

# Hotspots of damage by antagonists shape the spatial structure of plant–pollinator interactions

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**Abstract.** The balance between mutualistic and antagonistic plant–animal interactions and their spatial variation results in a highly dynamic mosaic of reproductive success within plant populations. Yet, the ecological drivers of this small-scale heterogeneity of interaction patterns and their outcomes remain virtually unexplored. We analyzed spatial structure in the frequency and intensity of interactions that vertebrate pollinators (birds and lizards) and invertebrate antagonists (florivores, nectar larcenists, and seed predators) had when interacting with the insular plant *Isoplexis canariensis*, and their effect on plant fitness. Spatially autocorrelated variation in plant reproductive success (fruit and viable seed set) emerged from the combined action of mutualists and antagonists, rather than reflecting the spatial pattern of any specific animal group. However, the influence of antagonists on plant fitness was stronger primarily due to the florivores' action on earlier reproductive stages, consuming and damaging floral structures before the arrival of pollinators. Our results indicate that the early action of antagonists creates hotspots of increased plant damage, where the effects of later acting mutualists are not translated into increased reproductive benefits. We foresee the potential for antagonists to shape the intra-population mosaics of plant fitness in situations where antagonists outnumber mutualists, when their interactions occur before those of mutualists, and when mutualists can detect and avoid damaged plants while foraging. Severely damaged plants in antagonistic hotspots might be excluded from the mating network and render a limited production of viable seeds, reducing both the growth rate of the plant population and the effective population size.

**Key words:** *bird pollination; Canary Islands; floral herbivory; Isoplexis canariensis; lizard pollination; mark correlation function; nectar larceny; plant–animal interactions; plant reproductive success; point pattern analysis; predispersal seed predation; spatial pattern.*

## INTRODUCTION

Complexity within plant–animal mutualistic interactions not only depends on the diversity of interacting partners (Bascompte and Jordano 2014), but also on the ecological context in which these interactions occur (Bronstein 1994, Chamberlain et al. 2014). Context dependency often involves another functional group, namely antagonists, that may constrain and potentially lead to the breakdown of mutualism-derived benefits (Bronstein et al. 2003, Gómez 2008, Chamberlain and Holland 2009). Moreover, these opposed biotic interactions generally vary across space, frequently resulting in cold and hotspots of plant reproductive success (PRS) that favor divergent selective trajectories among plant populations (Thompson 1994, 2005, 2013, Wilson et al. 2003, García et al. 2011). However, the determinants of spatial variation of interactions with both mutualists and antagonists within populations are largely unknown. Combined interactions with both agents generate variation in reproductive outcomes, and are thus a

central driver of coevolutionary processes (Thompson 1999).

Plants are largely sessile organisms, a condition that restricts their ecological context. The spatial position determines the local microclimate, habitat structure, and plant community composition to which an individual plant is exposed (e.g., conspecific and heterospecific competition for resources), and each of these factors has the potential to influence PRS. The spatial distribution of plants can also strongly affect the behavior of interacting animals (e.g., Ghazoul 2005, Rossi et al. 2011). However, their foraging behaviors and movement patterns not only track the distribution of plants, but also respond to abundances and characteristics of alternate food sources, competing and predator species, and to specific abiotic conditions (Nathan et al. 2008). This means that plant–animal interactions, and their outcomes in terms of PRS, will be largely conditioned by both plant distribution and the diversity of animal foraging strategies, promoting the emergence of spatially structured interaction outcomes (Carlo et al. 2007, Nattero et al. 2011).

Considering the wide variation in life histories of animal assemblages, we might expect that mutualists

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and antagonists (e.g., vertebrates vs. invertebrates) of a given plant species would be differentially influenced by the heterogeneous distribution of plant resources, plant signals, and their ecological context (e.g., Chamberlain and Holland 2008, García et al. 2011, Schaefer and Ruxton 2011). For example, animals with restricted mobility may tend to use area-limited searching within the plant population, increasing the likelihood that their effects will be restricted to a few plant individuals, or even unique plants, for long periods of their lifetime (i.e., high interaction intimacy; Pires and Guimarães 2013). This limited mobility would promote closely growing plants to have more similar interaction patterns and derived effects (i.e., spatially autocorrelated; e.g., Rossi et al. 2011) compared to interactions involving highly mobile animals, for which we might expect stronger spatial signals at broader scales. Additionally, this spatial signal in interaction effects may also depend on the temporal context in which they occur. Therefore, animal partners interacting at different temporal stages throughout plant ontogeny may influence the action of the rest of the interacting agents. For instance, antagonists can create mosaics of high and low plant attractiveness for beneficial partners if their interactions occur before those of mutualists (e.g., Krupnick et al. 1999, Gómez 2008). Mutualists may tend to visit plants free from critical damage, a situation that ultimately promotes a significant patchiness in their beneficial interactions.

A crucial consequence of structured plant–animal interactions is the emergence of fitness gradients within plant populations that may also appear highly structured (e.g., Araki et al. 2007, García-Meneses and Ramsay 2012), at least in those species for which plant–animal interactions determine PRS more than other factors (e.g., plant community composition). If the effects produced by mutualists or antagonists are strongly aggregated in space, their interactions might result in distinct regions of beneficial and detrimental outcomes for plant reproduction. Such spatial signals have a strong potential to influence phenotypic selection scenarios, genetic structure, and gene flow dynamics via local adaptation (e.g., García et al. 2007, Gómez et al. 2009); spatial signals are therefore essential to understand coevolution in action. However, the extent to which the balance between the effects of mutualists and antagonists at small spatial scales generates coevolutionary mosaics of cold and hotspots on broader scales remains largely unexplored, especially when the interacting assemblages include animal species with diverse life histories (Thompson 1994, 2005, 2013). Considerable attention has been paid to characterizing the spatial structure of plant–animal interactions within plant populations (e.g., Waser and Mitchell 1990, Nattero et al. 2011). However, as far as we know, no previous study has attempted to separate the relative importance of mutualistic and antagonistic partners on PRS on this

small a spatial scale, while accounting for the spatially explicit distribution of plants.

Here we investigate whether plant–animal interactions occurring during pollination act as underlying sources of the spatial variation in plant reproductive success of the insular plant *Isoplexis canariensis* (L.) J. W. Loudon (Plantaginaceae). We selected this study system for the relative simplicity of its animal assemblage with highly contrasting life forms (vertebrates vs. invertebrates), for which we would expect divergent spatial patterns in their interactions. We specifically ask: (1) Are interaction effects of mutualists and antagonists spatially structured, and is there any spatial association between these interaction effects? (2) Are their spatial patterns conditioned by the characteristics of the plant? (3) Are plant reproductive outcomes spatially structured, and if so, how do the spatial association of mutualistic and antagonistic effects determine the spatial variation in PRS? Finally, (4) which functional group has a greater relative effect on PRS? In the study system, mutualists are opportunistic, nectar-feeding vertebrates (passerine birds and lacertid lizards) with a larger body size, higher mobility, and later acting shorter interaction timing relative to the invertebrate antagonists (moths, ants, and beetles). We expect that the effects of antagonists will be more aggregated in space than those of mutualists due to their restricted movement during interactions, which occur primarily during the larval stages. Moreover, spatial patchiness in the antagonistic interactions will determine the spatial structure of later acting mutualists. Higher intimacy of interaction with plants, along with their precedent action, will favor a greater dependence of antagonists on plant characteristics and stronger relative effects on the spatial variation of PRS compared to mutualists.

## METHODS

### *Study system*

*Isoplexis canariensis* is an endemic perennial shrub from the Canary Islands, with a candelabra-shaped growth. This species is generally associated with canopy openings, showing a patchy distribution in the laurel and pine forests on the island of Tenerife (ATLANTIS 3.1, *available online*)<sup>2</sup>. Although plants can produce seeds through spontaneous autogamy, their legitimate pollinators (passerine birds and lacertid lizards) significantly increase fruit and viable seed production (Rodríguez-Rodríguez and Valido 2008, Ollerton et al. 2009, Rodríguez-Rodríguez et al. 2013). Its fruits are multi-seeded capsules that remain attached to the infructescence from one season to another, and seed dispersal occurs mainly due to gravity.

The animal assemblage that interacts during the predispersal stage is composed of two functional groups, mutualists and antagonists, both of which include

<sup>2</sup> <http://www.biodiversidadcanarias.es/atlantiss/>

several animal guilds. On one hand, mutualist guilds include legitimate bird (e.g., *Phylloscopus canariensis* [Hartwig, 1886], Phylloscopidae) and lizard pollinators (*Gallotia galloti* [Oudart, 1839], Lacertidae), and facultative bird pollinators that occasionally rob nectar (e.g., *Cyanistes teneriffae* [Lesson, 1831], Paridae). On the other hand, antagonist guilds include floral herbivores (Lepidoptera larvae), nectar larcenists (Formicidae), and predispersal seed predators (Coleoptera larvae; Appendix A).

#### *Study site*

The study was done in the protected area of Teno Rural Park from May to September 2008 (Teno Alto, 870 m above sea level, NW Tenerife). We selected this area for two main reasons: (1) plant patches are relatively large and representative of the natural distribution of *I. canariensis*, and (2) lizard activity is high compared to other populations covered by denser canopies. The local plant community is classified as a fayal-brezal forest, with *Erica arborea* L. (Ericaceae), *Morella faya* (Aiton) Wilbur (Myricaceae), and *Ilex canariensis* Poir. (Aquifoliaceae) as the dominant tree species. The insect-pollinated shrub *Cistus monspeliensis* L. (Cistaceae) is the primary co-flowering species (Appendix B: *Habitat*).

