Article

FLOWER-VISITING LIZARDS AS KEY ECOLOGICAL ACTORS FOR AN ENDEMIC AND CRITICALLY ENDANGERED PLANT IN THE CANARY ISLANDS

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Abstract—Oceanic islands are places where biological assemblages are relatively simple, as compared to the mainland. On islands, however, pollinator assemblages may to be composed of a taxonomically disparate group of organisms (e.g. insects, lizards, and birds), some of them with opportunistic nectar-feeding behaviour. Here we investigated some components of pollination effectiveness of *Lotus maculatus* (Fabaceae), an endangered Canary Islands endemic. In a flower exclusion experiment, we bagged flowers and compared their subsequent fruit and seed set to that of control flowers. Number of interactions with vertebrate and invertebrate flower visitors was counted and it was recorded whether interactions were legitimate (potentially pollinating) or non-legitimate (nectar robbing). Additionally, we estimated pollen loads on lizards and looked for any relationship between reproductive success of individual plants and number of visits made by the top three flower-visiting species (in terms of both frequency of occurrence at censuses and number of floral visits). Bagged flowers fruited less and with fewer seeds than control flowers. The only observed flower-visiting vertebrate was the Tenerife lizard *Gallotia galloti*, whose interactions were always legitimate and with around a half of captured individuals carrying pollen grains. The most frequent flower-visiting insect was the honeybee *Apis mellifera* followed by the solitary bee *Lasioglossum arctifrons*. The honeybee, however, was only a nectar robber, and the solitary bee was not an effective pollinator, but rather a pollen gatherer. Fruit set by individual plants was positively related only to frequency of visits by the lizard. Thus, the lizard seems to play a key role in the conservation management of *L. maculatus*.

Keywords—endemic mutualism, *Gallotia galloti*, *Lotus maculatus*, Macaronesia, oceanic island, pollination effectiveness

INTRODUCTION

Mutualisms have been highlighted as a key process in species coevolution and biodiversity maintenance (Thompson 2005). For instance, plant-pollinator interactions are crucial to the reproductive success of 87.5% of all flowering plant species (Ollerton et al. 2011). In the case of islands, their isolation is a barrier to species colonization, leading to a depauperate and disharmonic biota compared to nearby mainland (Carlquist 1974), which in turn leads insular plant-pollinator networks to include relatively few species and to have different species groups being under- or over-represented (Olesen & Jordano 2002). Consequently, island plant species include new interactions (that are more rarely seen on mainland) with opportunistic vertebrates like insect-eating passerine birds and lizards, which include nectar or pollen into their diet (e.g. Elvers 1977; Pérez-Mellado & Casas 1997; Traveset & Sáez 1997; Rodríguez-Rodríguez & Valido 2008; Hansen & Müller 2009; Siverio & Rodríguez-Rodríguez 2012; García & Vasconcelos 2017; Abrahamczyk 2019; Fuster et al. 2019).

In the Macaronesian Islands, flowers of several species of the genus *Canarina* L. (Campanulaceae), *Echium* L. (Boraginaceae), *Isoplexis* Lindl. Ex Benth.

(Scrophulariaceae) and *Lotus* L. (Fabaceae), among others, are visited and pollinated by opportunistic birds (Vogel et al. 1984; Olesen 1985; Valido et al. 2004; Rodríguez-Rodríguez & Valido 2008; Ollerton et al. 2009; Ortega-Olivencia et al. 2012). These plants have been included in the so-called 'Macaronesian bird-flower element' (Vogel et al. 1984; Olesen 1985; Ojeda Alayón 2013); a group of around 16 plant species that seem to have converged to ornithophily because of a set of floral traits like corolla colour, large volume of dilute and hexose-rich nectar, absence of scent, and a trend towards losing papillate cells in the epidermis of petals (e.g. Dupont et al. 2004; Valido et al. 2004; Ojeda et al. 2016). Besides birds, lizards have also been reported as flower visitors for some of these plant species (Elvers 1977; Olesen & Valido 2003; Rodríguez-Rodríguez & Valido 2008; Ortega-Olivencia et al. 2012; Siverio & Rodríguez-Rodríguez 2012; Esposito et al. 2021).

To our knowledge, no bird species has yet been recorded in the wild as flower visitor of any of the five Canary endemic and threatened *Lotus* species (section *Rhyncholotus* (Monod) D. D. Sokoloff): *L. pyranthus* P. Pérez and *L. eremiticus* A. Santos (La Palma), *L. berthelotii* Masf. and *L. maculatus* Breitf. (Tenerife), and *L. gomerythus* A. Portero, J. Martín-Carbajal & R. Mesa (La Gomera). The only exception is a record of visits by two passerine bird species to *L. berthelotii* in an urban garden (Ollerton et al. 2009). On the other hand, the Tenerife lizard *Gallotia galloti* (Oudart, 1839), an endemic to Tenerife and La Palma, has been recorded as a frequent visitor of *L. maculatus* (Siverio & Rodríguez-Rodríguez 2012) and sporadic visitor of *L. berthelotii* (Ollerton et al. 2009), which suggests this reptile might be a pollinator candidate of these plants. However, pollination effectiveness of this lizard to these *Lotus* species remains to be assessed.

Here, we describe the pollination of *L. maculatus*, a threatened Tenerife endemic. Although *G. galloti* visits flowers of *L. maculatus* frequently (Siverio & Rodríguez-Rodríguez 2012), its importance as effective pollinator has yet to be corroborated. Our aims are to 1) evaluate the ability of *L. maculatus* flowers to fruit without flower visitation; 2) compare contribution to the quantity component of pollination effectiveness provided by different flower-visiting animals to *L. maculatus*; 3) assess if *G. galloti* could contribute to pollination of *L. maculatus* by carrying pollen grains; and 4) compare the contribution of different flower-visiting animals to the fruit set of *L. maculatus*. This information is vital to the conservation management of this extremely threatened plant species.

MATERIALS AND METHODS

NATURAL HISTORY OF *LOTUS MACULATUS*

Lotus maculatus is a prostrate or pendant legume that produce a greatly variable number of zygomorphic flowers (mean: 48.9 flowers per plant, range: 7-360; *unpublished*). Flowers are yellow tending towards orange with a dark brown stripe in the banner and have a nectar reservoir at the base. These flowers have a lifespan of seven days (range: 6-8 d; *unpublished*). Pollen presentation to flower visitors follows the piston mechanism typical in legumes, which depends on animal-exerted pressure for anthers and stigma exposure and pollen release. Fruits have a highly variable number of ovules (mean: 6.8 ovules per fruit, range: 1-23; *unpublished*), viable seeds (mean: 4.9 seeds per fruit, range: 1-17; *unpublished*), as well as seed to ovule ratio (mean: 0.85, range: 0.36-0.88; *unpublished*). Further details about the plant and a description of the flower can be found in Breitfeld (1973) and Hind (2008). It is endemic to Tenerife, listed as 'Critically Endangered' (IUCN 2023), and included in both national and regional catalogues of threatened species as well as in the Annex I of the Bern Convention. Two natural populations are or were known from Tenerife: the first one (i.e. its *locus classicus*) is at the coast of the northern municipality of El Sauzal (Breitfeld 1973), and the second one is on a rocky islet off the north-east coast of the island (Hernández 1993). This latter one may have disappeared (but see Rodríguez Navarro & Fariña Trujillo 2011). At present, we regard the population from El Sauzal as the only natural one. In addition, about ten plantings have been established by the conservation authorities on Tenerife (Cabildo Insular de Tenerife) to ensure its survival.

