexual reproduction was related to flower production, that was estimated to vary on average between 15 and 3978 flowers per plant among the populations. Variation had a strong regional component, as plants in the NE region produced 8.6- and 13.5-fold fewer flowers than those in the NW (Fig. 3B) and S regions (see Alonso and Herrera, 2001), respectively. Such huge difference in flower production in a highly synchronously flowering species should have consequences for the plant–pollinator interaction, since the size of floral display also influences pollinator attraction and behaviour in gynodioecious species (e.g. Eckhart, 1991; Ashman and King, 2005). Geitonogamy, the transfer of self pollen between flowers within an individual plant, usually increases with floral display (Harder and Barrett, 1995). This relationship may explain why, at the interspecific level, dioecy tends to evolve more frequently in clades with many-flowered inflorescences in some groups (Vamosi *et al.*, 2003). At the intraspecific level, the results for *D. laureola* support a relationship between floral display size and the evolution of gynodioecy. On one hand, previous studies conducted

on S populations characterized by large floral displays suggested that geitonogamy is frequent and, thus, male sterility would be a mechanism for preventing selfing, particularly advantageous because it is coupled with strong inbreeding depression (Medrano *et al.*, 2005). On the other hand, the NE hermaphroditic populations were characterized by very small floral displays and thus presumably were the ones least susceptible to geitonogamous pollinations. These results suggest that the benefits of male sterility obtained through outbreeding might not be substantial enough to favour a gynodioecious breeding system in populations characterized by small plants and reduced floral display. The observed higher maternal pollination success of hermaphrodites in the monomorphic populations (see below) is consistent with this suggestion.

After statistically accounting for the effects of regional variation, female plants were 1.6 times larger and, as a consequence, produced 1.5 times more flowers than hermaphrodites in the gynodioecious populations. This finding is consistent with the generalized notion that male function is costly and females re-invest the saved resources in modular growth and, subsidiary, flower production (see Eckhart and Seger, 1999); this aspect does not seem to have often gained support from studies on vegetative traits in gynodioecious species (Ashman, 2005). Indeed, the pattern was not upheld in all of the study populations (Supplementary Information available online; see also Alonso and Herrera, 2001), highlighting that the cost of male function and the magnitude of gender dimorphism might also be context dependent (Alonso *et al.*, 2005; Delph and Wolf, 2005).

Pollination success

In early blooming species like *D. laureola*, characterized by extraordinarily low pollinator visitation rates (Alonso, 2004), direct estimates of identity, abundance and behaviour of pollinators are impractical at the broad geographic scale of this study. The scarcity of floral visitors was confirmed during the visits to *D. laureola* populations in all the study regions, and supported by the high percentages of unfertilized flowers, which ranged between 31.9 and 83.3% and 8.3 and 65.3% in female and hermaphrodite individuals of the gynodioecious populations, respectively, and between 8.6 and 30.8% in hermaphroditic populations. Pollination success of individual plants was thus estimated using three different, complementary parameters: mean stigmatic pollen loads; mean number of pollen tubes per ovule; and the proportion of unfertilized flowers within a plant. We are confident that these parameters provide a comprehensive description of the 'pollinating environment' faced by the *D. laureola* populations studied, and the last is also a good correlate of individual fruit set in females (Alonso, 2005). In a population studied in two other years (Roblehondo site in Alonso, 2005), estimates of pollination success were similar to those of the present study. This suggests yearly consistency in the pollinating environment and that the geographic variation observed in 2003 could be representative of prevailing conditions.

As in other gynodioecious species, differences among the sites in the composition and abundance of the pollinator assemblage, as well as in overall pollen availability resulting from differences in sex ratio, could both contribute to spatial variation in pollination success (e.g. Delph, 1990; Eckhart, 1991; Ashman, 2000; Case and Barrett, 2004). In addition, abiotic conditions and particularly temperature could determine the activity and fertilization effectiveness of insect pollinators (e.g. Totland, 2001). The positive relationship between mean annual temperature and pollination success at the population level observed here (Fig. 5) confirms previous findings suggesting that abiotic conditions determine the reproductive success of this early flowering species more strongly than the sex ratio (Alonso, 2005). Regarding variation in pollinator abundance and species composition, and because of the difficulty in characterizing pollinator assemblages mentioned above, it can only be speculated at present that *Meligethes* pollen beetles seem to be relatively common only in the southern *D. laureola* populations. In these populations, the pollen beetles are found at all altitudes, and occur in both hermaphroditic and gynodioecious populations (Alonso, 2004; and unpubl. data), while they have never been observed during the sampling visits to the northern populations. Along with the higher quantitative estimates of pollination success obtained in southern populations, in which on average only 14% of ovules were not fertilized at intermediate altitudes compared with the >33% observed in the northern populations, the present findings suggest that the abundance of pollen beetles is probably a major determinant of seed set in *D. laureola*.

