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Influence of reproductive traits on pollination success in two *Daphne* species (Thymelaeaceae)

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Abstract Taxonomically related species can differ in a number of reproductive traits, which may translate into a differential mating system and pollination success. Here we compare two hermaphroditic insect-pollinated *Daphne* species (*D. rodriguezii* and *D. gnidium*) which differ in distribution (island endemic vs. mediterranean) and floral traits (long- vs. short-tube corolla). We investigated their mating system and pollen limitation by means of hand-pollination experiments and quantified the diversity and abundance of flower visitors by direct observations. Plant size and five reproductive traits (flower production, proportion of viable anthers, pollen production, flower tube length and tepal area) were studied to assess how they contribute to reproductive success, measured as proportion of pollen grains germinated per stigma and fruit set. Selfing was very low and pollen limitation existed in both species, though was higher in *D. rodriguezii* probably due to the scarcity of flower visitors. The low fruit set in both species suggests that most of the pollen grains found on stigmas are self-pollen. Pollinators appeared to favour some floral traits (specifically, flower tube length or tepal area) in both species, although flower crop in *D. rodriguezii* was the only reproductive trait influencing fruit set. In both species, the highest variability in reproductive traits and pollination success was within individuals. Our findings suggest that

despite both species showed similar mating system, dependency on outcrossing pollen and selection of floral traits, pollen limitation was higher in *D. rodriguezii*, probably as a higher proportion of self-pollen arrives to its stigmas.

Keywords *Daphne rodriguezii* · *Daphne gnidium* · Flower traits · Mating system · Phenotypic selection · Pollen deposition · Insect pollination · Species comparison

Introduction

Pollinators can select for different floral characters, such as flower tube, petal width and spur length, as well as for flower production (Harder and Johnson 2009, and references therein). The selection they exert for specific floral traits, however, may depend on the plant mating system. Self-incompatible plants, for instance, are expected to display more extreme values of floral traits (e.g. flower production and density, flower size, flower longevity, duration of flowering period) in order to attract larger numbers of pollinators and to assure successful reproduction (Valido et al. 2002; Momose 2004). The selection of floral traits may be especially important in pollen-limited populations (Knight et al. 2005), as is the case of those flowering-early species (Schemske et al. 1978; Alonso 2004), since low pollinator visitation may lead to selection of those characters with proportionally higher floral reward. However, some of the selected floral traits (e.g. floral display) may promote high insect visitation rates that, in turn, result into a lower quality and/or quantity of offspring. This happens, for instance, when geitonogamous crosses are increased in self-compatible plants (De Jong et al. 1993) and/or when there is high pollen clogging in

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species with low ovule-seed ratio (Holland and Chamberlain 2007). Plants with low flower visitors tend to show greater variation in pollination success than pollinator-generalist plants (Knight et al. 2005), suggesting that selection on floral traits may be a spatio-temporal stochastic process when pollinator visits are scarce.

Since the reproductive output of many plant species depends upon the diversity and reliability of pollinators (Kearns et al. 1998 and references therein), a decline in such pollinator diversity is expected to have important negative consequences on pollination service to plants, especially for already rare and/or endangered plants (Potts et al. 2006; Ebeling et al. 2008). However, plants may also have compensatory mechanisms to assure reproductive output when pollinators are scarce (Bond 1994). In self-compatible plants, an increased selfing rate is commonly observed in species that evolved in or colonized habitats with depauperate pollinator faunas, such as islands or high-mountain areas (Baker 1955) and/or in those flowering during seasons with low pollination service (see review in Traveset and Jakobsson 2007 and references therein). Nevertheless, selfing may also involve a reduction of offspring quality due to high levels of inbreeding depression (Barrett 1998 and references therein) and/or a decrease in male fitness or ‘pollen discounting’ (e.g. Fishman 2000). Thus, opposing forces may act on plant species to reduce dependency on pollinators and, at the same time, to avoid reductions in fitness (Barrett 1998; Traveset and Jakobsson 2007).

In the present study, our goal was to compare the mating system and pollination success of two congeneric species in the genus *Daphne* (Thymelaeaceae) and to assess whether they have a differential selection on plant and flower traits. The two species differ in their distribution. The first, *D. rodriguezii*, is endemic to Menorca island (Balearic Islands, W. Mediterranean) whereas the second, *D. gnidium*, is widespread throughout the Mediterranean area. They also differ in flowering time, flower production and flower size. Given that *D. rodriguezii* is an island endemic, we might expect that it has developed reproductive traits adapted to such depauperated habitats, in which probably there is a greater scarcity of pollinators than in larger islands or in continental areas (Baker 1955). Our specific objectives were the following:

- (1) To test whether the two species have different selfing rates and levels of pollen limitation. We expect the island-endemic species to have greater levels of selfing and/or to be more pollen-limited than the mediterranean species.
- (2) To quantify the diversity and abundance of flower visitors between species; the species with long-tube corolla (i.e. *D. rodriguezii*) probably receive lower

diversity of flower visitors than the species with short-tube corolla (i.e. *D. gnidium*).

- (3) To evaluate the importance of different factors affecting the pollination success of each *Daphne* species. Specifically, we wanted to test if reproductive traits such as flower production, proportion of viable anthers, pollen production, and flower size (flower tube length and tepal area) affect their reproductive success, estimated as (1) number and fraction of germinated pollen grains per stigma and (2) number and set of fruits. For each reproductive trait, we estimated the strength of phenotypic selection at the flower and plant level. We further explored whether such traits are in turn influenced by plant size (i.e. ‘budget effect’; De Jong and Klinkhamer 2005).
- (4) To quantify the degree of phenotypic variation of each reproductive trait through different biological levels, from flower to species, in order to explore at which biological level there might be selection on reproductive traits.

Materials and methods

Study species

The genus *Daphne* (Thymelaeaceae) consists of 70 shrub species which are mainly distributed in Europe and in temperate and subtropical Asia (Brickell and Mathew 1976). The main floral reward is nectar, which is produced at the bottom of tube-like corolla, being mainly accessible to long-tongued insects, such as butterflies and moths (Brickell and Mathew 1976). Most *Daphne* species are hermaphroditic, although some are gynodioecious (Brickell and Mathew 1976; Kikuzawa 1989; Alonso and Herrera 2001).

