

RESEARCH PAPER

Reproductive success of the Canarian *Echium simplex* (*Boraginaceae*) mediated by vertebrates and insects

J. Jaca¹ , M. Nogales²  & A. Traveset¹ 

¹ Global Change Research Group, Institut Mediterrani d'Estudis Avançats IMEDEA (CSIC-UIB), Esporles, Mallorca, Balearic Islands, Spain

² Island Ecology and Evolution Research Group, Instituto de Productos Naturales y Agrobiología (CSIC-IPNA), C/ Astrofísico Fco, La Laguna, Canary Islands, Spain

Keywords

Canary Islands; *Echium*; opportunistic vertebrate pollinators; ornithophily; pollination effectiveness; reproductive biology; saurophily.

Correspondence

J. Jaca, Global Change Research Group, Institut Mediterrani d'Estudis Avançats IMEDEA (CSIC-UIB), C/ Miquel Marqués 21, E07190-Esporles, Mallorca, Balearic Islands, Spain.
E-mail: juliajaca@hotmail.com

Editor

G. Scopece

Received: 16 July 2018; Accepted: 11 October 2018

doi:10.1111/plb.12926

ABSTRACT

- Oceanic island ecosystems favour the appearance of novel interactions as a consequence of their depauperate and disharmonic flora and fauna. We investigated *Echium simplex*, endemic to the Anaga Biosphere Reserve in NE Tenerife, Canary Islands, belongs to the Canarian bird–flower element.
- Along two flowering seasons, we studied the breeding system of *E. simplex*, identified the floral visitors and compared the pollination effectiveness of different animal guilds (insects *versus* vertebrates) by means of selective exclosures.
- *E. simplex* is self-compatible but selfing significantly reduced fruit set. The flowers were visited by five bird species (mostly *Phylloscopus canariensis* and *Serinus canarius*, but also *Cyanistes teneriffae*, *Sylvia atricapilla* and *Sylvia melanocephala*), a lizard species (*Gallotia galloti*) and over a hundred insect species (mainly hymenopterans and coleopterans). Flying insects increased fruit set whereas small flower dwellers (mostly beetles) decreased both fruit and seed set. Vertebrates had a negligible effect on reproductive success.
- We conclude that although the floral resources provided by *E. simplex* may be important to some vertebrate species, these do not appear to contribute to increase fitness of the plant, which was more dependent upon flying insects for fruit and seed set. We additionally found that plant reproductive structures are heavily damaged by feral goats, which threaten the maintenance of this Canarian endemic species.

INTRODUCTION

Due to their geographic isolation and relatively small size compared to inland areas, oceanic island ecosystems have unique evolutionary histories (Warren *et al.* 2015), often containing a high number of endemic species (Carlquist 1974; Kier *et al.* 2009). Such isolation hinders colonisation and dispersal processes, leading to depauperate and disharmonic biota (Gillespie & Roderick 2002). Successful colonisation and establishment on islands for any group of organisms is influenced by their life histories and reproductive systems (Gillespie & Roderick 2002). Thus, for instance, the requirement of specialised animal pollinators for some plant species may be an impediment to their establishment (Barrett 1996). The usually high incidence of wind-pollinated plants on oceanic islands led to the hypothesis on the benefits of wind pollination on islands (Carlquist 1974; Crawford *et al.* 2011 and references therein), and there is evidence that several originally specialist insect-pollinated plant lineages switched to wind or generalist pollination after island colonisation (Crawford *et al.* 2011 and references therein). As a consequence of the depauperate and disharmonic flora and fauna, island plant–pollinator networks are much poorer in species and less complex than those in mainland areas, especially oceanic islands (Traveset

et al. 2015). Moreover, oceanic island networks often contain supergeneralist species, *i.e.* those that interact with a disproportionate number of species (Olesen *et al.* 2002). Such supergeneralists are important elements of island ecosystems and favour novel mutualistic interactions.

Novel pollination interactions between plants and opportunistic, food-generalist vertebrates, such as birds and lizards, are particularly prevalent in island systems (*e.g.* Grant & Grant 1981; Olesen 1985; Traveset & Sáez 1997; Olesen & Valido 2003). This phenomenon has been especially documented from the tropics (Anderson 2003), and more recently also from temperate regions (da Silva *et al.* 2014), which has been interpreted as the response of such vertebrates to density compensation and trophic niche expansion in both bird (Wright 1980 and references therein) and lizard (Rodda & Dean-Bradley 2002 and references therein) populations in island areas. Under food shortage, flower rewards such as nectar, pollen, floral oil, petals, water and flower-visiting insects attract potential vertebrate pollinators (Cecere *et al.* 2011), which can be essential for both specialised and non-specialised nectarivorous species (Cronk & Ojeda 2008; Cecere *et al.* 2011).

These vertebrates may be more trustworthy pollinators than insects under particular ecological circumstances, specifically when the latter are scarce and have low population densities,

e.g. in high-altitude ecosystems, bad weather conditions, isolated islands and for winter-flowering plants (Cronk & Ojeda 2008 and references therein). Typical ornithophilous (bird-visited) flowers, in particular, possess a set of features (bird pollination syndrome) such as scarlet, red or orange colour, absence of scent and nectar guides, large, funnel-like flowers and abundant and dilute nectar (Faegri & van der Pijl 1966; Rausher 2008). This syndrome has evolved independently in many groups of plants from bee-pollinated systems, ornithophily being a dead evolutionary end, and where turn-around is uncommon and unlikely (Rausher 2008; Van der Niet & Johnson 2012; Ojeda 2013). Usually, the transition in a bird pollination system starts with the flower pigmentation changing towards red, as in *Lotus*, *Mimulus* and *Ipomoea* (Cronk & Ojeda 2008). Such an evolutionary dead end implies highly specialised interactions, making these plants more vulnerable to extinction than more generalised plants (Ojeda 2013).

The Macaronesian islands have received much attention regarding flower visitation by generalist passerines since the late 19th century (Porsch 1924 citing observations of Lowe in 1896; Schmucker 1936; Ojeda 2013). Nearly 20 plant species, most of them endemic, from different families have been reported to be ornithophilous. The assemblage of such species is actually referred as the 'Macaronesian bird-flower element', which includes plant genera such as *Isoplexis*, *Scrophularia*, *Echium*, *Canarina*, *Aeonium*, *Lotus*, *Anagyris*, *Lavatera*, *Euphorbia*, *Teucrium*, *Rhamnus*, *Scrophularia* and *Navaea* (Valido & Olesen 2010). Generalist vertebrates that often visit flowers for nectar include bird species in the genera *Phylloscopus*, *Cyanistes*, *Sylvia*, *Serinus* and *Fringilla* among birds, and lizard species in the genus *Gallotia*. Most ornithophilous plants in the Canary Islands, in particular, have been shown to descend from continental ancestors that had entomophilous flower traits (Valido *et al.* 2004). One exception, however, is the *Canarina* genus in which ornithophily already evolved in continental ancestors in response to specialised nectarivorous sunbirds (Olesen *et al.* 2012). No evidence of sunbirds, present in the past on the mainland (Mayr & Wilde 2014), exists from the fossil record of the Canarian archipelago (Valido *et al.* 2004).

The effectiveness of generalist vertebrates as pollinators has been confirmed so far only in a few studies in the Canarian archipelago: *Isoplexis canariensis* (Rodríguez-Rodríguez & Valido 2008), *Canarina canariensis* (Rodríguez-Rodríguez & Valido 2011) and *Navaea phoenicea* (Malvaceae) (Fernández de Castro *et al.* 2017). With the main goal of providing further insights on the role of vertebrates in plant reproductive success in the Canarian archipelago, and on evolution of the bird pollination syndrome in Macaronesia, we selected the endemic *Echium simplex* (Boraginaceae) which belongs to the Canarian bird-flower element (Valido & Olesen 2010) and for which there is no information on its breeding and pollination system. The main objective of our study was to experimentally determine the effectiveness of vertebrates (birds and lizards, specifically) as pollinators and compare it to that of insects. We first assessed the breeding system of the plant and then evaluated the relative effectiveness of the different groups of flower visitors as pollinators in terms of fruit and seed production and seed germination. In addition, as naturalised goats were

observed feeding on reproductive *E. simplex* plants, at different stages of inflorescence development and often causing severe plant damage, we quantified the level of herbivory in this endemic plant whose most abundant populations are found in the study area.

