CONSEQUENCES OF PLANT-POLLINATOR AND FLORAL-HERBIVORE INTERACTIONS ON THE REPRODUCTIVE SUCCESS OF THE CANARY ISLANDS ENDEMIC CANARINA CANARIENSIS (CAMpanionulaceae)

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- **Premise of the study:** Pollination is a critical phase for plant reproduction, but ecological and evolutionary outcomes by pollinators may be counteracted by floral herbivores. These interacting assemblages may also be altered (directly or indirectly) by introduced species, especially on oceanic islands. In this study, we analyzed the effects of opportunistic nectar-feeding passerine birds and native (semi-slugs) and introduced (rats) floral herbivores on the reproductive success of *Canarina canariensis*.

- **Methods:** Manual pollination experiments were conducted to determine plant breeding system and pollen limitation. We also identified floral visitors and their visitation frequencies by censuses. Bird pollination effectiveness was evaluated by selective exclosures. The intensity of floral herbivory by native vs. introduced herbivores and its effect on plant fitness was estimated in different areas within the Canary island of Tenerife.

- **Key results:** *Canarina canariensis* had a very low spontaneous selfing ability and high pollen limitation, despite being self-compatible. Birds increased fruit set and the percentage of viable seeds per fruit, while florivores, the principal floral visitors, reduced them. Semi-slugs mainly consumed male reproductive organs, while rats preferred female. There was a strong within-island spatial variation in the herbivory intensity.

- **Conclusions:** Opportunistic nectar-feeding birds increase the production of viable seeds in *C. canariensis*, but their beneficial effects are counteracted by the high incidence of floral herbivory. Because native semi-slugs damaged anthers more frequently than introduced rats, these florivores may differ in their effects on male and female plant reproductive success.

**Key words:** bird pollination; breeding system; *Canarina*; *Cyanistes*; floral herbivory; *Phylloscopus*; *Plutonia*; pollen limitation; pollination effectiveness; *Rattus*.

Plant reproductive success is the result of the effects of both biotic interactions (e.g., pollination, herbivory) and abiotic conditions (e.g., resource availability). Pollination represents a critical phase in plant reproduction, in which interacting mutualistic agents influence the quantity and quality of pollen reaching stigmas (e.g., Herrera, 1987, 1989; Proctor et al., 1996). Interacting floral herbivores may also affect plant reproduction directly through loss of pollen and ovules or indirectly by deterring pollinators, which may avoid damaged flowers (e.g., Krupnick and Weis, 1999; Gómez, 2005). Thus, antagonistic agents may constrain the ecological and evaporationary outcomes of plant–animal interactions in ways that are unpredictable with studies limited to mutualistic interactions alone (e.g., Herrera and Pelmyr, 2002; Gómez, 2005; Strauss and Whittall, 2006).

This interacting assemblage of pollinators and floral herbivores may vary across the landscape, resulting in shifting scenarios of plant reproductive success and trait selection (e.g., Gómez and Zamora, 2000; Thompson and Fernandez, 2006). One of the most remarkable examples of this geographic variation is found on oceanic islands, in which pollination biology of insular plants differs from continental relatives due to the novel ecological conditions operating on them (Barrett, 1996; Olesen and Jordano, 2002). For example, the presence of a depauperate and disharmonic fauna on islands may promote a lower and more sporadic plant visitation frequency by pollinators compared to mainland relatives (e.g., Linhart and Feinsinger, 1980; Inoue et al., 1996; Bernardello et al., 2001). Pollinator scarcity and unpredictability may translate into a lower quantity and reduced quality of pollen dispersed among plants, and ultimately, to pollen limitation and a lower reproductive output (Spear, 1987; Rathcke, 2001; but see exceptions in, e.g., Larson and Barrett, 1998). Under these circumstances, dependence from animal pollination (e.g., Barrett, 1996; McMullen, 1987; Sakai et al., 1995; Bernardello et al., 2001) or pollination by novel and generalist (broad-diet) floral visitors may be favored (Olesen et al., 2002; Olesen and Valido, 2003; Valido et al., 2004).

On the other hand, floral herbivory also plays a key role in plant reproductive success (Crawley, 1989; McCall and Irwin,
2006) that may differ for islands: present evidence suggests a lower herbivore pressure on island plants than on mainland plants (e.g., Bowen and van Vuren, 1997). However, the ways that insular conditions affect floral herbivore communities and their consequences on the native flora, remain almost unknown (but see Cooper and Wookey, 2003; Kelly et al., 2008).

The composition and magnitude of insular native assemblages of pollinators and floral herbivores on plant fitness may be altered by introduced species through direct (consuming floral resources and reproductive tissues) and indirect effects (preying upon or competing with native pollinators) (e.g., Traveset and Richardson, 2006). Rats (Rattus spp.), among the most invasive species, have spread to at least 80% of the world’s island groups, where they threaten native biodiversity (e.g., Atkinson, 1977; Hernández et al., 1999), ecosystem functioning (e.g., Pisanu et al., 2011), and mutualistic interactions (e.g., Atkinson, 1985). Introduced rats may also act as potential pollinators (e.g., Ecroyd, 1996) and floral herbivores (e.g., Campbell et al., 1984), but their effects on insular pollination have been sparsely studied compared to their negative effects on seed dispersal (Delgado, 2002; Drake and Hunt, 2009).