Both *L. maculatus* and its close relative *L. berthelotii* seem to need pollinators to ensure fruit set as they rarely self-pollinate (Owens 1985; Calero & Santos 1988). The former is visited by insects and lizards, but also a single visit by the introduced house mouse *Mus musculus* has been observed (Siverio & Rodríguez-Rodríguez 2012).

STUDY SITES

The study was carried out during the plant's flowering season, between March and July 2016 at Punta Puertito de El Sauzal (the plant's *locus classicus*), and between March and May 2017 at five different localities from northern Tenerife: 1, the already mentioned Punta Puertito de El Sauzal (13 m a.s.l., area *c.* 0.2 ha, and with 22 and 18 flowering individuals within a population of 40 and 48 individuals in 2016 and 2017, respectively); 2, Punta de El Clavo (36 m a.s.l., 0.6 ha, and 6 individuals); 3, Punta del Sol (28 m a.s.l., 0.06 ha, and 15 individuals); 4, La Sabinilla (30 m a.s.l., 0.02 ha, and 12 individuals); and 5, La Fajana (7 m a.s.l., 0.03 ha, and 3 individuals) (Fig. 1). The four latter localities were among the plantings established by the Cabildo Insular de Tenerife. At three of them, all individuals flowered, except for La Fajana, where two out of three individuals produced flowers.

At all study sites, the habitat is characterized by xerophytic and shrubby plants, like *Euphorbia lamarckii* Sweet, *Artemisia thuscula* Cav., and *Kleinia neriifolia* Haw., and especially by halophilous species like *Astydamia latifolia* (L. f.) Baill., *Salsola divaricata* Masson ex Link in Buch, and *Schizogyne sericea* (L. f.) DC. Mean annual rainfall and temperature were similar at all the study sites and ranged between 200-300 mm and 16-20 ºC (Marzol 2000).

VISITOR-EXCLUSION EXPERIMENT

To test if *L. maculatus* can produce fruits without animal visitation, we made an exclusion experiment in the natural population (Punta Puertito de El Sauzal). In 2016 we selected 10 flowering plants. Although 12 more individuals flowered in 2016, they did so at the end of the study period (late June) and with a very low number of flowers, thus it was not possible to increase sample size without altering the balanced experimental design (i.e. same number of flowers per treatment and individual). In 2017 the experiment was repeated with 10 new individuals that did not flower in 2016. However, flowering was poor, which hampered us to use more plants without affecting the hand-pollination experiment (see below) and censuses. On each of the total 20 plants from the two years, we randomly selected six flowers ($N = 120$ flowers). Three of these ($N = 60$ flowers for 20 plants) were enclosed in muslin bags to exclude animals (exclusion treatment) and the

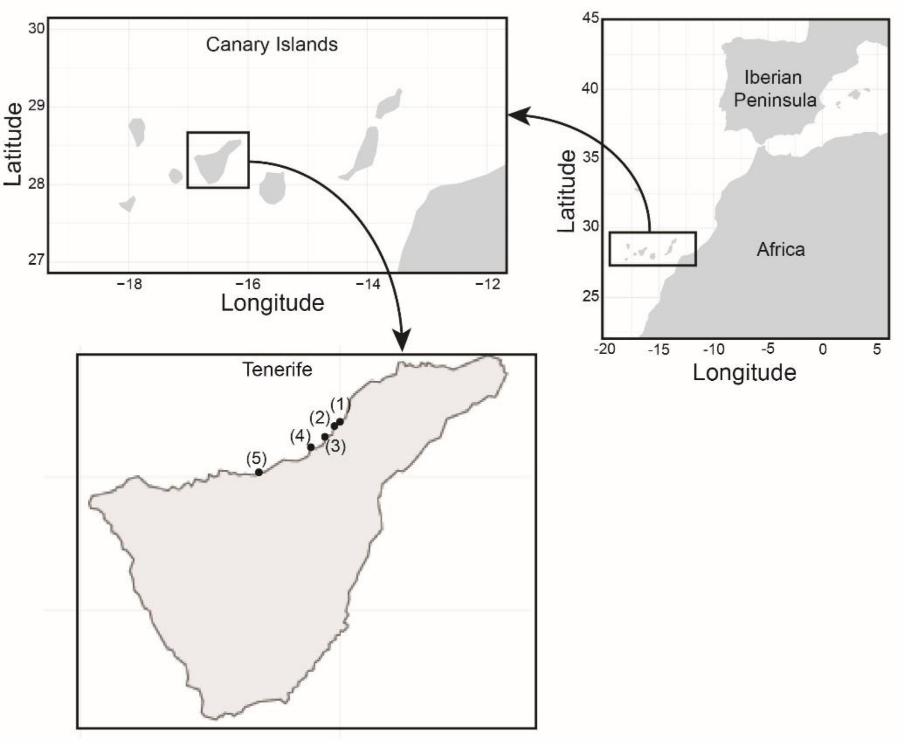

Figure 1. Map showing the location of the study sites on the island of Tenerife. The study site codes refer to: Punta de El Clavo (1), Punta Puertito de El Sauzal (2), Punta del Sol (3), La Sabinilla (4), and La Fajana (5).

remaining three (*N* = 60) were left exposed to flower visitors (control treatment). Treatment assignment to each flower was also made randomly. Later, we estimated fruit set (i.e. number of flowers that became fruit). From the sample of fruits, we also estimated seed set (i.e. number of seeds per fruit).

In 2017 we also initiated a hand-pollination experiment to describe the breeding system. We made three experimental treatments in the natural population: (1) 'A', autogamous crossing, where flowers were bagged and left unmanipulated to test for autonomous self-pollination; (2) 'G', geitonogamous crossing with pollen from another flower but on the same plant; and (3) 'X', xenogamous crossing with pollen from other individuals located at distances ranging between 5.98 m and 16.18 m from recipient plants, but in the same population. Lastly, we used a group of untreated flowers left exposed to flower visitors as a control 'C'. Unfortunately, flowering was scarce and reduced the sample size to six individuals. In addition, the flowers are fragile, and many were aborted due to manipulation. Thus, our results of this experiment were dropped out of the study.