MATERIALS AND METHODS

Study species

The giant rosette plant *Echium simplex* DC. (Boraginaceae), locally known as 'tajanaste blanco', is endemic to the Anaga Biosphere Reserve in NE Tenerife (Canary Islands). This area encompasses one of the most recent zones of the island, a 4.9–3.9 million-year-old basaltic massif (Guillou *et al.* 2004). It is considered a vulnerable species in the Red List of the Spanish vascular flora (Moreno 2008), with very few, reduced and isolated populations. The species is one of the three monocarpic *Echium* species in the Canary Islands, together with *E. wildpretii* in La Palma and Tenerife, and *E. pininana* in La Palma, and grows for 5–9 years before producing a single inflorescence (Stöcklin & Lenzen 2013). Reproductive individuals reach a height of up to 3 m, of which the long inflorescence – composed of scorpioid cymes – can contribute up to 1.5 m. The inflorescence height is directly proportional to the rosette diameter. The cymes are double coiled and the largest plants may have three to four branches per cyme. After a successful pollination event, a flower develops into a fruit which consists of a maximum of four nutlets. The number of cymes and flowers per wrap increases along the inflorescence. The smallest of our examined plants had an average of 12 flowers per wrap, whereas the largest had 51. The number of mature subfruits per flower (one to three, on average) also increased along the length of the inflorescence. Hence, the number of potential seeds produced increases enormously in relation to the size of the inflorescence, ranging from 4560 to 234 000 (Stöcklin & Lenzen 2013). Preliminary observations on flower visitors of *E. simplex* noted insect species, mostly honeybees and wild bees, beetles and ants (Stöcklin & Lenzen 2013), and some passerine birds (Valido & Olesen 2010), but neither quantitative nor qualitative data existed prior to this present study.

Study area

The study site is located northwest of Chamorga village, north-east Tenerife (Canary Islands). The population of *E. simplex* is at ca. 250 m a.s.l. and occupies an area of ca. 1 km². There are also scattered individuals along the trail of the north coast. The location has a warm coastal climate with average temperatures between 17 and 19 °C in winter and between 20 and 25 °C in summer. The summer is very dry and most rain falls in winter, but only in small quantities. The area is exposed to the north-east wind, which is responsible for the lush green vegetation of Anaga Mountains. The vegetation is shrubby–herbaceous, dry Mediterranean and characterised by numerous endemic species such as *Artemisia thuscula*, *Descurainia millefolia*, *Aeonium canariense*, *Asphodelus tenuifolius*, *Achyranthes aspera* and *Galactites tomentosa*. Fieldwork was conducted during the reproductive season of *E. simplex*, between April and June 2015 and 2016.

Plant breeding system

Hand-pollination experiments were performed on a total of 30 randomly selected plants. In each individual plant, we randomly assigned flowers to five different treatments: (i) *autogamy*: flowers were bagged (with muslin) before anthesis and left until fruits were produced to evaluate the autonomous selfing capacity; (ii) *apomixis*: flowers were emasculated and bagged to assess their capacity to produce fruits without pollen; (iii) *anemogamy*: flowers were emasculated and bagged with nylon mesh allowing pollen but not insects to pass through; (iv) *xenogamy*: a fresh pollen mix source (from two to three plants in the same population) was applied to the stigmas of flowers which had been also previously emasculated; and (v) *open pollination*: a group of flowers from each plant was kept as control treatment, leaving flowers open to natural pollination.

The apomixis and anemogamy treatments were performed only in 2015 on one flower per plant. In contrast, the autogamy and xenogamy treatments were conducted both in 2015 (on three flowers per plant) and 2016 (on four flowers per plant). Each year, the control flowers were marked simultaneously as pollination treatments were performed.

Flowers used for the autogamy and xenogamy treatments were kept in muslin bags to exclude any animals or potential airborne pollen grains. Bags were removed and fruits collected when ripe. Fruit set was calculated as the proportion of flowers that developed fruit, whereas seed set as the amount of viable seeds produced per fruit. Seeds were regarded as non-viable (aborted) based on characteristic greyness and smaller size. A germination experiment was conducted in order to test the accuracy of this assumption. A total of 313 seeds were randomly selected from a pool that had been classified as aborted and were left to germinate. Only 4.15% of those seeds germinated successfully, in contrast to the 77.02% of the seeds considered as viable, thus we considered our classification as valid.

Flower visitors and visitation frequency

To identify flower visitors and determine their visitation frequency, a total of 143 individual plants, chosen randomly, were observed during focal censuses. Vertebrates (birds and lizards) were monitored for 30 min per census from a place 5–10 m away from the plant and using binoculars. Flying insects were observed for 10–15 min per census at a shorter distance (0.5 m) from the plant. Insects of all species or morphospecies were captured and taken to the lab for identification. Flower-dweller insects on each censused plant were counted after flying insect observations. These direct observations were supplemented by video recording and photographs. Animals were considered as flower visitors whenever they touched the flower, as sexual organs are exerted from the corolla. For each flower visitor, we recorded: species identity (sometimes family or order for insects), number of flowers visited (for insects and birds when possible) and time on the inflorescence (for birds and lizards). For the vertebrate censuses, we monitored 41 plants over 33.75 h of diurnal observations ($N = 72$; 15–30 min periods) in 2015 and 41 plants over 104.58 h of diurnal observations ($N = 217$; 10–30 min periods) in 2016. For the insect censuses, we monitored 35 plants over 10.84 h of diurnal observations ($N = 65$; 10-min periods) throughout the flowering season of 2015, and 31 plants for 31 h of diurnal

observations ($N = 120$; 15–30 min periods) in 2016. All censuses began in early April and lasted until early June.

Relative effectiveness of flower visitors as pollinators

Based on observations of flower visitors, we conducted experiments to study the pollination importance of different guilds of pollinators (passerine birds, lizards, flying insects and flower dwellers). Five randomly assigned exclusion treatments were performed on each individual plant: (i) *total exclusion*: the whole inflorescence was bagged (with muslin bags) to exclude any type of flower visitor; (ii) *bird exclusion*: the plants were entirely covered with a 5×2 cm plastic mesh to avoid birds accessing the flowers; (iii) *lizard exclusion*: a 30-cm diameter acetate funnel was placed at the base of the inflorescence to avoid lizards climbing into flowers; (iv) *vertebrate (bird + lizard) exclusion*: plants were covered with the cage and a basal funnel was placed at their base; and (v) *only access by flower dwellers*: plants were covered with a nylon mesh bag with 3×3 mm openings that allowed small insects (mainly small beetles and ants) to enter and crawl between flowers. Another group of plants were used as controls, i.e. they were left open to all flower visitors. A total of 10–15 individuals was used for each treatment. The plants were inspected daily to guarantee that the enclosures did not interfere with flower visitor foraging.

Fruits were collected when ripe and taken to the laboratory where seed viability was evaluated using the procedure described above. In 2015, all treatments were performed, but in 2016 we only repeated treatments 1 (total exclusion) and 5 (only flower dwellers exclusion), plus the control group. For each treatment, we again assessed fruit and seed set.

Different pollinators may deposit pollen of different quality (Ne'Eman *et al.* 2010), which may lead to different germinability. In order to test for differences in germination patterns (germinability and germination rate) among treatments, we carried out germination trials in a greenhouse in Tenerife. A total of 2245 viable seeds were sown in early October (both 2015 and 2016) into trays filled with a 1:2:1 mixture of peat, common agricultural soil and ravine sand. Trays were periodically watered every 2 days to ensure that the soil was constantly moist, and seedling emergence was recorded every 5 days for 3 months until January, when the germination experiment concluded after no seed germination for >25 days. Germinability refers to the fraction of seeds that germinated, whereas germination rate is the number of days elapsed since seed sowing to seedling emergence. Sown seeds of each treatment were previously weighed to the nearest 0.1 mg.

Herbivory levels

Most reproductive individuals in the population were checked for herbivore damage in late June 2015 and 2016 and, when this was present, it was classified into four types depending on the stage at which it was produced and on the extent of damage: (i) at early stage with total or partial herbivory of the flower meristem, causing total lack of flowers, or delayed and ball-shaped flowering; (ii) at intermediate stage with apical herbivory of the growing inflorescence stem, causing short/square-shaped flowering; and (iii) at final flowering stage, causing normal-shaped but with some grazed parts of the inflorescences. In this last case, the percentage of the plant that was affected was also recorded.

Statistical analyses

We used GLMM in R version 3.3.3 (R Core Team 2018) followed by a Tukey test of multiple comparisons. For the hand-pollination, plant exclusion and germination experiments, each estimate of plant reproductive success (*i.e.* fruit set, seed set, seed weight, germinability and germination rate) was analysed separately as a dependent variable. Treatment and year were used as fixed factors, and seed weight and inflorescence height were also included in the models as covariables; these were removed from such models when non-significant. Individual plant was used as random effect to control for lack of independence among flowers on the same individual plant. Differences in fruit set and germinability were estimated using a binomial error distribution and logit link function, whereas a Poisson family was used to test for differences in seed set and germination rate (as data were a discrete count of seeds or days, respectively). Seed weight was normally distributed and, for this variable, we thus adjusted errors to a Gaussian distribution.