The endemic bellflower Canaria canariensis (L.) VATKE. (Campanulaceae) is an example of an insular plant receiving flower visitation by introduced rats. This plant species belongs to the Canarian bird–flower element (Vogel et al., 1984; Olesen, 1985), a group of endemic species in the genera Echium (Boraginaceae), Teucrium (Lamiaceae), Scrophularia (Scrophulariaceae), Isoplexis (Scrophulariaceae), Navaea (Malvaceae), and Lotus (Fabaceae), apart from Canaria (Campanulaceae), that are visited by opportunistic nectar-feeding passerine birds (Phylloscopus, Cyanistes, Sylvia, Serinus, and Fringilla). Some of these plants constitute prominent examples of pollinator shift from insects to birds after island colonization (Valido et al., 2004; Rodríguez-Rodríguez and Valido, 2008; Ollerton et al., 2009). Pollination transition of C. canariensis is supposed to represent a less dramatic shift from its mainland ancestors (from specialist nectarivorous sunbirds to opportunistic nectar-feeding birds; Valido et al., 2004; J. M. Olesen et al., University of Aarhus, unpublished manuscript). However, birds are not the sole floral visitors of these plants. They are also seriously affected by the negative impact of introduced species (e.g., honeybees and rats; Hohmann et al., 1993; Valido et al., 2002; Valido and Olesen, 2010).

The flower visitors of C. canariensis include various vertebrates and invertebrates that may act as potential pollinators; but some visitors, e.g., native semi-slugs and introduced rats, consume floral tissues and/or steal floral resources. The importance of birds as pollinators for this species has been previously suggested, but their impact on plant fitness, along with introduced species’ effect, has not been well studied (see however, Hedberg, 1961; Olesen, 1985; Dupont et al., 2004; Ollerton et al., 2009). To achieve an integrative perspective, we analyzed the direct effects of the mutualistic passerine pollinators and the antagonistic native and introduced floral herbivores on plant reproductive success. Previous research suggested that passerine birds may act as effective pollinators that increase male and female plant fitness (Ollerton et al., 2009), but this positive effect may be counteracted by floral herbivory. To investigate this possibility, we addressed the following questions: (1) What is the breeding system and level of pollen limitation? (2) What is the composition and visitation rate of native and introduced floral visitors? (3) What is the relative contribution of floral visitors (potential pollinators) to plant reproductive success? (4) What are the levels of floral damage by native vs. introduced floral herbivores and their within-island spatial variation? (5) What are the consequences of floral herbivory on fruit and seed production?

**MATERIALS AND METHODS**

**Study area**—The present study was conducted in two protected areas far apart from each other (Anaga and Teno) on the island of Tenerife (Canary Islands) during two consecutive flowering periods (2008–2009). In each area, we selected two Canaria populations: Monte del Agua [MA; 740 m a.s.l.] and Barranco de Cuevas Negras [CN; 500 m a.s.l.] in Teno (northwestern part of Tenerife), and Las Vueltas de Taganana [VT; 635 m a.s.l.] and El Pijaral [PI; 718 m a.s.l.] in Anaga (northeastern part of Tenerife; Fig. 1). Both areas harbor relatively well-preserved laurel forest remains, viz. a Mediterranean Tertiary relict evergreen flora restricted to Macaronesia (Santos, 1990).

**Study organism**—Canaria canariensis is a Canary endemic perennial herb inhabiting the islands of El Hierro, La Palma, La Gomera, Tenerife, and Gran Canaria, growing mainly in the laurel forest (500–1000 m a.s.l.). Its inclusion in the early-diverging platycodonoids clade, together with the other Canaria species from East Africa (Canaria abyssinica Engl. and Canaria eminii Asch. and Schweinf.), suggests a reticulate position of the genus within the family (Haberle et al., 2009). It is prostrate and creeping, up to 3 m long, with multiple hollow and laticiferous stems that lead to the formation of expanded mats with overlapping individuals. Propagation is by seeds and shoots from its tuber (Bramwell and Bramwell, 2001). The plant blooms from early winter (November) until late spring (May), with red-orange, bell-shaped flowers borne in a three-flower dichasium at the end of stems. Individual flower longevity is 18.1 ± 4.4 d (Ollerton et al., 2009). A diluted and hexose-dominated nectar (12.2–16.4% sugar; Dupont et al., 2004; Ollerton et al., 2009; present study) is concealed by expanded stamen bases and produced in larger quantities in the morning (109.8 ± 75.6 µL; Ollerton et al., 2009). The flowers are protandrous and have secondary pollen presentation. When stigma is receptive, the recurvates of the stigmatic lobes to the sides of the style, where the pollen has been retained by pollen-collecting hairs (PCBs), may allow autonomous autogamy (M.C.R., personal observation). The ovary, with many ovules borne on the basal placenta and fused with the calyx, develops after fertilization into an edible and ovoid-shaped reddish-orange berry consumed principally by native birds and lizards (Valido, 1999; Rodríguez et al., 2008), but also by introduced rats and rabbits (A. González, IPNA-CSIC, personal communication; present study).