Geographical variation in the quality of pollination, and particularly its correlations with geitonogamy and resulting inbreeding, may be another factor contributing to the observed broad-scale variation in breeding system in gynodioecious species (e.g. Ramsey *et al.*, 2006). Previous studies on *D. laureola* have shown that spontaneous autogamy is extraordinarily infrequent (Alonso and Herrera, 2001), but seed progeny of hermaphrodites are characterized by extremely low outcrossing rates in S gynodioecious populations (Medrano *et al.* 2005). In the NE hermaphroditic populations, where geitonogamy is expected to be much less frequent due to reduced floral display, individuals received on average 1.5 times more pollen grains and twice as many tubes per pistil than the hermaphrodite individuals in gynodioecious populations. A higher pollen tubes/grains ratio in hermaphroditic populations would indicate increased quality of the pollen received (Alonso, 2005). Furthermore, the increased opportunity for pollen tube competition would eventually lead to increased offspring quality and might consequently reduce inbreeding depression (e.g. Lankinen and Armbruster, 2007). Such results support the hypothesis that hermaphrodite individuals of monomorphic populations should have higher maternal fitness in order to preclude the spread of females. It should be noted here that in two of the NE hermaphroditic populations studied, one single female individual was found (see Supplementary Information for details), and thus the male sterility mutation was not totally absent

from these populations. Reduced floral display might thus contribute to the relative success of hermaphroditism in the NE because it apparently increased pollination quality without reducing the proportion of visited flowers per plant. To test rigorously for the importance of geitonogamy as a determinant of breeding system evolution in this species, the mating system and inbreeding depression should also be estimated in the purely hermaphroditic populations.

Combining the average population estimates of flower production and pollination failure shown above, upper estimates of total seed production per sex and site were obtained. Despite the fact that the females produced more flowers in all of the northern populations studied (see Supplementary Information), their flower production was insufficient to compensate for their much lower proportion of fertilized flowers in three out of five populations. The two exceptions are from the NE region: in population #16 average seed production was estimated as 65.6 seeds/plant in females and 41.8 seeds/plant in hermaphrodites, and in population #19 the corresponding estimates were 70.0 vs. 62.8 seeds/plant. Excluding these two sites, estimates of female seed production relative to hermaphrodites ranged between 0.34 and 0.95. Furthermore, the absence of a significant correlation between female frequency and the proportion of unfertilized flowers of both female and hermaphrodite individuals supports previous findings suggesting that differences between sexes in seed output in *D. laureola* are insensitive to variation in sex ratio (Alonso, 2005; Medrano *et al.*, 2005).

Conclusions

The present results highlight the contribution of both abiotic conditions and pollinator service to intraspecific geographic variation in plant breeding systems. Contrary to the most common rule for sub-dioecious species, hermaphroditic populations were located at warmer sites and female percentage decreased with local temperature. The findings do not support the idea that enhanced female seed production through resource compensation, and frequency-dependent selection, are important factors contributing to the maintenance of gynodioecy in *D. laureola* in the Iberian Peninsula. However, inbreeding avoidance coupled with strong inbreeding depression could be crucial factors in some regions where the populations are characterized by large plants and floral displays. This hypothesis, however, does not hold over the large geographical study area. In a few southern populations hermaphroditism was not associated with small plant size and low flower production (C. Alonso, unpubl. data). Ongoing studies on the mating system of these hermaphroditic populations should explain whether this inconsistency might result from a reduced geitonogamy associated with differences in pollinator service and/or a lower inbreeding depression associated with local abiotic conditions.

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SUPPLEMENTARY INFORMATION

Supplementary information is available online at <http://aob.oxfordjournals.org/> and gives average estimates of plant and floral display sizes recorded in the ten *Daphne laureola* northern populations that were studied.