CENSUSES OF FLOWER VISITORS

In 2016, preliminary censuses of flower visitors were made at El Sauzal. However, the diversity of potential flower visitors was too low. Therefore, we re-arranged censuses in 2017, expanding our observations to more localities (i.e. to planting sites). From March to May, we regularly visited each study site and made censuses from 08:00 to 18:15 to quantify animal flower visitation. Working within this range of times allowed us to match with the activity of potential flower visitor groups (i.e. birds, lizards, and insects). We made 15-minute censuses of floral visitors at a total of 53 individually marked plants from all study sites. However, number of individuals surveyed had to vary across sites from two at La Fajana to 18 at El Sauzal. In total, we made 233 censuses for insects and 226 for vertebrates, encompassing 114.7 hours (ranging from 15.0 to 34.7 hours at La Fajana and Punta del Sol, respectively). Across-site variation in observation time was due to differences in flowering phenology length. After each 15-minutes census, we randomly moved to other plants and alternated between censuses for vertebrate and insect visitors so as not to introduce any bias between visitor groups.

For vertebrates, censuses were carried out with binoculars and/or a field scope (20–60×) at a prudential distance so as not to interfere with their behaviour near the focal plant. Censuses for insects were made *c.* 1.5 m from the focal plant. Small invertebrates may spend much time (i.e. longer than 15 minutes) inside flowers. Thus, after each census we inspected the interior of 10-20 flowers of the focal plant for small insects, e.g. ants and small beetles, and the number of flowers with animals was recorded. At each census, we recorded the total number of open flowers of the focal plant and the number of flowers visited by animals. To standardize the number of flower visits across censuses, we divided it by the number of open flowers and the time in minutes each census lasted. Additionally, during 2016 we accumulated 130 hours of observation by nightly census and camera traps. However, they were performed just in the *locus classicus* and we observed no flower visits.

Each flower visit by an animal was categorized as either legitimate or illegitimate. In the case of *Lotus* spp., visit legitimacy is influenced by flower size and petal arrangement. Species taxonomically grouped within the section *Rhyncholotus*, like *L. maculatus*, have relatively large flowers and the keel (where anthers and stigma are hidden) is displayed in upper position, with a space of *c.* 30 mm between tip of the keel and nectar reservoir (Fig. 2A). On the other hand, other *Lotus* species, like those within section *Pedrosia* (Lowe) Valdés (Fig. 2B), have relatively small flowers, the keel is displayed in lower position, and space between keel and nectar reservoir is shorter than in *Rhyncholotus* flowers. When animals visit flowers like those within section *Pedrosia*, they alight on the keel and their body weight is enough to release the piston mechanism for anthers and stigma exposure and pollen release, putting them in contact with the animal's body. Meanwhile, in the case of *Rhyncholotus*, flower visitors accessing nectar must be large and heavy enough to reach the keel and to provoke the piston mechanism. Given this context, flower visits were categorized as legitimate if the animal contacted the keel, hence potentially touching anthers and stigma, acting therefore as a potential pollinator. When insects alighted on the flower keel to gather pollen, visits were also

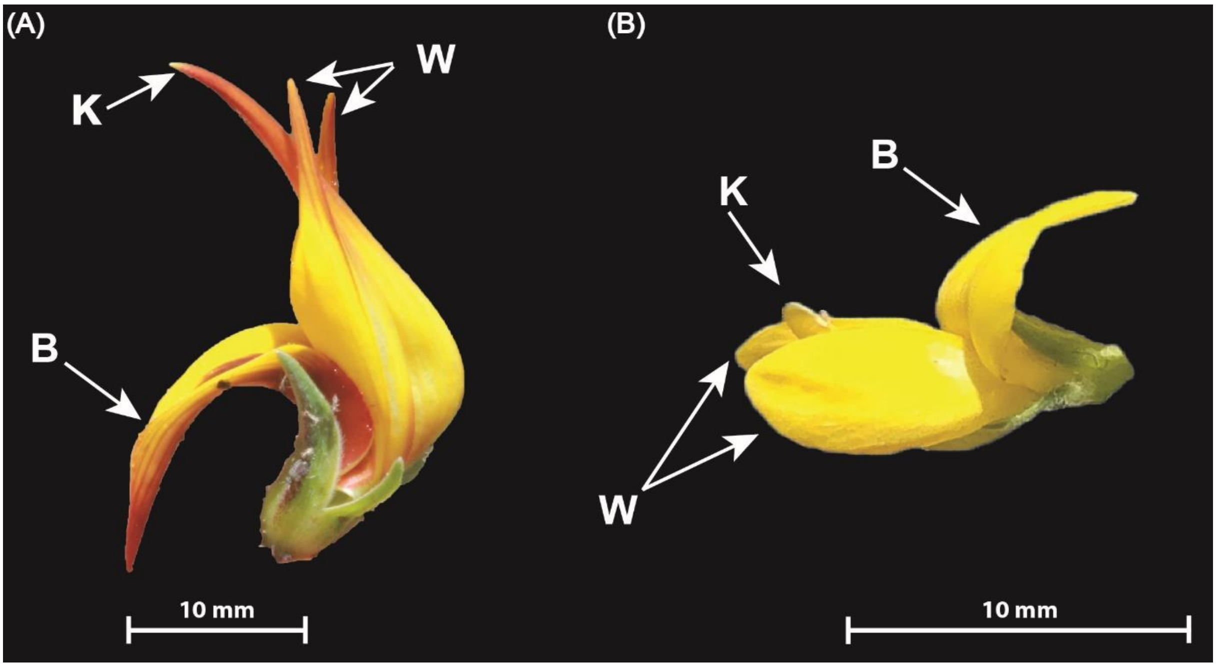

Figure 2. The differential flower size and arrangement of petals within the sections *Rhyncholotus* (A) and *Pedrosia* (B) (*Lotus* spp.). Flowers are shown in vertical (A) or horizontal (B) position as they naturally appear displayed to animals. Letters refer to the banner (B), the keel (K), and the wings (W). *Rhyncholotus* flowers are larger than *Pedrosia* ones. The keel is displayed in upper position in *Rhyncholotus* and in a lower position in *Pedrosia*. Notice that given the size of flowers, *Rhyncholotus* needs to be visited by relatively larger animals than *Pedrosia* for the anthers and stigma to be contacted by animals when they access the nectar through the space between keel and banner.

considered as legitimate because they might potentially transfer pollen between sexual parts of flowers. Visits were considered as illegitimate when the animal accessed the nectar at the base of flowers via the space between sepals (or by piercing them), thus without any contact with anthers and stigma, acting as nectar robber.

FLOWER VISITORS AS POLLINATORS

Gallotia galloti was the only vertebrate performing flower visits, and we assessed if pollen grains got attached to its scales. We did not analyse pollen loads of insects, because we assumed that these animals are capable of carrying pollen on their body surface or in their corbiculae and scopae when they perform legitimate visits.