The primary bird flower visitors to C. canariensis are the opportunistic, native passerine birds Phylloscopus canariensis Hartwig (Sylviidae) and Cyanistes caerulesus L. (Paridae) and three species of warblers, Sylvia melanocephala Gmelin, S. atricapilla L., and S. conspicillata Temminck (Sylviidae) (Valido et al., 2004). The endemic Canarian lizard Gallotia galloti Oudart (Lacertidae) also visits C. canariensis flowers in open areas such as forest borders (F. Siverio, Alas Cinematográfica, personal communication). Insect flower visitors are the introduced honeybee Apis mellifera L. (Apidae), wasp Paravespula germanica Fabricius (Vespidae), Canary Island Brimstone Gonepteryx cleobule Hübner (Pieridae), and several species of solitary bees in the genus Lasigosum (Halictidae; Hohmann et al., 1993; Valido et al., 2004; Ollerton et al., 2009; present study). Other visitors include the endemic semi-slug Plutomia spp. (Vitrinidae) and introduced rats Rattus rattus L. (Muridae); as we will show, both consume floral tissues. The introduced house mouse Mus musculus L. (Muridae) may also sporadically visit the flowers (F. Siverio, personal communication). During fieldwork in 2008, an important event of leaf herbivory by the moth Mecyna axinides Hübner (Crambidae) was detected in El Pijaral, in which some of the plants were completely defoliated.

**Floral traits**—A total of 276 open flowers from 100 individual plants (Table 1) were sampled to describe various floral traits. For each flower, we scored the phenological phase (male or female) and estimated floral rewards (nectar volume and sugar concentration). For this estimation, flowers were depleted of nectar and bagged during 24 h, after which nectar was extracted with 50-µL micropipettes to measure the volume produced during this period. The concentration was measured with a handheld refractometer (Bellingham & Stanley, Tunbridge Wells, Kent, UK).

**Breeding system and pollen limitation**—Hand self- (autogamy and geitonogamy) and hand cross-pollinations (xenogamy) were conducted to estimate the
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They were placed in muslin bags before and after hand pollination, except for flowers used in treatments C and SP (no bags) and AN (enclosed in nylon mesh bags with 1×1 mm openings, which excluded insects but allowed airborne pollen to pass). Flowers were protected with chicken wire to avoid fruit loss by animal consumption. Bags were completely removed when corollas withered, then fruits were collected in May–June 2008 for measurements and comparisons of fruit and seed production among treatments. The values of the analyzed variables obtained from C and SP treatments were used to calculate the cumulative pollen limitation index (PLI; see e.g., González-Varo et al., 2009), defined as: $\text{PLI cumulative} = 1 - \frac{C_{\text{fruit set}}C_{\text{seed set}}C_{\text{viable seed set}}}{SP_{\text{fruit set}}SP_{\text{seed set}}SP_{\text{viable seed set}}}$, where C is the value for the control and SP is the value for pollen-supplemented treatments; PLI values may vary from 0 (no pollen limitation) to 1 (maximum pollen limitation). After obtaining a cumulative value of pollen limitation using the percentage of flowers that set fruit (fruit set), the total number of seeds per fruit (seed set), and the percentage of viable seeds per fruit (viable seed set) simultaneously, we applied the same formula separately to each variable included in the PLI equation. Thus, $\text{PLI}_{\text{varX}} = 1 - \frac{C_{\text{fruit set}}}{SP_{\text{fruit set}}}$, in which varX was the analyzed plant-fitness variable.

Fig. 1. Map of Tenerife (Canary Islands) showing actual and potential areas with laurel forest (modified from Santos, 1990) and the studied populations, with their respective percentage of flowers with and without floral damage by semi-slugs, rats, or both from January to March 2009. Values are from 450 flowers per population (punctual floral surveys; see Material and Methods for details).
<table>
<thead>
<tr>
<th>Flower trait</th>
<th>X ± SD</th>
<th>Range</th>
<th>N</th>
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<tbody>
<tr>
<td>Corolla length (mm)</td>
<td>38.7 ± 4.4</td>
<td>28.6–51.4</td>
<td>276</td>
</tr>
<tr>
<td>Corolla bell diameter at opening (mm)</td>
<td>57.5 ± 7.9</td>
<td>39.1–77.7</td>
<td>276</td>
</tr>
<tr>
<td>Corolla bell diameter at nectary level (mm)</td>
<td>15.7 ± 1.7</td>
<td>11.3–19.5</td>
<td>276</td>
</tr>
<tr>
<td>Corolla–stigma distance (mm)</td>
<td>13.1 ± 2.1</td>
<td>6.8–18.6</td>
<td>276</td>
</tr>
<tr>
<td>Stigmatic lobe length (mm)</td>
<td>8.5 ± 1.2</td>
<td>6.3–12.0</td>
<td>101</td>
</tr>
<tr>
<td>Stigmatic lobe length (mm)</td>
<td>4.2 ± 0.5</td>
<td>3.4–5.9</td>
<td>101</td>
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<tr>
<td>Number of sepal</td>
<td>6.0 ± 0.4</td>
<td>4–8</td>
<td>276</td>
</tr>
<tr>
<td>Number of petals</td>
<td>6.0 ± 0.3</td>
<td>5–7</td>
<td>276</td>
</tr>
<tr>
<td>Number of stamens</td>
<td>6.0 ± 0.4</td>
<td>4–7</td>
<td>101</td>
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<tr>
<td>Number of stigmatic locbes</td>
<td>6.0 ± 0.3</td>
<td>5–7</td>
<td>101</td>
</tr>
<tr>
<td>Nectar volume (µL)</td>
<td>86.8 ± 70.3</td>
<td>0–300.8</td>
<td>206</td>
</tr>
<tr>
<td>Sugar concentration (%)</td>
<td>14 ± 6</td>
<td>5–49</td>
<td>252</td>
</tr>
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**Identification and visitation rate of main floral visitors**—To characterize quantitatively and qualitatively the native and introduced diurnal flower visitors of *C. canariensis*, we divided them into five functional groups: birds, insects, rodents, semi-slugs, and litter-dwelling arthropods (Table 1). Twelve floral patches (15–35 open flowers/patch) were selected for census in El Pijaral at Anaga in 2008. For birds, insects, and rodents, visits were noted for 30-min periods (12–15 periods/patch) from 0800 to 2030 hours. After each 30-min period, flowers were inspected for invertebrates. We calculated visitation rate as number of visits per 30 min per flower for all floral visitors. This variable may be an underestimate due to nocturnal visitation by, e.g., rats and semi-slugs, which were not censused.