LITERATURE CITED

- Alonso C. 2004. Early blooming' challenges: extended flowering season, diverse pollinator assemblage, and the reproductive success of gynodioecious *Daphne laureola*. *Annals of Botany* **93**: 61–66.
- Alonso C. 2005. Pollination success across an elevation and sex ratio gradient in gynodioecious *Daphne laureola*. *American Journal of Botany* **92**: 1264–1269.
- Alonso C, Herrera CM. 2001. Neither vegetative nor reproductive advantages account for high frequency of male-steriles in southern Spanish gynodioecious *Daphne laureola*. *American Journal of Botany* **88**: 1016–1024.
- Alonso C, Pérez R, Nieto PM, Delgado J. 2005. Gender dimorphism and altitudinal variation of secondary compounds in leaves of the gynodioecious shrub *Daphne laureola*. *Journal of Chemical Ecology* **31**: 139–150.
- Ashman T-L. 2000. Pollinator selectivity and its implications for the evolution of dioecy and sexual dimorphism. *Ecology* **81**: 2577–2591.
- Ashman T-L. 2005. The limits on sexual dimorphism in vegetative traits in a gynodioecious plant. *American Naturalist* **166** (Supplement): S5–S16.
- Ashman T-L. 2006. The evolution of separate sexes: a focus on the ecological context. In: LD Harder, Barrett SCH, eds. *Ecology and evolution of flowers*. Oxford: Oxford University Press, 204–222.
- Ashman T-L, King EA. 2005. Are flower-visiting ants mutualists or antagonists? A study in a gynodioecious wild strawberry. *American Journal of Botany* **92**: 891–895.
- Asikainen E, Mutikainen P. 2003. Female frequency and relative fitness of females and hermaphrodites in gynodioecious *Geranium sylvaticum* (Geraniaceae). *American Journal of Botany* **90**: 226–234.
- Bailey MF, Delph LF, Lively CM. 2003. Modeling gynodioecy: novel scenarios for maintaining polymorphism. *American Naturalist* **161**: 762–776.
- Barrett SCH. 1995. Mating-system evolution in flowering plants: micro- and macroevolutionary approaches. *Acta Botanica Neerlandica* **44**: 385–402.
- Barrett SCH. 2002. The evolution of plant sexual diversity. *Nature Reviews Genetics* **3**: 274–284.
- Barrett SCH, Dorken ME, Case AL. 2001. A geographical context for the evolution of plant reproductive systems. In: Silvertown J, Antonovics J, eds. *Integrating ecology and evolution in a spatial context*. Oxford: Blackwell Science, 341–364.
- Bellot F. 1978. *El tapiz vegetal de la Península Ibérica*. Madrid: Blume Ediciones.
- Brickell CD, Mathew B. 1976. *Daphne. The genus in the wild and in cultivation*. Surrey: The Alpine Garden Society.
- Bustamante J. 2003. Cartografía predictiva de variables climáticas: comparación de distintos modelos de interpolación de la temperatura en España peninsular. *Graellsia* **59**: 359–376.