To test for differences in flower visitation rates, we built a model including as response variable the number of flower visits per unit time and per flower, whereas the predictor variables were: animal group, year, day, time of day and number of flowers per plant. Besides differences between animal groups, we thus aimed to detect differences in the pattern of flower visitation between years, within season and during the day; we further included individual flower crop as this might be an important factor determining the visits of a given guild of pollinators. We performed separate analyses considering the different types of census carried out, *i.e.* for insects and for vertebrates. We evaluated collinearity by means of the variance inflation factor (VIF), and variables with $VIF > 3$ were removed from the model (Zuur *et al.* 2009). To find the best model, we used model selection with the dredge function in the R package *MuMIn* (multimodel inference) and adjusted data to a gamma error distribution. Again, observed plant was used as random effect. The VIF analysis showed that all predictors had low collinearity (<3) and thus were included in the models.

Pollination effectiveness (PE) was calculated and pollination landscape built using the methodologies developed by Reynolds & Fenster (2008) and Schupp *et al.* (2010). Only the major groups of flower visitors (*i.e.* birds, lizards, flower insect dwellers and flying hymenoptera) were considered in this analysis. The quantitative component (QNC) was considered as number of visits per 500 flowers per hour, whereas the qualitative component (QLC) was percentage fruit set. Mean and SE of each component was estimated using 500 simulations on bootstrap resamples of 80 empirical data in order to combine estimates of QNC and QLC subcomponents obtained in different sets of field observations and experiments. The final QNC and QLC subcomponents were obtained as the average across resampling trials to evaluate the stability of the estimation, and pollination effectiveness was calculated as the product of QNC and QLC subcomponents. Throughout the paper, all means are ± 1 SE unless otherwise indicated.

RESULTS

Flower characteristics

Flowers are protandrous and open for 2–3 days ($N = 90$). The carpel elongates and splits becoming longer than the anthers

during the female phase. The flowers open successively from the proximal to the distal part of the cyme. The total flowering time of a plant is 3–5 weeks. We studied the nectar standing crop in 20 plants, finding that it varied significantly during flower ontogeny ($\chi^2 = 6.53$, $df = 2$, $P = 0.04$), with male and transitional flowers producing more nectar (2.05 ± 0.46 ml, $N = 23$; and 1.89 ± 0.48 ml, $N = 28$, respectively) than females flowers (1.09 ± 0.24 ml, $N = 37$). However, we did not detect significant differences ($\chi^2 = 4.72$, $df = 2$, $P = 0.09$) in sugar concentration between flower phases; this was $15.85 \pm 0.93\%$ in male flowers ($N = 22$), $18.18 \pm 0.74\%$ in transitional flowers, ($N = 23$) and $16.71 \pm 0.73\%$ in female flowers ($N = 28$).

Breeding system

No fruits were obtained by apomixis and thus those flowers were removed from subsequent analyses. Likewise, fruit set resulting from anemogamy was negligible (4%) and might be due to accidental pollen contamination or even pollination by tiny insects, such as thrips or tiny ants (Fig. 1). Fruit set varied with the hand-pollination treatment, and the effect was consistent for the two study years. An average of 70% of flowers open to pollinators produced fruits, and this was not significantly different from the xenogamy treatment, suggesting no pollen limitation. By contrast, autogamous flowers produced significantly less fruits (*ca.* 20%) than controls, showing the benefit of animal pollination. Fruit set was independent of inflorescence height (Table 1).

Regarding seed set, this was similar across treatments, and was also consistent in time (Table 1). Hence, fruits from autogamous flowers produced a similar number of seeds per fruit to either xenogamous or control flowers. The mean number of seeds per fruit was 1.25 ± 0.07 ($N = 125$ fruits).

Floral visitors and visitation rates

Five species of passerine bird were observed visiting flowers of *E. simplex*, in order of importance: *Phylloscopus canariensis*

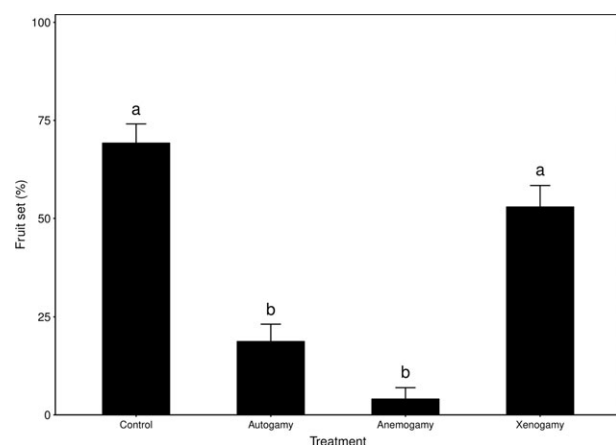


Fig. 1. Mean (\pm SE) percentage of flowers that set fruit for hand-pollination treatments (Control, $N = 91$ flowers; Autogamy, $N = 80$ flowers; Anemogamy, $N = 49$ flowers; Xenogamy, $N = 85$ flowers) in *E. simplex*. Different letters indicate significant differences among treatments using Tukey's test after GLMM.

Table 1. Mixed model analysis of the effects of year, pollination and inflorescence height on fruit set and seed set in *E. simplex*.

source of variation	fruit set			seed set		
	df	χ^2	P	df	χ^2	P
Year	1	1.142	0.285	1	1.552	0.213
Treatment	3	88.903	<0.001	3	3.630	0.304
	Treatment	Mean \pm SE	N			
	Control	69.23 \pm 4.86	91			
	Autogamy	18.75 \pm 4.39	80			
	Anemogamy	4.08 \pm 2.86	49			
	Xenogamy	52.94 \pm 5.45	85			
Inflorescence height	1	0.493	0.493	1	0.868	0.352
Year \times treatment	3	2.4132	0.4827	3	4.868	0.182

Bold indicates P-value ($P < 0.05$).

**Fig. 2.** Images of different vertebrate flower visitors of *E. simplex*: (A) *Phylloscopus canariensis*; (B) *Serinus canarius*; (C) *Gallotia galloti*; (D) *Cyanistes teneriffae*. Photo credits: Beneharo Rodríguez.

Hartwig, *Serinus canarius* L., *Cyanistes teneriffae* Lesson, *Sylvia atricapilla* L. and *Sylvia melanocephala* Gmelin. The mean number of flowers contacted by birds per unit time was 0.66 ± 0.02 visits \cdot h $^{-1}$ \cdot flower $^{-1}$ (N = 14). Although much more rarely and only in 2016, a lizard species – *Gallotia galloti* Oudart – was also seen lapping nectar from this plant (Fig. 2).

Vertebrate species and year had an interactive effect in flower visitation rate ($\chi^2 = 109.21$, df = 4, $P < 0.001$). Data for both *Sylvia* species were pooled for the analysis as identification to species level was not always possible. Flower visitation rate was consistent in time within species except for *P. canariensis*, with

more visits in 2016 compared to 2015 ($\chi^2 = 12.22$, df = 1, $P < 0.001$; Table 2). *Cyanistes teneriffae* was the most frequent visitor in 2015 whereas it was *Phylloscopus canariensis* in 2016. Only one observation of *Gallotia galloti* on the flowers was recorded during the censuses in 2016 (Table 2), although this interaction was also observed on several occasions outside census periods during June 2016.

Regarding insects, all floral visitors were clustered into seven groups: (1) Coleoptera (29 species), (2) Diptera (25 species), (3) Formicidae (seven species), (4) Hymenoptera (23 species; Formicidae excluded), (5) Hemiptera (12 species), (6) Lepidoptera (three species), and (7) Other (including anecdotal visits of different species of Thysanoptera, Araneae, Blattaria and Dermaptera species; Fig. 3). A list of all identified species of insect floral visitors is given in Appendix S1. The most frequent insect groups were hymenopterans and coleopterans, especially in 2015, followed by dipterans and ants (Fig. 3B). There was an interactive effect of group and year ($\chi^2 = 182.69$, df = 6, $P < 0.001$). Overall, coleopterans, ants and flying hymenopterans predominated on the flowers ($\chi^2 = 77.24$, df = 1, $P < 0.001$; $\chi^2 = 10.74$, df = 1, $P < 0.001$; $\chi^2 = 58.71$, df = 1, $P < 0.001$, respectively; Fig. 3B).

Pollination effectiveness of the different flower-visitor groups

Data on fruit set from treatments performed in both years (*i.e.* total exclusion, only flower dwellers and control) were pooled as no significant effect of year ($\chi^2 = 0.02$, df = 1, $P = 0.96$) and no interactions between treatment and year ($\chi^2 = 0.56$, df = 2, $P = 0.75$) were found. Although the exclusion of both birds and lizards produced a slightly lower fruit set than in control flowers, differences were not significant (Fig. 4A). When both vertebrates and insects were excluded (*i.e.* flowers could only be self-fertilised), however, fruit set was much lower, consistent with the breeding system data. On the other hand, insect flower dwellers played a negligible role as pollinators; fruit set in this treatment was similar to that observed when all flower visitors were excluded (Fig. 4A). Hence, flying insects were the most effective pollinators contributing to fruit set.