Daphne rodriguezii Teixidor is an endemic and threatened species from Menorca island (Balearic Islands), currently restricted to a few sites in the east and south of the island and to a small islet (<60 ha) located off the North-eastern coast. It is a perennial shrub, up to 1 m tall, which grows in coastal shrublands. Inflorescences, located in terminal position of branches of the previous year, are rapidly overtaken by new branches growing during flowering period (Castroviejo et al. 2001). Flowers, opening from below to above of the inflorescence are arranged in groups of one to three flowers, and remain open for 7–10 days (pers. obs.). They are hermaphroditic, cream-coloured, and have a sweet smell. Flowers consist of a corolla tube (4–10 mm) with four lobes (Fig. 1) and bear eight stamens arranged in two whorls of four stamens each. The ovary has a single locule and ovule (a common trait in the genus *Daphne*), and the style is very short or non-existent.

Daphne gnidium L. is also a perennial shrub up to 1.5 m tall, which has a wide distribution encompassing the entire Mediterranean Basin, from the Iberian Peninsula to Greece, and even extending to the Canary Islands (Castroviejo et al. 2001). Flowers, whose position is terminal from inflorescences located in the apical part of new branches (Roccoliello et al. 2009), open less synchronously than *D. rodriguezii* (i.e. the number of receptive flowers per plant of *D. gnidium* at flowering peak is less than 10%, compared with more than 50% in *D. rodriguezii*; pers. obs.). They are arranged in inflorescences of 10 to 60 flowers. Flowers are hermaphroditic bearing, like *D. rodriguezii*, eight stamens and one ovary with a single locule, they release also a sweet smell, and have a tube-like corolla, but smaller than in *D. rodriguezii* (i.e. 3–5 mm; Fig. 1). Flower lifespan is shorter (3–4 days; pers. obs.) than in *D. rodriguezii*. The style is short, and the ovary is located at the bottom of the flower tube. *D. gnidium* is present in most of Balearic Islands (Castroviejo et al. 2001), being locally common in Mallorca. In Menorca, it is found scattered in open habitats at the west and centre of the island, never coexisting with *D. rodriguezii* (P. Fraga, pers. comm.).

Study sites

The study with *D. rodriguezii* was conducted in four populations located in coastal shrubland, three in the north

of Menorca (Mesquida, Favàritx and Pudent; Fig. 2) and one in the south (Porter). Population size varies between 50 (e.g. Porter and Mesquida), ca. 150 (e.g. Favàritx) and ca. 300 reproductives (e.g. Pudent). The flowering period of this species lasts from February to mid April, occurring about 2 weeks earlier in the southern population than in the northern ones. More detailed information on each of these study sites and on several aspects of its reproductive biology is available in Traveset and Riera (2005).

Regarding *D. gnidium*, we chose two populations in two contrasting habitats of Mallorca: one in an abandoned olive grove at the western side of the island (Deià) and the other in a fixed dune shrubland in the north (Ca'n Picafort, CPF hereafter). The size of both populations is equivalent, with ca. 200 reproductive plants (unpubl. data). *D. gnidium* flowers from July to September, beginning earlier and lasting for longer in Deià than in CPF.

Hand-pollination experiments

The levels of selfing and the effect of pollen limitation were investigated in 2003 in two populations of *D. rodriguezii* (i.e. Porter and Pudent) and in the two populations of *D. gnidium*. We used 5–12 plants in each population, depending upon availability of individuals with enough flowers to carry out the following treatments which depend on the pollen source: (a) spontaneous selfing; flower buds were bagged and remained unmanipulated through the entire flowering period (i.e. letting pollination to occur from self-pollen), (b) intra-population outcrossing (xenogamy I); flowers were hand-pollinated with pollen from dehiscent anthers from other individuals in the same population, and (c) buds were marked but left to open pollination; this was used as the control (i.e. both selfing and outcrossing pollination). In the case of *D. rodriguezii*, and due to their small population sizes which might promote high inbreeding levels, we included another treatment: (d) inter-population outcrossing (xenogamy II); flowers from Porter were pollinated with pollen from Pudent, and viceversa. To minimize the effect of loss of pollen viability, these pollinations were performed within 2 h after pollen collection. Treatments were blocked within individuals; a total of 20 and 80 flowers (from different branches) of *D. rodriguezii* and *D. gnidium*, respectively, were used for each treatment and plant. All treatments were performed during the flowering peak of each population, during late March to early April in *D. rodriguezii* and during August to September in *D. gnidium*. Given the small flower size and the low pollen production (see results), pollinations were done by touching the stigmas directly with anthers (held by forceps), checking if pollen was deposited over the stigma afterwards by means of a magnifying glass. For both species, we opened laterally the outcrossing-pollinated flowers, as the tube-like

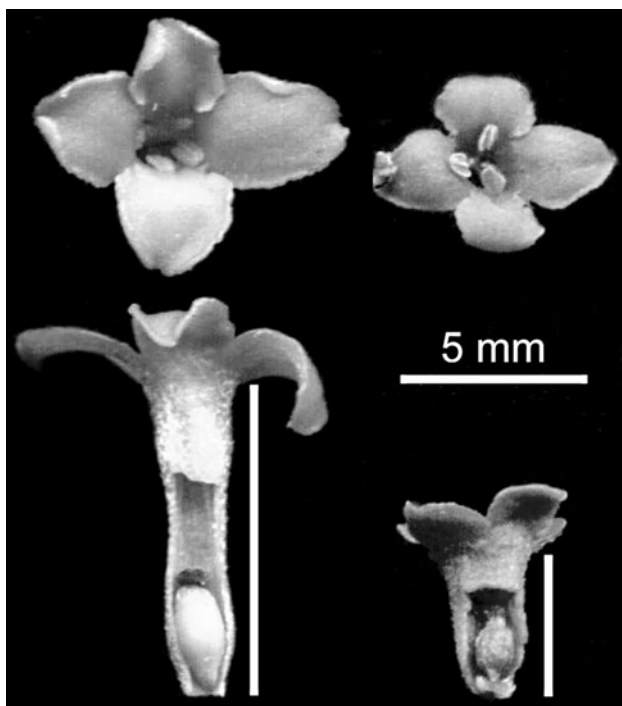
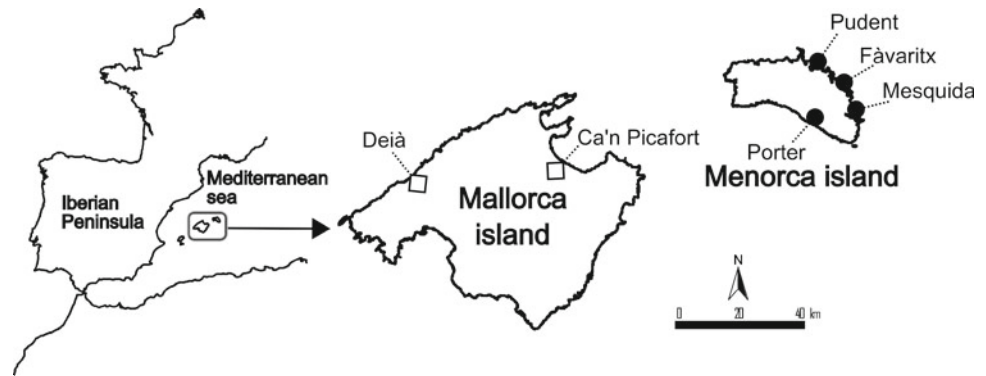