- Capel Molina JJ. 1981. *Los climas de España*. Barcelona: Oikos-Tau Ediciones.
- Case A, Barrett SCH. 2004. Floral biology of gender monomorphism and dimorphism in *Wurmbea dioica* (Colchicaceae) in Western Australia. *International Journal of Plant Science* 165: 289–301.
- Charlesworth D. 1999. Theories of the evolution of dioecy. In: Geber MA, Dawson TE, Delph LF, eds. *Gender and sexual dimorphism in flowering plants*. Berlin: Springer-Verlag, 33–60.
- Charlesworth D, Charlesworth B. 1978. A model for the evolution of dioecy and gynodioecy. *American Naturalist* 112: 975–997.
- Delph LF. 1990. Sex-ratio variation in the gynodioecious shrub *Hebe stric-tissima* (Scrophulariaceae). *Evolution* 44: 134–142.
- Delph LF, Carroll SB. 2001. Factors affecting relative seed fitness and female frequency in a gynodioecious species, *Silene acaulis*. *Evolutionary Ecology Research* 3: 487–505.
- Delph LF, Wolf DE. 2005. Evolutionary consequences of gender plasticity in genetically dimorphic breeding systems. *New Phytologist* 166: 119–128.
- Eckhart VM. 1991. The effect of floral display on pollinator visitation vary among populations of *Phacelia linearis* (Hydrophyllaceae). *Evolutionary Ecology* 5: 370–384.
- Eckhart VM, Seger J. 1999. Phenological and developmental costs of male sex function in hermaphroditic plants. In: Vuorisalo TO, Mutikainen PK, eds. *Life history evolution in plants*. Dordrecht: Kluwer, 195–213.
- Harder LD, Barrett SCH. 1995. Mating cost of large floral displays in hermaphrodite plants. *Nature* 373: 512–515.
- Herrera CM, Castellanos MC, Medrano M. 2006. Geographical context of floral evolution: towards an improved research programme in floral diversification. In: Harder LD, Barrett SCH, eds. *Ecology and evolution of flowers*. Oxford: Oxford University Press, 278–294.
- Kikuzawa K. 1989. Floral biology and evolution of gynodioecism in *Daphne kamtschatica* var. *jezoensis*. *Oikos* 56: 196–202.
- Lankinen Å, Armbruster WS. 2007. Pollen competition reduces inbreeding depression in *Collinsia heterophylla* (Plantaginaceae). *Journal of Evolutionary Biology* 20: 737–749.
- Lewis D. 1941. Male sterility in natural populations of hermaphrodite plants. The equilibrium between females and hermaphrodites to be expected with different types of inheritance. *New Phytologist* 40: 56–63.
- Littell RC, Milliken GA, Stroup WW, Wolfinger RD. 1996. *SAS system for mixed models*. Cary, NC: SAS Institute.
- McCauley DE, Taylor DR. 1997. Local population structure and sex ratio: evolution in gynodioecious plants. *American Naturalist* 150: 406–419.
- Medrano M, Alonso C, Herrera CM. 2005. Mating system, sex ratio, and persistence of females in the gynodioecious shrub *Daphne laureola* L. (Thymelaeaceae). *Heredity* 94: 37–43.
- Nilsson E, Agren J. 2006. Population size, female fecundity, and sex ratio variation in gynodioecious *Plantago maritima*. *Journal of Evolutionary Biology* 19: 825–833.
- Obeso JR. 2002. The costs of reproduction in plants. *New Phytologist* 155: 321–348.
- Pannell J. 1997. The maintenance of gynodioecy and androdioecy in a metapopulation. *Evolution* 51: 10–20.
- Peinado Lorca M, Rivas-Martínez S, eds. 1987. *La vegetación de España*. Madrid: Universidad de Alcalá de Henares.
- Ramsey M, Vaughton G, Peakall R. 2006. Inbreeding avoidance and the evolution of gender dimorphism in *Wurmbea biglandulosa* (Colchicaceae). *Evolution* 60: 529–537.
- Sakai AK, Weller SG. 1999. Gender and sexual dimorphism in flowering plants: a review of terminology, biogeographic patterns, ecological correlates, and phylogenetic approaches. In: Geber MA, Dawson TE, Delph LF, eds. *Gender and sexual dimorphism in flowering plants*. Berlin: Springer-Verlag, 1–31.
- SAS Institute. 2002. *SAS for Windows* (version 9-13). Cary, NC: SAS Institute.
- Shykoff JA, Kolokotronis S, Collin CL, López-Villavicencio M. 2003. Effects of male sterility on reproductive traits in gynodioecious plants: a meta-analysis. *Oecologia* 135: 1–9.
- Tøtland O. 2001. Environment-dependent pollen limitation and selection on floral traits in an alpine species. *Ecology* 82: 2233–2244.
- Vamosi JC, Otto SP, Barrett SCH. 2003. Phylogenetic analysis of the ecological correlates of dioecy in angiosperms. *Journal of Evolutionary Biology* 16: 1006–1018.
- Vaughton G, Ramsey M. 2004. Dry environments promote the establishment of females in monomorphic populations of *Wurmbea biglandulosa* (Colchicaceae). *Evolutionary Ecology* 18: 323–341.
- Webb CJ. 1999. Empirical studies: evolution and maintenance of dimorphic breeding systems. In: Geber MA, Dawson TE, Delph LF, eds. *Gender and sexual dimorphism in flowering plants*. Berlin: Springer-Verlag, 61–95.
- Weiblen GD, Oyama RK, Donoghue MJ. 2000. Phylogenetic analysis of dioecy in monocotyledons. *American Naturalist* 155: 46–58.
- Williams CF, Kuchenreuther MA, Drew A. 2000. Floral dimorphism, pollination, and self-fertilization in gynodioecious *Geranium richardsonii* (Geraniaceae). *American Journal of Botany* 87: 661–669.
- Wolfe LM, Shmida A. 1997. The ecology of sex expression in a gynodioecious Israeli desert shrub (*Ochradenus baccatus*). *Ecology* 78: 101–110.