To estimate the lizards' capacity to carry pollen grains, we visited each site once, except for Puertito de El Sauzal (visited twice), and placed two pitfall traps baited with tomatoes to capture lizards. Pitfall traps were operative from 11:30 to 17:00, when activity of lizards was the highest (*unpublished*). To avoid direct contact between lizards and tomatoes in the trap, we placed a false bottom made of metal mesh upon which lizards stayed, whereas the tomato bait was below the mesh. In order to avoid pollen transfer between lizards or pollen loss, traps were continuously monitored with binoculars, and whenever a lizard was caught the trap was immediately removed, and the lizard inspected for pollen. In accordance with the protocol by Pérez-Mellado et al. (2000), we pressed transparent adhesive tape to the neck, head, and throat of the lizard. Then, the strip of tape was placed on a microscope slide for later study under an optical microscope (20× and 40×). We also measured the lizard snout-vent length (hereafter, SVL), noticed its sex (i.e. male, female, or undetermined), age (i.e. juvenile or adult), and marked the lizard with xylene-free paint to avoid repeated samples. In general, the whole sampling process took less than ten minutes and lizards were released at the same site with no damage.

To assess the relative contribution of *G. galloti* and insects to the fruit set of *L. maculatus*, we correlated the reproductive success of each individual plant monitored during censuses with the total number of flower visits it received by lizards and the two most frequent flower-visiting insect species, i.e. *Lasioglossum arctifrons* (Saunders, 1903) and the honeybee *Apis mellifera* Linnaeus,

1758. Reproductive success of individual plants was estimated by following a similar procedure to that shown by Gómez (2003). At each individual used for flower-visitor censuses, we counted the number of flowers and fruits once per week until the plant stopped flower and fruit production. As flowers and fruits are very fragile and tend to be dropped if manipulated, we could not mark them to control for aborted flowers and fruits. In addition, it is difficult to monitor all abortion on the ground, because of the dense prostrate growth of the species. Therefore, we divided the maximum number of fruits (i.e. corresponding to the week with the highest number of fruits on its branches) by the maximum number of flowers produced (corresponding to the week with the highest number of flowers) to estimate the fruit set as a measure of reproductive success of each individual plant.

STATISTICAL ANALYSES

Regarding the exclusion experiment, to test for any differences in fruit set and seed set from the exclusion and control treatments, two Generalized Linear Mixed Models (GLMM) were performed by using the 'lme4' package (Bates et al. 2015) for R (R Core Team 2023). The first GLMM was performed with a binomial error distribution, where the response variable was the success (1) or failure (0) of each target flower to fruit. The second GLMM was made with a Poisson error distribution for count data, where the number of seeds per fruit was the response variable. In both GLMMs the exclusion treatment (i.e., exclusion *vs*. control) was the explanatory variable, and the identity of each individual plant was the random factor. To test for the effect of exclusion treatment, we used the function 'Anova' from the R package 'car' (Fox & Weisberg 2019).

We are aware that our sample size (three flowers per treatment and individual plants) might be considered too small to make reliable statistical inference. Therefore, we used a null model based on permutations to make statistical inference (Anderson 2001). This null model was made under the assumption that experiment outputs (proportion of flowers setting fruits and number of seeds per fruit) occur randomly across different levels of the exclusion treatment. Permutations of data were run 9999 times and the GLMM was run on each simulated dataset, so with the 'Anova' function we obtained 9999 simulated Chi-squared values (χ^2_s) for exclusion treatment as explanatory variable. Then, to calculate the Monte Carlo p-value for exclusion treatment, the χ^2 calculated on the original dataset was compared to the distribution of χ^2_s values obtained from permutations as

$$(1) \qquad P = \frac{K+1}{R+1}$$

There, K is the number of permutations leading to a χ^2_s value equal or higher to the χ^2 calculated on the original data, and R is the total number of permutations.

To assess if different flower visitors differed regarding the legitimacy of their visits (i.e. potentially pollinating visits), we performed a G-test for count data in a contingency table by using the 'DescTools' package (Signorell 2023) for R.

For captured lizards that carried pollen grains, we tested for the relationship between lizard traits (i.e. SVL, sex and age) and amount of pollen sampled from their body by using a Generalized Linear Model (GLM) with normal error distribution. For some individuals, we could not to determine sex or age. Therefore, to have a sample size as large as possible to assess the effect of each explanatory variable, the GLMs to test for each of them were run separately. For this analysis we excluded individuals from La Fajana. We did so because we observed that lizards in the planting at La Fajana were prevented from interacting with *L. maculatus* flowers due to protection fences, and lack of pollen grains could be due to these fences rather than any lizard traits. Significance of sex and age was estimated with the function 'Anova' from the R package 'car' (Fox & Weisberg 2019). As number of pollen grains in samples was very variable, we log-transformed the variable to ensure its normality.

Lastly, to assess the relationship between number of floral visits by animals to each individual plant and its reproductive success (i.e. fruit set) we performed a GLM with binomial error distribution for proportion data, with plant reproductive success as a response variable and number of visits made by lizards, *L. arctifrons,* and *A. mellifera* as explanatory variables. Due to great variability in the number of visits by each flower visitor, explanatory variables were log-transformed. When individual plants grew very close to each other, it was difficult to assign floral visits to a given individual. This may affect our estimation of the relationship between number of floral visits received by a given plant and its reproductive success. Therefore, for this particular analysis we excluded observations to groups of individuals growing close together. It resulted in a sample size of 23 individual plants.

RESULTS

VISITOR-EXCLUSION EXPERIMENT

Exclusion of flower visitors resulted in significantly less fruit and seed set than the control (χ^2 = 10.59; *D.F.* = 1; *P* = 0.001; *Monte Carlo-P* = 0.0012; Fig. 3A, and χ^2 = 26.51; *D.F.* = 1; *P* < 0.001; *Monte Carlo-P* = 0.02; Fig. 3B, respectively).

FLOWER VISITORS

During the entire study, vertebrates made 183 flower visits and invertebrates 433 visits. *Gallotia galloti* was the only flower-visiting vertebrate; no birds visited the flowers although they were active around the plants. Among the invertebrates, *A. mellifera* and *L. arctifrons* made 79.5% and 16.6% of all flower visits, respectively, whereas the remaining 3.2% of visits were mainly by flies (Diptera), ants (Formicidae), and beetles (Coleoptera). Each flower received several visits by the same flower visitor species, both within each census period and between different censuses.