#### *Plant monitoring*

At the beginning of flowering period, we selected two patches of *I. canariensis* as replicate plots located ~50 m apart (patch 1, 8.9 × 13.7 m,  $n = 67$  plants; patch 2, 17.5 × 19.70 m,  $n = 52$  plants; Appendix B: *Replicate patches*). Inside each studied patch, we mapped all the reproductive plants to the nearest centimeter by laying out two perpendicular measuring tapes to record the  $x$ - and  $y$ -coordinates. In order to estimate the consequences of plant–animal interactions for each plant, we monitored all tagged individuals through the flowering period every three to four days until no open flowers remained within each patch (18 June to 15 August,  $n = 119$  plants). Each individual was surveyed 1–18 times, depending on flowering phenology. We tagged all inflorescences produced by a plant from the start of flowering period onwards and assigned each inflorescence a unique numeric identifier (total  $n = 384$  inflorescences). Anthesis proceeds from the bottom to the top of the inflorescence; as the lowest flowers wither and drop, new buds at the top of the inflorescence unfold. At the start of the study, inflorescences either had no open flowers, or had several basal flowers that were already open. In the first situation, all floral pedicels were tagged and included in the monitoring. In the second situation, flowers that were already open or withered were discarded and the remaining floral pedicels in bud stage were tagged. We also measured three plant characteristics (height, floral nectar production, and sugar concentration; Appendix C: *Methods*) to explore their potential influence on the spatial pattern of plant–animal interactions.

After the flowering season, we protected the infructescences with chicken wire cages (25 × 25 × 40 cm, 2.2 cm mesh) to estimate PRS and the losses derived from predispersal seed predators. Cage holes were large enough to allow Coleoptera to enter and potentially consume seeds, but small enough to prevent fruit predation by introduced rats (Appendix A: *Antagonists*). Once fruits ripened, they were collected immediately before capsule dehiscence, stored separately in paper bags, and taken to the laboratory.

#### *Definition of plant–animal interaction strengths*

For each animal guild, we estimated interaction strength as the frequency with which the specific guild interacted with individual plants, multiplied by its intensity. This definition captures both the frequency at which interactions occurred and the per-plant interaction effect (Vázquez et al. 2005). Both of these components (frequency and intensity) were expressed as proportions to facilitate inter-plant comparisons. Once the interaction strength was determined for the six animal guilds (see *Study system*), we estimated the interaction strength for each individual plant. The interaction strength between individual plants and the mutualist functional group was estimated as the sum of interactions with the three plant pollinators, and the interaction strength with the antagonist functional group was estimated as the sum of interactions with florivores, nectar larcenists, and predispersal seed predators.

#### *Interaction strength with mutualists*

The frequency and intensity of interactions were obtained from data collected by two alternative methods: focal and spot censuses. For focal censuses, each individual plant was observed at a minimum distance of ~3–15 m, with the observer camouflaged by the surrounding vegetation and equipped with binoculars. The observer was located at fixed positions in the patch corners to minimize their influence on pollinator behavior. Individual plants were watched during 30-min periods from 09:00 to 21:00 hours (range: 25–271 periods/plant). In total, over 12 580 individual plant censuses were conducted. For spot censuses, the observer stood in a corner of the patch at different times of the day and took a visual snapshot of all plant–pollinator interactions occurring within the patch at that moment (range: 25–186 snapshots/plant). In this case, 13 782 spot censuses were conducted. In both types of methods, the range in the number of censuses among individual plants was large as a result of the variable duration of plant flowering phenologies. In addition, we recorded the proportion of flowers probed by the individual pollinator per plant visit in relation to the total number of open flowers on the plant whenever possible.

For each plant, we compiled both the total number of focal and spot censuses (for frequency estimation), and

the total plant visits in which we recorded the proportion of flowers probed by the pollinator (for intensity estimation). In doing so, the frequency of interaction was defined as the proportion of total censuses in which the pollinator guild fed on the plant. The intensity of interaction was defined as the average proportion of flowers probed per plant visit, calculated across all plant visits recorded.

*Interaction strength with floral herbivores and nectar larcenists*

For the estimation of the frequency and intensity of antagonistic interactions, we used data collected from the periodical plant surveys (see *Plant monitoring*). In each plant survey, we recorded: (1) the total number of open flowers, (2) the number of inflorescences with at least one open flower, (3) the presence or absence of antagonistic damage, (4) the number of flowers affected by floral herbivores, and (5) the number of inflorescences affected by nectar larcenists. Floral herbivory was visually identified by the existence of chewing damage on floral reproductive organs, silk and frass remains, corolla holes, and/or the presence of caterpillars inside the flower. Nectar larceny was identified by the presence of at least one ant feeding on nectar. We recorded the effect of nectar larceny at the inflorescence instead of the floral level because ants, when present, often visit all flowers within the inflorescence.

From the recorded information, we estimated the frequency of interaction as the proportion of surveys in which floral herbivory or nectar larceny was detected in the individual plant. The estimation of the intensity of interaction varied depending on the antagonistic guild. In the case of floral herbivores, we estimated the intensity of interaction as the average proportion of damaged flowers with respect to the total number of open flowers, calculated across all plant surveys. In the case of nectar larcenists, we estimated the intensity of interaction to be the average proportion of inflorescences with at least one ant consuming nectar with respect to the total number of inflorescences, calculated across all plant surveys.

*Interaction strength with predispersal seed predators*

To estimate seed predation, we used resampling techniques on infestation data obtained from the harvested fruits at the end of fieldwork. To obtain data on infestation rates, we chose a subset of fruits per plant located at basal positions in the infructescences ( $n = 2042$ ; range: 3–23 fruits/plant; 34% of plants produced less than 20 fruits) and identified those that were infested ( $n = 185$  fruits). We selected basal fruits to avoid PRS underestimation caused by the heterogeneous resource allocation within the inflorescence due to positional effects. Fruit infestation was visually recognized by the presence of larvae (dead or alive), frass remains, partially consumed seeds, and/or holes in the capsule. Then, we estimated the proportion of viable seeds

consumed by seed predators with respect to the initial number of viable seeds produced by the fruit (Appendix D: *Methods*).

Once the levels of infestation in the field were determined, we obtained “simulated surveys” by resampling the data associated with the collected fruits (infested and noninfested) from the same plant; measurements included the presence or absence of fruit predation, and the respective proportion of viable seeds consumed. The simulated surveys were repeated as many times as the number of real periodical surveys done on the plant, and each simulated survey contained the same number of harvested fruits per plant. For each simulated survey, we calculated the proportion of infested fruits and the average proportion of seeds consumed per fruit. At the end of all surveys, we defined the frequency of interaction as the average proportion of infested fruits, and the intensity of interaction as the average proportion of viable seeds consumed per fruit; these values were also calculated across all the surveys.

*Plant reproductive success*

We estimated plant reproductive success (PRS) as the product of two female fitness components: fruit set  $\times$  viable seed set. Both components were expressed as proportions to facilitate inter-plant comparison. The fruit set was estimated as the proportion of monitored floral pedicels that set fully developed fruits that were either infested by floral herbivores or noninfested ( $n = 8478$  floral pedicels, range: 8–606 pedicels/plant). The number of floral pedicels was obtained by counting the floral attachment points present on the dried infructescences, which corresponded to the sum of aborted floral buds and open flowers.

The viable seed set was estimated as the average proportion of viable seeds produced per fruit, that were either infested by seed predators or noninfested. For this estimation, we used the same subset of fruits collected per plant to determine the interaction strength with predispersal seed predators. We counted the number of viable and aborted seeds inside the fruit (see Rodríguez-Rodríguez and Valido 2008 for determination of seed viability). We then calculated the proportion of those seeds ( $P_{vs}$ ) that were viable as  $P_{vs} = S_v / (S_v + S_a)$ , where  $S_v$  is the number of viable seeds, and  $S_a$  the number of aborted seeds. Finally, we determined the viable seed set as the average  $P_{vs}$  calculated across all fruits analyzed in the plant.

*Data analysis*

We analyzed the data under the statistical framework of spatial point pattern analysis with Programita software (Wiegand and Moloney 2004, 2014), and that of generalized linear mixed-effects regression models with R software (R Development Core Team 2013). Throughout the paper, mean values are accompanied by their standard deviations unless otherwise indicated.



TABLE 1. Questions, predictions, null models, and methods used to study the spatial structure of plant–animal interaction strengths and their plant reproductive outcomes.

Question	Prediction	Analysis	Null model	Statistic	Variable and Fig. reference
1) Are plants randomly distributed?	Plants would be highly aggregated at close distances as a consequence of seed dispersal by gravity.	Univariate SPP	HPP	$O(r)$	$x$ - and $y$ -coordinates of plant spatial position (1A)
2) Are plant–animal interaction strengths spatially structured?	Antagonistic interactions would show stronger positive autocorrelation than mutualistic effects due to the more restricted mobility of invertebrate antagonists compared to vertebrate pollinators.	Univariate MCF	IM	$I_{m1m1}(r)$	mutualistic IS (1B) antagonistic IS (1C) legitimate bird pollinator IS (E2A) facultative bird pollinator IS (E2B) legitimate lizard pollinator IS (E2C) floral herbivore IS (E2D) nectar larcenist IS (E2E) predispersal seed predator IS (E2F)
3) Are mutualistic and antagonistic interaction strengths spatially associated?	Both interaction effects would be negatively correlated due to the earlier action of antagonists before pollinator activity; pollinators may avoid plant damage.	Bivariate MCF	IM	$I_{m1m2}(r)$	mutualistic IS and antagonistic IS (1D)
4) Are plant characteristics spatially structured?	Plant characteristics would be positively correlated at close distances due to, e.g., similar ecological context.	Univariate MCF	IM	$I_{m1m1}(r)$	plant height (E3A) nectar production (E3D) sugar concentration (E3G)
5) Are plant–animal interaction strengths conditioned by the spatial structure of plant characteristics?	Antagonistic interactions would show a stronger association with plant characteristics than mutualistic ones due to the higher intimate interaction of antagonists with plants.	Bivariate MCF	IM	$I_{m1m2}(r)$	mutualistic IS and plant characteristic (E3B, E, H) antagonistic IS and plant characteristic (E3C, F, I)
6) Are plant reproductive outcomes spatially structured?	Plant reproductive success would be structured primarily due to the spatial pattern of antagonistic effects.	Univariate MCF	IM	$I_{m1m1}(r)$	PRS (2A)
7) Is the spatial variation in plant reproductive outcomes associated with the spatial structure of plant–animal interaction strengths?	Plant reproductive success would be positively correlated with mutualistic effects, and negatively with antagonistic effects.	Bivariate MCF	IM	$I_{m1m2}(r)$	mutualistic IS and PRS (2B) antagonistic IS and PRS (2C)

*Notes:* For each analysis and study patch, we set as maximum  $r$  a distance not much longer than half of the length of the shortest side of each study patch. In our case, the shortest side was found in patch 1 (900 cm). We assigned a maximum  $r$  of 500 cm and estimated the summary statistics at distance bins of 10 cm and a 110 cm ring width for dr. Abbreviations are as follows: spatial point pattern, SPP; heterogeneous Poisson process, HPP; mark correlation function, MCF; independent marking, IM; interaction strength, IS; and plant reproductive success, PRS.