**Relative contribution of the floral visitors to plant reproductive success**—The pollination efficiency of floral visitors (mainly focusing on birds) was ascertained from fruit and seed set via experimental exclusions. Sixty plants selected at El Pijaral (Anaga, winter–spring 2008) were separated enough (always >10 m distance) to avoid replicating experiments on the same gene. These plants were not the same ones used for hand pollinations, but were selected following the same protocol. Two flowers per treatment and per plant were used (N = 120 flowers per treatment), assigned randomly to a treatment: (1) all visitors (Co), control flowers open to all visitors; (2) flying insects (I), flowers protected against semi-slug and rat visits by elevation from the ground (pendent flowers without avian perch) and by application of glue (Tree Tanglefoot, Tanglefoot Co., Grand Rapids, Michigan) to the stems, and against bird visits by chicken wire with 2.2-cm diameter mesh; (3) flying insects and birds (IB), flowers protected against semi-slug and rat visits as described for the previous treatment (I); (4) semi-slugs and litter-dwelling arthropods (S), flowers on the ground and protected against visits by flying insects, birds, and rats using a closed-woven-cage (minute hole diameter) elevated 3 cm from the ground over the flower; (5) spontaneous autogamy (SA), bagged flowers to estimate the natural level of self-pollination. A selective enclosure type allowing only rat visitation was logistically impossible. All flowers were emasculated during the bud phase before secondary pollen presentation occurred, except those from the C and SA treatments. After the corolla withered, fruits were protected with chicken wire to avoid fruit loss by animal consumption and collected in May—June 2008 for estimation of fruit and seed production among treatments.

**Level of floral damage by native vs. introduced herbivores and consequences on plant fitness**—To determine the level of floral herbivory (percentage of damaged flowers) and the effects on plant reproductive success (fruit and seed set) at a within-island spatial scale, we conducted punctual (explained later) and long-term floral surveys in four populations (Fig. 1). For the level of floral herbivory, we surveyed 150 flowers to check the presence/absence of floral herbivory signs along a linear transect per population and per month, during 3 months (from January to March in 2009, N_{total} = 1800 flowers). To assure data independence and avoid flower re-use, we chose independent linear transects each month, so each flower was surveyed once during its lifespan (i.e., punctual floral survey).

To examine the effects on plant reproductive success, we tagged a different subset of flowers to evaluate the consequences of floral damage on plant fitness and checked them four times during their lifespan (once per month, from January to April in 2009) until the flower withered or produced a fruit (i.e., long-term floral survey). For this, a total of 140 randomly selected individual plants were chosen in the same four populations (Monte del Agua = 30; Barranco de Cuevas = 40; Las Vueltas de Taganana = 30; El Pijaral = 40), and three floral buds per plant (N_{total} = 420 flowers) to record final fruit and seed set.

In both observational studies (level of floral herbivory and its effects on plant reproductive success), we recorded the floral position (on ground or hanging) and herbivory condition for each survey of a flower using the following criteria: (1) presence or absence of floral herbivory signs; (2) damage produced only by semi-slugs or rats or by both; (3) floral structures with herbivory: damage in the corolla, androecium (anthers, deposited pollen on the style, filaments) and/or gynoecium (stigmatic lobes and style). Sepal damage was not observed in the field.

**Statistical analysis**—All analyses were performed with R software version 2.11.1 (R Development Core Team, 2010). Differences in floral traits between the male and female stages were analyzed with paired t tests (square-root and arcsine transformation applied to nectar volume and sugar percentage, respectively). To test for among-treatment differences in hand pollinations, selective exclosures, and floral herbivory (long-term floral surveys), we analyzed five variables related to female fitness: (percentage of flowers that set fruit, fruit length and width, total number of seeds (viable and aborted), and percentage of viable seeds per fruit) using generalized linear models (GLM). Differences in fruit biometry (log transformation, except in hand pollination experiments) and in total number of seeds (square-root transformation) were estimated using a Gaussian error distribution. To determine differences in the percentage of flowers that set fruit per plant and viable seeds per fruit among treatments, as well as the percentage of damaged flowers between areas and floral herbivores (punctual floral surveys), we used a binomial error distribution. The binomial family was also used to estimate differences in the percentage of flowers that set fruit between flowers with and without damage and between flowers damaged only by slugs and only by rats. Finally, differences in the number of damaged flowers depending on floral position (punctual floral surveys) were analyzed with a Poisson family. Before the data analysis, we explored which link function was best adapted, using the model with the lowest Akaike information criterion (AIC) and the quasi-binomial or quasi-Poisson family to deal with overdispersion when necessary (Crawley, 2007). Multiple comparisons of regression coefficients among treatments were applied with adjusted probability values by the single step method using packages multcomp (Hothorn et al., 2008) and sandwich (Zeileis, 2006). Throughout the paper, all means are accompanied with their standard deviations unless otherwise indicated.

