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To be or not to be better pollinated: Differences between sex morphs in marginal gynodioecious populations[1]

Antonio R. Castilla[2,3], Conchita Alonso[2], and Carlos M. Herrera[2]

[2]Estación Biológica de Doñana, Consejo Superior de Investigaciones Científicas (CSIC),
Avenida Américo Vespucio s/n 41092 Sevilla, Spain

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[3] Author for correspondence (e-mail: acastilla@utexas.edu), phone: +1-512-471-5858; present address: Section of Integrative Biology, University of Texas at Austin, 401 Biological Laboratories 1 University Station, Austin, Texas 78712 USA

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PREMISE OF THE STUDY: Changes in the pollinator communities of marginal plant populations can affect their pollination quantity or quality. Geographic variation in pollination success can alter the reproductive advantage that female plants require to persist within gynodioecious populations. Particularly valuable is determining the pollination success at the prezygotic stage in self-compatible gynodioecious species whose females do not exhibit enhanced seed production.

METHODS: In core and marginal populations of *Daphne laureola*, we analyzed the differences between hermaphrodites and females in the proportion of flowers visited, the stigma pollen loads, and the quantity of pollen tubes in styles. We also examined the relationship between the number of pollen tubes in styles vs. the number of pollen grains on stigmas using piecewise regression and binomial generalized linear mixed models.

KEY RESULTS: Pollinators deposited larger pollen loads on flowers in marginal populations. In marginal populations, female flowers received more pollinator visits and more pollen grains on their stigmas, and they had more pollen tubes in their styles than did female flowers in core populations. Both piecewise regression and binomial GLMM analyses showed that females in marginal populations had a lower proportion of grains that developed tubes than females in the core populations, which suggests decreased pollination quality.

CONCLUSIONS: More efficient pollination services in marginal populations decreased the overall differences in the prezygotic pollination success between the sex morphs. Our results also suggest that pollination quality is lower in females of marginal populations, which could be counteracting the increased pollination in females in marginal populations.

KEY WORDS: *Daphne laureola*; gynodioecy; margins of distribution; pollen tubes; pollen grains; piecewise regression; pollinator fauna; Thymelaeaceae

Declining reproductive success is a common explanation for limits in the distribution of plant species (Gaston, 2009; Hargreaves and Eckert, 2014). In fact, inadequate pollen receipt may be the most prominent cause of reproductive impairment in marginal populations (Aguilar et al., 2006; Levin, 2012). Changes in pollinator community composition toward margins of plant distributions have been reported in the literature (e.g., Silva-Montellano and Eguiarte, 2003; Moeller, 2006; Castilla, 2011; Chalcoff et al., 2012). Shifts in the identity of floral visitors leading to an overrepresentation of low-quality pollinators can result in inefficient pollen transfer, low pollen tube survival and zygote death (Herrera, 1987; Gómez et al., 2010; Harder and Aizen, 2010). Furthermore, these shifts in the pollinator community may have important consequences for the mating system within marginal populations (Hargreaves and Eckert, 2014; Bontrager and Angert, 2015). Variation in mating systems toward margins of distribution can be particularly relevant for gynodioecious species with self-compatible hermaphroditic plants because the two sex morphs have contrasting mating strategies (i.e., self-compatibility in hermaphroditic and strict self-incompatibility in female plants).

Gynodioecy, the co-occurrence of females and hermaphrodites within the same population, is a relatively common sexual system that constitutes one of the most likely steps in the pathway from hermaphroditism to dioecy in plants (Webb, 1999; Weiblen et al., 2000; Dufay et al., 2014). To cope with this 50% reduction in gene transmission due to the lack of male function, female plants must exhibit a reproductive advantage over hermaphrodites to persist within populations (Lewis, 1941; Charlesworth and Charlesworth, 1978; Dufay and Billard, 2012). The magnitude of reproductive advantage needed to maintain female plants, depends on the genetic control of sex and can be gained by producing higher quantity or quality of seeds (Charlesworth and Charlesworth, 1978; Bailey et al., 2003; Dufay and Billard, 2012). Because pollination constitutes the first stage in the reproductive process, the study of the relative pollination success of each sex is an essential first step to understand how sex morphs coexist within populations and how the female frequency can vary among populations (Ashman, 2006; Alonso et al., 2007; Ramula et al., 2007; Alonso and Herrera, 2011). Furthermore, examining the quality of pollination may be particularly important to understand those situations where the female reproductive advantage is not achieved by increased seed production or where such reproductive advantage varies among populations (Shykoff et al., 2003; Dufay and Billard, 2012; Molano-Flores and Faivre, 2014).