Fig. 1 Upper and lateral view (down) of *D. rodriguezii* (left) and *D. gnidium* (right) flowers. Measures of flower tube length are shown. The area included in the flower contour from the upper view is considered as the tepal area

Fig. 2 Location of study sites of *D. rodriguezii* and *D. gnidium*. Black dots and white squares showed names and locations of study populations of *D. rodriguezii* (Menorca Island) and *D. gnidium* (Mallorca Island), respectively



corolla (especially that of *D. rodriguezii*) prevented to pollinate intact flowers. Due to the difficulty of bagging individual flowers, entire inflorescences were usually used for the same pollination treatment. Branches containing hand-pollinated flowers were bagged before anthesis (in order to avoid natural pollination) and also after manipulation, until fruits had ripened. Fruit set in each treatment was recorded on early June in *D. rodriguezii* and from September to November in *D. gnidium*.

In 2004, we wanted to assess whether self-pollen clogging on the stigma might cause the low fruit set found in *D. rodriguezii* (Traveset and Riera 2005). We tested such effect in 10 plants from the population of Pudent. For each plant, we considered two factors in a complete block design: (1) pollination treatment (induced selfing vs. intra-population outcrossing) and (2) emasculation (flowers with removed vs. intact anthers). Flowers used in the emasculation treatment were laterally opened, with a sharp forceps, before anther dehiscence. For the non-emasculation treatment (intact anthers), we chose flowers the anthers of which had already dehisced (i.e. 2–3 day-old flowers), and thus, stigmas bore large amounts of self-pollen (Roccoliello et al. 2009). Furthermore, we opened laterally all treated flowers in order to control for the possible effect of flower manipulation on fruit set. All these flowers were bagged before and after the pollination treatment in order to avoid natural pollination. A control group of flowers, neither emasculated nor laterally opened, was left to open pollination. For each combination of factors, we treated, on average, 10.8 flowers per individual plant. The experiment was performed on April 5–8, and fruit set was recorded a couple of months later, on June 11.

Flower visitors

During the flowering period of 2004 and 2005, diurnal observations on flower visitors were performed in all populations (i.e. four for *D. rodriguezii* and two for *D. gnidium*). The presence of insects was monitored from 10:00 to 19:00 h every 15 min on randomly selected 10

plants per population. On average, eight and 18 observation hours per year were performed for *D. rodriguezii* and *D. gnidium*, respectively.

Reproductive traits and pollination success

We assessed reproductive traits (on a flower basis) on 8–12 plants per population on each of the four populations of *D. rodriguezii* and the two of *D. gnidium*. We collected a random sample of 10 buds (closed, but about to open) and 10 open flowers per plant from different inflorescences. Both buds and flowers were kept in 70% ethanol until the time of dissection in the laboratory. We recorded male investment from collected buds by counting the number of viable (filled) anthers relative to the total present. By means of a particle counter (Coulter[®] Counter Z2), we also recorded the number of pollen grains per flower from all viable anthers, after squashing and diluting them in 35 ml of ISOTON II. The particle counter was previously calibrated to measure pollen grains between 16 and 30 μm , a range which encompasses the pollen size of both species. Mean number of pollen grains per flower was obtained by averaging the values from five aliquots (0.5 ml each) per sample. From such values we further obtained the pollen grains per anther.

From dehisced flowers, we estimated flower tube length and tepal area (Fig. 1), number of pollen grains per stigma and the fraction of germinated pollen grains on each stigma. Measures on flower tube length (to the nearest 0.01 mm) and tepal area (mm^2) were obtained by scanning 10 dehisced flowers and using the SigmaScan Pro 5.0 software. Flower pistils from such flowers were softened in 1 N NaOH at 65°C for 20 min, rinsed with distilled water, and stained for 20 min at 65°C in decolorized aniline blue, following the protocol used by Alonso (2004). Both pollen grains and developed pollen tubes on the stigmas were counted by means of an epi-fluorescence microscope, following the methodology in Kearns and Inouye (1993).

On a plant basis, we also recorded maximum plant height (± 1 cm) and flower production. After the flowering

period, we measured fruit production and fruit set (proportion of fruits relative to total number of flowers produced).

Data analyses

Data were analyzed by means of Generalized Linear Models and likelihood-ratio test III, using the GENMOD procedure in SAS 9.0 (SAS Institute 2000). Unless otherwise indicated, average values are reported as mean \pm standard error ($1 \pm$ SE).

Mating system

A repeated measurements design was employed for data on the hand-pollination experiments: treatment was nested within individual, which was in turn nested within population. Population was also nested within species. Treatments (i.e. pollination and/or emasculation treatments), species and population were fixed factors whereas individual was a random factor. We fitted binomial distribution and logit link functions to fruit set (i.e. treated flower set or not set fruit). Moreover, deviances were re-scaled using the root-square of the ratio deviance/degrees of freedom in order to correct for over-dispersion. Multiple pair-wise contrasts, corrected by sequential Bonferroni (Rice 1989), were performed whenever significant effects of fixed factors were detected.

Effect of reproductive traits on pollination success

A repeated measurements analysis was carried out to test for differences between species and among populations (fixed factors) of each individual reproductive trait, including individual as a subject (random) factor and species and population as within-subject fixed factors. We used the error distributions and link functions that best fitted our data: (1) binomial distributions for fruit set and proportion of viable anthers, (2) normal distributions for flower tube length and number of fruits, (3) gamma distributions for flower production and tepal area, and (4) negative binomial for number of pollen grains per anther and number of germinated pollen grains per stigma; in this model, tube length and tepal area were included as covariates after testing for homogeneity of slopes. The logit link function was used for the first model whereas the log link was used for the rest of models. For the binomial distribution models, deviances were re-scaled using the root-square of the ratio deviance/degrees of freedom. Multiple pair-wise contrasts were again performed when necessary (see previous section).