**RESULTS**

**Floral traits**—Most flowers (>90%) examined were hexameros. Analyzing those plants that presented both male and female flowers on the same individual, flowers in the male phase produced more nectar (121.3 ± 45.2 µL) than those in the female phase (38.7 ± 50.9 µL) (N = 30 flowers per phase, paired t test, t = 7.45, df = 24, P < 0.001), whereas nectar sugar concentration was the same in the two phases (paired t test, t = −2.01, df = 27, P = 0.055).

**Breeding system and pollen limitation**—No fruits were obtained by apomixis (AP) or our tests of anemophily (AN), and they were removed from the subsequent analysis. In all treatments, <50% of the flowers per plant set fruit (except for flowers that received supplemental pollen) and differed significantly among the treatments (χ² = 26.30, df = 4, P < 0.001; Fig. 2A).

With respect to the SI estimation, fruits from self-pollen treatments (autogamy [A] and geitonogamy [G]) were similar in all measured variables (P > 0.05 in all cases). Both treatments also produced fruits with a length and number of seeds per fruit similar to those in the cross-pollen treatment (X) (P > 0.05 in all cases). However, results differed for the rest of the...
analyzed variables when each self-pollen treatment was compared separately to xenogamy (X). Autogamy had a lower percentage of flowers that set fruit per plant compared to the xenogamous treatment (Z = 3.49, df = 1, P = 0.004; Fig. 2A), and fruits were narrower (Z = 2.79, df = 1, P < 0.04) and had a lower percentage of viable seeds (Z = 7.95, df = 1, P < 0.001; Fig. 2B). On the contrary, geitonogamy only differed from xenogamous treatment in the percentage of viable seeds per fruit (Z = 3.79, df = 1, P = 0.001; Fig. 2B).

Respect to the pollen limitation, the addition of pollen (SP) significantly increased the percentage of flowers that set fruit per plant (Z = 3.40, df = 1, P = 0.006; Fig. 2A) and the total number of seeds per fruit compared to control flowers (C) (Z = 6.11, df = 1, P < 0.001). However, the percentage of viable seeds per fruit was similar (Z = 1.15, df = 1, P = 0.76; Fig. 2B). The cumulative PLI value (0.85) indicated strong pollen limitation, as well as index values when fitness variables were analyzed individually (PLI > 0.5), except for the percentage of viable seeds per fruit (PLI = 0.06).

**Identification and visitation rate of main floral visitors**—We monitored 227 flowers for 77.5 h of diurnal observations (N = 155 30-min periods). Floral visitors included four invertebrate and three vertebrate species, all native species except *R. rattus* and the pillbug *Armadillidium vulgare* Latreille (Table 2). Floral herbivores (semi-slugs *Plutonia* spp. and rats) and pollen collectors (solitary bees: *Lasiosglossum viride* Brullé) represented 89% of all visits (N = 113 visits), birds constituted 5% of the visits (*P. canariensis* and *C. caeruleus*, N = 7), butterflies 2% (*G. cleobule*, N = 3) and 3% by *A. vulgare* (N = 4). Birds and butterflies consumed only nectar, in contrast to semi-slugs and rats, which consumed floral tissues besides nectar. Solitary bees harvested only pollen, whereas *A. vulgare* was always observed quiet inside the bell flower, apparently for shelter (no exploitation of floral resources).

Flowers were mainly visited by floral herbivores and pollen collectors, instead of opportunistic birds. As the most frequent visitors, semi-slugs and solitary bees had a visitation rate twice as high as rats (Table 2). Rats, however, probed more flowers per visit (2.2 ± 0.9 flowers/visit) than did semi-slugs (one for all visits) and solitary bees (1.1 ± 0.4). In 28% of the visited flowers (N = 14), rats touched the reproductive organs without any detrimental effects, a percentage impractical to detect for semi-slugs because of their long lasting visits (>30 min was once recorded). The passerines *P. canariensis* and *C. caeruleus* visited five times fewer flowers than did the herbivores (Table 2).

**Relative contribution of the floral visitors to plant reproductive success**—The low relative visitation rate by birds at El Pijaral (Anaga) during 2008 resulted in a low fruit production (N = 47) in the exclusion experiment (600 emasculated flowers; Fig. 3A). The open pollinated controls (Co) had the highest percentage of flowers that set fruit per plant (16 ± 25%). No differences were detected in this percentage between the flying insect and bird treatment (IB) and those where birds had been excluded, that is, flying insects (I), semi-slugs and litter-dwelling arthropods (S), and spontaneous autogamy (SA) (P > 0.05 in all cases; Fig. 3A).

However, the flying insect and bird treatment resulted in larger fruits (length $F_{3,39} = 26.30$, $P < 0.001$; width $F_{3,39} = 18.17$, $P < 0.001$) compared to bird-exclusion treatments (I and S). With respect to the percentage of viable seeds per fruit, fruits from spontaneously self-pollinated flowers did not produce any practical number of viable seeds (Fig. 3B) and were excluded from the analysis. In the rest of the treatments, flowers visited by birds (Co and IB) presented a greater percentage of viable seeds per fruit than in those visited by flying insects and semi-slugs ($\chi^2 = 36.02$, df = 3, $P < 0.001$; Fig. 3B).