Contrasting results between years were found regarding seed set. In 2015, both the lizard exclusion and flower dweller treatments produced fruits with less seeds than in other treatments ($\chi^2 = 19.95$, df = 5, $P < 0.01$; Fig. 3B). In 2016, in contrast, seed set was not affected by treatment ($\chi^2 = 1.96$, df = 2, $P = 0.38$),

Table 2. Flower visitation rate (visits · h⁻¹ · flower⁻¹) in 2015 and 2016 for each animal group or species. Asterisks indicate significant differences between years in the visitation rate. Hymenoptera group is Hymenoptera other than Formicidae

	2015	2016
Invertebrates		
Coleoptera*	0.28 ± 0.037	0.017 ± 0.0027
Diptera	0.015 ± 0.0040	0.022 ± 0.0049
Formicidae*	0.044 ± 0.0086	0.021 ± 0.0026
Hemiptera	0.003 ± 0.0010	0.003 ± 0.0006
Hymenoptera*	0.46 ± 0.065	0.23 ± 0.030
Lepidoptera	0.0002 ± 0.0001	0.0006 ± 0.0003
Other	0.0003 ± 0.002	0.0002 ± 0.0001
Vertebrates		
Serinus	0.015 ± 0.007	0.017 ± 0.002
Sylvia	0.011 ± 0.006	0.005 ± 0.001
Cyanistes	0.036 ± 0.021	0.012 ± 0.03
Phylloscopus*	0.008 ± 0.05	0.03 ± 0.005
Gallotia	0	0.0003 ± 0.0003

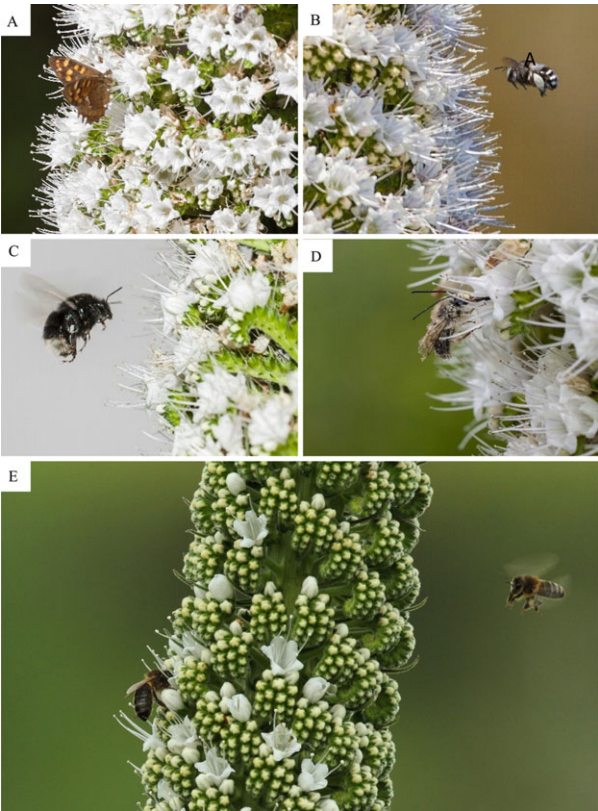


Fig. 3. Images of invertebrate flower visitors to *E. simplex*: (A) *Pararga xiphioides*; (B) *Anthophora alluaudi*; (C) *Bombus canariensis*; (D) *Eucera gracillipes*; (E) *Apis mellifera*. Photo credits: A-D, Juan Curbelo; E, Beneharo Rodríguez.

but a significant negative effect of inflorescence height on this variable was detected ($\chi^2 = 4.11$, $df = 1$, $P = 0.04$), *i.e.* longer inflorescences set less seeds per fruit than shorter ones. Control flowers set slightly more seeds in 2015 than in 2016 (1.54 ± 0.03

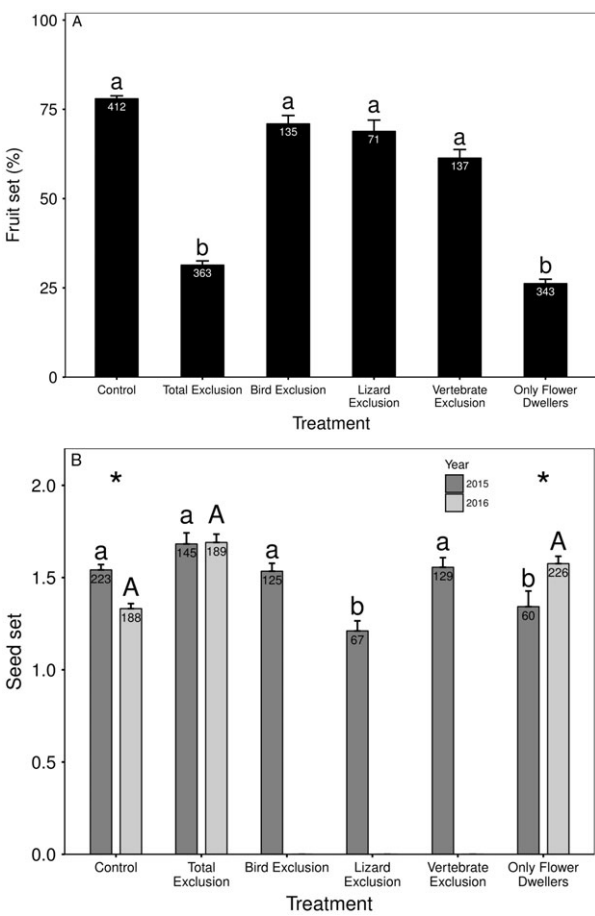


Fig. 4. Mean (\pm SE) (A) percentage of flowers that set fruit for exclusion experiments and (B) number of viable seeds per fruit for exclusion experiments in 2015 and 2016. Numbers in each bar are samples sizes. For each year, letters indicate significant differences between treatments; and for each treatment, asterisks indicate significant differences among years using Tukey's test after GLMM.

versus 1.33 ± 0.03 seeds·fruit⁻¹, respectively; $\chi^2 = 4.31$, $df = 1$, $P = 0.04$); the 'flower dwellers' treatment, however, showed the opposite pattern (1.30 ± 0.08 *versus* 1.58 ± 0.04 seeds·fruit⁻¹, respectively; $\chi^2 = 7.92$, $df = 1$, $P < 0.01$). Flowers totally excluded of visitors set a similar number of seeds per fruit in the 2 years, an average of 1.67 ± 0.04 seeds·fruit⁻¹ (Fig. 4B).

Flying hymenopterans were, by far, the most effective pollinators (PE = 5662.78) whereas birds, lizards and insect flower dwellers had a PE close to 0 (PE = 7.04; 0.86 and 2.17, respectively; Fig. 5). Flying hymenopterans showed both high QNC and QLC but relative strengths of the components depended on pollinator identity of the other groups. Regarding lizards, fruit set was the major determinant (7.6%), whereas for insect flower dwellers the visitation frequency was the dominant factor (34.73 visits·h⁻¹·500 flowers⁻¹) and the other components were null. The higher PE of birds compared to lizards and insect flower dwellers was explained by both modest QNC and QLC.

Seed weight varied significantly among treatments ($\chi^2 = 22.59$, $df = 5$, $P < 0.001$). Interestingly, the 'total exclusion' and 'flower dwellers' treatments produced heavier seeds