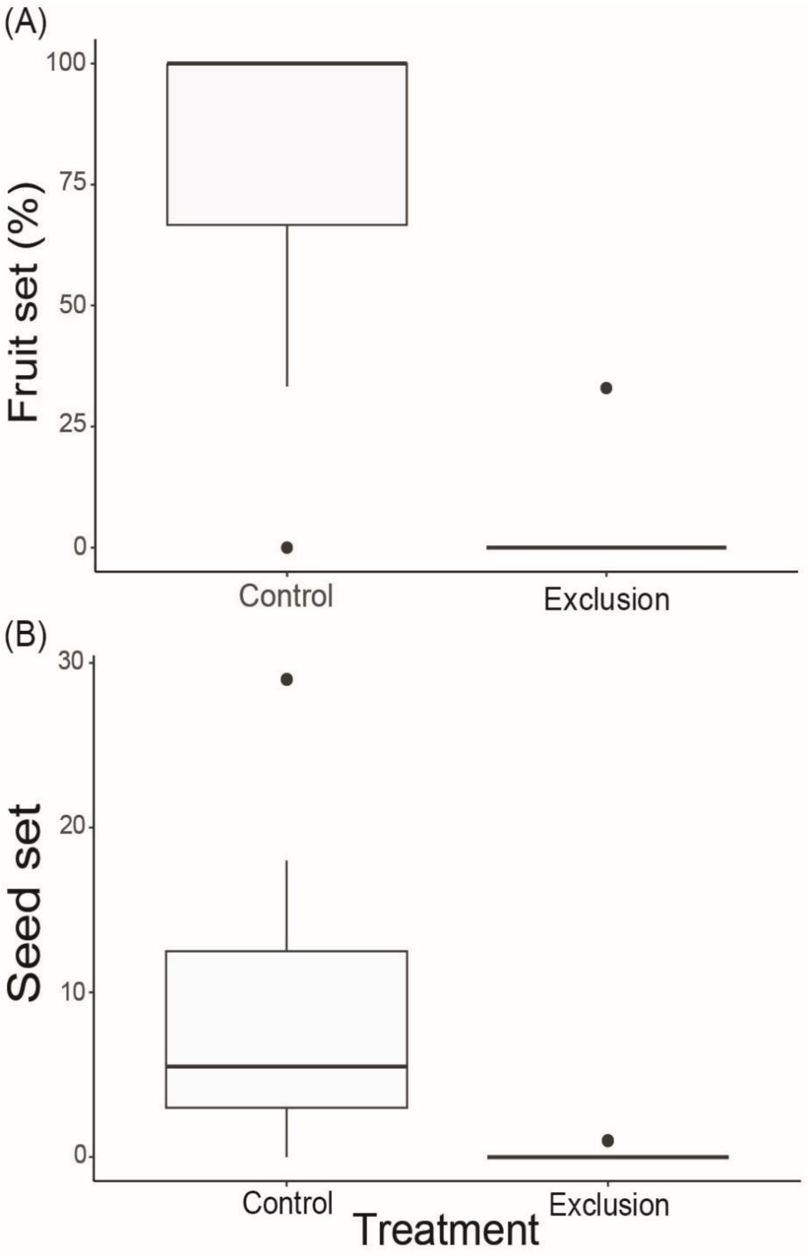

Figure 3. Results of an exclusion experiment to assess the effect of enclosing flowers on fruit set (A; expressed as the percentage of flowers that produced fruits) and seed set (B; as number of seeds per fruits). Horizontal thick lines in each box indicates the median; lower and upper box limits refer to first and third quartile, respectively; vertical lines are the 1.5 interquartile range; and dots are outliers.

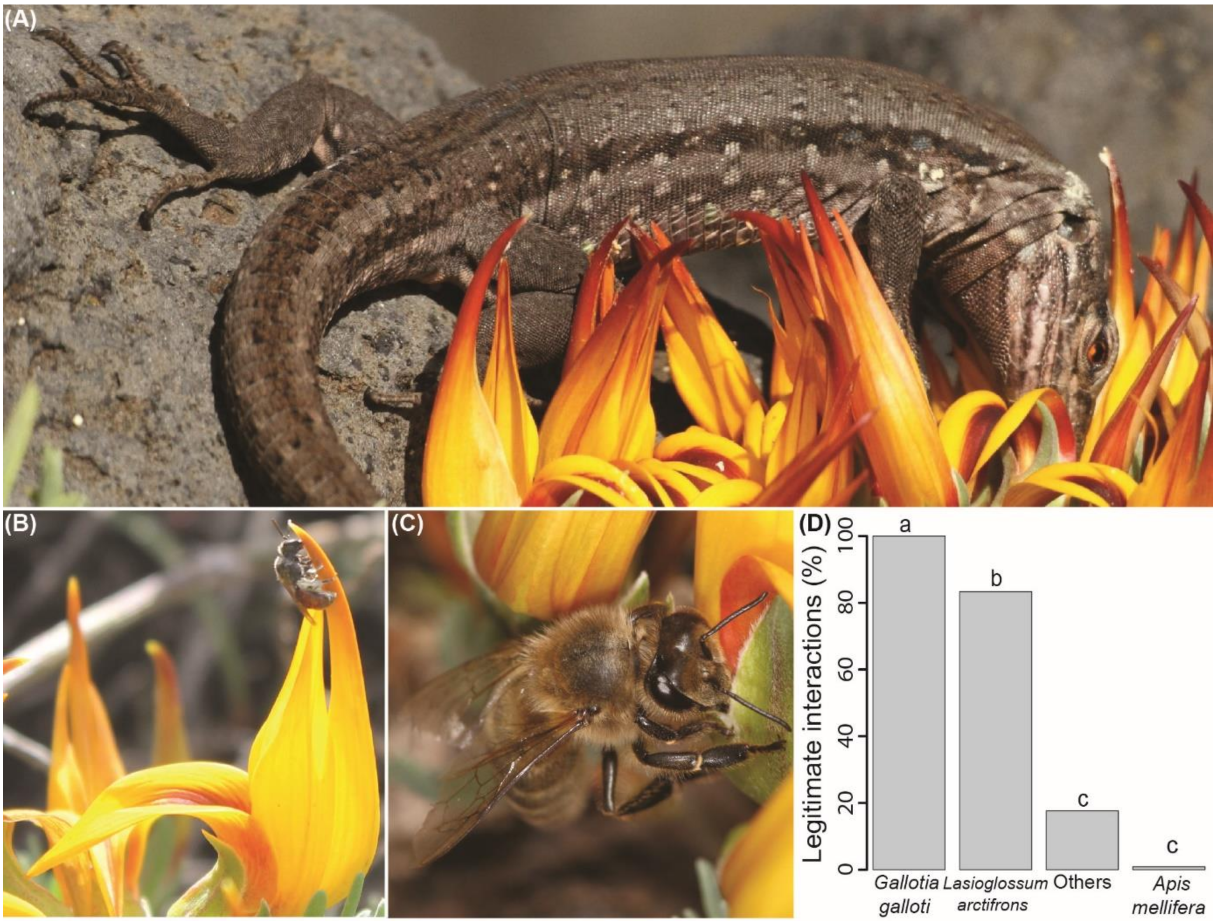

Figure 4. Flower visits were considered as legitimate when the animal accessed the nectar through the space between banner and keel or touched the keel, where anthers and stigma are hidden. The endemic Tenerife lizard *Gallotia galloti* (A) always access the nectar legitimately. *Lasioglossum arctifrons* (B) tended to alight on the keel seeking for pollen, so it potentially touches the reproductive parts of the flower and could be a pollinator. Honeybees *Apis mellifera* (C) mostly accessed the nectar at the base of the flower, acting as nectar robber. After applying Bonferroni's correction factor, the proportion of legitimate visits (D) was significantly different among animals, as shown by different letters at the top of bars. Photo credits: Beneharo Rodríguez (A and C) and Yurena Gavilán (B).