At the flower level, the fitness of each reproductive trait was estimated for each species from the partial-regression

coefficients of a linear regression (Arnold and Wade 1984). Instead of considering fruit set at plant level as the only measure of reproductive fitness, we used the number and fraction of germinated pollen grains per stigma as a proxy of reproductive fitness at flower level. We again approached a repeated measurements design, including individual plant (nested within population) as subject (random) and population (nested within species) as within-subject fixed factor. In order to get comparable estimates of regression coefficients among populations, relative fitness (i.e. standardized trait for each population, see Maad and Alexandersson 2004) was used instead of absolute fitness. In each model, all possible effects were fitted using the various combinations of reproductive variables with their interactions. We considered the best models as those with the lowest AIC (Akaike's Information Criterion) score. These analyses were performed using STATISTICA 6.0 (Statsoft Inc. 2005). Spearman-rank correlations were used to test for relationships among reproductive traits, averaging variables by individual plant whenever necessary.

Variation of reproductive traits at different levels

The degree of variability of each reproductive trait at different levels (species, population, plant and flower) was obtained by calculating the variance components from the deviance (based on the likelihood-ratio test I). Error distributions and link functions of each reproductive trait were fitted and analysed as in the previous section (see above). The error deviance denotes the variation among flowers in each reproductive trait. Percentage of variance was fitted to a gamma distribution and a log link function.

Results

Mating system

Fruit set differed significantly among pollination treatments ($F_2 = 12.96$, $p = 0.002$; Fig. 3), being higher for xenogamous crosses than for the open pollination and spontaneous selfing treatments (i.e. the three treatments were significantly different from each other). In both species, stigmas of the two latter treatments were completely covered by pollen once anthesis, suggesting that the pollen covering stigmas of open pollination treatments was mostly self-pollen. Treatments were consistent among populations ($F_2 = 3.83$; $p = 0.147$), but not between species (interaction treatment \times species: $F_1 = 6.93$; $p = 0.031$). Xenogamous crosses of *D. rodriguezii* produced 30 times as many fruits as open-pollinated flowers; by contrast, pollen supplementation in *D. gnidium* increased fruit set only by three times (Fig. 2). At least for *D. rodriguezii*, the effect of

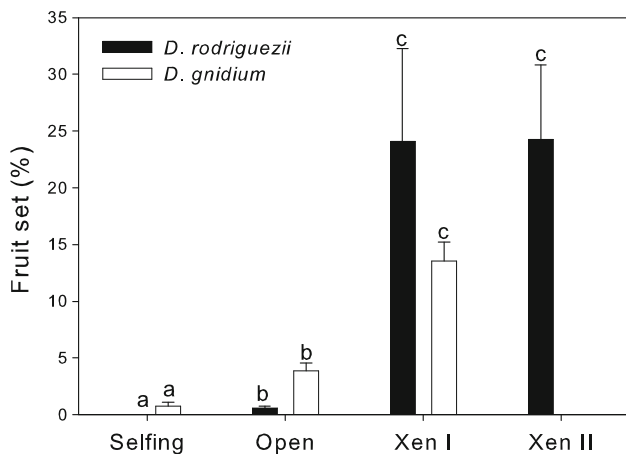


Fig. 3 Fruit set (proportion of fruits in respect to flowers) for each pollination treatment and for each *Daphne* species. Treatments were: spontaneous selfing (Selfing), open or natural pollination (Open) and intra-population outcrossing (Xen I). For *D. rodriguezii*, another treatment inter-population outcrossing (Xen II) was also performed. For each species, bars with the same letter above are not significantly different among treatments ($p < 0.05$; sequential Bonferroni correction)

xenogamy was the same regardless the origin of pollen, i.e. either from the same or from another population (Fig. 2). Spontaneous selfing was negligible in *D. gnidium*, but not nil (Fig. 2); in *D. rodriguezii*, this treatment produced no fruits at all (Fig. 2).

No effect on pollen clogging was detected in *D. rodriguezii*, as fruit set did not differ between emasculated and non-emasculated flowers (on average, $19.2 \pm 4.3\%$; $F_{1,53} = 0.02$; $p = 0.890$), and this was consistent between treatments (i.e. the interaction between pollination and emasculating treatment was not significant; $F_{2,53} = 0.31$; $p = 0.732$). Significant differences ($F_{2,53} = 15.5$; $p < 0.0001$) were again observed in fruit set among treatments. Fruit set was lowest for open pollination ($1.6 \pm 1.2\%$), it increased when selfing was induced ($4.4 \pm 2.3\%$) and it was about ten times higher when xenogamous crosses ($39.2 \pm 8.3\%$) were compared to open pollination. These results, thus, confirmed that this island-endemic species have low levels of self-compatibility and high pollen limitation.

Flower visitors

Direct observations showed important differences of flower visitors between *Daphne* species. In the case of *D. rodriguezii*, they were extremely rare (Table 1): we only observed small ants (*Plagiolepis pygmaea*) inside the flowers in two of the four populations and, in one population, *Tropinota hirta* (Cetoniidae) was feeding on anthers and tepals. In *D. gnidium*, flower visitors were more abundant, belonging mostly to Hymenoptera and Diptera (Table 1). Considering *D. gnidium*, hymenoptera were

Table 1 Flower visitors of *D. rodriguezii* and *D. gnidium* during 2004 and 2005 flowering period

Plant species	Population	Visitor order	N species	N visits
<i>D. rodriguezii</i>	Porter	Hymenoptera	1	1
		Mesquida	0	0
	Pudent	Coleoptera	1	4
		Favàritx	Hymenoptera	1
<i>D. gnidium</i>	Deià	Hymenoptera	6	47
		Diptera	8	18
		Thysanoptera	1	2
	CPF	Hymenoptera	11	28
		Diptera	8	30

Number of visits was quantified in 10 flowering plants for time-spans of 15 min

more diverse in CPF whereas they performed most of the visits in Deià. The last population showed a little lower flower-visitor diversity than CPF (15 vs. 19 species, respectively), whereas visits were a little higher (i.e. 67 vs. 58, respectively). The most common flower visitors in Deià were *Halictus* sp. (Halictidae), *Apis mellifera* (Apidae) and one unidentified dipter (21, 19 and 5 visits, respectively), whereas *Eristalinus aeneus* (Syrphidae), *Dasyscolia ciliata* (Scolidae), *Megachile* sp. (Megachilidae) and one unidentified syrphid were common visitors in CPF (16, 5, 5 and 5 visits, respectively). The rest of flower visitors of *D. gnidium* were observed less than five times.