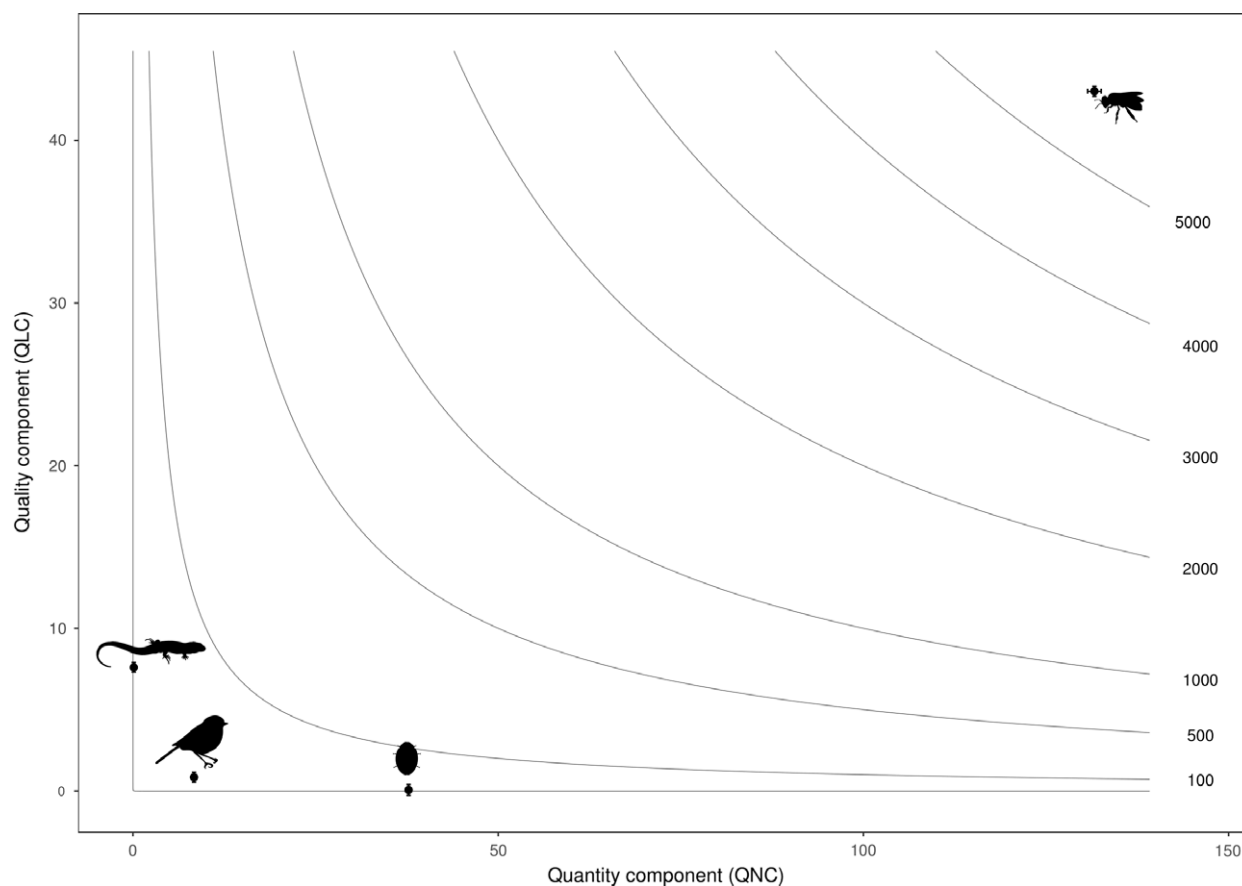


Fig. 5. Mean (\pm SE) pollination efficiency (PE) values for the pollinator guilds for the quantitative (x-axis) and the qualitative (y-axis) components. Isoclines represent all combinations of quantity and quality components with the same PE measured as visit rate (visits·h⁻¹·flower⁻¹) and fruit set, respectively. Images depict: flying insects; insect flower dwellers; *Gallotia galloti* lizards; and birds.

(with thicker seed walls) than control and bird-excluded plants. In contrast, seeds resulting from vertebrate exclusions had similar weights to those from the other treatments (Fig. 6A); for this analysis, data from the 2 years were pooled as there was no effect of year ($\chi^2 = 0.02$, $df = 1$, $P = 0.88$) and no interaction between treatment and year ($\chi^2 = 2.72$, $df = 1$, $P = 0.26$).

Regarding germination patterns, the effect of exclusion treatment was highly significant ($\chi^2 = 12.85$, $df = 5$, $P = 0.02$), but varied depending on seed weight, both in 2015 ($\chi^2 = 13.80$, $df = 5$, $P = 0.017$) and 2016 ($\chi^2 = 10.75$, $df = 2$, $P = 0.004$). Given that there was a significant triple interaction (treatment \times seed weight \times year) on germinability ($\chi^2 = 6.62$, $df = 2$, $P < 0.05$), seed germination data were separately analysed for each year (Appendix S2). In 2015, control seeds germinated more than selfed seeds, whereas in 2016, all treatments had similar germinability (Fig. 6B).

When comparing only the treatments performed in the 2 years (*i.e.* control, total exclusion and flower dwellers), germinability was higher in 2015 than in 2016 ($78.67 \pm 0.02\%$ and $70.78 \pm 0.01\%$, respectively; $\chi^2 = 4.79$, $df = 1$, $P < 0.05$; Fig. 7B). Nevertheless, there were no significant differences in germination rate among treatments, either in 2015 ($\chi^2 = 7.31$, $df = 5$, $P > 0.05$) or 2016 ($\chi^2 = 3.36$, $df = 2$, $P > 0.05$). Interestingly, there was an interaction effect of seed weight \times treatment ($\chi^2 = 39.90$, $df = 2$, $P < 0.001$ and $\chi^2 = 21.47$, $df = 5$, $P < 0.001$,

respectively), *i.e.* the effect of treatment depended again on seed weight (Appendix S2). Germination rate was higher in 2015 (12.20 ± 0.31 days) than in 2016 (16.12 ± 0.49 days; $\chi^2 = 17.73$, $df = 1$, $P < 0.001$), both for control and selfed seeds ($\chi^2 = 7.68$, $df = 1$, $P = 0.006$ and $\chi^2 = 8.17$, $df = 1$, $P = 0.004$, respectively).

Herbivory by goats

In 2015, ca. 50% of the 464 reproductive plants examined to assess herbivory by goats was consumed by these animals to some extent. In 2016, the level of herbivory was even higher: ca. 99% of the 835 adult plants examined were damaged. In this year, at least 30% of the inflorescences were totally consumed and thus no fruits and seeds were produced. Table 3 shows the number of plants affected for each category of damage (Fig. 7). In both years, most damaged plants had a ball-shaped inflorescence, thus also losing a high proportion of the flowers.

DISCUSSION

Echium simplex has only been anecdotically reported as an ornithophilous species (Valido & Olesen 2010; Ojeda 2013; Mittelbach *et al.* 2015), but in this study we show that it is visited by a large number of bird species and even, though rarely,

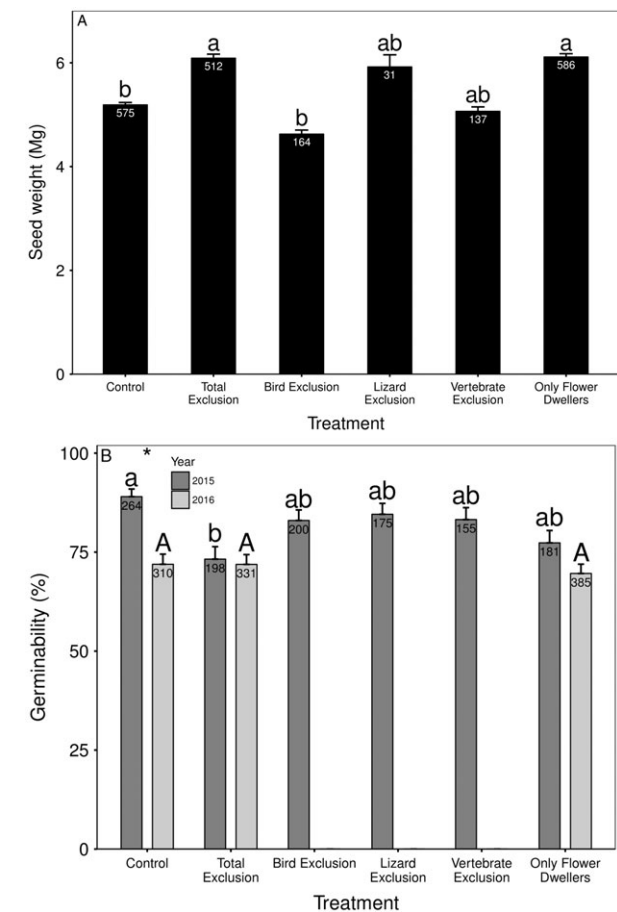


Fig. 6. Mean (\pm SE) (A) seed weight per treatment in 2015 and 2016 and (B) seed germinability percentage per treatment and year. Numbers in each bar are sample sizes. For each year, letters indicate significant differences between treatments; and for each treatment, asterisks indicate significant differences among years using Tukey's test after GLMM.

by the lizard *Gallotia galloti*. Despite the flowers can self-fertilise, the plant seems to rely mostly on insects for its reproductive success, although birds represent a non-irrelevant number of the total visits to flowers. Our findings indicate that the flowers of *E. simplex* constitute a food and water source for birds and lizards, whereas the plant does not benefitted as much from visits of these vertebrates to its flowers.

Breeding system

Echium simplex can self-fertilise although it is not agamospermic. Nor is the role of wind for seed production relevant.