Gallotia galloti always made legitimate visits (Fig. 4A). Almost always, corolla parts returned to their normal position after visits by lizards, but sometimes the corolla was left disarticulated after a visit by lizards. Most of the visits by *L. arctifrons* were legitimate, as it tended to alight on the keel seeking for pollen (Fig. 4B). *Apis mellifera* always made illegitimate visits, thus acting as a nectar robber (Fig. 4C). This difference across flower visitors in proportion of legitimate visits was highly significant ($G_3 = 716.1$; $P < 0.001$; Fig. 4D).

QUALITY OF FLOWER VISITORS AS POLLINATORS OF *LOTUS MACULATUS*

Considering data from all five study sites, 34 lizards were captured, and we obtained pollen grains from 18 of them (Table 1; Fig. 5A). It is noteworthy that in the natural population at Punta Puertito de El Sauzal, we sampled the highest number of pollen grains from 63% of captured individuals (Table 1). The amount of pollen varied widely among individuals (range 1-1004 pollen grains), but there was no relationship with lizard body size (parameter estimate = -0.014; *T-value* = -1.05; $P = 0.3$). Although samples from males tended to have less pollen grains than females, we found no difference between sexes ($\chi^2 = 2.73$; $D.F. = 1$; $P = 0.1$) or between juvenile and adult lizards ($\chi^2 = 0.03$; $D.F. = 1$; $P = 0.87$).

Reproductive success (i.e. fruit set) of each individual plant was positively related to number of visits by lizards (parameter estimate = 1.46; $Z = 26.74$; $P < 0.001$; Fig. 5B), not related to number of visits by *L. arctifrons* (parameter estimate = 0.04; $Z = 1.26$; $P = 0.21$; Fig. 5C), and negatively

Table 1. Sampling of pollen grains of *Lotus maculatus* on lizards. Number of individuals of Tenerife lizard *Gallotia galloti* captured (*N*) at each study site, number of individuals carrying pollen grains (*NIp*), and number of pollen grains (*Np*) counted per individual (mean ± standard deviation). *The natural population and *locus classicus* of *L. maculatus*.

Study site	Study site code	*N*	*NIp*	*Np*
Punta de El Clavo	1	8	4 (50%)	7.13 ± 11.58
Punta Puertito de El Sauzal*	2	19	12 (63.2%)	167.47 ± 304.73
Punta del Sol	3	1	1 (100%)	1
La Sabinilla	4	1	1 (100%)	4
La Fajana	5	5	0 (0%)	-

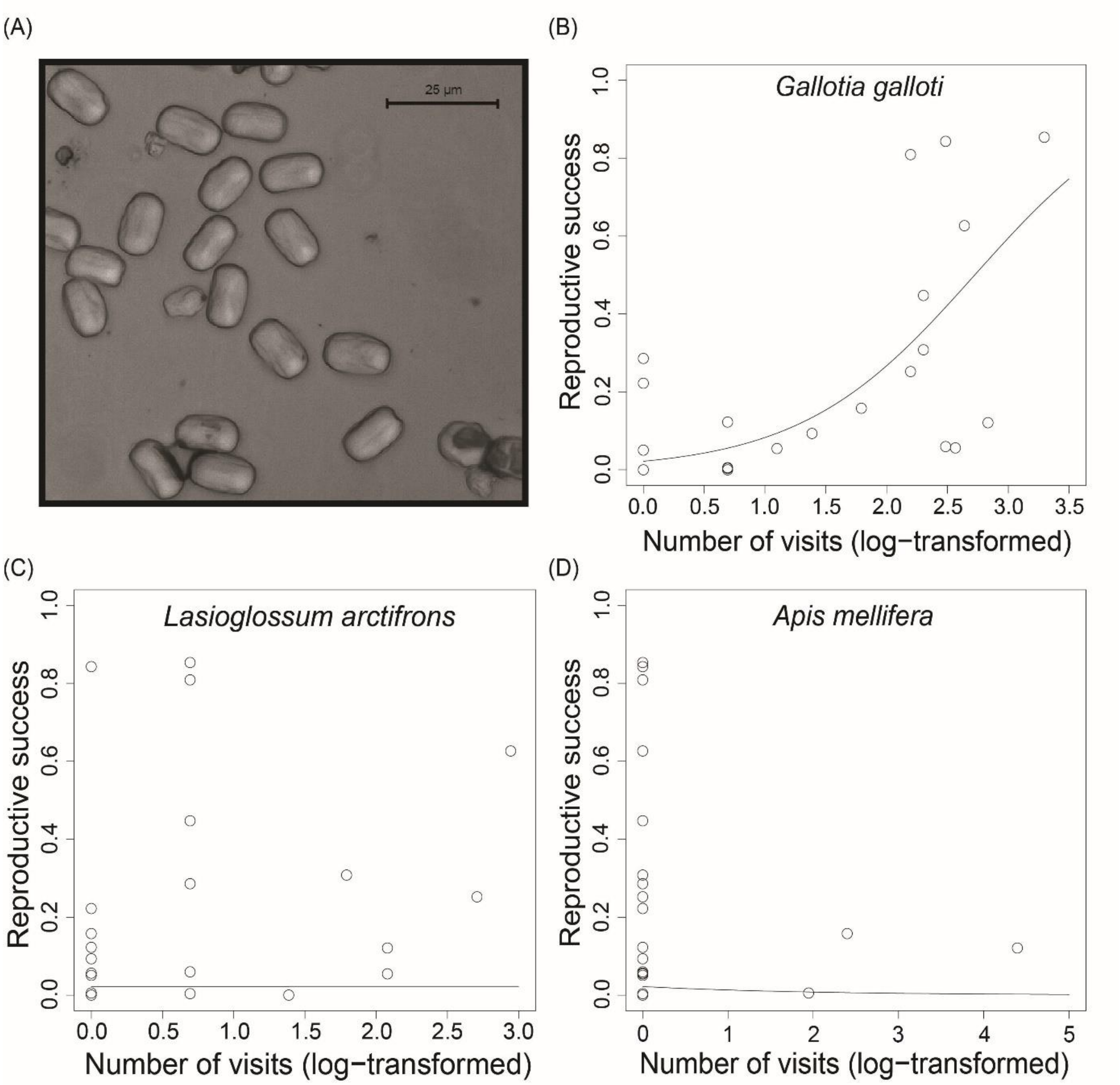

Figure 5. Pollen grains of *Lotus maculatus* (A) under an optical microscope (40×). The panels show the relative reproductive success of each individual plant (dots) against the number of visits by Tenerife lizard *Gallotia galloti* (B), *Lasioglossum arctifrons* (C) and honeybee *Apis mellifera* (D). Solid line in panels B-D represents the reproductive success predicted by the model for each visitor. Photo credits: Aarón González-Castro (A).

related to number of visits by *A. mellifera* (parameter estimate = -0.62; Z = -25.32; P < 0.001; Fig. 5D).

DISCUSSION

In this study we show that the Tenerife endemic and threatened *L. maculatus* flowers rarely set fruit by spontaneous self-pollination, that *G. galloti* might be an effective (and perhaps the most important) pollinator, and that visitation by honeybees has a negative impact upon fruit set.