The reproductive advantage of female plants varies among populations in many gynodioecious species (Dufay and Billard, 2012). However, few studies have explored the processes involved in the reduction of the fecundity of female plants (Van Etten and Chang, 2014). Pollinator discrimination against female plants can constitute an important factor decreasing their fecundity (reviewed by Delph, 1996). Pollinator discrimination against females can be caused by the lower attractiveness of female flowers to pollinators due to their smaller size, number (Delph, 1996; Shykoff et al., 2003) and reduced floral rewards compared with hermaphroditic flowers (no pollen and less nectar, reviewed by Delph, 1996; Talavera et al., 1996; Eckhart, 1999; Molano-Flores, 2002). Shifts in the pollinator community composition may alter the magnitude of pollinator discrimination against females and thus the difference in the pollination success between sex morphs (Van Etten and Chang, 2014).

The analysis of pollen grain–pollen tube dose-response curves of naturally pollinated, wilted flowers constitutes a suitable tool to study the quantity and quality of the pollination (Aizen and Harder, 2007; Alonso et al., 2012, 2013). Recently, Alonso et al. (2012) proposed that changes in the relative importance of quantity and quality of pollen limitation determine the shape of natural pollen grain–pollen tube dose-response curves. Their relative importance in determining pollination success can be quantified by using piecewise regression analysis (Alonso et al., 2012). This analytical framework excludes the confounding effects of resource allocation during the post-pollination processes involved in the more traditional hand-pollination experiments (Knight et al., 2006; Aizen and Harder, 2007; Wesselingh, 2007; Alonso et al., 2012).

In this study, we analyzed geographic variation in the pollination intensity and quality of the gynodioecious shrub *Daphne laureola* in the southern Iberian Peninsula. In core populations, the main pollinator is a small pollen-beetle that visits female plants less frequently than hermaphrodites, although it deposits higher-quality pollen in female flowers (Alonso, 2004, 2005; Alonso et al., 2012). In these populations, the persistence and relative high frequency of females is accounted for by a combination of extensive selfing in hermaphrodites and strong inbreeding depression in their offspring (Medrano et al., 2005). Marginal populations exhibit more diverse pollinator communities with several bee and bumble bee species visiting flowers of *D. laureola* (Castilla et al., 2011). These pollinators seem to provide more efficient pollination service given the higher fruit set of plants in marginal populations (Castilla et al., 2011). However, information about the pollination success and mating system of the plants in marginal populations is not fully characterized. Here, we explore variation in the quantity and quality of pollination success in a geographic context and focus on the pollination in marginal disjunct populations. We expected that marginal disjunct populations of *D. laureola*, which had more diverse pollinator communities, exhibited (1) higher quantity and quality of pollination and (2) decreased differences in pollination success between sex morphs.