*Spatial point pattern analysis.*—We used marked point pattern analysis to resolve our central question as to what processes are behind the spatial variation of PRS. For this purpose, we created a data set for each replicate patch that included plants as a series of mapped point locations, and the estimated variables as quantitative marks assigned to each point. Data sets included plant characteristics, plant–animal interaction strengths, and PRS estimates.

We carried out analyses in two steps. First, we explored the spatial distribution without taking into account the marks associated with the plant to estimate the level of plant aggregation (Table 1, analysis 1; Appendix E: *Plant spatial distribution*). Based on plant distribution, we then analyzed the spatial structure of the quantitative marks in isolation (univariate pattern, e.g., PRS) or associated by pairs (bivariate pattern, e.g., mutualistic interaction strength and PRS) using mark

correlation functions (Table 1, analyses 2–7). We chose the Schlather's Index  $I(r)$  as the summary statistic for the correlations (Schlather et al. 2004, Wiegand and Moloney 2014), which is an analog of the classical Pearson coefficient and similar to Moran's  $I$ . In the first step, all pairs of plants separated by distances within a specific interval ( $r - dr/2, r + dr/2$ ) are determined, where  $dr$  is the ring width. For each pair of plants, indexed by  $k$ , we have a corresponding pair of marks ( $m_{k_i}, m_{k_j}$ ) where  $m_{k_i}$  is the mark of the first plant  $i$ , and  $m_{k_j}$  is the mark of the second plant  $j$ . Schlather's  $I$  is then the Pearson correlation coefficient of the two variables  $m_{k_i}$  and  $m_{k_j}$ , taken over all  $k$  pairs at the distance  $r$ . The correlation coefficient is then estimated for different values of  $r$  to obtain the final functional summary statistics  $I(r)$ . In the univariate version,  $m_{k_i}$  and  $m_{k_j}$  values are taken from the same mark in two different plants, denoted by  $I_{m1m1}(r)$ . In the bivariate version, the  $m_{k_i}$  is the second mark of the second plant  $j$ , denoted by  $I_{m1m2}(r)$  (Appendix E: *Mark correlation functions*).

Independently of the spatial analysis, we used noncumulative second-order statistics (Appendix E: *Technical settings*). We calculated the summary statistic per patch and combined the results from the two patches into a weighted mean value. The empirical values for each statistic were compared with theoretical values from the chosen null expectation following the Monte Carlo simulation. Significance was assessed by comparing the observed data with simulation envelopes from 999 simulated patterns of the null model ( $P = 0.05$ ). Since we simultaneously tested the null hypothesis at several scales of distance  $r$ , we used a goodness-of-fit test that collapses the scale-dependent information contained in the test statistics into a single index  $u_i$  to avoid Type I error inflation. There is a significant departure from the null model when the index rank of the observed pattern  $u_0$  is  $>950$  with  $\alpha = 0.05$  among all  $u_i$  (Appendix E: *Goodness-of-fit test*).

*Generalized linear mixed-effects regression models.*—After the spatial analysis of plant–animal interaction strengths and their outcomes, we were interested in determining the relative importance of animal functional groups on the spatial variation of PRS. We hypothesized that antagonists would have a greater impact on plant fitness than mutualists would due to their earlier action and higher interaction intimacy with plants. For this, we linked the variation in plant reproductive outcomes with the interaction strengths via spatially explicit, generalized linear mixed models (GLMMs, nlme package; Pinheiro et al. 2013). We fit two independent models that had PRS as the response variable. One model used the interaction strengths with the two animal functional groups (mutualists and antagonists) as regressors, while the other model used the interaction strengths with the six animal guilds as regressors. Prior to model fitting, interaction strengths were  $z$  score relativized and tested for multi-collinearity via variance inflation factors (VIF, HH package; Heiberger 2013); VIF values were less than

two for all predictor variables. We assumed a normal distribution of errors with an identity link function, and we specified a replicate patch as a random factor to account for the disjunct distribution of plants. Plant coordinates were included in an exponential correlation function. This procedure allows us to accommodate plot differences into autocorrelation distances, and assumes autocorrelation only between plants within the same plot (e.g., Dormann et al. 2007; Appendix F: *Methods*).

## RESULTS

### *Spatial pattern of mutualistic and antagonistic interaction strengths and their association*

*Isoplexis canariensis* plants were more densely distributed than expected by chance between 0 and 40 cm (rank = 965,  $P = 0.036$ ; Fig. 1A). Most plants (84%) interacted with both mutualists and antagonists ( $n = 100$  plants; Appendix D: Table D1, Appendix E: Fig. E1A–C). The univariate spatial correlation analysis revealed that the interaction strengths with mutualists and antagonists were significantly structured (Fig. 1B, C). Plants separated 30–290 cm were more similar in their interaction strength with mutualists than randomly expected ( $P < 0.05$  for all  $r$  distances; Fig. 1B), showing a strong positive correlation (rank = 998,  $P = 0.003$ ). Plants were also positively correlated in their antagonistic interactions within a similar range of distances ( $P < 0.05$  for all  $r$  distances; Fig. 1C), but the spatial signal was less marked than the mutualistic interaction (rank = 981,  $P = 0.020$ ). When considering the relationship of both interaction types, we found a significant negative association between the spatial patterns of mutualistic and antagonistic interaction strengths (rank = 998,  $P = 0.003$ ), up to 290 cm ( $P < 0.05$  for all  $r$  distances; Fig. 1D).

### *Spatial pattern of PRS and its association with plant–animal interaction strengths*

Female plant fitness (fruit set  $\times$  viable seed set) was moderate in the study population ( $43\% \pm 20\%$  viable seeds per plant, range = 0–91%). The univariate spatial correlation analysis of PRS detected a significant deviation from the null model (rank = 995,  $P = 0.006$ ; Fig. 2A), where plants separated between 40 and 270 cm were more similar in female PRS than expected by chance ( $P < 0.05$  for all  $r$  distances).

When we compared the mutualistic interaction strength with PRS by bivariate correlation (rank = 999,  $P = 0.002$ ), we found a significant positive association up to 310 cm ( $P < 0.05$  for all  $r$  distances; Fig. 2B). For the antagonistic interaction strength (rank = 1000,  $P = 0.001$ ), we detected a significant negative correlation to PRS of up to 270 cm ( $P < 0.05$  for all  $r$  distances; Fig. 2C).

### *Relative importance of animal assemblage composition on PRS*

Differences among *Isoplexis* individuals in their plant–animal interaction strengths translated into a

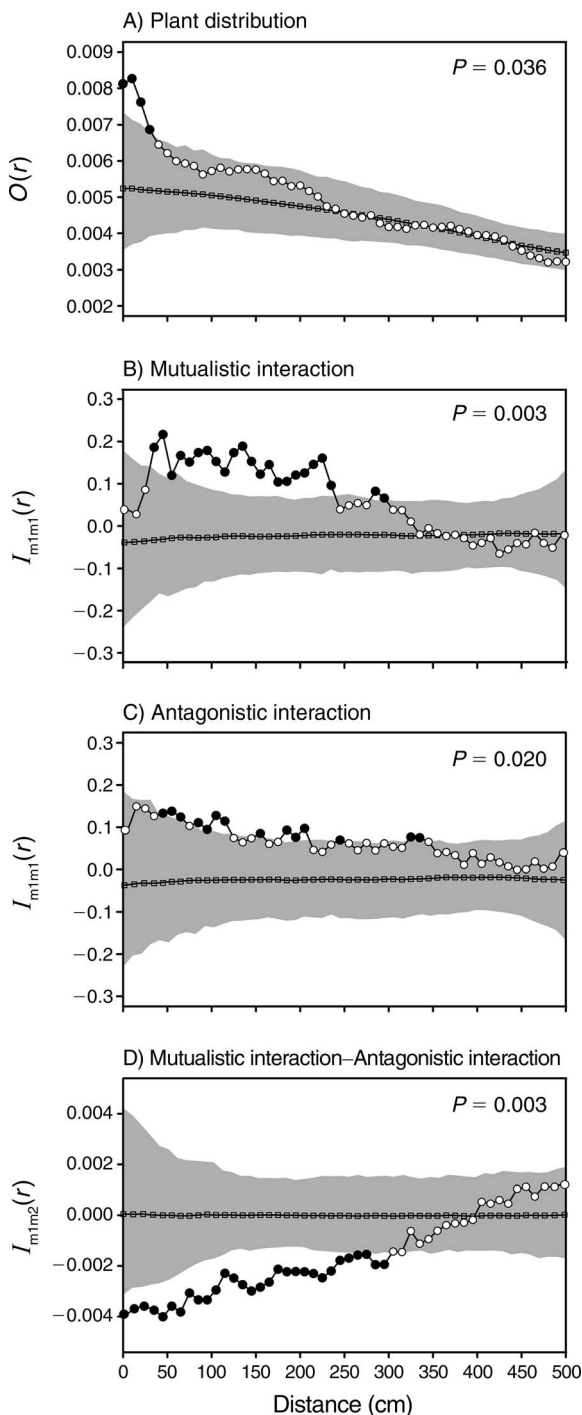


FIG. 1. Spatial analysis of *Isoplexis canariensis* (Plantaginaceae) distribution in the study population and associated quantitative marks. For mark correlation analysis, we used Schlather's  $I$ , which is analogous to the Pearson correlation coefficient of two values  $m_{k_i}$  and  $m_{k_j}$  from the same (univariate correlation) or different variables (bivariate correlation), taken over all  $k$  pairs of plants at the distance  $r$ . (A) Univariate point pattern analysis of plants with the  $O$ -ring statistic  $O(r)$  using Heterogeneous Poisson Process as the null model. (B, C) Univariate mark correlation analysis with Schlather's Index  $I_{m1m1}(r)$  of the interaction strength with (B)

difference in maternal fitness. For the year studied, the effects of the mutualistic and antagonistic interaction strengths on PRS were statistically significant. The two functional groups had coefficients with opposing signs, and the effect of antagonists ( $\beta = -0.086$ ,  $P < 0.001$ ) was  $>2.5$  times that of mutualists ( $\beta = 0.035$ ,  $P = 0.032$ ; Appendix F: Results).