Our results of the visitor-exclusion experiment agree with previous studies on lizard-pollinated species, showing that flowers prevented from visits by animals have a lower reproductive success than control flowers (e.g. Pérez-Mellado & Casas 1997; Traveset & Sáez 1997). It was surprising that one out of 60 excluded flowers fruited. The result might be a consequence of an error while manoeuvring flowers to be bagged or the fact that self-pollination in *Rhyncholotus* is quite difficult but not impossible (Owens 1985). This very low spontaneous self-pollination is partly in agreement with Calero & Santos (1988) and might be explained by the late-acting self-incompatibility found in other *Lotus* species (Lundqvist 1993; Ollerton & Lack 1998). Like other Fabaceae, *L. maculatus* has a stigmatic cuticle, preventing the entrance of the pollen tube into the stigma (Owens 1985; Rodríguez-Riaño et al. 2004; Valtueña et al. 2010; Ojeda & Santos-Guerra 2011). Effective pollination observed in the closely related *L. berthelotii*, as well as in other Fabaceae, depends on the rupture of the stigmatic cuticle (Heslop-Harrison & Heslop-Harrison 1983; Owens 1985; Heenan 1998). Contact of control flowers with animals might have facilitated cuticle rupture, thus breaking down self-incompatibility and enhancing fruiting success. Consequently, foreign pollen may perhaps not be a prerequisite for fruiting, but spontaneous self-pollination needs animals to break the stigmatic cuticle.

VERTEBRATES AS POLLINATORS OF *LOTUS MACULATUS*

The only vertebrate recorded as a flower visitor of *L. maculatus* was *G. galloti*. Lizard pollination of an island plant is not surprising as it has been observed worldwide (Olesen & Valido 2003). Besides *G. galloti*, the nocturnal Delalande's gecko *Tarentola delalandii* (Duméril & Bibron, 1836), an endemic to Tenerife and La Palma, is also a flower visitor for different plants in the Canary Islands (Hernández-Teixidor et al. 2020; Fariña & Mangani 2020; Koppetsch et al. 2020), like other *Tarentola* species in Macaronesia (Pinho et al. 2018) and other geckos worldwide (Olesen & Valido 2003). However, after 130 hours of night observation and use of camera traps in 2016, we did not record any flower visits made by geckos (*unpublished*). Therefore, *G. galloti* seems to be the most probable reptile pollinator of *L. maculatus*, although the role of Delalande’s gecko as a pollinator should not be completely disregarded.

Although some *G. galloti* lizards consume flowers of *L. maculatus*, most recorded interactions during our study were legitimate pollinations. Lizards accessed the nectar through the front of the flower, between keel and banner petals, pressing the flower down and provoking the piston mechanism to release the pollen and cause the stigma to emerge, and thus touching anthers and stigma with head and neck, causing pollination. Therefore, the high frequency of legitimate interactions, together with the fact that they can carry pollen grains and the positive relationships between number of flower visits and reproductive success of plants, strongly suggest that *G. galloti* is currently the most important and perhaps sole pollinator of *L. maculatus*. Numerous studies demonstrate that lizards might be effective pollinators, as they visit flowers and are able to carry pollen (e.g. Elvers 1977; Pérez-Mellado & Casas 1997; Traveset & Sáez 1997; Rodríguez-Rodríguez & Valido 2008; Hansen & Müller 2009; Ortega-Olivencia et al. 2012; García & Vasconcelos 2017; Jaca et al. 2018; Pinho et al. 2018; Hernández-Teixidor et al. 2020; Koppetsch et al. 2020; Esposito et al. 2021). However, data about the contribution of Macaronesian reptiles to plant reproductive success or their ability to carry pollen grains are still scarce (but see Rodríguez & Valido 2008; Jaca et al. 2018; Hernández-Teixidor et al. 2020).

The lack of any relationship between lizard traits and pollen load might be explained by the wide body size span of male, female, and juvenile lizards. Adult males, for example, may exert too much pressure on flowers, forcing them backward, and thus reducing the possibility for pollen to pop out or for the lizard to touch the keel. Nonetheless, the low number of pollen grains found in some samples is noteworthy (Table 1). Although low number of pollen grains may be considered as contamination (Romero-Egea *et al*. 2023), in the picture shown in Fig. 4A it is possible to see a relatively large pollen spot on the lizard's head. Therefore, the low number of pollen grains might be due to low adherence of pollen to animal scales rather than contamination. Besides, samples containing between one and six pollen grains could be considered as contaminated (Romero-Egea *et al*. 2023). Although contamination of sampling tools (e.g. adhesive tape or microscope slide) might have occurred, it is necessary to highlight that five out of our 18 pollen-occurring samples contained less than ten pollen grains, and four of them were from localities where *L. maculatus* has been planted, where lizard activity can be lower, or plant protection fences tend to prevent flower visit by lizards (see below). If manipulation-related contamination were a pervasive issue in the study, samples with less than six pollen grains (i.e. potentially contaminated) would occur at similar proportions across all study sites.

It was surprising that no flower visits by birds were recorded, although this plant traditionally has been proposed to be putatively ornithophilous (Olesen 1985; Dupont et al. 2004). The explanation may be that the habitat is not suitable for passerine birds (Siverio & Rodríguez-Rodríguez 2012). This may be true for the only natural population, at El Sauzal, but in the present work, interactions with birds were not observed at any study site, even in some localities (i.e. the plantings at La Fajana, La Sabinilla, Punta del Sol, and Punta de El Clavo), where abundance of opportunistic passerine birds is high.

Ollerton et al. (2009) recorded flower visits by two passerine species –mainly the Canary Islands chiffchaff *Phylloscopus canariensis*– to *L. berthelotii* in a gardened area. As both *Lotus* species produce large volume of dilute and hexose-rich nectar (Dupont et al. 2004), nectar composition does not seem to be the reason for the absence of visits by birds to *L. maculatus*. A plausible explanation might be the different colours of flowers, because *L. berthelotii* produces intensely red flowers, a common (though not exclusive) colour among ornithophilous plants (Proctor et al. 1996), whereas *L. maculatus* flowers are yellow towards orange. Despite birds are attracted to yellow flowers, this trend towards orange-yellowish colours and a changing contrast to the green foliage background could make *L. maculatus* less prone than *L. berthelotii* to receive visits by birds (Ollerton et al. 2009). More studies on flower colorimetry of *Lotus* species within the section *Rhyncholotus*, as well as focusing on other traits that do not fit the ornithophilous syndrome, may improve our understanding of this interspecific difference in attractiveness of *Lotus* flowers to birds (e.g. Ollerton 2024; Rodríguez-Sambruno et al. 2024).