MATERIALS AND METHODS

Study species—

Daphne laureola L. (Thymelaeaceae) is an evergreen shrub with a disjunct Palearctic distribution associated with the main calcareous mountain ranges of Europe and northern Africa. In the Iberian Peninsula, it has a disjunct distribution, being abundant in the northern Cantabrian Range and the Pyrenees and also in the southern Baetic Ranges (Alonso et al., 2007). This study was conducted in the Baetic Ranges (Appendix S1, see Supplemental Data with the online version of this article), where the species flowers from January to April, a period characterized by low temperatures with frequent rains and irregular snowfalls. Plants of core populations are larger and produce more flowers and fruits than those of marginal populations (Castilla et al., 2011). Within each region, hermaphroditic and female plants are similar in size and flower and fruit production (Alonso and Herrera, 2001; Castilla et al., 2011). Each plant produces a large number of small, tubular, green-yellowish flowers aggregated into several compact inflorescences per stem. Hermaphroditic flowers have longer corollas than female flowers, although the magnitude of the difference between sex morphs is higher in core populations (Castilla et al., 2015). Individual flowers of both sex morphs have a single ovule and a very short style, which cannot be detached from the ovary without damaging it. Each flower remains open and functional for up to 1 month (Alonso, 2004). Hermaphroditic plants are fully self-compatible, but fruit production requires flower visitation by pollinators, and an excess of pollen may clog the stigma, reducing fruit set (Alonso and Herrera, 2001). The small beetle *Meligethes elongatus* Rosenhauer is the main pollinator in core populations (Alonso, 2004). During four consecutive flowering seasons (2007–2011), this pollen beetle was never observed visiting *D. laureola* flowers in the marginal disjunct populations studied here (Castilla, 2011; Castilla et al., 2011). Instead, several bee and bumble bee species were the main floral visitors in marginal populations (Castilla, 2011).

Study area—

Across the Baetic Ranges, *D. laureola* is especially abundant in the Sierra de Cazorla, where populations are largely connected and occupy a broad altitudinal range (Appendix S1; Castilla et al., 2012). The spatial isolation of populations increases toward the western and eastern margins of the Baetic Ranges, especially in western populations that are analyzed here (Appendix S1; Castilla et al., 2012). Furthermore, western marginal populations are strongly genetically differentiated from core populations of *D. laureola* in the southern Iberian Peninsula, even though they do not differ in their overall genetic diversity (Castilla et al., 2012). Therefore, in this study, we considered the Sierra de Cazorla as the local continuous core (“core region” hereafter) and populations at the western edge as local disjunct margin (“marginal region” hereafter). We studied three accessible populations per region (Table 1;[ID]TBL1[/ID] see Castilla et al., 2011, for a more detailed description of study populations). All study populations were gynodioecious with the proportion of females ranging from 10 to 25.5%. The proportion of females did not differ significantly between core and marginal populations, although it tended to be lower in the marginal ones (Mann–Whitney U test; $Z = 1.75$, $P = 0.08$; Table 1). In this study, we randomly marked 25 hermaphroditic plants and 15 female plants in each study population to avoid potential artifacts due to uncontrolled variation in sex ratio among populations.

Pollination and reproductive success—

In 2007, 12 wilted flowers per marked plant were collected at the end of the flowering period (N = 2880 flowers). 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 (0.1% aniline blue in 0.1 M K_3PO_4). Pollen grains on the stigma and pollen tubes at the base of the style were counted for each flower using a fluorescence microscope.

We explored the relationship between pre- and postzygotic fitness using Pearson correlations between the mean numbers of pollen grains deposited on the stigma, pollen tubes in the style, and the fruit set of each individual plant. Since the style cannot be detached from the ovary without damaging it, we used two different sets of inflorescences for each plant; one for the monitoring of pollination success (i.e., 12 inflorescences and 1 flower per inflorescence), and another for the monitoring of fruit set (i.e., 5 inflorescences; see Castilla et al., 2015 for details).