**Level of floral damage by native vs. introduced herbivores and consequences on plant fitness**—When pooling across all surveyed flowers in 2009, 52% of the total (N = 1800) were damaged from herbivory by semi-slugs (N = 720), rats (N = 120), or both (N = 100, Fig. 1). Semi-slugs damaged proportionally more flowers than rats (Z = 6.05, df = 1, P < 0.001).

![Fig. 2. Mean (±SE) (A) percentage of flowers that set fruit per plant and (B) percentage of viable seeds per fruit for hand pollination treatments. Numbers below the bars are sample sizes (number of fruits). C, control; A, autogamy; G, geitonogamy; X, xenogamy; SP, pollen supplementation. See Materials and Methods for treatment details.](image-url)
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set than those not damaged (Z = −2.35, df = 1, P = 0.02) (Table 3). In addition, fruits from damaged flowers were smaller and produced fewer seeds (P < 0.05) than those from undamaged flowers (Table 3). However, the percentage of viable seeds was similar (Z = −0.35, df = 1, P = 0.73) (Table 3). Analyzing each herbivore separately, flowers damaged only by semi-slugs had a moderately higher fruit set (17%) than did flowers damaged only by rats (14%) (Z = 0.25, df = 1, P = 0.81).

DISCUSSION

The Canarian bellflower, C. canariensis, has been frequently reported as one of the classical bird-flower elements from the Macaronesian archipelagoes, but no conclusive evidence about the impact on plant reproductive success of its opportunistic nectar-feeding bird visitors has previously been published. Despite potential self-fertilization of the flowers, we documented by controlled pollination and exclusion experiments that this plant relies on passerine birds for reproductive success. We also found that the studied plant population was pollen-limited. However, the most frequent flower visitors were herbivores (native semi-slugs and introduced rats), which clearly decreased plant reproductive output and presented an important within-island spatial variation in herbivory intensity.

Fig. 3. Mean (±SE) (A) percentage of flowers that set fruit per plant and (B) percentage of viable seeds per fruit for exclusion treatments. Numbers below the bars are sample sizes (number of fruits). Co, Control: no visitors excluded; IB, all visitors excluded except flying insects and birds; I, all visitors excluded except semi-slugs; SA, spontaneous autogamy. See Materials and Methods for treatment details.

Table 2. Floral visitors of Canarina canariensis and their visitation rate during diurnal censuses. Data were compiled from 77 h 30 min of census in 227 flowers from El Pijaral (Anaga, Tenerife). During monitoring, 127 visits to floral patches were recorded, with 172 flowers visited (No. flowers). Visitation rate is expressed as the number of visits per 30-min period per flower (X ± SD).

<table>
<thead>
<tr>
<th>Visitor sp. (Order)</th>
<th>Functional group</th>
<th>Ecological role</th>
<th>No. visits (%)</th>
<th>Visit rate</th>
<th>No. flowers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plutonia spp. (Pulmonata)</td>
<td>Semi-slugs</td>
<td>Floral herbivores, low-efficiency pollinators</td>
<td>45 (35)</td>
<td>0.015 ± 0.028</td>
<td>45 (26)</td>
</tr>
<tr>
<td>Lasiosglossum viride (Hymenoptera)</td>
<td>Flying insects</td>
<td>Pollen collectors, low-efficiency pollinators</td>
<td>44 (35)</td>
<td>0.016 ± 0.043</td>
<td>48 (28)</td>
</tr>
<tr>
<td>Rattus rattus (Rodentia)</td>
<td>Rodents</td>
<td>Floral herbivores, low-efficiency pollinators</td>
<td>24 (19)</td>
<td>0.008 ± 0.023</td>
<td>51 (30)</td>
</tr>
<tr>
<td>Phylloscopus canariensis (Passeriformes)</td>
<td>Birds</td>
<td>High-efficiency pollinators</td>
<td>4 (3)</td>
<td>0.004 ± 0.014</td>
<td>16 (9)</td>
</tr>
<tr>
<td>Armadillidium vulgare (Isopoda)</td>
<td>Litter-dwelling arthropods</td>
<td>Unknown role</td>
<td>4 (3)</td>
<td>0.001 ± 0.009</td>
<td>4 (2)</td>
</tr>
<tr>
<td>Cyanistes caeruleus (Passeriformes)</td>
<td>Birds</td>
<td>High-efficiency pollinators</td>
<td>3 (2)</td>
<td>0.001 ± 0.008</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Gonopteryx clebeola (Lepidoptera)</td>
<td>Flying insects</td>
<td>Nectar consumer, low-efficiency pollinators</td>
<td>3 (2)</td>
<td>0.0007 ± 0.006</td>
<td>5 (3)</td>
</tr>
</tbody>
</table>
Table 3. Percentage of flowers that set fruit (fruit set) and percentage of viable seeds/fruit (viable seed set) obtained from damaged (semi-slugs, rats, and both pooled) and undamaged flowers per population and area (long-term floral surveys; see Material and Methods for details). Means are accompanied with their standard deviations.