When analyzed by animal guild (Fig. 3), we found that only the facultative bird pollinators increased PRS among mutualists ( $P = 0.006$ ). The estimated effects of legitimate bird and lizard pollinators were not significant ( $P > 0.05$ ). Among antagonists, floral herbivores had the greatest negative effect on PRS ( $P < 0.001$ ). The incidence of nectar larceny was also significantly negative ( $P = 0.032$ ), although relatively lower than floral herbivory. In contrast, the effect of predispersal seed predators was not strong enough to produce a significant impact ( $P > 0.05$ ).

#### DISCUSSION

The outcomes of plant–animal interactions can display a marked spatial structure due to two main influences: the spatial distribution of reproductive plants, and the spatial foraging patterns of interacting animals. We found that the interaction outcome (i.e., female PRS) was spatially structured within the studied plant population. Our results indicate that the combined interaction strengths between plants and their animal mutualists and antagonists contributed to this spatial pattern, rather than PRS reflecting the action of any specific animal partner. However, the influence of antagonists on PRS was more marked, especially that of floral herbivores, which damaged reproductive structures well before interactions with pollinators actually occurred. Thus, the earlier timing of antagonistic interactions ultimately shapes the later acting effects of mutualists, with clear consequences on the spatial variation of PRS.

#### *Spatial pattern of mutualistic and antagonistic interaction strengths and their association*

The positive autocorrelation detected in the mutualistic interaction strength closely reflects the spatial signal

mutualists and (C) antagonists, where subscript  $m1m1$  refers to the same mark in two different plants. (D) Bivariate mark correlation analysis with Schlather's Index  $I_{m1m2}(r)$  between the mutualistic and antagonistic interaction strengths, where subscript  $m2$  refers to the second mark of the second plant. We used independent marking as the null model in panels (B–D). Dots represent the mean-weighted summary statistic of the data, where black dots indicate values that are statistically different from the null model ( $P < 0.05$ ) and white dots indicate values that are statistically similar to those expected under the null model. Squares represent the expectation under the null model, and gray shading represents simulation envelopes marking the 25th lowest and highest values taken from 999 simulations of the goodness-of-fit test.

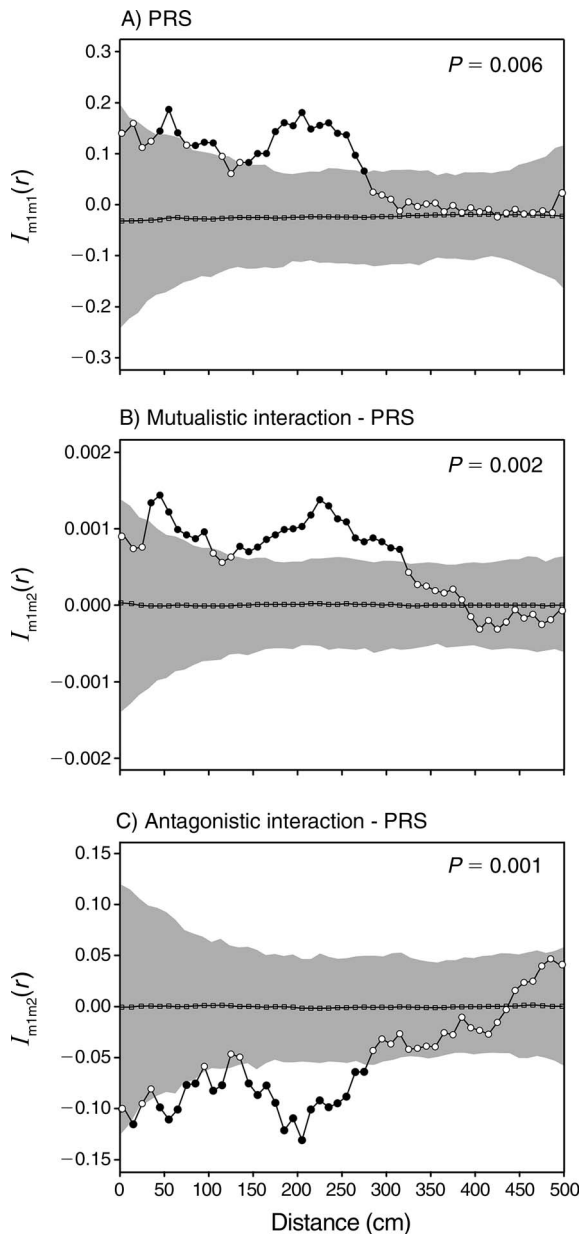


FIG. 2. (A) Univariate mark correlation analysis using Schlather's Index  $I_{m1m1}(r)$  of plant reproductive success (PRS). (B, C) Bivariate mark correlation analysis using Schlather's Index  $I_{m1m2}(r)$  between PRS and (B) the mutualistic interaction strength, and (C) the antagonistic interaction strength. See Fig. 1 for null models and symbol interpretations.

of legitimate pollinator activity by birds (Appendix E: Fig. E2A). This result, however, does not mirror the fine-scale plant aggregation revealed by the  $O$ -ring analysis, which identified small clumps of plants at  $<40$  cm (Fig. 1A, B). We can consider several, non-exclusive, explanations for the observed pattern in the mutualistic interaction strength. First, plant height, a surrogate of floral display size, was the unique plant characteristic positively correlated with the mutualistic

interaction strength up to 490 cm (Appendix E: Fig. E3B). This spatial association suggests a general preference for larger plants by pollinators, with more visible signals and abundant rewards (e.g., Brody and Mitchell 1997, Nattero et al. 2011). However, this correlation only coincided with the mutualistic signal up to 290 cm (Fig. 1B). A second explanation relates to ecological, morphological, and physiological restrictions on these pollinators during foraging (e.g., Leisler and Winkler 1991, Marchetti et al. 1995). Bird pollinators, especially *Phylloscopus canariensis*, frequently move with flights of 100–200 cm in length when visiting consecutive *Isoplexis canariensis* plants (Appendix G), a distance that overlaps the spatial signal in the mutualistic interaction strength. By maintaining this spacing of inter-plant movements, birds can maximize their rate of energy intake and avoid long, costly flights while balancing the required metabolic demand (e.g., Zimmerman 1981). Finally, the mutualistic signal may be explained more parsimoniously by the earlier action of antagonists that alters plant attractiveness for pollinators. It is known that bird pollinators can detect the detrimental effects on plants of antagonists (e.g., Irwin 2000), such that inter-plant movements may be spatially constrained to avoid plants with damaged flowers. The resulting negative correlation between the mutualistic and antagonistic interactions up to 300 cm supports this hypothesis (Fig. 1D; Appendix E: Fig. E1B–C).

The antagonistic interaction strength was also spatially structured, but this signal was less pronounced compared to that of mutualists. Contrary to our expectation, we found a weak autocorrelation over short distances. This result contrasts with frequent reports of markedly structured distribution of antagonistic interactions involving invertebrates (e.g., Rausher et al. 1981, Rossi et al. 2011), though several explanations may account for this difference. For example, plant characteristics influence the spatial pattern of plant selection by antagonists (e.g., Gómez et al. 2009, Muola et al. 2010). In our system, we detected that the antagonistic interaction was negatively correlated with plant height and positively correlated with nectar production at the same distance interval as the spatial signal of its interaction strength (Appendix E: Fig. E3C, F; Fig. 1C). These opposing associations may constrain the emergence of a stronger spatial structure in the damage caused by antagonists. An alternative explanation may lie in the level of phenological synchrony among plants, which can influence the use of hosts by insects (e.g., Russell and Louda 2004). The flowering phenology of *I. canariensis* was considerably synchronized among conspecifics (flowering synchrony index, adapted from Augspurger [1983]: patch 1 = 0.80; patch 2 = 0.69). This synchronization may favor the propagation of damage within the patch. High antagonistic abundance can also account for the observed pattern since we found that virtually all individual plants (97%) interacted with these detrimental agents. The high



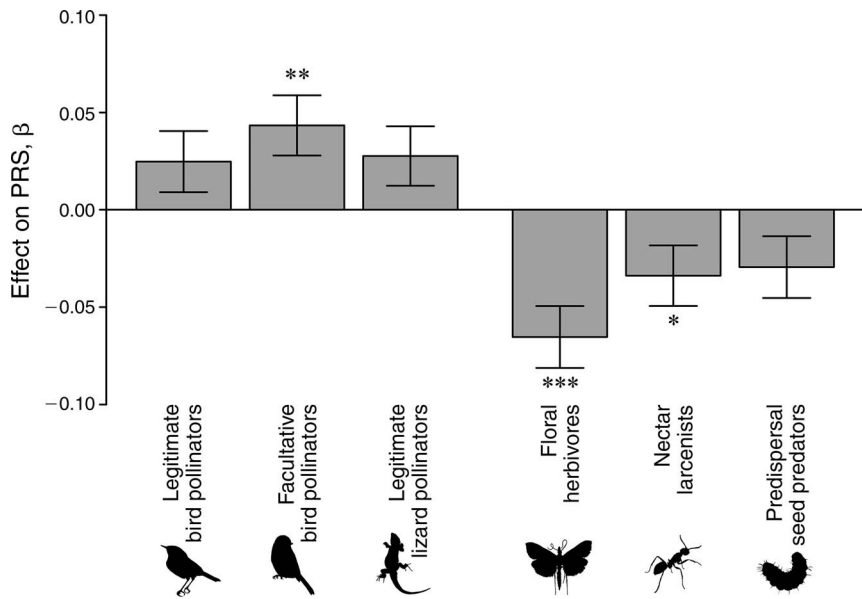


FIG. 3. Estimated effects of the interaction strengths of mutualistic and antagonistic animal guilds on plant reproductive success (PRS) of *Isoplexis canariensis*, obtained with spatially explicit, generalized linear mixed regression. Values represent the regression coefficients ( $\beta$ ) of the individual interaction strengths accompanied by their standard error ( $\pm$ SE). Statistical significance is indicated as: \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ . See Appendix A for the taxonomic composition of animal guilds.

prevalence of antagonists may counterbalance the marked positive correlation at short distances expected by damage caused by less mobile larvae.