Effect of reproductive traits on pollination success

Most reproductive traits differed significantly between both *Daphne* species (Table 2). Specifically, flowers of *D. rodriguezii* were longer, showed a larger tepal area, and produced a higher number of pollen grains per anther than *D. gnidium* flowers. On the contrary, *D. gnidium* produced a higher number of flowers per plant and had more viable anthers per flower. Stigmatic pollen loads were high both in *D. rodriguezii* (83% of stigmas bore at least one pollen grain) and *D. gnidium* (92%), not differing between species (Table 2). Fruit set was consistently low in both species in all six populations (Table 2). However, it was somewhat higher in *D. gnidium* despite the similar fraction of germinated pollen grains per stigma in the two species (Table 2).

When comparing the populations of *D. rodriguezii*, we found no association between any of the reproductive traits and measures of pollination success (Table 2), which agrees with the low variability explained at the population level (see below). The longest flower tubes and largest flower production were found at Porter and Pudent, respectively, but this did not translate into higher fruit set

in these populations (Table 2). Fruit set was greater in Mesquida and Favàritx than in the other two populations, but, again, this estimate of reproductive success was not associated to any particular reproductive trait. When pooling data from all four populations, taller and more fecund plants of *D. rodriguezii* showed to have higher fruit sets; interestingly, fruit set was also correlated, but

negatively, with the proportion of viable anthers, which suggests that individuals with higher height might be allocating more to female than to male function (Table 3). Also, individual flowers with longer tubes bore more pollen grains on their stigmas (Table 4a), which resulted into higher a fraction of germinated pollen grains, at plant (Table 3) and at flower level (Table 4c). On a plant basis,

Table 2 Reproductive traits (mean ± SE) of each *Daphne* species and population

Traits	<i>D. rodriguezii</i>				<i>D. gnidium</i>		Diff. between species χ^2
	Mesquida (n = 8)	Porter (n = 11)	Favàritx (n = 9)	Pudent (n = 13)	Deià (n = 12)	Ca'n Picafort (n = 12)	
Flower production × 100	9.77 ± 3.78 ab	6.47 ± 2.87 ab	4.21 ± 1.41 a	14.53 ± 3.06 b	166.9 ± 39.0 a	208.1 ± 33.4 a	596.5***
Flower tube length (mm)	7.35 ± 0.29 ab	8.44 ± 0.44 a	7.03 ± 0.48 b	7.46 ± 0.19 ab	4.59 ± 0.10 a	3.91 ± 0.08 b	47.5***
Flower tepal area (mm ²)	0.93 ± 0.03 a	1.05 ± 0.04 b	0.92 ± 0.03 a	1.04 ± 0.03 b	0.81 ± 0.02 a	0.73 ± 0.01 b	41.1***
Viable anthers/flower	7.7 ± 0.1 a	7.9 ± 0.0 a	7.5 ± 0.5 a	7.7 ± 0.1 a	8.0 ± 0.0 a	7.9 ± 0.1 a	5.02*
Pollen grains/anther	1,242 ± 94 a	1,450 ± 126 a	1,431 ± 156 a	1,517 ± 122 a	1,154 ± 49 a	1,115 ± 69 a	10.8**
Pollen grains/stigma	61.8 ± 24.8 a	85.9 ± 18.6 a	41.7 ± 16.7 a	48.6 ± 18.7 a	89.6 ± 24.7 a	35.6 ± 9.5 b	0.02 n.s.
Germ. pollen grains/stigma (%)	8.96 ± 5.23 ab	18.26 ± 5.18 a	7.55 ± 2.96 ab	7.14 ± 3.27 b	5.14 ± 2.46 a	8.69 ± 3.39 a	1.08 n.s.
Fruit production	55.5 ± 37.6 a	10.5 ± 5.4 a	17.0 ± 6.2 a	12.4 ± 5.6 a	208.8 ± 66.6 a	261.7 ± 150.9 a	10.7**
Fruit set (%)	2.65 ± 1.22 a	0.83 ± 0.30 b	3.05 ± 0.79 a	0.62 ± 0.20 b	3.60 ± 0.60 a	2.27 ± 0.56 b	30.4***

Within each species and for each trait, populations with the same letter did not differ significantly ($p < 0.05$). For differences between species, population was included as a within-subject fixed factor. Multiple pair-wise contrasts were corrected by sequential Bonferroni (Rice 1989) n.s. non-significant; † $p < 0.1$; * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$

Table 3 Spearman-rank correlation coefficients among reproductive traits and pollination success for *Daphne rodriguezii* (above-right diagonal; $n = 41$ plants) and *D. gnidium* (below-left diagonal; $n = 24$ plants)

	<i>Daphne rodriguezii</i>								
	PMH	FPR	FTL	FTA	VAF	PGA	NPG	PGP	FRS
<i>Daphne gnidium</i>									
PMH	–	<i>0.446</i>	0.053	–0.133	–0.420	–0.073	–0.215	–0.246	0.388
FPR	<i>0.634</i>	–	0.034	–0.388	–0.198	–0.062	–0.121	–0.200	<i>0.448</i>
FTL	–0.047	–0.123	–	0.297	0.006	0.065	0.278	0.106	0.207
FTA	0.003	–0.034	0.112	–	0.082	0.170	0.177	0.144	–0.178
VAF	0.232	<i>0.457</i>	0.054	0.071	–	0.008	0.111	0.062	–0.007
PGA	–0.229	–0.189	0.121	0.097	–0.082	–	0.071	0.022	–0.030
NPG	–0.227	0.050	–0.152	0.230	0.267	0.177	–	<i>0.805</i>	–0.007
PGP	–0.338	0.060	–0.376	0.103	0.110	0.080	<i>0.650</i>	–	–0.007
FRS	0.104	–0.109	0.255	0.131	–0.170	–0.036	–0.031	–0.486	–

For each species, population-dependent relative fitness (i.e. standardized trait for each population, see “Material and methods”) was used instead of absolute fitness. Correlations were performed on a plant basis; some variables were thus averaged for each plant (FTL, FTA, VAF, PGA, NPG, PGP). Multiple pair-wise contrasts were corrected by sequential Bonferroni (Rice 1989)

PMH plant’s maximum height, FPR flower production, FTL flower tube length, FTA flower tepal area, VAF viable anthers per flower, PGA pollen grains per anther, NPG number of pollen grains per stigma, PGP proportion of germinated pollen grains per stigma, FRS fruit set

Significance: italic, $p < 0.05$

Table 4 Results of the Generalized Linear Model analysis on the number (a, b) and proportion of germinated pollen grains (c, d) per stigma in *D. rodriguezii* ($n = 368$) and in *D. gnidium* ($n = 240$)