<table>
<thead>
<tr>
<th>Population</th>
<th>Fruit set</th>
<th>Viable Seed set</th>
<th>Nfr</th>
<th>Seed set</th>
<th>Nfr</th>
<th>Viable Seed set</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA</td>
<td>43</td>
<td>456 ± 664 (6)</td>
<td>38</td>
<td>428 ± 58 (11)</td>
<td>36</td>
<td>346 ± 36 (13)</td>
</tr>
<tr>
<td>CN</td>
<td>90</td>
<td>452 ± 478 (6)</td>
<td>11</td>
<td>117 ± 20 (11)</td>
<td>14</td>
<td>37 ± 17 (11)</td>
</tr>
<tr>
<td>VT</td>
<td>50</td>
<td>248 ± 108 (10)</td>
<td>14</td>
<td>40 ± 15 (5)</td>
<td>15</td>
<td>35 ± 10 (5)</td>
</tr>
<tr>
<td>PI</td>
<td>72</td>
<td>166 ± 48 (6)</td>
<td>7</td>
<td>40 ± 6 (1)</td>
<td>15</td>
<td>35 ± 10 (5)</td>
</tr>
<tr>
<td>Total Teno</td>
<td>93</td>
<td>248 ± 108 (10)</td>
<td>17</td>
<td>70 ± 20 (10)</td>
<td>15</td>
<td>35 ± 10 (5)</td>
</tr>
<tr>
<td>Total Anaga</td>
<td>122</td>
<td>166 ± 48 (6)</td>
<td>8</td>
<td>40 ± 6 (1)</td>
<td>15</td>
<td>35 ± 10 (5)</td>
</tr>
<tr>
<td>Grand total</td>
<td>215</td>
<td>248 ± 108 (10)</td>
<td>14</td>
<td>70 ± 20 (10)</td>
<td>15</td>
<td>35 ± 10 (5)</td>
</tr>
</tbody>
</table>

- **Note:** Nfr, no. of fruits from which means and standard deviations were calculated (some fruits produced were lost). MA, Monte del Agua; CN, Barranco de Cuevas Negras; VT, Las Vueltas de Taganana; PI, El Pijaral.

**Breeding system**—Self-fertilization is possible, but not spontaneously and likely not through agamospermy. The origin of the pollen was relevant for plant reproductive success since xenogamy treatment produced the highest values of fruit and seed production in general. In addition, outcrossed-pollinated flowers (X) showed a significantly higher percentage of viable seeds compared to the selfed (A, G) (Fig. 2B). Among the underlying mechanisms explaining this lower reproductive output in self-treatments may be the existence of self-incompatibility systems or inbreeding depression, as described for several species of *Campanula* (e.g., Stephenson et al., 2000; Good-Avila et al., 2001). However, additional studies would be required to discern between them.

The observed low reproductive success in selfed flowers might be also promoted by protandry and secondary pollen presentation via pollen collecting hairs (PCHs, e.g., Nyman, 1993a, b). Within the bellflower family, the degree to which stigmatic branches recurve varies (e.g., Anderson et al., 2000), as does the retraction of hairs into their basal cavities during the female phase (Nyman, 1993a, b). We did not analyze these two processes, but an incomplete recurvature of stigmatic branches (without any stylar contact) and/or the fixed retraction of PCHs before stigmatic receptivity might be involved. A temporal mismatch between pollen viability and stigma receptiveness should also be considered (e.g., *Campanula* spp.; Nyman, 1992).

On the basis of our results, *C. canariensis* is self-compatible, but selfing depresses its reproductive success. The level of self-compatibility, along with the cited protandry and PCHs’ mechanism, may have evolved in a different direction under insular conditions (e.g., SI-to-SC shift in *Campanula punctata*; Inoue and Amano, 1986). Unfortunately, the breeding system of the East African *Canarina* relatives is almost unknown and cannot help us clarify the influence of island colonization on the evolution of this genus.

**Pollen limitation**—The lower fruit and seed set obtained in the control (C) compared to pollen-supplemented (SP) flowers may be due to pollinator limitation and/or differential shortage of resources among experimental plants. Our experimental set-up does not allow us to discern between these possibilities. However, since control and supplemented flowers had a similar relative position within an individual plant, our results suggest that pollinator service might be the main cause. In addition, the area with the lowest bird activity on the flowers (M. C. Rodríguez, personal observation) and with the highest level of floral herbivory (Anaga) also had a lower fruit set. Thus, the combination of low bird activity and frequent damage in the androecium by floral herbivores might limit pollen transfer. It is interesting that pollen supplementation did not increase the percentage of viable seeds per fruit, but did increase the total number of seeds per fruit. Although we did not measure the number of pollen grains or pollen tubes present in the different hand-pollination treatments, these results suggest that control flowers probably received (by pollinators) high-quality pollen (e.g., Aizen and Harder, 2007), but not in sufficient amounts compared to supplemented flowers.

In summary, in the studied population (El Pijaral, Anaga), pollen limitation could be due to the independent or combined effects of the low spontaneous self-pollination (Figs. 2A, B; 3A, B), the low visitation rate of avian pollinators (Table 2), and the highest percentage of damaged flowers of all studied populations (Fig. 1). The temporal and spatial variation of these pollen-limiting factors may create different “pollen limitation
Identification and visitation rate of main floral visitors—The most frequent floral visitors in Anaga were native semi-slugs and introduced rats along with pollen collectors (solitary bees). Although up to four (Anaga) and three (Teno) species of the semi-slug *Plutonia* have been recorded in the laurel forest (Kappes et al., 2009), only *P. lamarckii* was recorded as a floral visitor in our visual censuses. In terms of field observations, rats made fewer visits than semi-slugs, and rat visitation did not always result in herbivory on reproductive organs.