SUPPLEMENTARY INFORMATION

Average estimates of plant and floral display sizes recorded in the ten northern *Daphne laureola* populations studied at the Iberian Peninsula, differing in breeding system and sex ratio. See Fig. 1 for population number and exact location. Standard deviation is given in parentheses

Region	NE										NW					
	26	27	24	18	19	14	16	1	BP*	6	1	2	3	4	5	6
Population #	26	27	24	18	19	14	16	1	BP*	6	1	2	3	4	5	6
% females	0	1	1	0	7.84	44.0	54.0	39.4			39.4					41.76
Sex	Herm	Herm	Herm	Herm	Herm	Fem	Herm	Fem	Herm	Fem	Herm	Fem	Herm	Fem	Herm	Fem
Sample size	40	40	40	40	36	4	20	20	20	20	20	20	20	20	20	20
No. stems	7.33 (5.47)	1.58 (0.90)	9.30 (10.93)	2.38 (1.64)	2.58 (2.64)	13.00 [†] (7.21)	10.25 (8.85)	13.75 (14.15)	6.80 (9.34)	15.65 (18.71)	26.80 (31.80)	25.75 (22.12)	13.10 (17.87)	26.40 (32.25)	86.10 (68.84)	88.75 (112.49)
No. flowering stems	–	1.23 (0.53)	7.45 (8.97)	2.08 (1.44)	2.06 (1.64)	14.75 (14.01)	6.45 (6.85)	5.75 (7.00)	2.20 (1.44)	5.95 (11.99)	23.20 (30.02)	22.55 (20.82)	9.95 (16.13)	15.70 (17.28)	74.80 (51.31)	75.70 (88.66)
% flowering stems	–	88.33 (24.45)	84.49 (24.66)	91.23 (17.95)	90.17 (17.91)	51.09 (19.98)	66.85 (28.64)	53.14 (31.79)	60.47 (35.04)	44.50 (31.54)	79.82 (20.36)	81.26 (15.96)	74.09 (24.96)	67.77 (24.52)	92.00 (9.40)	88.84 (18.04)
Inflorescences per stem	4.06 (1.46)	2.47 (1.56)	7.28 (3.80)	6.05 (2.02)	6.49 (2.13)	4.50 (1.11)	5.29 (1.83)	5.93 (2.69)	4.93 (2.95)	3.83 (1.96)	6.62 (2.11)	7.58 (2.21)	6.53 (4.56)	7.31 (4.39)	9.68 (2.48)	7.90 (2.34)
Flowers per inflorescence	5.62 (1.40)	5.39 (1.10)	5.78 (0.85)	5.91 (0.68)	6.31 (0.76)	6.33 (0.98)	6.18 (0.85)	6.48 (1.57)	6.29 (1.35)	5.98 (1.41)	6.92 (1.28)	7.45 (1.25)	5.30 (1.54)	6.13 (1.45)	6.05 (1.10)	5.80 (1.09)
Flower	115.9	14.8	441.1	78.9	84.8	420.2	251.9	259.2	72.5	290.1	1079.8	1119.5	606.9	875.9	4477.9	3478.0

production[§] (130.0) (14.6) (677.5) (70.6) (76.1) (438.3) (386.2) (409.7) (79.1) (884.3) (1428.3) (894.7) (1544.4) (1215.0) (4226.6) (4347.9)

* Bosque de Peloño, gynodioecious population close to population # 10 for which only an incomplete sex ratio was obtained and thus was not included in the sex ratio study

† Some of the scarce female plants found in this population were extremely large and one individual with 165 stems and most of them without flowers were excluded from calculations

§ Estimated multiplicatively per plant by firstly averaging no. inflorescences/stem × no. flowers/inflorescence, and subsequently multiplying it by the no. of flowering stems. In population # 26 in which the number of flowering stems per plant was not recorded, this was estimated from the average percentage of flowering stems observed in the two closest populations (27 and 24 = 86.41 %)