Data analyses—

The analyses were performed in R 3.1.1 (R Development Core Team, 2010), except for the piecewise regression, which was conducted in SAS 9.2 (SAS Institute, Cary, North Carolina, USA). The quantitative component of pollination success was evaluated by generalized linear mixed models (GLMM) using the lme4 package (glmer function; Bates et al., 2014) and three response variables; proportion of flowers visited, pollen receipt, and number of pollen tubes in the styles. First, for each plant, we quantified the proportion of flowers with at least one pollen grain on their stigmas (“proportion of flowers visited” hereafter), which was analyzed using a GLMM with a binomial error distribution that accounts for differences in sample size [i.e., R glmer: cbind (s, n-s) ~ β_i, where n = number of trials, s = number of successes, β_i = explanatory variables]. In our model, the number of trials is the number of flowers assessed per plant, and the number of successes is the number of flowers visited per plant. Second, the number of pollen grains on the stigma was used to estimate the quantity of pollen received ("pollen receipt" hereafter), and it was analyzed with a Poisson error structure and a log link function. Third, the number of pollen tubes at the base of the style was used as the most proximate estimate of quantitative prezygotic pollination success (Herrera, 2004; Burd et al., 2009), and it was modeled with a Poisson error structure and a log link function. In all three models, we included sex, region, and their interaction as fixed factors. In addition, we included population as a random factor nested within region. We quantified the pollen receipt and pollen tubes in multiple flowers within individuals, and thus, we included individual as a random factor nested within population in these two analyses. All data were over-dispersed (i.e., greater variance relative to the expectation in the modeled distribution). To account for the over-dispersion, we included an observational-level effect where each data point receives a unique level of a random effect that models the extra variation present in the data (Bolker et al., 2009; Harrison, 2014). We used Tukey contrast tests for all pairwise comparisons among groups.

The quality component of pollination was analyzed as the functional relationship between pollen tubes and pollen grains of flowers visited using two different approaches. First, we used the "piecewise" approach of Alonso et al., (2012) to analyze the functional relationship between pollen tubes and pollen grains in those flowers with at least one pollen grain on their stigmas (i.e., flowers visited). Linearity of the relationship between pollen tubes and grains was tested using generalized additive models (procedure GAM). A significant spline component indicates improvement of fit when the model includes a nonlinear local component, in which case, a piecewise regression was conducted. Piecewise regression allows the inclusion of a breakpoint in a linear regression when an abrupt change in the slope of the relationship between the two variables is predicted. In the pollen tubes–pollen grains relationship, the breakpoint (c) has been interpreted as a shift in the relative importance of quantitative (Qt) and qualitative (Ql) components of pollination success along the pollen receipt axis. The width of the confidence interval around *c* defines the range of pollen load sizes for which Qt and Ql contribute similarly to pollination success (Alonso et al., 2012). The model is defined by the initial linear slope (*b1*), the breakpoint (*c*) and the second linear slope (*b2*), with $b1 > b2$. Following the description of Alonso et al. (2012), for comparative analysis higher initial slope (*b1*) reflects receipt of higher-quality pollen. Piecewise models were fitted using the NLIN procedure in SAS 9.2 (SAS Institute) and confidence intervals (BCa, hereafter) for all three parameter estimates (*b1*, *b2*, and *c*) in the piecewise regression were calculated using nonparametric bootstrapping ($N = 1000$). We applied the method to female and hermaphroditic samples separately within each region (core and marginal) but merging all populations within each region to reach the required sample sizes after excluding flowers with zero pollen grains. Comparisons between sexes in core and marginal populations with regard to the slopes of the piecewise regressions (*b1*, *b2*) were then conducted by splitting the data sets at *c* and applying a test of slope (ANCOVA) on each data subset (see Alonso et al., 2012 for details).

Second, we analyzed the proportion of pollen grains that developed pollen tubes (i.e., stigmatic germinability sensu Dafni and Firmage, 2000) using a GLMM with a binomial error distribution that accounts for differences in sample size (i.e., R glmer: cbind (s, n-s) ~ β_i, where n = number of trials, s = number of successes, β_i = explanatory variables). In our model, the number of trials represents the number of pollen grains on the stigma of each flower, and the number of successes represents the number of pollen grains that developed pollen tubes. Differences between regions and sexes in the probability of tube development were analyzed including sex, region, and their interaction as fixed factors. In addition, we included population as a random factor nested within region and individual as a random factor nested within population.