Reproductive traits	Estimate parameter	df	χ^2
(a) Pollen grains/stigma in <i>D. rodriguezii</i>			
Flower tube length	0.016 ± 0.007	1	4.45*
Flower production	-0.001 ± 0.001	1	3.52 [†]
(b) Pollen grains/stigma in <i>D. gnidium</i>			
Population (CPF)	0.264 ± 0.193	1	1.82 n.s.
Flower production	0.003 ± 0.002	1	1.53 n.s.
FPR*Pop. (CPF)	-0.003 ± 0.002	1	1.50 n.s.
(c) Germinated pollen grains in <i>D. rodriguezii</i>			
Pollen grains/stigma	0.058 ± 0.003	1	9.90**
Flower production	-0.001 ± 0.001	1	3.53 [†]
(d) Germinated pollen grains in <i>D. gnidium</i>			
Flower tepal area	3.316 ± 0.451	1	6.09*
Flower production	0.405 ± 0.058	1	5.85*
Pollen grains/stigma	0.592 ± 0.076	1	5.28*
FTA*FPR	-0.036 ± 0.005	1	6.02*
FTA*Pop. (CPF)	0.661 ± 0.122	1	6.68*
FPR*Pop. (CPF)	-0.062 ± 0.012	1	5.51*

Data for each reproductive trait were based on standardized estimates within each population and species (see “Material and methods”). For each analysis, we show those reproductive traits included in the model with the lowest AIC score. We did not include flower tepal area in (c) analyses, since this effect was not significant in the previous one (a). Likewise, flower tube length was removed from (d) analyses because it was not significant in the previous one (b). Estimate parameters (mean ± SE), degrees of freedom (*df*) and significance (symbols as in Table 2) are shown. Same acronyms as in Table 3 are used

however, such greater pollen germination in flowers with long-tubed corollas did not render into a higher fruit set (Table 3).

Regarding *D. gnidium*, some floral traits (i.e. tube length, tepal area, and number of pollen grains per stigma) showed higher values in Deià than in CPF (Table 2). Fruit set was also higher in the former population, although it was not significantly associated to any of those floral traits on a plant basis. On a plant basis, flower production was again positively associated with plant height but, unlike in *D. rodriguezii*, it was not associated with other flower traits (Table 3). We also detected a correlation between the number and germinated pollen grains per stigma (Table 3). Despite the number of pollen grains on stigmas (at the flower level) was not influenced by any reproductive traits (Table 4b), the proportion of pollen grains germinated on the stigmas showed to be significantly and positively influenced by tepal area, flower production and number of pollen grains on stigmas (Table 4d). Moreover, the significant interaction between flower production and tepal area (Table 4d) suggests that plants producing more flowers tended to have greater pollen germinability in their

Table 5 Percentage of the relative variance for reproductive traits and pollination success measured for each biological level (flower, plant, population and species)

Trait	df	Species	Population	Plant	Flower
(a) Reproductive traits					
Flower production	1, 4	47.0	1.1	51.9	–
Flower tube length	1, 4, 60	38.7	2.4	5.3	53.6
Flower tepal area	1, 4, 60	26.7	6.0	8.2	59.1
Viable anthers/flower	1, 4, 57	4.9	3.8	22.0	69.2
Pollen grains/anther	1, 4, 57	6.6	8.6	52.6	32.2
(b) Pollination success					
Pollen grains/stigma	1, 4, 60	0.0	0.5	3.6	95.9
Germ. pollen grains/stigma	1, 4, 60	0.5	2.7	29.4	67.4
Fruit production	1, 4	13.5	0.6	86.0	–
Fruit set	1, 4	48.2	1.1	50.7	–

For each reproductive trait at each biological level, relative variance was obtained from the deviance based on the type I likelihood-ratio test. For flower production, fruit production and fruit set, the flower level was obviously not included in the model. We presented the degrees of freedom (*df*) for species, population and plant, respectively

stigmas but this effect was reduced when flower tepal area increased. Nevertheless, the effect of tepal area and flower production on the proportion of germinated pollen grains varied across populations (Table 4d).

Variation of reproductive traits at different levels

The variance partitioning of reproductive traits differed significantly across biological levels ($\chi^2_3 = 54.1$, $p < 0.0001$; Table 5): flower (65%), plant (20%), species (11%) and population (4%). Such variance was dependent on each reproductive trait ($\chi^2_8 = 16.3$, $p < 0.038$) and there was also a ‘reproductive trait × biological level’ interaction ($\chi^2_{20} = 34.5$, $p = 0.023$). Thus, most variation in flower production, flower tube length, tepal area and fruit set was found at the level of species; number of pollen grains per anther was mostly variable at the population level, whereas number of viable anthers and pollen grains per stigma varied at flower level (Table 5). By contrast, traits describing pollination success (i.e. proportion of germinated pollen grains per stigma, fruit set and total number of fruits) varied mostly at within-population level (i.e. plant and/or flower).

Discussion

Mating and reproductive traits influencing pollination success

A number of studies have reported that closely related species show greater levels of selfing in island than in

mainland ecosystems (Barrett 1998), which is usually attributed to the more depauperate insular pollinator faunas (Inoue et al. 1996). The two *Daphne* species studied here do not fit into this rule since both showed low levels of fruit set in the spontaneous selfing treatment (i.e. less than 5%). Thus, the degree of change in mating systems may vary much among taxa, and has actually been found to depend upon the phylogenetic inertia of each taxonomic group (Armbruster 2002). Stigmatic pollen loads were high in both species (i.e. more than 80% of stigmas bore at least one pollen grain), which agrees with values obtained in the congeneric *D. laureola* (Alonso 2005). Nevertheless, we found in either species no relationship between fruit set and pollen-grain germinability, as presumably very low fractions of outcrossing pollen grains appear to reach their stigmas. In both species, stigmas are completely covered by self-pollen after anthesis and, thus, it is likely that although fertilization may eventually occur, some self-incompatible mechanisms prevent fruit set when outcrossing pollen does not arrive to flowers, as has been suggested to occur in *D. gnidium* (Roccoliello et al. 2009). There were no differences between self-induced and self-spontaneous hand-pollinations in other *Daphne* species (present work; Alonso and Herrera 2001), which suggests that spontaneous selfing treatment could be a proxy to detect self-incompatibility in hermaphrodite *Daphne* species. Since we did not detect in *D. rodriguezii* any effect of self-pollen clogging effect on stigmas, its low fruit set under natural conditions may be more consequence of the low arrival of outcrossing pollen.