The high visitation rate of herbivores contrasted dramatically with the relatively small number of bird visitors. Our recorded frequency of avian visits is even lower than previously recorded with the relatively small number of bird visitors. Our recorded visits by only semi-slugs (S) or only solitary bees (I) resulted in herbivory on reproductive organs. The rats made fewer visits than semi-slugs, and rat visitation did not always result in herbivory on reproductive organs.

Relative contribution of the floral visitors to plant reproductive success—Visits by only semi-slugs (S) or only solitary bees (I) resulted in extremely low fruit set and viable seed set, with values similar to spontaneous autogamy treatment (SA) (Fig. 3A, B). Because the pillbug *A. vulgare* was never observed in contact with reproductive organs and this species had the lowest visitation rate, all seeds produced in the S treatment are considered to have been sired exclusively by semi-slugs. These experimental results point out the lower contribution of native semi-slugs as effective pollinators compared with birds, so they may be considered as low-quality pollinators. The negligible contribution of semi-slugs may be due to the spatial or temporal mismatch between their foraging activity and the sexual receptivity pattern of the flowers (e.g., a higher floral visitation frequency in male phase as suggested by floral surveys; see Results).

Birds (IB) did not significantly increase the fruit set in the studied populations (El Pijaral, Anaga), but the fruits produced had the highest percentage of viable seeds of all exclusion treatments (Fig. 3B). Their importance as pollinators is stressed by the low spontaneous selfing ability (SA) of *Canarina* to set fruits in the absence of floral visitors and by the almost complete lack of viable seeds in these fruits (Fig. 3A, B). This result was also obtained by Ollerton et al. (2009), but interestingly, they recorded higher natural fruit (62 ± 14%) and seed set (1164 ± 496 seeds/fruit) from open-pollinated flowers (Anaga, *N* = 8 populations) compared with our results (C treatment in Fig. 2A, B; Co treatment in Fig. 3A, B; long-term floral surveys, Table 3). The spatial and temporal variation of the assemblage of interacting agents (including passerine birds) may account for this difference, even within the same area (e.g., Anaga).

Despite the increase in viable seed production, birds may not be able to mitigate the pollen limitation suffered by the studied population (El Pijaral). Compared to the cross-pollinated flowers from hand pollinations (X and SP), bird-visited flowers from selective exclosures (Co and IB) seem to have produced fewer fruits per plant (Figs. 2A, 3A) and fewer seeds per fruit, but the percentage of viable seeds per fruit was similar (Figs. 2B, 3B). Thus, birds may counteract inbreeding effects that mediate long-distance pollen flow, but at levels that are insufficient to achieve the best pollination service (quantity limitation), at least in the studied population.

The introduced rats, although they are predominantly floral herbivores (see below), might also act as pollen vectors of *C. canariensis*, a possibility suggested for other insular plant species (Vitousek et al., 1987; Ecroyd, 1996). In fact, 28% of flowers visited by rats during our diurnal censuses were not damaged. However, our results suggest that rats are not contributing much (if at all) to pollination relative to birds, because open-pollinated flowers (Co), the only flowers that rats had access along with birds, did not differ in any variable of female fitness from bird-visited flowers (IB), which are inaccessible for rats (Fig. 3A, B). Nevertheless, these results are not conclusive, and specific rat exclusion experiments are needed to clarify their potential role as pollinators.

Level of floral damage by native vs. introduced herbivores and consequences on plant fitness—Half of all surveyed flowers suffered from herbivory, with a higher percentage of damaged flowers in the Anaga populations than in the Teno in general. When each floral herbivore was analyzed separately, floral damage was also greater in percentage in Anaga, a result that can partially be attributed to the relative abundances of rats and semi-slugs in these two areas: *Plutonia* semi-slugs are equally abundant (Kappes et al., 2009), but the rats are relatively more abundant in Anaga than Teno (Hernández et al., 1999).

When compared to each other, floral herbivores differed in their pattern and intensity. Because semi-slugs principally affected male reproductive organs, the semi-slugs may have a greater impact via pollen consumption on the reduction of pollen availability (Krupnick and Weis, 1999; Mothershead and Marquis, 2000) and probably on the induction of pollen limitation (Bertness and Shumway, 1992; Cunningham, 1995). Rats may also promote pollen limitation but to a lesser degree than semi-slugs because rats consume pollen secondarily deposited on the style (always correlated with style consumption) and did not always damage visited flowers during our censuses.

Although our data cannot answer to what extent the difference in florivory intensity of the floral herbivores is also maintained for plant reproductive success, the results showed that floral damage in general clearly decreased fruit and seed production. However, our measure of herbivory is solely based on maternal fitness. As mentioned, floral damage almost certainly reduces male fitness (e.g., pollen transfer among plants) because both floral herbivores, especially semi-slugs, eat pollen.

Conclusion—Opportunistic nectar-feeding birds facilitate the reproductive success of *C. canariensis* via efficient pollination, counteracted by the decrease of fruit and seed production by floral herbivory. Although this plant species appears to have evolved under a naturally low rate of bird visitation, the high incidence of floral herbivory may cause pollen limitation (as
shown by our results), a circumstance aggravated by the incapacity of spontaneous selfing. Native semi-slugs have evolved with *C. canariensis*; however, introduced rats have increased this herbivory level to the detriment of reproduction. Differences between native and introduced herbivores in their damage to reproductive organs and herbivory intensity suggest that they may differ in their effects (e.g., altering pollinator attraction) on male and female plant reproductive success. Elucidating how these effects are translated into seed production and population dynamics will let us understand the real extent of the rat impact on this native system.

**LITERATURE CITED**