RESULTS

***Proportion of flowers visited, pollen receipt, and pollen tubes*—**

The proportion of flowers visited (i.e., pollen grains > 0) was higher for hermaphroditic than for female plants in both regions, although the difference between sex morphs was significantly greater in core populations (region × sex: $\chi^2 = 6.43$, df = 1, $P < 0.05$; Fig. 1A).[ID]FIG1[/ID] More specifically, females had a higher proportion of flowers visited in marginal populations ($z = 6.81$, $P < 0.0001$; Fig. 1A), while hermaphrodites presented similar proportion of flowers visited in core and marginal populations ($z = 1.49$, $P = 0.4$; Fig. 1A).

Flowers of hermaphroditic plants exhibited greater pollen receipt than did females' flowers, the difference being significantly larger in core populations (region × sex: $\chi^2 = 65.06$, df = 1, $P < 0.0001$; Fig. 1B). These results were consistent when considering only flowers visited, i.e., excluding zero pollen loads (region x sex: $\chi^2 = 22.88$, df = 1, $P < 0.0001$; Appendix S2, see online Supplemental Data). Both female and hermaphrodite flowers received more pollen in marginal populations than in core populations ($z = 11.31$, $P < 0.001$ and $z = 4.02$, $P < 0.001$, respectively; Fig. 1B).

Flowers of hermaphroditic plants had a higher number of pollen tubes in their styles compared with flowers of female plants in both regions, although the difference between sex morphs was greater in the core populations (region × sex: $\chi^2 = 98.3$, df = 1, $P < 0.0001$; Fig. 1C). Female flowers of marginal populations exhibited a higher number of pollen tubes in the style than did female flowers of core populations ($z = 4.64$, $P < 0.001$; Fig. 1C). In contrast, the mean number of pollen tubes in hermaphroditic flowers did not differ between regions ($z = -2.27$, $P = 0.09$; Fig. 1C).

***Functional relationship between pollen tubes and grains of visited flowers*—**

Piecewise regression analysis showed that the slope of the initial relationship between pollen grains and tubes (*b1*) was nearly twice as high in females than in hermaphrodites in core populations, suggesting that hermaphrodites received lower-quality pollen in core populations (sex × pollen receipt: $F_{1,\,508} = 5.08$, $P < 0.05$; Table 2, Fig. 2).[ID]TBL2[/ID][ID]FIG2[/ID] In contrast, *b1* was similar for the two sex morphs in marginal populations (sex × pollen receipt: $F_{1,\,369} = 2.02$, $P = 0.16$; Table 2, Fig. 2). The slope of the second relationship (*b2*) did not differ significantly from zero in any case (Table 2, Fig. 2), suggesting that styles were fully saturated with pollen tubes in all cases. The number of tubes corresponding to the breakpoint was considerably higher than the single ovule per flower in all cases (16 and 8 tubes for hermaphrodites and females, respectively, in core populations; 7 and 13 for hermaphrodites and females, respectively, in marginal ones).

Binomial response analyses revealed that hermaphroditic flowers had a lower proportion of pollen grains developing pollen tubes than did female flowers in core and marginal populations ($z = -9.26$, $P < 0.001$ and $z = -17.24$, $P < 0.001$ respectively; Fig. 3).[ID]FIG3[/ID] The difference between sex morphs with respect to the proportion of pollen grains developing pollen tubes was greater in marginal populations than in core populations (region × sex: $\chi^2 = 4.44$, df = 1, $P < 0.05$; Fig. 3). In marginal populations, flowers of female plants exhibited a lower proportion of pollen grains developing tubes than in females in core populations ($z = -2.65$, $P < 0.05$; Fig. 3). Similarly, flowers of hermaphroditic plants had a lower proportion of grains developing tubes than those of hermaphrodites in core populations ($z = -3.99$, $P < 0.001$ respectively; Fig. 3).

Relationship between pre- and postzygotic fitness—

Both the mean number of pollen grains on the stigma and the mean number of pollen tubes in the style were positively correlated with fruit set per plant ($r_p = 0.36$, $P < 0.0001$; $r_p = 0.13$, $P < 0.05$, respectively; online Appendix S3).