Another plausible explanation for the lack of visits by birds might be that localities where birds were abundant correspond to non-natural populations of *L. maculatus*. At these planting sites, the number of *L. maculatus* individuals is lower than in the natural population, thus birds may be habituated to exploit other food resources than nectar of *L. maculatus*, especially if ecological interactions may depend on individual bird behaviour (Aplin et al. 2013, 2014). In this sense, it is possible that interactions with a "new" species in non-natural plantings might depend on the presence of "bold" individual birds willing to explore this new resource, which thereby would trigger the use of *L. maculatus* nectar by "shy" individuals in the population. The natural population at El Sauzal, is perhaps the last stronghold, at the margin of the original distribution of *L. maculatus*, where the plant might have been relegated due to effect of alien herbivores (i.e. rabbits and goats). Previous populations at higher altitudes –where birds are abundant– might have interacted with pollinating birds. Therefore, we cannot disregard the possibility that interactions of *L. maculatus* with flower-visiting birds have been lost before any of mutualists have gone extinct, as reported for seed dispersal mutualisms (McConkey & O'Farrill 2016).

INSECTS AS POLLINATORS OF *LOTUS MACULATUS*?

The probably introduced *A. mellifera* was the most frequently recorded flower visitor, but it only made illegitimate visits. It accesses the nectar at the base of the flower, thus acting as a nectar robber. Indeed, the relationship between number of honeybee visits and plant reproductive success was negative. If a bee made any attempt to access the nectar through the front of the flower, between keel and banner petals (in a similar way to legitimate visits made by lizards), it was too light to make enough pressure to trigger pollen release. Furthermore, *A. mellifera* is too small to reach the anthers and stigma in case they were already exposed outside the keel after a visit by lizards. Therefore, apparently legitimate visits by bees would hardly imply an effective pollination of *L. maculatus*. This contrasts with legitimate honeybee visits to flowers of *Lotus tenellus* (Lowe) Sandral, A. Santos & D. D. Sokoloff (*unpublished*), a species sympatric with *L. maculatus* in some of our study sites but taxonomically grouped in section *Pedrosia*. *Lotus tenellus* has smaller flowers than *L. maculatus* and its keel, as in other Fabaceae, is arranged downward (Fig. 2). When a honeybee visits a flower of *L. tenellus* it alights on the keel and its body weight is large enough to release the pollen and force the anthers and stigma out of the keel. Thus, here the honeybee might act as a pollinator of *L. tenellus*, as also in other *Lotus* species (e.g. Benachour et al. 2007; Siqueira et al. 2018). Therefore, the legitimacy of *A. mellifera* as a flower visitor of *Lotus* spp. seems to be more dependent on flower size and petal arrangement, rather than on an intrinsic stereotyped feeding behaviour of bees. A similar conclusion may be reached for other insects (i.e. ants, beetles, and flies) which weigh too little to trigger the pollen release, despite these animals sometimes accessing the nectar in a way that could be considered as apparently legitimate.

The native, solitary bee *L. arctifrons* made >80% of visits that could be considered as apparently legitimate. It alights on the keel –where the stigma and anthers are hidden– to collect, and probably eat, pollen (Fig. 3C). However, we found no effect of number of visits by this insect on plant reproductive success. Therefore, although *Lasioglossum* spp. and other halictid bees have been reported as pollinators of other species (Singer & Cocucci 1999; Howard et al. 2021), including some Fabaceae (Gros 2001), it seems unlikely that *L. arctifrons* was a quantitatively important pollinator of *L. maculatus*, though it may happen for flowers previously visited by lizards. Sometimes, after visits by lizards, flowers may be left with anthers and stigma exposed, then it may be possible that pollen grains carried by *L. arctifrons* to be transferred to a stigma. Nonetheless, such a sequential double interaction has not been corroborated and remains hypothetical.

RECOMMENDATION TO ENSURE THE REPRODUCTIVE SUCCESS OF *LOTUS MACULATUS*

Although other vertebrates cannot be disregarded as pollinator candidates of *L. maculatus*, our results suggest that the endemic *G. galloti* might be currently the most important and perhaps the sole pollinator of this threatened species. Nonetheless, to compare the effectiveness and definitive importance of *G. galloti* as pollinator of *L. maculatus* it would be desirable to perform some additional experiments that we could not carry out due to the relatively large number of flowers needed for exclusions, which was incompatible with the threatened status of *L. maculatus*. Beyond of visitor-exclusion experiments, it would be required additional experiments to selectively exclude lizards and invertebrate visitors, as well as single-visit experiments, where recently open flowers are observed until a visit is recorded, and subsequently bagged to exclude further visitors. Whereas all these experiments are quite difficult to be addressed in the wild, they could be easily performed in greenhouses.

In any case, conservation of *L. maculatus* is urgent and any information gathered from studies like ours should be useful for conservation of this endangered plant. Despite local administration conducting several plantings of *L. maculatus* throughout the northern coast of Tenerife, it is difficult to witness natural regeneration by seedling recruitment. Study sites where lizards performed flower visits more often were also the sites where plants showed a higher fruit production. Therefore, to enhance the success of conservation actions made by the local administration, it is necessary to ensure this mutualistic interaction.

Since the flowering of *L. maculatus* mostly occurs in winter and spring, plantings should be made in zones exposed to sunlight –e.g. coastal tips of land and rocky islets–, where lizard activity can be high; but without disregarding other zones inland at slightly higher elevations, where interactions with potential flower-visiting birds could be enhanced. Also, the design of protection fences for plants against introduced herbivores should allow lizards to get into the fenced areas and be planned with space enough for the plants to grow prostrate to the ground, facilitating lizard access to flowers, instead of allowing plants to climb the fences as occurred at several planting sites. Indeed, it has been demonstrated that protection fences may have negative consequences for threatened species (Lorite et al. 2021), so that it is necessary to keep monitoring fenced plants. Also, it is important to ensure diverse and abundant populations of potential flower visitors, because nectar of *L. maculatus* is a novel resource for lizards and birds in human-made plantings, and the occurrence of these plant-vertebrate interactions might rely on the presence of bold individuals willing to explore this new resource and transmit their feeding behaviour through the population (see Aplin et al. 2013, 2014; Pérez-Cembranos & Pérez-Mellado 2014).

Acting mostly as nectar robbers for *L. maculatus*, the role of *A. mellifera* –apart from having a "sterile" interaction with the plant– appears to be unfavourable to this threatened species, as honeybees deplete nectar resource level in flowers and may exclude other pollinators from the community (e.g. Carbonari et al. 2009; Valido et al. 2019). Therefore, it would be desirable to avoid honeybee hives in places close to populations of *L. maculatus*. Lastly, it is known that *G. galloti* and other lizard species from Canary Islands are exposed to introduced predators like feral cats and snakes (Medina & Nogales 2009; Piquet & López-Darias 2021) and it has been demonstrated that key mutualistic interactions disappear even before the interacting species become extinct (McConkey & O'Farrill 2016). Therefore, it is necessary to prevent both competition and predation risk to lizards to ensure the further maintenance of pollination and hence the survival of *L. maculatus*.

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AUTHOR CONTRIBUTION

AGC and FS conceived the idea, designed the study, and performed fieldwork. AGC led statistical analyses and manuscript writing with substantial input of FS. Both authors read and give approval to the final version of the manuscript.

DISCLOSURE STATEMENT

Authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets analysed for this study can be found in the figshare repository at doi: 10.6084/m9.figshare.25559724.

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