Table 3. Types of herbivory damage by feral goats recorded in 2015 and 2016 in *E. simplex*.

damage type	year	
	2015	2016
No flowers	31 (13.25%)	247 (29.94%)
Short inflorescence	58 (24.79%)	5 (0.61%)
Ball-shape	130 (55.55%)	569 (68.94%)
Some grazed parts (mean \pm SE, range)	15 (6.41%) (44.67 \pm 7.21%, 10–100%)	4 (0.48%) (85 \pm 9.57%, 60–100%)

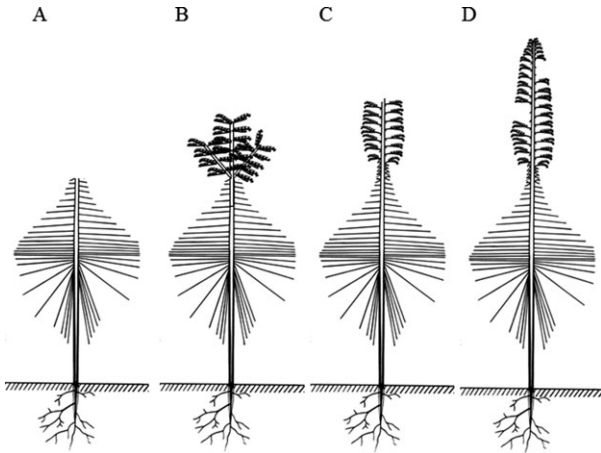


Fig. 7. Classification of the different types of herbivory that feral goats can exert on inflorescences of *E. simplex* (A) lack of flowers, (B) ball-shaped inflorescence, (C) short inflorescence and (D) well-shaped but with some grazed parts inflorescence. Adapted from Stöcklin & Lenzin 2013.

Selfed flowers produce fewer fruits than flowers open to pollination, as reported for other *Echium* species (Bramwell 1972; Sedlacek 2009); this might be due to the existence of self-incompatibility systems or inbreeding depression, as suggested by Bramwell (1972), and/or due to protandry. Male flowers open before female ones, as in *E. wildpretii* (Olesen 1988), although both phases coexist during day 2 of anthesis, and this is why autogamy is possible. Xenogamy produced as many fruits as the open pollination treatment, which suggests that pollen is not limiting even though we cannot discard an outbreeding phenomenon or some imperceptible damage to flowers during hand-pollination. On the other hand, the fact that seed set was similar among treatments indicates that seed formation is controlled by resource-based constraints rather than by pollen limitation (Yang *et al.* 2005).

The nectar concentration and volume vary among flower phases, population and time of day in *Echium* species (Olesen 1988; Kraemer & Schmitt 1997; Dupont *et al.* 2004; Mittelbach *et al.* 2015). In general, male flower phases produce more nectar than female flowers, and during the third day no nectar is produced (Olesen 1988; Kraemer & Schmitt 1997). Mittelbach *et al.* (2015) found higher nectar volumes and lower sugar concentration in *E. simplex* than found in our study. Differences in nectar composition are usual among populations (Mittelbach *et al.* 2015), whereas differences in nectar standing crop varies depending on the nectar consumed by flower visitors (Kraemer & Schmitt 1997); on the other hand, sugar concentration depends heavily on ambient relative humidity and the rate of photosynthesis (Corbet & Delfosse 1984).

Flower visitors

The extremely high diversity of flower visitors found in this study contrasts with that found in other *Echium* species (but see Bramwell 1972). For *E. wildpretii*, Valido *et al.* (2002, 2004) reported three bird species (*Cyanistes caeruleus*, *Serinus canarius* and *Phylloscopus canariensis*), one lizard (*Gallotia galloti*) and 16 species of bee, butterfly and fly visiting its flowers. The previous observations for *E. simplex* reported only a few insects (Dupont & Skov 2004; Stöcklin & Lenzin 2013), all of which have been recorded in our study, and three bird species (*Serinus canarius*, *Phylloscopus canariensis* and *Cyanistes caeruleus*; Valido & Olesen 2010; Mittelbach *et al.* 2015). *P. canariensis* is the most common visitor in ornithophilous plant species in the Canary Islands (Valido *et al.* 2004) and is widely distributed in Tenerife, especially in Anaga (Carrascal & Palomino 2005).

Insects outnumbered birds as flower visitors of *E. simplex*. Hymenopterans, in particular, were up to 31 times more frequent than birds. However, at least for certain species in both years, flower visitation rate was higher for birds than for dipterans species. Bird visitation rate in *E. simplex* was lower than that found by Ollerton *et al.* (2009) for *Canarina canariensis*, but higher than that recorded by Rodríguez-Rodríguez & Valido (2011) in the same species and in others such as *Isoplexis canariensis*, *Lotus berthelotii* (Rodríguez-Rodríguez & Valido 2008; Ollerton *et al.* 2009), and even in the same *E. simplex* (Mittelbach *et al.* 2015).

Relative contribution of different floral visitors to plant reproductive success

Contrary to our expectations, we found no evidence for an increase in reproductive success of the flower visits by vertebrates, as reported in other studies (Ratto *et al.* 2018 and references therein), which also include species of the bird–flower Canarian element (Rodríguez-Rodríguez & Valido 2008, 2011; Fernández de Castro *et al.* 2017). Hence, vertebrates play a minor role in the reproduction of *E. simplex*.

Flying insects, especially bees, were the most frequent pollinators and the most effective, *i.e.* those contributing most to the reproductive success of *E. simplex*. In contrast, insect flower dwellers, mainly ants and small beetles, did not increase fruit set and indeed reduced seed set relative to selfed flowers, suggesting that they consume pollen and by doing so reduce final reproductive success (Kevan & Baker 1983). Pollination by beetles and ants has mainly been documented in tropical plant families, and in pollinator-depauperated and environmentally stressful areas (Bawa 1990; Gómez *et al.* 1996).

The annual differences in seed set are attributed to lower insect visitation rates – mainly of bees and flies – in 2016, probably due to a lower insect abundance or a higher flower availability. Fluctuations in insect populations are usual and often associated with inter-annual variation in climate conditions, as these can have a substantial effect on insect survival or overwintering (Chown & Terblanche 2007 and references therein).

Seeds of *E. simplex* from selfed flowers were heavier than those resulting from cross-pollination. The reason is that the former have a thicker coat, while embryo size is similar between the two treatments. Such difference is consistent with that found in another study of *E. vulgare* (Melser *et al.* 1997) and also with studies of other species (Kudo & Maeda 1998;

Hudewenz *et al.* 2014). However, it contrasts with many other studies that either found the opposite (Galen *et al.* 1985; Navarro & Guitián 2002) or no differences in seed weight between selfed and crossed flowers (Eckert & Barrett 1994; Abdelgadir *et al.* 2009). Regarding germination, crossed and lighter seeds of *E. simplex* germinated more and faster than selfed seeds; moreover, within the control seeds, heavier seeds performed better than lighter ones, in agreement with findings from other studies (Schemske 1983; Navarro & Guitián 2002). The thicker coat of the seeds from selfed flowers is probably the reason for slower germination (Crocker 1906; Miyoshi & Mii 1988). This has indeed been reported in at least one species, *Sinapis arvensis* (Paolini *et al.* 2001).

Impact of feral goats on plant performance

Introduced mammals are major drivers of extinctions (Elton 1958; Gurevitch & Padilla 2004 and references therein). Those mammal species acting as herbivores are known to have strong deleterious effects on plant growth, reproduction and even survival (Crawley 1989; Marquis 1992). Feral goats (*Capra hircus*), in particular, can have a devastating impact in island ecosystems (Donlan *et al.* 2002; Gurevitch & Padilla 2004). The intense damage that goats produced on *E. simplex*, particularly in the second year of the study, not only caused delayed flowering but also reduced the recruitment probabilities for this endemic species. A high incidence of herbivory by feral cattle have been reported for other plant species in some plant communities, where up to 96% of plants were consumed. Reproduction may be totally impeded in many individuals, and initial herbivore damage might also weaken individuals and increase their susceptibility to other stress agents (Chynoweth *et al.* 2013 and references therein).

Damage by herbivores can modify flower characteristics and decrease overall plant attractiveness to pollinators (McCall & Irwin 2006), greatly reducing the opportunity for pollinators to select between plants (Strauss & Zangerl 2002). In general, plants have evolved a wide variety of rapid, inducible responses to herbivory that allow their survival (Strauss & Agrawal 1999). However, oceanic island ecosystems have usually evolved under very low pressure from herbivory (*e.g.* Van Vuren & Bowen 1999). Hence, endemic plants such as *E. simplex* are not expected to have evolved any compensatory mechanism to efficiently limit mammalian herbivores. Further studies are needed to quantify how the extent of herbivory upon *E. simplex* is altering its population dynamics in the long term, but we foresee that feral goats may intensely alter this isolated, fragile and exclusive island habitat, in which *E. simplex* is one of the dominant species in the community. We argue that only with effective control campaigns that minimise the effects of this invasive herbivore can this endemic species be maintained in the most natural state possible.

Concluding remarks

Despite the abundant bibliography on endemic Canarian flora visited by vertebrates, very few studies have yet examined their role as legitimate pollinators. Our study contributes to fill this gap in information. We have focused on a narrow endemic, *E. simplex*, finding that despite its floral resources might be

important to some bird and lizard species, these do not appear to notably contribute to increase plant fitness, which is effectively pollinated by a wide assemblage of flying insects. We additionally found that feral goats represent a serious threat to the survival of this endemic species, as they damage a large fraction of the reproductive structures. We thus argue that these alien animals should be controlled in this highly valuable Biosphere Reserve.