DISCUSSION

This study revealed that pollination services were not worse in marginal populations of the gynodioecious shrub *D. laureola* than in the core populations. Both sexes had higher pollination intensity in marginal than in core populations. However, this increase was more marked in flowers of female plants, which reduced the differences in the relative pollination success between the two sex morphs in marginal populations. Despite receiving more pollen grains on their stigmas, the flowers of female plants had a lower proportion of pollen grains developing pollen tubes in marginal than in core populations. This interesting finding suggests a lower pollination quality in female plants of marginal populations compared with those of core populations, although further studies are necessary to validate this hypothesis. In the following paragraphs, we will discuss these findings in detail.

Pollination service in marginal populations—

Depleted reproduction of marginal populations could be caused by marked pollinator declines and ensuing pollen limitation associated with the loss of the most effective pollinators (Levin, 2012). However, the empirical support for this prediction remains elusive with only two of three studies reporting evidence for increased pollen limitation in marginal populations (Moeller et al., 2011; Chalcoff et al., 2012; Hargreaves et al., 2015). A previous study reported increased fruit set (i.e., proportion flowers setting fruits) in marginal populations of *D. laureola* suggesting more efficient pollination services in these populations (Castilla et al., 2011). Results from the current study confirmed the preceding prediction and did not support depleted pollination service in individuals of marginal populations, regardless of their sex. Both sex morphs receive more pollen in marginal populations. In addition, hermaphroditic flowers were similarly visited and had similar pollen tube quantities in both regions, whereas female flowers were more frequently visited and had larger pollen tube loads in marginal populations. Hargreaves et al. (2015) reported the absence of pollen limitation despite pollen receipt decline in marginal populations of *Rinanthus minor*. However, our study is the first to report higher pollen receipt and pollen tube formation in marginal plant populations, calling into question the generality of pollination deficits in marginal populations of plants.

Geographic variation in the relative pollination success between sex morphs—

Geographic changes in pollinator composition can alter the relative pollination success between morphs of sexually polymorphic plants (Case and Barrett, 2004; Ashman, 2006). Our results confirmed the reduction of pollination quantity in female flowers compared with hermaphroditic flowers in core populations of *D. laureola* (Alonso, 2005) and revealed smaller differences between sex morphs regarding the pollination quantity in marginal populations. Shifts in pollinator community composition can lead to changes in pollinator discrimination against female plants in populations of gynodioecious plants. For instance, Van Etten and Chang (2014) reported increased pollinator discrimination against female plants of *Geranium maculatum* when the pollinator fauna changed from being dominated by small bee species to large and medium bee species. Interestingly, in core populations of *D. laureola* where the small pollen-beetle *M. elongatus* is the main pollinator, female flowers were less frequently visited. In contrast, both sex morphs were similarly visited in marginal populations dominated by bees and bumblebees. Some studies have suggested that reduction in visual floral display (petal size and flower number) or rewards (pollen and nectar) are responsible for the females' lower attractiveness to pollinators relative to male-fertile conspecifics (i.e., males or hermaphrodites; Bell, 1985; Delph and Lively, 1992; Ashman et al., 2000). In *D. laureola,* smaller differences between sexes in the corolla tube length have been reported in marginal populations, where the discrimination against females is also lower (Castilla et al., 2015). Controlled experiments on pollinator behavior could test whether removal of fertile anthers, a difference in corolla length, or both are cues to discriminate between female and hermaphroditic flowers by different pollinators.

The decreased pollinator discrimination against females in marginal *D. laureola* populations is accompanied by more similarity of sex morphs with respect to quantity of stigma pollen and pollen tubes per style through a more marked increase of pollination intensity in females than hermaphrodites of marginal populations. In the core region, seed production in female plants is mostly limited by pollen deposition (Alonso, 2005; Alonso et al., 2012). Therefore, the increase in the quantity of pollination and opportunities for gametophyte competition may facilitate the maintenance of female plants in the marginal region, as long as this improved pollination leads to more or better quality of the offspring. Our results revealed a positive relationship between our estimates of prezygotic pollinations success and the fruit set at the plant level. Nevertheless, a comprehensive evaluation would ideally require the use of controlled hand pollination experiments to apply different amounts of pollen to female flowers and track the effects in terms of the seed production, seed mass, seed germination, and seedling survival.