Furthermore, both *Daphne* species were found to be strongly pollen-limited (<5% of the flowers set fruit in the open pollination treatment). Fruit sets reported for other hermaphroditic congeneric species, like *D. kamtchatica* (Kikuzawa 1989) and *D. laureola* (Alonso and Herrera 2001) are higher (>10%), although still not very high. Therefore, it is possible that a low fruit set is a characteristic of the genus *Daphne*, although not of other genera in the family, as for instance *Thymelaea velutina* (>50% fruit set; De la Bandera and Traveset 2006). In a recent study, Roccoliello et al. (2009) point out that the low fruit set (5–18%) in *D. gnidium* seems to result from an embryo collapse during fruit development (i.e. 75% of immature fruits die out), which is likely derived from an apomictic process of self-pollination. On the other hand, *D. gnidium* seems to be more resource limited than *D. rodriguezii*, as the former produced 40% less fruits in hand-cross pollinations than the latter. *D. gnidium* pistils can wither at early stages of flower development, which explains the reduction of the functionality of female organs (Roccoliello et al. 2009). *D. gnidium* blooms during a period (from late summer to early autumn) when pollination service is much more reliable to allow outcrossing pollinations. Conversely, *D. rodriguezii*

was much more pollen limited than *D. gnidium* and received lower flower visitors during its flowering period (as previously observed by Traveset and Riera 2005), probably because it flowers during late winter–early spring when there are still few insect pollinators available (pers. obs.). In the case of *D. gnidium*, its flowers receive greater diversity of visits by diurnal insects (more than 15 species, mainly belonging to the orders Hymenoptera and Diptera). In other mainland populations, this species is also visited by an equivalent community of flower visitors (Herrera 1987; Roccoliello et al. 2009), which suggest that island populations are at least as limited by flower visitors as the rest of mainland populations. Inbreeding depression is a less likely mechanism to explain the low fruit set in *D. rodriguezii*, since there were no differences in fruit set between intra- and inter-population crosses. Interestingly, larger and more fecund plants of *D. rodriguezii* had lower number of viable anthers per flower. This reduction of the male function might be interpreted as a mechanism to actually decrease self-pollen deposition (and, consequently, self-pollen interference) as Holland and Chamberlain (2007) have previously reported in *Pachycereus schottii* (Cactaceae).

Selection on floral traits

It is widely accepted that variation in floral traits influences plant reproductive success due to differential selection by pollinators (Harder and Johnson 2009). Moreover, in pollen-limited populations, pollinators may select for individuals with proportionally higher floral reward (Schemske et al. 1978; Murray et al. 2002; Alonso 2004). In *D. gnidium*, individuals with larger flower crops and/or larger tepal areas showed higher proportions of pollen germination that might suggest greater visitation rates by pollinators (i.e. pollinators might be selecting for such traits). Nevertheless, such greater germination did not translate into higher fruit set, probably because most of that germinated pollen was self-pollen (Roccoliello et al. 2009). In *D. rodriguezii*, on the other hand, we detected more pollen grains on the stigmas of flowers with long-tube corollas (as also found in other studies; see review in Harder and Johnson, 2009); despite such flowers had greater proportions of germinated pollen grains, plants were no more likely to set fruits. Flower production appeared to be the only plant trait correlated with pollination success in *D. rodriguezii*; that is, larger floral displays translated into higher fruit sets. This actually has been found in many flowering plants (e.g. Johnston 1991) because larger floral displays are more attractive and proportionally more visited by pollinators (Ohashi and Yahara 1999 and references therein). In the case of *D. rodriguezii*, diurnal flower visitors were extremely rare and were not performed by long-proboscis insects, suggesting that other undetected and

highly-rare flower visitors may perform such detected selection on flower traits. For instance, S. Pons (pers. com.) observed at night unidentified moths on *D. rodriguezii* flowers, which suggests that nocturnal flower visitors could be also important on its pollination biology. In other congeneric species, Alonso (2004) detected that day-active flower visitors were more infrequent in *D. laureola* whereas nocturnal pollinators were relatively more abundant but inefficient. Certainly, future research on the flower-trait selection of *D. rodriguezii* needs to carefully decompose the importance of each pollinator assemblage (i.e. diurnal vs. nocturnal).

The variability of floral traits and the selection that pollinators exert on them may differ among populations due to climatic or ecological factors prevailing in them (e.g. Herrera 1995; Sánchez-LaFuente et al. 2005). Moreover, plants relying on few pollinators tend to show greater variation in pollination success than pollinator-generalist plants (Knight et al. 2005). For *D. rodriguezii*, the relationship between reproductive traits and reproductive output was similar in all populations. By contrast, such relationship differed between the two study populations of *D. gnidium*, probably as differences in flower-visitor assemblages. Nevertheless, the observed differences in pollination success for both species (and especially for *D. rodriguezii*) were, in fact, unlikely to be the result of idiosyncratic differences among populations (i.e. pollinator diversity and abundance, plant resources). Population and within-individual variance were the factors accounting for the lowest and highest variability in reproductive traits and pollination success, suggesting that selection of floral traits may be spatially stochastic and dependent on the within-population differences between reproductive plants. This is actually consistent with most studies that have examined variability of plant traits at different levels (Herrera 2009).

In conclusion, both species of *Daphne* showed a similar mating system despite they differ, not only in their distributions, but in other reproductive traits. Contrary to our expectations that the endemic species might have greater selfing levels, *D. rodriguezii* showed higher pollen limitation and a similar dependency on outcrossing pollen for seed production as the widespread species *D. gnidium*. The levels of spontaneous selfing were very low in both species, suggesting that there might be taxonomic constraints precluding the evolution of selfing capacity to overcome such pollen limitation. Outcrossing pollen arriving to stigmas was presumably low in both species, but is probably lower in *D. rodriguezii* due to the scarcity of pollinators during its flowering period. In both species, pollinators appeared to favour within-population variability in floral traits (specifically, flower tube length or tepal area), whereas, in *D. rodriguezii*, flower production was the only trait associated to reproductive output. Our findings, thus, suggest

that despite both species showed a similar mating system, dependency on outcrossing pollen and (low) selection of floral traits, pollen limitation was higher in the low-flower-visited species *D. rodriguezii*, probably due to a lower proportion of outcrossing pollen arriving to its stigmas.