ACKNOWLEDGEMENTS

The authors thank Antonio Pérez Delgado for insect identification; Benito Pérez Vispo and Jaume Seguí Colomar for technical assistance in the field; Ricardo Mesa for information on *Echium* localities; and Juana Pérez López for providing logistical support in Chamorga. We are also grateful to Servicio Administrativo de Medio Ambiente, Excmo, Cabildo Insular de Tenerife for permission (2016-01704) to work in Anaga Biosphere Reserve, Tenerife. Author Contributions: JJ, AT and MN conceived and designed the experiments; JJ and MN performed the experiments; JJ analysed the data, and JJ and AT wrote the paper. The study was funded by a predoctoral fellowship to Julia Jaca from the Ministerio de Educación, Cultura y Deporte [FPU13/05880] and framed within a project financed by Ministerio de Economía, Industria y Competitividad [CGL2013-44386-P] to Anna Traveset.

REFERENCES

- Abdelgadir H.A., Johnson S.D., Van Staden J. (2009) Pollinator effectiveness, breeding system, and tests for inbreeding depression in the biofuel seed crop, *Jatropha curcas*. *Journal of Horticultural Science and Biotechnology*, **84**, 319–324.
- Anderson S.H. (2003) The relative importance of birds and insects as pollinators of the New Zealand flora. *New Zealand Journal of Ecology*, **27**, 83–94.
- Barrett S.C.H. (1996) The reproductive biology and genetics of island plants. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences*, **351**, 725–733.
- Bawa K.S. (1990) Plant–pollinator interactions in tropical rain forests. *Annual Review of Ecology and Systematics*, **21**, 399–422.
- Bramwell D. (1972) Breeding systems in Canary Islands *Echium* species. *International Organization of Plant Systematics Newsletter*, **6**, 2–9.
- Carlquist S.J. (1974) *Island biology*. Columbia University Press, New York, USA.
- Carrascal L.M., Palomino D. (2005) Preferencias de hábitat, densidad y diversidad de las comunidades de aves en Tenerife (Islas Canarias). *Animal Biodiversity and Conservation*, **28**, 101–119.
- Cecere J.G., Spina F., Jenni-Eiermann S., Boitani L. (2011) Nectar: an energy drink used by European songbirds during spring migration. *Journal of Ornithology*, **152**, 923–931.
- Chown S.L., Terblanche J.S. (2007) Physiological diversity in insects: ecological and evolutionary contexts. *Advances in Insect Physiology*, **33**, 50–152.
- Chynoweth M.W., Litton C.M., Lepczyk C.A., Hess S.C., Cordell S. (2013) Biology and impacts of Pacific island invasive species. 9. *Capra hircus*, the feral goat (Mammalia: Bovidae). *Pacific Science*, **67**, 141–156.
- Corbet S.A., Delfosse E.S. (1984) Honeybees and the nectar of *Echium plantagineum* L. in south-eastern Australia. *Australian Journal of Ecology*, **9**, 125–139.
- Crawford D.J., Anderson G.J., Bernardello G. (2011) The reproductive biology of island plants. In: Bramwell D., Caujapé-Castells J. (Eds), *The biology of island floras*. Cambridge University Press, New York, USA, pp 11–36.
- Crawley M.J. (1989) Insect herbivores and plant population dynamics. *Annual Review of Entomology*, **34**, 531–564.
- Crocker W. (1906) Role of seed coats in delayed germination. Contributions from the Hull Botanical Laboratory. *Botanical Gazette*, **42**, 265–291.
- Cronk Q., Ojeda I. (2008) Bird-pollinated flowers in an evolutionary and molecular context. *Journal of Experimental Botany*, **59**, 715–727.
- Donlan C.J., Tershy B.R., Croll D.A. (2002) Islands and introduced herbivores: conservation action as ecosystem experimentation. *Journal of Applied Ecology*, **39**, 235–246.
- Dupont Y.L., Skov C. (2004) Influence of geographical distribution and floral traits on species richness of bees (Hymenoptera: Apoidea) visiting *Echium* species (Boraginaceae) of the Canary Islands. *International Journal of Plant Sciences*, **165**, 377–386.
- Dupont Y.L., Hansen D.M., Rasmussen J.T., Olesen J.M. (2004) Evolutionary changes in nectar sugar composition associated with switches between bird and insect pollination: the Canarian bird-flower element revisited. *Functional Ecology*, **18**, 670–676.
- Eckert C.G., Barrett S.C.H. (1994) Inbreeding depression in partially self-fertilizing *Decodon verticillatus* (Lythraceae): population-genetic and experimental analyses. *Evolution*, **48**, 952–964.
- Elton C.S. (1958) *The ecology of invasions by animals and plants*. Butler and Tanner, London, UK.
- Faegri K., van der Pijl L. (1966) *The principles of pollination ecology*. Pergamon Press, Oxford, UK.
- Fernández de Castro A.G., Moreno-Saiz J.C., Fuertes-Aguilar J. (2017) Ornithophily for the nonspecialist: differential pollination efficiency of the Macaronesian island paleoendemic *Navaea phoenicea* (Malvaceae) by generalist passerines. *American Journal of Botany*, **104**, 1556–1568.
- Galen C., Plowright R.C., Thomson J.D. (1985) Floral biology and regulation of seed set and seed size in the lily, *Clintonia borealis*. *American Journal of Botany*, **72**, 1544–1552.
- Gillespie R.G., Roderick G.K. (2002) Arthropods on islands: colonization, speciation, and conservation. *Annual Review of Entomology*, **47**, 595–632.
- Gómez J.M., Zamora R., Hódar J.A., García D. (1996) Experimental study of pollination by ants in Mediterranean high mountain and arid habitats. *Oecologia*, **105**, 236–242.
- Grant B.R., Grant P.R. (1981) Exploitation of *Opuntia* cactus by birds on the Galápagos. *Oecologia*, **49**, 179–187.
- Guillou H., Carracedo J.C., Paris R., Torrado F.J.P. (2004) Implications for the early shield-stage evolution of Tenerife from K/Ar ages and magnetic stratigraphy. *Earth and Planetary Science Letters*, **222**, 599–614.
- Gurevitch J., Padilla D.K. (2004) Are invasive species a major cause of extinctions? *Trends in Ecology & Evolution*, **19**, 470–474.
- Hudewenz A., Pufal G., Bögeholz A.L., Klein A.M. (2014) Cross-pollination benefits differ among oil-seed rape varieties. *Journal of Agricultural Science*, **152**, 770–778.
- Kevan P.G., Baker H.G. (1983) Insects as flower visitors and pollinators. *Annual Review of Entomology*, **28**, 407–453.
- Kier G., Kreft H., Lee T.M., Jetz W., Ibsch P.L., Nowicki C., Mutke J., Barthlott W. (2009) A global assessment of endemism and species richness across island and mainland regions. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 9322–9327.
- Kraemer M., Schmitt U. (1997) Nectar production patterns and pollination of the Canarian endemic *Echium wildpretii* Pearson ex Hook. fil. *Flora*, **192**, 217–221.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Flower visitors of *Echium simplex* found during 2015 and 2016.

Appendix S2. Effect of pollination treatment on germination patterns depending on the seed weight.

Figure S1. GLMM predicted probabilities of germination (A and B) and days to germination (C and D), in seed weight for each exclusion treatment in 2015 (A and C) and 2016 (B and D). Sample sizes from germinability studies were: Total Exclusion 2015 and 2016, N = 198 and N = 331 seeds, respectively; Birds Exclusion, N = 200 seeds; Lizards Exclusion, N = 175 seeds; Vertebrates Exclusion, N = 155 seeds; Flower Dwellers 2015 and 2016, N = 181 and 385 seeds, respectively; and Control 2015 and 2016, N = 264 and N = 310 seeds, respectively. Sample sizes for germinated seeds were Total Exclusion 2015 and 2016, N = 140 and N = 268 seeds, respectively; Birds Exclusion, N = 166 seeds; Lizards Exclusion, N = 148 seeds; Vertebrates Exclusion, N = 129 seeds; Flower Dwellers 2015 and 2016, N = 140 and 268 seeds, respectively; and Control 2015 and 2016, N = 235 and N = 223 seeds, respectively.