Interestingly, the increase in the pollen deposition on female flowers of marginal populations did not lead to an equivalent increase in pollen tube production, but it reduced the female advantage in pollen quality observed in core populations (Alonso, 2005; Alonso et al., 2012). Our results of the piecewise regression and the binomial GLMM analyses similarly suggested a decreased ability of pollen grains to produce tubes in female flowers of the marginal populations compared with those in core populations. This result requires further discussion because female and hermaphroditic plants do not differ in seed production (Alonso and Herrera, 2001; Castilla et al., 2011), but differences in seed quality seem to be essential for successful offspring recruitment at least in core populations (Medrano et al., 2005). Therefore, low quality pollen could counteract the higher pollination intensity experienced by females of marginal populations if they produce low quality offspring.

In a recent review, Losdat et al. (2014) found support for a negative impact of inbreeding on pollen performance. Strong fine-scale spatial genetic structure with large genetic patch size enhances biparental inbreeding by promoting mating between more related individuals in a population (Williams, 2007; Zhao and Lu, 2009; Castilla et al., 2016). A stronger fine-scale spatial genetic structure has been reported in marginal compared with core populations for a number of plant species (e.g., Gapare and Aitken, 2005; Pandey and Rajora, 2012), but it has not been investigated in *D. laureola* yet. While biparental inbreeding is expected to limit the reproduction of both sex morphs in gynodioecious species, several studies have reported stronger negative effects on females' offspring (Keller and Schwaegerle, 2006; Collin et al., 2009). For instance, Collin et al. (2009) reported that sib crosses performed worse than outcrosses in females of the gynodioecious *Dianthus sylvestris*, whereas for hermaphrodites both sib- and outcross progenies performed well. Similarly, Keller and Schwaegerle (2006) found that female mothers of *Silene acaulis* experienced significant biparental inbreeding depression, whereas sib-crossed hermaphroditic mothers did not differ from within-population outcrosses. Therefore, increased biparental inbreeding in marginal populations with differential effects on the pollination success of female and hermaphroditic plants could account for the lower quality of pollination in females of marginal populations. Alternatively, multiple visits to female flowers in marginal populations could lead to a lower proportion of the pollen grains deposited, resulting in pollen tubes in the style because of competition between pollen grains deposited at different times. Sequential competition could occur if tubes grow into the style region where resources have been depleted by tubes from pollen grains deposited earlier. This low-resource style environment may cause a deceleration in the proportion of pollen grains developing tubes (Harder et al., 2016; and references therein). Further studies on fine-scale spatial genetic structure and mating system parameters using highly polymorphic genetic markers and specific hand-pollination crosses as described by Collin et al. (2009) could help to elucidate the mechanisms behind the observed pattern.

Concluding remarks—

The results of this study challenge the generality of the prediction that marginal populations will suffer from depleted pollinator service relative to core populations. Instead, our results reveal higher pollination intensity in marginal populations, with this increase being particularly marked in female plants. As a consequence and contrary to theoretical expectations, the differences between sex morphs are reduced in marginal populations relative to core populations. Therefore, our results provide empirical support for a relevant role of pollinators promoting geographic variation in the relative pollination success between sex morphs in gynodioecious species and encourage further studies linking pre- and postzygotic variation in pollination quality.

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Appendix S1. (a) Distribution range of *Daphne laureola* in the Iberian Peninsula. (b) *D. laureola* populations across the Baetic Ranges. Populations: core, filled circles; marginal, black triangles. (c, d) Detailed distribution of the study populations in the core and marginal regions, respectively. Intervals of altitude (m a.s.l.) in the study area are represented with a grey scale (see legend).