Earlier theoretical studies suggest that antagonists can influence the spatial pattern of later acting mutualists (e.g., Wilson et al. 2003), despite having a weaker spatial signal (present study). Most plants within the patches (84%) interacted with both functional groups, but the highest values of the mutualistic interaction strength showed a marked spatial segregation from the highest values of antagonistic interactions. These results suggest that antagonists create hotspots of damaged plants that deter later acting pollinators. Bird pollinators tend to avoid floral displays damaged by invertebrate antagonists (Irwin 2000). This would make plants with mixed assemblages (vertebrate mutualists, invertebrate antagonists) more likely to experience stronger negative effects compared to plants with solely invertebrate assemblages (Irwin et al. 2001).

#### *Spatial pattern of PRS and its association with animal interaction strengths*

Plant reproductive success showed a strong spatial signal that did not significantly match the spatial pattern of any specific animal functional group, rather reflecting their combined interaction effects. Two main results support this inference. First, the spatial patterns of mutualistic and antagonistic interactions were significantly associated with that of PRS at distances that matched the autocorrelation signal in PRS (up to 300 cm). Second, mutualists and antagonists showed a

negative spatial correlation in their interactions in the same interval. This can be explained by the temporal sequence of their interactions. The earlier action of antagonists allows them to shape the fine-grained spatial template of plant attractiveness on which mutualists later act. Thus, antagonists are crucial in determining small-grained spatial variation in plant fitness.

Our initial hypothesis that antagonists would have a greater influence on shaping the spatial variation of PRS was also supported by the regression analysis. The negative effects of antagonists had a stronger combined impact on PRS than that of mutualists, which had marginal but positive effects on PRS. However, not all mutualistic or antagonistic animal guilds were equally important when determining small-scale heterogeneity in plant fitness. Among the mutualists, the legitimate bird pollinator *P. canariensis*, had the highest interaction strength but a very limited effect on PRS. In contrast, the facultative bird pollinator *Cyanistes teneriffae*, was the sole mutualist with a significant beneficial effect on PRS, despite being an occasional nectar robber. Legitimate visitation by *C. teneriffae* resulted in a higher percentage of viable seeds per floral visit ( $57.7\% \pm 38.04\%$  viable seeds/fruit,  $n = 16$  fruits) than *P. canariensis* ( $35.7\% \pm 42.2\%$ ,  $n = 29$ ). Furthermore, the behaviorally flexible *C. teneriffae* may generate a lower predictability in its interaction strength and have a greater impact on PRS compared to fully legitimate bird and lizard pollinators.

Among antagonists, the three animal guilds had contrasting impacts in which earlier interaction timing

was associated with a greater impact on PRS. Thus, floral herbivores had the greatest negative effect on PRS, acting from floral buds to developing fruits, followed by nectar larcenists, whose effects occur simultaneously with pollinator activity. The effect of the late-acting agents, i.e., predispersal seed predators, was not large enough to result in a strong limitation of PRS. This ranking of relative effects has also been found in other systems in which floral herbivores have stronger effects on PRS than other subsequent antagonists and pollinators (e.g., Adler et al. 2001). The temporal sequence of interactions likely favors the detrimental effects of florivores to modulate the spatial variation in the strength and fitness consequences of plant–pollinator interactions. This influence can occur through the direct consumption of pollen grains and ovules, as well as by making flowers less attractive or accessible to their mutualistic partners (e.g., Krupnick et al. 1999).

### Conclusion

Our results provide novel insights into the importance of the ecological context in which plant–animal interactions occur as a determinant of reproductive outcomes for individual plants. The spatial structure in PRS emerged from the combined interactions with mutualists and antagonists, rather than from an intimate spatial association with any specific functional group. Antagonists create a complex landscape of hot and cold spots of plant attractiveness for mutualists that ultimately shape the spatial structure of plant fitness. We foresee the potential of antagonists in conditioning the small-scale heterogeneity of PRS to be especially important when: (1) the detrimental interactions of antagonists occur at earlier stages of the plant reproductive period compared to mutualists; (2) antagonists outnumber mutualists and interact with the majority of plants in the population, thereby promoting a high prevalence of floral damage that overrides the benefits provided by pollinators; and (3) mutualists (e.g., vertebrates) are highly mobile organisms with the ability to detect and avoid plants affected by antagonists. Plants located in antagonistic hot spots might be excluded from the mating network, with reduced seed production and potentially negative effects on plant population growth rate. Moreover, plant damage by antagonists that results in reproductive failure would entail a decreased effective population size by limiting the number of individuals actually reproducing. Studies on other systems including animal partners with different foraging patterns to our study will clarify whether or not antagonists consistently constrain plant–pollinator interactions on a small scale.

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#### SUPPLEMENTAL MATERIAL

##### Ecological Archives

Appendices A–G are available online: <http://dx.doi.org/10.1890/14-2467.1.sm>

María C. Rodríguez-Rodríguez, Pedro Jordano, and Alfredo Valido. 2015. Hotspots of damage by antagonists shape the spatial structure of plant–pollinator interactions. *Ecology* 96:2181–2191.

<http://dx.doi.org/10.1890/14-2467.1>

APPENDIX A. Description of animal mutualists and antagonists included in the study.

The functional group of mutualists includes three animal guilds: bird and lizard legitimate pollinators, as well as facultative bird pollinators. Antagonists are represented by floral herbivores, nectar larcenists, and predispersal seed predators (see Table A1 for species identities).

### Mutualists

Pollinators are basically represented by three native species: the Canary Islands chiffchaff *Phylloscopus canariensis* (Hartwig, 1886) (Phylloscopidae) and the lacertid *Galloti galloti* (Oudart, 1839) (Lacertidae) as legitimate pollinators, and the African blue tit *Cyanistes teneriffae* (Lesson, 1831) (Paridae) as facultative bird pollinator that occasionally robs nectar (Fig. A1A–C; Rodríguez-Rodríguez and Valido 2008, Rodríguez-Rodríguez et al. 2013). Birds are more effective pollinators than lizards, primarily due to their higher visitation frequency. However, lizards are comparable to birds in their qualitative pollination effectiveness, especially the adult lizards. Thus, flowers visited only once by a bird or an adult lizard set fruits and viable seeds per fruit in similar proportions (Rodríguez-Rodríguez et al. 2013).

Bird species differ in their foraging behaviors. In contrast to *P. canariensis*, *C. teneriffae* occasionally robs nectar making a hole at the corolla base (primary nectar robbery), or simply revisiting previously made holes (secondary nectar robbery) (Fig. A2A). For this, the passerine sometimes tears the corolla to feed on nectar, a destructive behavior also recorded for other native Canarian plants (e.g., *Navaea phoenicea* (Vent.) Webb & Berthel., González and Fuertes 2011). This mixed mutualistic-antagonistic behavior appears to be not fixed in *C. teneriffae*. While some individuals always behave as pollinators or nectar robbers, others change from mutualistic to antagonistic foraging even from flower to flower within the same inflorescence. Both adults and juveniles show pure and mixed behaviors (*personal observation*).

Five more passerine bird species also visit the flowers of *Isoplexis canariensis*: *Sylvia melanocephala* (Gmelin, 1789), *S. atricapilla* (Linnaeus, 1758), *S. conspicillata* Temminck, 1820 (Sylviidae), *Fringilla coelebs* Linnaeus, 1758, and *Serinus canaria* (Linnaeus, 1758) (Fringillidae). However, their visitation rate is marginal or practically absent in the study area (Rodríguez-Rodríguez and Valido 2008). All these passerines also act as potential legitimate pollinators except *S. canaria*, which makes both legitimate and illegitimate floral visits as described for *C. teneriffae*.

### Antagonists

Floral herbivory is caused by Lepidoptera larvae (Fig. A1D). In our study area, we have recorded floral damage by three moth species: the native *Gymnoscelus rufifasciata* (Haworth, 1809) (Geometridae) and *Hypena obsitalis* (Hübner, [1813]) (Noctuidae), and the Canarian endemic *Choristoneura simonyi* (Rebel, 1892) (Tortricidae). Most of observed larvae during fieldwork belonged to *G. rufifasciata*. This species is distributed in Asia and Europe, including the Canary Islands, and adults may fly throughout the year (Argaman and Wysoki 1991, Báez 1998). This polyphagous moth also feeds on the close continental relatives of *Isoplexis* species (e.g. *Digitalis purpurea* L., Petschenka 2010). Although both plant genera are cardenolide rich, this generalist geometrid possibly detoxifies these compounds (Petschenka 2010). Female adults usually oviposit one egg per floral bud in *I. canariensis*, although eggs may be also present on other inflorescence parts. When feeding on buds, larvae usually ‘enclose’ the flower with silk so they prevent future floral opening and alter its development. The early instar caterpillars feed inside the anthers or the ovary, which are apparently used as protective sites when larvae are very small. As they grow up, larvae leave the reproductive organs and may feed on other floral tissues (e.g., filaments, style, corolla, sepals). Larvae may even move to mature flowers or unripe fruits, causing sometimes the complete loss of gametes and fertilized ovules on an inflorescence (Fig. A2B–E). Fully-grown caterpillars leave inflorescences to continue their development, as we have not seen pupae on the plant. As far as we have observed in the field, caterpillars feed exclusively on *I. canariensis* flowers, although they eat the leaves in captivity. Apart from Lepidoptera larvae, introduced mammal herbivores (e.g., goats, rabbits) and occasional human gathering (e.g., medicinal properties) also damage the inflorescences. However, both impacts were virtually absent in the study area.

Ants are frequent nectar larcenists during the flowering season, including the native species *Plagiolepis barbara* Santschi, 1911 and *Camponotus hesperius* Emery, 1893 (Fig. A1E), but also the introduced invader *Linepithema humile* (Mayr, 1868) (Formicidae). In their search for nectar, ants use holes previously made by nectar-robbing birds or florivorous caterpillars (secondary nectar robbing). They also visit the flower legitimately, but without contacting reproductive organs (nectar theft).

Coleoptera larvae consume developing and mature seeds prior to seed dispersal (probably fam. Kateritidae, Paolo Audisio *personal comment*; Fig. A1F). One larva is usually found per fruit, and before pupating, they excavate a tunnel later used to exit. Introduced rats (cf. *Rattus rattus* (Linnaeus, 1758), Muridae) are also predispersal seed predators on developing and ripe fruits. These rodents consume seeds on the infructescence or remove them from the plant, sometimes at further distances. Their damage can be so considerable that would prevent us to estimate the production of fruits and viable seeds in certain plants. This fact, along with their potential role as seed dispersers and introduced status, led us to exclude them from the present study.