- Kudo G., Maeda T. (1998) Size-dependent variation of phenotypic gender and functional gender of a spring ephemeral, *Anemone debilis* Fisch. *Plant Species Biology*, **13**, 69–76.
- Marquis R.J. (1992) The selective impact of herbivores. In: Fritz R.S., Simms E.L. (Eds), *Plant resistance to herbivores and pathogens. Ecology, evolution, and genetics*. University of Chicago Press, Chicago, IL, USA, pp. 310–325.
- Mayr G., Wilde V. (2014) Eocene fossil is earliest evidence of flower-visiting by birds. *Biology Letters*, **10**, 1–4.
- McCall A.C., Irwin R.E. (2006) Florivory: the intersection of pollination and herbivory. *Ecology Letters*, **9**, 1351–1365.
- Melser C., Rademaker M.C.J., Klinkhamer P.G.L. (1997) Selection on pollen donors by *Echium vulgare* (Boraginaceae). *Sexual Plant Reproduction*, **10**, 305–312.
- Mittelbach M., Yurkov A.M., Nocentini D., Nepi M., Weigend M., Begerow D. (2015) Nectar sugars and bird visitation define a floral niche for basidiomycetous yeast on the Canary Islands. *BMC Ecology*, **15**, 1–13.
- Miyoshi K., Mii M. (1988) Ultrasonic treatment for enhancing seed germination of terrestrial orchid, *Calanthe discolor*, in asymbiotic culture. *Scientia Horticulturae*, **35**, 127–130.
- Moreno J.C. (2008) *Lista Roja 2008 de la Flora Vascular Española*. Dirección General de Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino, y Sociedad Española de Biología de la Conservación de Plantas), Madrid, Spain.
- Navarro L., Guitián J. (2002) The role of floral biology and breeding system on the reproductive success of the narrow endemic *Petrocoptis viscosa* Rothm. (Caryophyllaceae). *Biological Conservation*, **103**, 125–132.
- Ne'Eman G., Jürgens A., Newstrom-Lloyd L., Potts S.G., Dafni A. (2010) A framework for comparing pollinator performance: effectiveness and efficiency. *Biological Reviews*, **85**, 435–451.
- Ojeda D.I. (2013) The Macaronesian bird-flower element as a model system to study the evolution of ornithophilous floral traits. *Vieraea*, **41**, 73–89.
- Olesen J.M. (1985) The Macaronesian bird-flower element and its relation to bird and bee opportunists. *Botanical Journal of the Linnean Society*, **91**, 395–414.
- Olesen J.M. (1988) Floral biology of the Canarian *Echium wildpretii*: bird-flower or a water resource to desert bees? *Acta Botanica Neerlandica*, **37**, 509–513.
- Olesen J.M., Valido A. (2003) Lizards as pollinators and seed dispersers: an island phenomenon. *Trends in Ecology & Evolution*, **18**, 177–181.
- Olesen J.M., Eskildsen L.I., Venkatasamy S. (2002) Invasion of pollination networks on oceanic islands: importance of invader complexes and endemic super generalists. *Diversity and Distributions*, **8**, 181–192.
- Olesen J.M., Alarcón M., Ehlers B.K., Aldasoro J.J., Roquet C. (2012) Pollination, biogeography and phylogeny of oceanic island bellflowers (Campanulaceae). *Perspectives in Plant Ecology, Evolution and Systematics*, **14**, 169–182.
- Ollerton J., Cranmer L., Stelzer R.J., Sullivan S., Chittka L. (2009) Bird pollination of Canary Island endemic plants. *Naturwissenschaften*, **96**, 221–232.
- Paolini R., Bàrberi P., Rocchi C. (2001) The effect of seed mass, seed colour, pre-chilling and light on the germination of *Sinapis arvensis* L. *Italian Journal of Agronomy*, **5**, 39–46.
- Porsch O. (1924) Vogelblumenstudien I. *Jahrbücher für Wissenschaftliche Botanik*, **63**, 553–706.
- R Core Team (2018) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria [online]. Available from <https://www.r-project.org/> (accessed 14 April 2018).
- Ratto F., Simmons B.I., Spake R., Zamora-Gutierrez V., MacDonald M.A., Merriman J.C., Tremlett C.J., Poppy G.M., Peh K.S.-H., Dicks L.V. (2018) Global importance of vertebrate pollinators for plant reproductive success: a meta-analysis. *Frontiers in Ecology and the Environment*, **16**, 92–90.
- Rausher M.D. (2008) Evolutionary transitions in floral colour. *International Journal of Plant Sciences*, **169**, 7–21.
- Reynolds R.J., Fenster C.B. (2008) Point and interval estimation of pollinator importance: a study using pollination data of *Silene caroliniana*. *Oecologia*, **156**, 325–332.
- Rodda G.H., Dean-Bradley K. (2002) Excess density compensation of island herpetofaunal assemblages. *Journal of Biogeography*, **29**, 623–632.
- Rodríguez-Rodríguez M.C., Valido A. (2008) Opportunistic nectar-feeding birds are effective pollinators of bird-flowers from Canary Islands: experimental evidence from *Isoplexis canariensis* (Scrophulariaceae). *American Journal of Botany*, **95**, 1408–1415.
- Rodríguez-Rodríguez M.C., Valido A. (2011) Consequences of plant–pollinator and floral–herbivore interactions on the reproductive success of the Canary Islands endemic *Canarina canariensis* (Campanulaceae). *American Journal of Botany*, **98**, 1465–1474.
- Schemske D.W. (1983) Breeding system and habitat effects on fitness components in three Neotropical *Costus* (Zingiberaceae). *Evolution*, **37**, 523–539.
- Schmucker T. (1936) Über die Blüten von *Canarina Campanula*. *Berichte der Deutschen Botanischen Gesellschaft*, **53**, 230–239.
- Schupp E.W., Jordano P., Gómez J.M. (2010) Seed dispersal effectiveness revisited: a conceptual review. *New Phytologist*, **188**, 333–353.
- Sedlacek J. (2009) *Demography and inbreeding depression in the rare, endemic Echium wildpretii* (Boraginaceae). M. Sc. dissertation, University of Zurich, Zurich, Switzerland
- da Silva L.P., Ramos J.A., Olesen J.M., Traveset A., Heleno R.H. (2014) Flower visitation by birds in Europe. *Oikos*, **123**, 1377–1383.
- Stöcklin J., Lenzen H. (2013) *Echium simplex*, ein seltener Schopffrossettenbaum auf Teneriffa. *Bauhinia*, **24**, 23–37.
- Strauss S.Y., Agrawal A.A. (1999) The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology & Evolution*, **14**, 179–185.
- Strauss S., Zangerl A.R. (2002) Plant–insect interactions in terrestrial ecosystems. In: Herrera C. M., Pellmyr O. (Eds), *Plant–animal interactions: an evolutionary approach*. Blackwell Scientific, Oxford, UK, pp. 77–154.
- Traveset A., Sáez E. (1997) Pollination of *Euphorbia dendroides* by lizards and insects: Spatio-temporal variation in patterns of flower visitation. *Oecologia*, **111**, 241–248.
- Traveset A., Tur C., Trøjelsgaard K., Heleno R., Castro-Urgal R., Olesen J.M. (2015) Global patterns of mainland and insular pollination networks. *Global Ecology and Biogeography*, **25**, 880–890.
- Valido A., Olesen J.M. (2010) Pollination on islands: examples from the Macaronesian archipelagos. In: Serrano A. R. M., Borges P. A. V., Boeiro M., Oromí P. (Eds), *Terrestrial arthropods of Macaronesia: biodiversity, ecology and evolution*. Sociedad Portuguesa de Entomologia, Lisboa, Portugal, pp. 249–283.
- Valido A., Dupont Y.L., Hansen D.M. (2002) Native birds and insects, and introduced honey bees visiting *Echium wildpretii* (Boraginaceae) in the Canary Islands. *Acta Oecologica*, **23**, 413–419.
- Valido A., Dupont Y.L., Olesen J.M. (2004) Bird–flower interactions in the Macaronesian islands. *Journal of Biogeography*, **31**, 1945–1953.
- Van der Niet T., Johnson S.D. (2012) Phylogenetic evidence for pollinator-driven diversification of angiosperms. *Trends in Ecology & Evolution*, **27**, 353–361.
- Van Vuren D., Bowen L. (1999) Reduced defenses in insular endemic plants: an evolutionary time frame. *Conservation Biology*, **13**, 211–212.
- Warren B.H., Simberloff D., Ricklefs R.E., Aguilée R., Condamine F.L., Gravel D., Morlon H., Mouquet N., Rosindell J., Casquet J., Conti E., Cornuault J., Fernández-Palacios J.M., Hengl T., Norder S.J., Rijdsdijk K.F., Sanmartín I., Strasberg D., Triantis K.A., Valente L.M., Whittaker R.J., Gillespie R.G., Emerson B.C., Thébaud C. (2015) Islands as model systems in ecology and evolution: prospects fifty years after MacArthur–Wilson. *Ecology Letters*, **18**, 200–217.
- Wright S.J. (1980) Density compensation in island avifaunas. *Oecologia*, **45**, 385–389.
- Yang C.F., Sun S.G., Guo Y.H. (2005) Resource limitation and pollen source (self and outcross) affecting seed production in two louseworts, *Pedicularis siphonantha* and *P. longiflora* (Orobanchaceae). *Botanical Journal of the Linnean Society*, **147**, 83–89.
- Zuur A.F., Ieno E.N., Walker N.J., Saveliev A.A., Smith G.M. (2009) *Mixed effect models and extensions in ecology with R*. Springer, New York, USA.