Appendix S2. Variation in mean pollen receipt (±SE) between sex morphs (hermaphrodites vs. females) and regions (core vs. marginal) of visited flowers of *Daphne laureola* in the southern Iberian Peninsula. Pollen receipts were calculated excluding flowers with zero pollen grains on their stigma.

Appendix S3. Correlations between the pre- and postzygotic fitness estimates. Mean pollen receipt represents the mean number of pollen grains deposited on the stigma of each plant. Fruit set refers the proportion of flowers setting fruits in each plant.

FIGURE 1. Variation in quantitative pollination success between sex morphs (hermaphrodites vs. females) and regions (core vs. marginal) of *Daphne laureola* in the southern Iberian Peninsula. In the box plots, horizontal lines represent the median, and boxes and whiskers the interquartile range and the nonoutlier range, respectively. Top row: Proportion of flowers visited (i.e., flowers with at least one pollen grain deposited on the stigma). Middle row: Natural logarithm of the number of pollen grains deposited on the stigma per flower (i.e., pollen receipt). Bottom row: Natural logarithm of the number of pollen tubes in the style per flower. In all cases, dots represent individual flowers (N = 469 and 444 female flowers in core and marginal populations; N = 748 and 738 hermaphroditic flowers in core and marginal populations, respectively).

FIGURE 2. Piecewise regression (solid line) of the relationship between the numbers of pollen grains and pollen tubes for female and hermaphroditic individuals in core and marginal populations of *Daphne laureola*. Each symbol corresponds to an individual flower. Vertical dashed lines denote the 95% approximate bootstrapping confidence intervals (BCa) of the breakpoint estimated by a piecewise regression with 1000 bootstraps. Bar graphs in the inserts depict the relative frequency of data points falling into each of the three regions defined by the confidence intervals around the breakpoint. Note that the x-axes were scaled to data.

FIGURE 3. Proportion of pollen grains developing pollen tubes in female and hermaphroditic individuals in core and marginal populations of *Daphne laureola*. Confidence intervals were calculated using 500 bootstraps of the values of predicted means. Dots indicate the observed pollen loads per flower. The dot size corresponds to different intervals of pollen load size.

TABLE 1. General features of the six *Daphne laureola* populations studied. Population size refers to the number of reproductive plants per population. Female frequency refers to the percentage of female plants in a sample of 100 randomly chosen plants per population.

Region	Population	Altitude (m.a.s.l.)	Population size	Female frequency (%)
Core	Cañada del Espino (CDE)	1575	178	20
	Fuente Bermejo (FBJ)	1513	2115	21
	Valdecuevas (VCV)	1380	2787	25.5
Marginal	Fuente Molina (FML)	1380	621	20
	Grazalema (GRZ)	1229	1580	10
	Cañada de las Animas (CDA)	1333	561	18.5

TABLE 2. Parameters of piecewise regression analysis of the relationship between numbers of pollen grains and pollen tubes for hermaphroditic and female individuals in core and marginal populations of *Daphne laureola*. The dependent variable is the number of pollen tubes in the styles, and the explanatory variable is the number of pollen grains deposited on the stigmas. The breakpoint indicates the number of pollen grains at which the slope of the relationship changes. Bootstrapping confidence intervals (BCa) were estimated with *n* = 1000 bootstraps. Slopes of the relationship before and after the breakpoint are represented by *b*1 and *b*2, respectively.

Region	Sex	*b*1 (CI)	Breakpoint (BCa)	*b*2 (CI)
Core	Female	0.57 (0.50–0.65)	14 (10–24)	0.02 (–0.11 to 0.15)
	Hermaphrodite	0.34 (0.30–0.40)	47 (37–56)	–0.01 (–0.03 to 0.01)
Marginal	Female	0.26 (0.22–0.34)	51 (29–71)	–0.06 (–0.16 to 0.02)
	Hermaphrodite	0.31 (0.20–0.37))	25 (20–43)	0.002 (–0.01 to 0.01)