TABLE A1. List of interacting animal species. We indicate their taxonomic status in the Canary Islands (Arechavaleta et al. 2010, Gill and Donsker 2014).

Functional group	Species	Family	Status
<b>Mutualists</b>			



Legitimate bird pollinators	<i>Phylloscopus canariensis</i>	Phylloscopidae	E
	<i>Sylvia melanocephala</i>	Sylviidae	N
	<i>Sylvia atricapilla</i>	Sylviidae	N
	<i>Sylvia conspicillata</i>	Sylviidae	N
	<i>Fringilla coelebs</i>	Fringillidae	N
Facultative bird pollinators	<i>Cyanistes teneriffae</i>	Paridae	N
	<i>Serinus canaria</i>	Fringillidae	N
Legitimate lizard pollinators	<i>Gallotia galloti</i>	Lacertidae	E
<b>Antagonists</b>			
Floral herbivores	<i>Gymnoscelis rufifasciata</i>	Geometridae	N
	<i>Hypena obsitalis</i>	Noctuidae	N
	<i>Choristoneura simonyi</i>	Tortricidae	E
Nectar larcenists	<i>Plagiolepis barbara</i>	Formicidae	N
	<i>Camponotus hesperius</i>	Formicidae	E
	<i>Linepithema humile</i>	Formicidae	I
Predispersal seed predators	NA	cf. Kateritidae	NA
	<i>Rattus rattus</i>	Muridae	I

Code: E, Endemic; N, Native; I, Introduced; NA, Not Available.

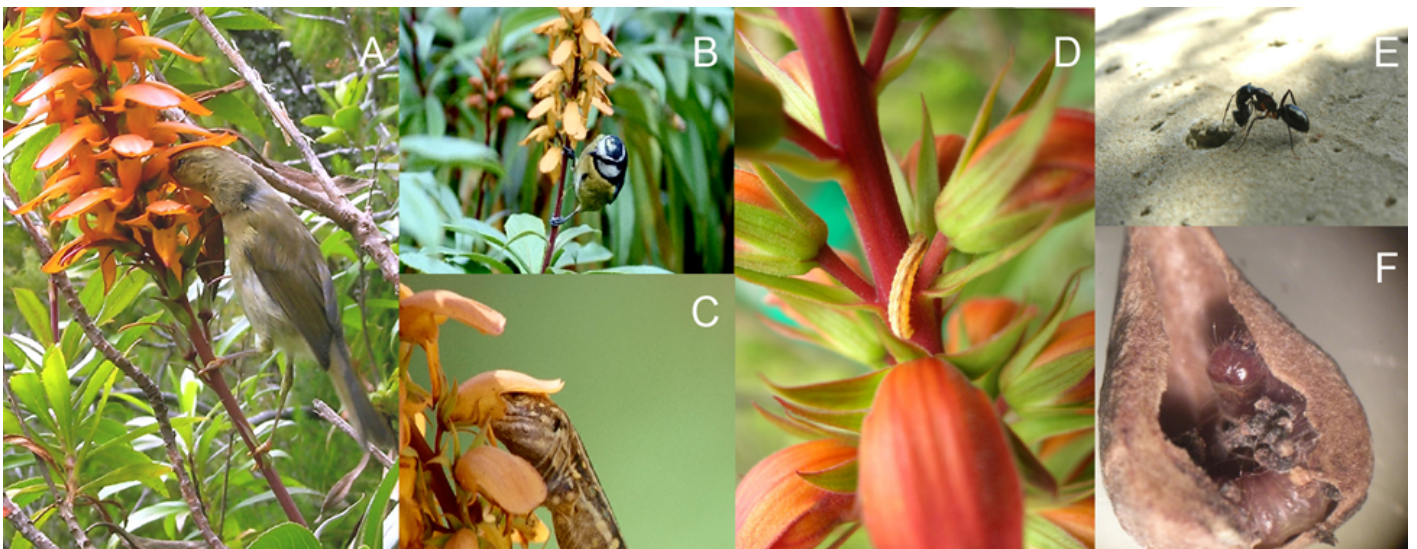


FIG. A1. Illustrations of the study interacting animals. Mutualists: (A) *Phylloscopus canariensis*, (B) *Cyanistes teneriffae*, (C) *Gallotia galloti*. Antagonists: (D) larvae of *Gymnoscelis rufifasciata*, (E) *Camponotus hesperius*, (F) Coleoptera larvae (cf. fam. Kateritidae). Photo credits: M. C. Rodríguez-Rodríguez (A, D–F), P. Felipe (B–C).

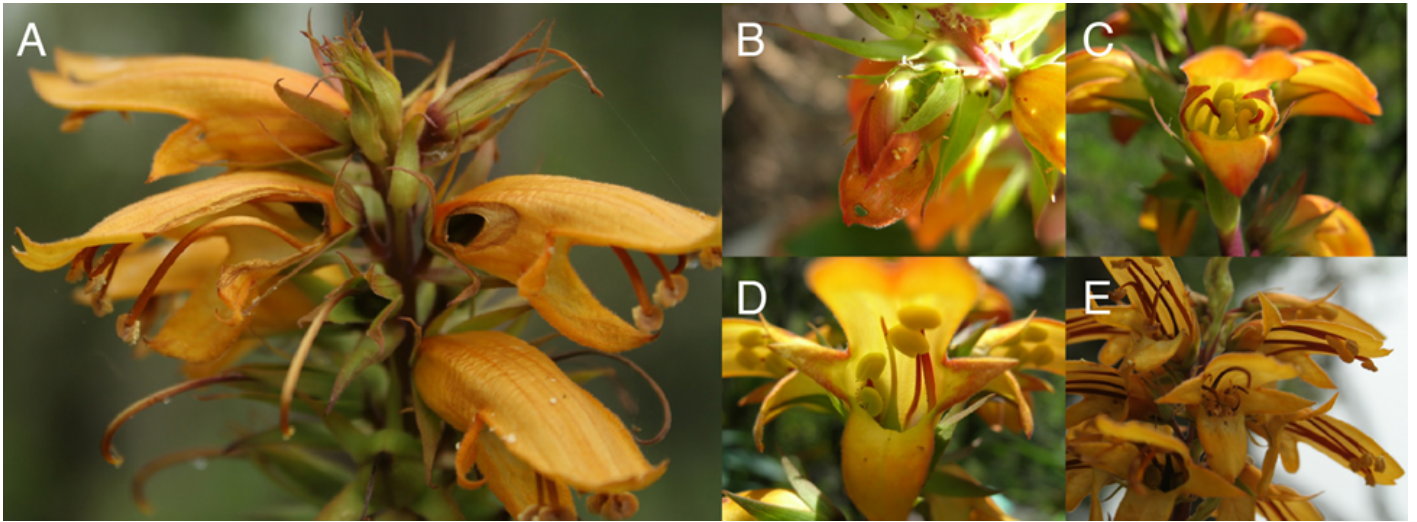


FIG. A2. Floral damage by the occasional nectar robber *Cyanistes teneriffae* (A) and floral herbivores (B-E) in different stages of floral development. Photo credits: M. C. Rodríguez-Rodríguez.

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APPENDIX B. Description of the study site and the replicate patches.

### *Habitat*

Teno Alto is located in the Teno massif, one of the oldest geological sectors of Tenerife (5.6–6.2 million years, Guillou et al. 2004). This locality, included in the protected area of Teno Natural Park, is characterized by an abrupt orography exposed to constant and intense winds and high insolation. Climate is Mediterranean, with hot and dry summers and mild winters. Mean annual precipitation is over 350 mm, with most of it falling between November and March. Annual average temperature is 19.4°C, with January being the coldest month (Marzol-Jaén, 1984).

The predominant vegetation is ‘fayal-brezal’ forest (Fig. B1). This forest represents a transitional stage of laurel forest located in its upper distributional limit. Its abundance is a consequence of the intense human disturbance (forest logging, farming and grazing activities) that swept away the original laurel forest in the locality. Thus, the ‘fayal-brezal’ has lower species richness compared to laurel forest, with a canopy dominated by the most pioneering tree species: *Erica arborea* L. (Ericaceae), *Morella faya* (Aiton) Wilbur (Myricaceae) and *Ilex canariensis* Poir. (Aquifoliaceae). Among the understorey species, the rockrose *Cistus monspeliensis* L. (Cistaceae) was the most frequent shrub coflowering with our study species, *Isoplexis canariensis* (L.) J. W. Loudon (Plantaginaceae). All plant species are pollinated by insects except *Canarina canariensis* (L.) Vatke (Campanulaceae), which is a vertebrate-pollinated creeper that flowers in winter (Rodríguez-Rodríguez and Valido 2011).

### *Replicate patches*

To collect the data, we mapped all the adult reproductive plants of *I. canariensis* present in two replicate patches, approximately 50 m apart (patch 1: 8.9 × 13.7 m, 67 individuals; patch 2: 17.5 × 19.70 m, 52 individuals; Fig. B2).

In the study area, there are several patches with similar conditions to the selected replicates. Most of them are very close together, although separated by natural barriers, e.g., rocky outcrops or forest fragments. We selected these close replicates for three main reasons: (i) to include a representative distribution of individual plants in nature (Fig. B2, see also Appendix E: Fig. E1A), (ii) to avoid highly contrasting conditions across *I. canariensis* individuals in climate, habitat structure, and composition of the surrounding plant community. In this way, we potentially control for these alternative factors and reduce their relative impact on PRS compared to the net effect of plant-animal interactions. Finally, (iii) for logistical reasons. Many patches are inaccessible because of the abrupt relief present in Teno massif, what makes it a region abundant in ravines and cliffs quite difficult for fieldwork.

Inside each studied patch, we mapped all the reproductive plants, excluding recruits, seedlings and juveniles from the analysis. Plants were spatially closer in patch 1 (averaged nearest neighbor distance: 54.6 ± 48.1 cm) than in patch 2 (90.6 ± 80.4 cm). In total, we monitored 119 adult plants, 384 inflorescences, and 8478 floral pedicels that included open flowers and floral buds that did not develop into open flowers.