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References

- Alonso C (2004) Early blooming’s challenges: extended flowering season, diverse pollinator assemblage and the reproductive success of gynodioecious *Daphne laureola*. *Ann Bot* 93:61–66
- Alonso C (2005) Pollination success across an elevation and sex ratio gradient in gynodioecious *Daphne laureola*. *Am J Bot* 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* (Thymelaeaceae). *Am J Bot* 88:1016–1024
- Armbruster WS (2002) Can indirect selection and genetic context contribute to trait diversification? A transition-probability study of blossom-colour evolution in two genera. *J Evol Biol* 15:468–486
- Arnold SJ, Wade MJ (1984) On the measurement of natural and sexual selection. *Theory Evol* 38:709–719
- Baker HG (1955) Self compatibility and establishment after long-distance dispersal. *Evolution* 9:347–349
- Barrett SCH (1998) The reproductive biology and genetics of island plants. In: Grant PR (ed) *Evolution on Islands*. Oxford University Press, Oxford, pp 18–34
- Bond WJ (1994) Do mutualisms matter?—assessing the impact of pollinator and disperser disruption on plant extinction. *Phil Trans R Soc Lond B* 344:83–90
- Brickell CD, Mathew B (1976) *Daphne*. The genus in the wild and cultivation. Alpine Garden Society, Birmingham
- Castroviejo S, Aedo C, Cirujano S, Laínz M, Montserrat P, Morales R, Muñoz Garmendia F, Navarro C, Paiva J, Soriano C (2001) *Flora Iberica*. Plantas vasculares de la península Ibérica e Islas Baleares. Real Jardín Botánico (CSIC), Madrid
- De Jong TJ, Klinkhamer PGL (2005) Evolutionary ecology of plant reproductive strategies. Cambridge University Press, Cambridge
- De Jong TJ, Waser NM, Klinkhamer PGL (1993) Geitonogamy—the neglected side of selfing. *TREE* 8:321–325
- de la Bandera M, Traveset A (2006) Breeding system and spatial variation in the pollination biology of the heterocarpic *Thymelaea velutina* (Thymelaeaceae). *Plant Syst Evol* 257:9–23
- Ebeling A, Klein AM, Schumacher J, Weisser WW, Tschardt T (2008) How does plant richness affect pollinator richness and temporal stability of flower visits? *Oikos* 117:1808–1815
- Fishman L (2000) Pollen discounting and the evolution of selfing in *Arenaria uniflora* (Caryophyllaceae). *Evolution* 54:1558–1565

- Harder LD, Johnson SD (2009) Darwin's beautiful contrivances: evolutionary and functional evidence for floral adaptation. *New Phytol* 183:530–545
- Herrera J (1987) Biología reproductiva de algunas especies del matorral de Doñana. *Anales Jard Bot Madrid* 44:483–497
- Herrera CM (1995) Floral biology, microclimate, and pollination by ectothermic bees in an early-blooming herb. *Ecology* 76:218–228
- Herrera CM (2009) Multiplicity in unity: plant subindividual variation and interactions with animals. University of Chicago Press, Chicago
- Holland JN, Chamberlain SA (2007) Ecological and evolutionary mechanisms for low seed:ovule ratios: need for a pluralistic approach? *Ecology* 88:706–715
- Inoue K, Maki M, Masuda M (1996) Evolution of campanula flowers in relation to insect pollinators on islands. In: Lloyd DG, Barrett SCH (eds) *Floral biology*. Chapman & Hall, New York, pp 377–400
- Johnston MO (1991) Natural-selection on floral traits in two species of *Lobelia* with different pollinators. *Evolution* 45:1468–1479
- Kearns CA, Inouye DW (1993) *Techniques for pollination biologists*. University press of Colorado, Niwot
- Kearns CA, Inouye DW, Waser NM (1998) Endangered mutualisms: the conservation of plant–pollinator interactions. *Annu Rev Ecol Syst* 29:83–112
- Kikuzawa K (1989) Floral biology and evolution of gynodioecism in *Daphne kamtchatica* var *jezoensis*. *Oikos* 56:196–202
- Knight TM, Steets JA, Vamosi JC, Mazer SJ, Burd M, Campbell DR, Dudash MR, Johnston MO, Mitchell RJ, Ashman TL (2005) Pollen limitation of plant reproduction: pattern and process. *Annu Rev Ecol Syst* 36:467–497
- Maad J, Alexandersson R (2004) Variable selection in *Platanthera bifolia* (Orchidaceae): phenotypic selection differed between sex functions in a drought year. *J Evol Biol* 17:642–650
- Momose K (2004) Plant reproductive interval and population density in aseasonal tropics. *Ecol Res* 19:245–253
- Murray BR, Thrall PH, Gill AM, Nicotra AB (2002) How plant life-history and ecological traits relate to species rarity and commonness at varying spatial scales. *Austral Ecol* 27:291–310
- Ohashi K, Yahara T (1999) How long to stay on, and how often to visit a flowering plant?—a model for foraging strategy when floral displays vary in size. *Oikos* 86:386–392
- Potts SG, Petanidou T, Roberts S, O'Toole C, Hulbert A, Willmer P (2006) Plant–pollinator biodiversity and pollination services in a complex Mediterranean landscape. *Biol Con* 129:519–529
- Rice WR (1989) Analyzing tables of statistical tests. *Evolution* 43:223–225
- Roccoliello E, Casazza G, Galli L, Cornara L, Moncalvo A, Minuto L (2009) The flower biology of *Daphne gnidium* L. (Thymelaeaceae). *Pl Syst Evol* 279:41–49
- Sánchez-Lafuente AM, Guitián J, Medrano M, Herrera CM, Rey PJ, Cerdà X (2005) Plant traits, environmental factors, and pollinator visitation in winter-flowering *Helleborus foetidus* (Ranunculaceae). *Ann Bot* 96:845–852
- SAS Institute (2000) *SAS/STAT® software: user's guide*. SAS Institute, Cary
- Schemske DW, Willson MF, Melampy MN, Miller LJ, Verner L, Schemske KM, Best LB (1978) Flowering ecology of some spring woodland herbs. *Ecology* 59:351–366
- Statsoft Inc. (2005) *STATISTICA for Windows*. Statsoft Inc., Tulsa
- Traveset A, Jakobsson A (2007) Ecology of plant reproduction: Mating systems and pollination. In: Pugnaire FI, Valladares F (eds) *Functional plant ecology*. CRC press, Boca Ratón, pp 515–548
- Traveset A, Riera N (2005) Disruption of a plant–lizard seed dispersal system and its ecological effects on a threatened endemic plant in the Balearic Islands. *Conserv Biol* 19:421–431
- Valido A, Dupont YL, Hansen DM (2002) Native birds and insects, and introduced honey bees visiting *Echium wildpretii* (Boraginaceae) in the Canary Islands. *Acta Oecol* 23:413–419