FIG. B1. Summits of Teno Alto covered by ‘fayal-brezal’ forest (Teno Rural Park, Tenerife, Canary Islands). Note the sea of clouds blown by the northeast trade winds. Photo credits: M. C. Rodríguez-Rodríguez.

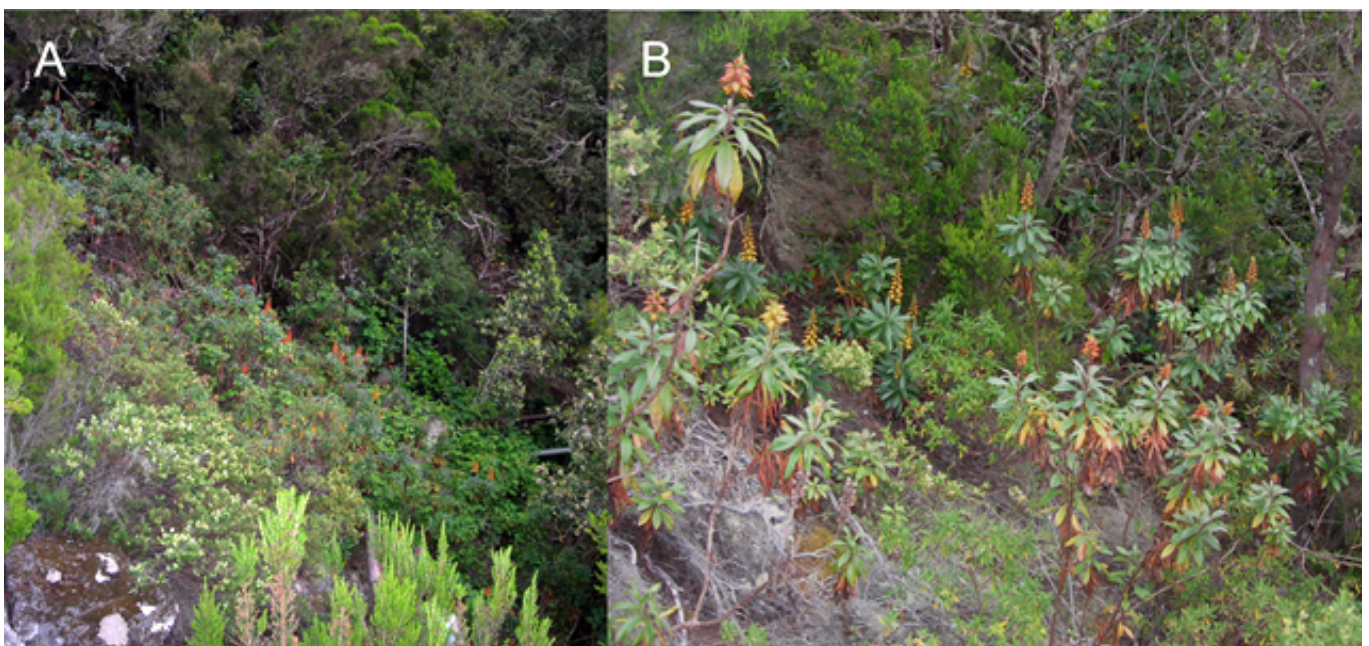


FIG. B2. Patchy distribution of *Isoplexis canariensis* in the replicate plots 1 (A) and 2 (B). The most frequent coflowering species was *Cistus monspeliensis* (Cistaceae) with white, insect-pollinated



flowers (foreground in patch 1). Photo credits: M. C. Rodríguez-Rodríguez.

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APPENDIX C. Description of the methodology used to estimate plant characteristics.

### *Methods*

For each individual plant, we measured its height and two variables related to animal reward: floral nectar production and nectar sugar concentration.

Plant height (cm) was measured as the distance from plant base to the tip of the highest inflorescence. We used this biometric variable as a surrogate of floral display because it was positively correlated with the number of inflorescences (Spearman's rank correlation,  $r_s = 0.69$ ,  $P < 0.001$ ) and total number of floral pedicels ( $r_s = 0.61$ ,  $P < 0.001$ ).

For the estimation of floral nectar production and nectar sugar concentration, we selected a subset of flowers located at basal positions in the inflorescences that we depleted and bagged during 24h ( $n = 790$  flowers, range: 5-31 flowers/plant). In each flower, we used 50- $\mu$ L microcapillary tubes to probe the corolla base until no more nectar could be removed. Then, we measured with dial calipers the distance nectar had migrated up the tubes and converted it to volumes ( $\mu$ L). We spotted the nectar within the tubes on a handheld refractometer (Bellingham & Stanley Ltd, Tunbridge Wells, Kent, UK) to record its sugar concentration (w/w% of sugar equivalents). Calculated over all flowers analyzed in the plant, we defined nectar volume as the average number of microliters produced per flower ( $\mu$ L/flower), and nectar sugar concentration as the average percentage of sugar per flower (w/w% sugar/flower).

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APPENDIX D. Description of the methodology used to estimate the proportion of viable seeds consumed per fruit by predispersal seed predators, plus a table containing the estimated interaction strengths per animal guild.

**Methods**

*Estimation of the proportion of viable seeds consumed per fruit by predispersal seed predators*

We defined the proportion of viable seeds consumed by seed predators ( $P_{sc}$ ) in the infested fruits as  $P_{sc} = (S_i - S_r) / S_i$ , where  $S_i$  is the initial number of viable seeds produced by the fruit, and  $S_r$  the number of remaining viable seeds after larvae predation.

The estimation of  $P_{sc}$  was done in three steps. First, we separated the infested fruits in the respective plant (total  $n = 185$  fruits from 71 plants) from the uninfested ones, and counted the number of remaining viable seeds within the infested fruits ( $S_r$ ). Second, we predicted the initial number of viable seeds present in the fruit before predation ( $S_i$ ) by linear regression. For this, we used data only from uninfested fruits produced by those plants that interacted with seed predators, instead of data from all studied plants. This procedure avoided bias in the estimation of  $S_i$  caused by mother plant effects. Concretely, we fit a zero-inflated model with the number of viable seeds per uninfested fruit as response variable, and fruit width as explanatory variable (negative binomial family, log link function). After model fitting, we predicted  $S_i$  for the infested fruits substituting their respective values of fruit width into the regression equation. Third, we estimated  $P_{sc}$  using the above-mentioned formula after obtaining  $S_i$  and  $S_r$  values.

**Results**

TABLE D1. Estimates of the interaction strengths between *Isoplexis canariensis* and its animal mutualists and antagonists, accompanied by the proportion of plants that interacted with each functional group, or any of their animal guilds. See [Appendix A](#) for the taxonomic composition of animal guilds.

Interacting agent	Interaction strength						Plants	
	$n_{freq}$	Frequency	$n_{int}$	Intensity	Frequency × Intensity <sup>a</sup>	CV	$n_{pl}$	Proportion
<b>Mutualists</b>								
Legitimate bird pollinators	119	0.028 ± 0.030	115	0.162 ± 0.178	(4.6 ± 6.1)·10 <sup>-3</sup>	132.6	99	0.83

Facultative bird pollinators	119	0.002 ± 0.003	117	0.053 ± 0.136	(0.3 ± 0.8)·10 <sup>-3</sup>	266.7	35	0.29	
Legitimate lizard pollinators	119	0.002 ± 0.006	115	0.031 ± 0.079	(0.3 ± 0.9)·10 <sup>-3</sup>	300	30	0.25	
All mutualists	-	-	-	-	(5.2 ± 6.2)·10 <sup>-3</sup>	119.2	103	0.87	
<b>Antagonists</b>									
Floral herbivores	119	0.66 ± 0.33	119	0.18 ± 0.17	0.16 ± 0.17	106.3	112	0.94	
Nectar larcenists	119	0.06 ± 0.14	119	0.03 ± 0.09	0.01 ± 0.04	400	24	0.20	
Predispersal seed predators	118	0.09 ± 0.13	113	0.40 ± 0.42	0.08 ± 0.12	150	72	0.61	
All antagonists	-	-	-	-	0.25 ± 0.24	96	115	0.97	

*Notes:* Data included in the table are means ± SD. Frequency indicates the proportion of plant censuses (mutualists) or surveys (antagonists) in which the plant-animal interaction was observed. Intensity represents the proportion of flowers probed by mutualists per plant visit, or the proportion of reproductive units (flowers/inflorescences/fruits) damaged by antagonists per plant survey; see *Methods* in the article. Coefficient of variation (%) is calculated for the product of frequency and intensity. Sample sizes indicate the number of plants in which the interaction frequency ( $n_{freq}$ ) and intensity ( $n_{int}$ ) was estimated for the respective animal functional group or guild. a Due to logistic constraints, the data set contained missing values ( $n = 16$  out of 714 values, 15 plants) in the interaction strengths of mutualists (legitimate bird pollinators  $n = 4$ , facultative bird pollinators  $n = 2$ , legitimate lizard pollinators  $n = 4$ ) and antagonists (predispersal seed predators  $n = 6$ ). These values were estimated by multiple imputation using Bayesian regression models (Gaussian family; *mi* package in R, Su et al. 2011). The sample size  $n_{pl}$  indicates the number of plants (out of 119) that had contact with the corresponding animal functional group or guild.

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APPENDIX E. Detailed description of spatial point pattern analyses, plus additional results from univariate and bivariate analyses of plant–animal interaction strengths and plant characteristics.

## Methods

### Plant spatial distribution

We characterized plant spatial distribution with the  $O$ -ring statistic  $O(r)$  as summary statistic (Wiegand and Moloney 2004, 2014). This statistic estimates the expected density of points at distance  $r$  from the typical point of the pattern. It is estimated as  $O(r) = \lambda g(r)$ , where  $\lambda$  is the intensity of points in the pattern, and  $g(r)$  the pair-correlation function (Wiegand and Moloney 2004). We used the estimator with edge correction, so the number of points in an incomplete circle was divided by the proportion of the area of the circle that lies within the study region. The empirical values for the statistic were compared with the theoretical values from the chosen null model, the *Heterogeneous Poisson Process* (HPP). We chose this null model because plants were not distributed within the patches with a constant intensity  $\lambda$  (first-order heterogeneity) due to the presence of gaps at the edges of the rectangular patches (Fig. E1). The presence of rocky outcrops and plant individuals of different species to *Isoplexis canariensis* (L.) J. W. Loudon (Plantaginaceae) caused these gaps. In the HPP model, the constant  $\lambda$  of the homogeneous Poisson process is replaced by a function  $\lambda(x, y)$ , but the occurrence of any point remains independent of that of any other. In our case, the intensity function was estimated with a non-parametric method using the Box Kernel function and circular moving windows of radius = 300 cm (Wiegand and Moloney 2014).

### Mark correlation functions

Mark correlation functions are summary statistics adapted for quantitatively marked patterns, where a spatial point pattern (e.g., individual plants of a given species) carries a quantitative mark (e.g., plant reproductive success). The rationale behind these functions is simple: they give the mean values of a test function calculated from the marks of all plant pairs  $i$  and  $j$  that are separated by distance  $r$  (Illian et al. 2008). The estimate of the mean value is then repeated for different  $r$  distances. Therefore, it is necessary to choose an appropriate test function that characterizes the relationship between the marks  $m_i$  and  $m_j$  of plants  $i$  and  $j$ . In our case, we applied two types of test functions depending on whether the marks belonged to the same or different variables.

When we analyzed the spatial relationship among marks from the same variable, we used the Schlather's Index  $I_{m_1 m_1}(r)$  (Schlather et al. 2004, Wiegand and Moloney 2014). In this case, the point pattern is univariate quantitatively marked because each plant has only one mark attached. The statistic  $I_{m_1 m_1}(r)$  looks at pairs of plants separated by distance  $r$  and compares the marks  $m_i$  from plant  $i$  and  $m_j$  from plant  $j$  for each plant pair with the actual mean mark resulting from all pairs of plants separated by distance  $r$ . A value of  $I_{m_1 m_1}(r) \sim 0$  would indicate there is absence of spatial correlation among the marks. In contrast, a value of  $I_{m_1 m_1}(r) > 0$  would indicate mutual stimulation, that is, plants are more similar in their mark values than expected by chance. Conversely, a value of  $I_{m_1 m_1}(r) < 0$  would indicate mutual inhibition.

When marks belonged to two different variables, we used the Schlather's Index  $I_{m_1 m_2}(r)$  (Schlather et al. 2004, Wiegand and Moloney 2014). The point pattern is bivariate quantitatively marked because each plant carries two marks instead of one. This approximation let us to explore if two joint properties (i.e., quantitative variables) of plants were spatially associated. Specifically, the statistic  $I_{m_1 m_2}(r)$  tests if the mark  $m_1$  at plant  $i$  (focal plant) and the mark  $m_2$  at plant  $j$  separated by distance  $r$  tend to be relatively similar or dissimilar compared to those of plant pairs taken at random. Again, a value of  $I_{m_1 m_2}(r) \sim 0$  would indicate there is absence of spatial autocorrelation among the marks  $m_1$  and  $m_2$ , but in this case the mark correlation function involves marks from different variables. In contrast, a value of  $I_{m_1 m_2}(r) > 0$  would indicate mutual stimulation, and a value of  $I_{m_1 m_2}(r) < 0$  would indicate mutual inhibition.

In both cases, we normalized the mark correlation function with the mark variance to make it independent of the distribution and values of the marks (Wiegand and Moloney 2014). In the univariate pattern, the normalization factor coincided with the variance of the variable  $\sigma$ , whereas in the bivariate pattern it represented the covariance of the marks  $\sigma_{12}$  that belong to different variables. The summary statistics were first estimated separately for each patch. Then, we combined them into a single mean-weighted spatial statistic because we were interested in the broad biological pattern rather than on the potential variability among replicate patches. The formulas used to combine mark-correlation functions are detailed in Wiegand and Moloney (2014). At the end, the empirical summary statistics were compared to those arising from the null model of *independent marking*, which shuffles the marks independently and randomly among all plant locations (Wiegand and Moloney 2014).

### Technical settings

Non-cumulative second-order statistics are based on the distance between all pairs of plants of a point spatial pattern, and they count the number of points in an annulus of radius  $r$  and width  $dr$  located at distance  $r$  from a focal plant, with  $r$  taking a range of scales. The advantage of these statistics is that the result at smaller scales does not bias the result at higher scales (Wiegand and Moloney 2004, 2014). In our case, we took into account that  $r < 1/2$  length of the shortest side of the study patch (patch 1, max  $r = 500$  cm), and estimated the summary statistics at distance bins of 10 cm and ring width of  $dr = 110$  cm. The bin value indicates the interval of distance at which the statistic is calculated from the focal point. We selected the

value of ring width to assure a minimum of 30 plant pairs per distance class. Note that we obtained similar results in all spatial analyses using a range of  $dr = 60\text{--}110$  cm.

### Goodness-of-Fit test

Independently of the spatial analysis, we compared the empirical values of the summary statistics to simulation envelopes arising from the corresponding null model. To create the envelopes, we used at every distance  $r$  the 25th highest and 25th lowest results for the chosen statistic (O-ring or Schlather's Index), calculated from 999 Monte Carlo simulations ( $P = 0.05$ ). The observed pattern is considered different to the simulated process if the results lie outside the envelope at any distance  $r$ . However, we cannot interpret the simulation envelopes as confidence intervals because we tested the null hypothesis at several distances simultaneously, and this approach greatly underestimates the type I error rate. As a complement to analyses based on simulation envelopes, we used a Goodness-of-Fit test (GoF). The GoF collapses the scale-dependent information contained in the test statistics into a single index  $u_i$  (Loosmore and Ford 2006). This index represents the total squared deviation between the observed summary statistic and the theoretical statistic under the null model across the distance interval analyzed (0–500 cm). The  $u_i$  was calculated for the observed data ( $i = 0$ ) and for the simulated data ( $i = 1 \dots 999$ ) and the rank of  $u_0$  among all  $u_i$  was determined. If the rank of  $u_0$  was  $> 950$ , there was a significant departure from the null model with  $\alpha = 0.05$ .

### Results

*Spatial representation of plants, plant–animal interaction strengths and associated plant reproductive outcomes.*

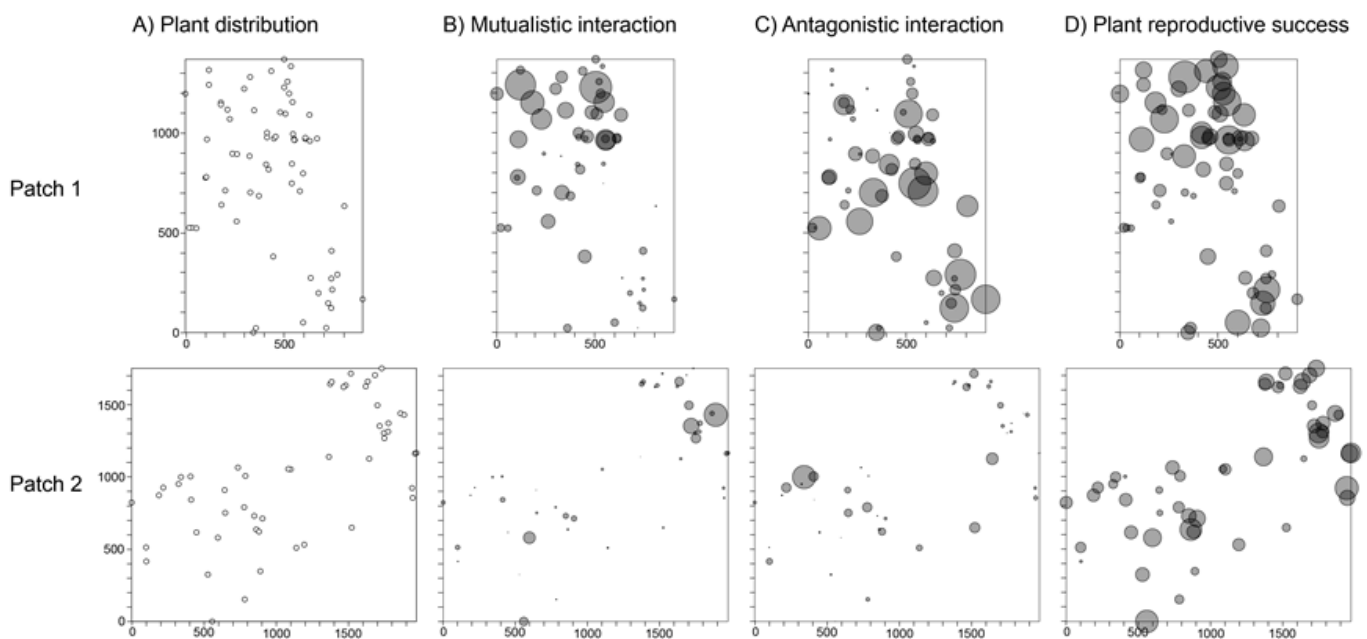


FIG. E1. (A) Spatial locations of individual *Isoplexis canariensis* adult plants in patch 1 ( $n = 67$ ) and patch 2 ( $n = 52$ ), along with their interaction strengths with mutualists (B) and antagonists (C), and associated plant reproductive success (PRS) (D). In (B–D), the circle size indicates the relative values in comparison to the largest value (mark) in the respective patch. This largest value was scaled to have a physical size of 80 units (*spatstat* package in R; Baddeley and Turner 2005). Patch dimensions are in cm.

### Spatial analysis of plant–animal interaction strengths per animal guild

When analyzed per mutualistic guild, we found that the interaction strengths with birds were spatially structured (Fig. E2A–B), but not that with lizards (i.e., *Gallotia galloti*; rank = 815,  $P = 0.186$ ) (Fig. E2C). In the interaction with legitimate bird pollinators (i.e., *Phylloscopus canariensis*, rank = 986,  $P = 0.015$ ), plants were positively correlated at 40–310 cm ( $P < 0.05$ , Fig. E2A). In the case of facultative bird pollinators (i.e., *Cyanistes teneriffae*, rank = 988,  $P = 0.013$ ), plants were positively correlated in their interaction strength at 0–20 cm and 240–280 cm, but negatively correlated at further distances 350–420 cm ( $P < 0.05$ , Fig. E2B).

A closer look to each antagonistic guild revealed the absence of spatial signal in the interaction strength with floral herbivores (rank = 453,  $P = 0.548$ ; Fig. E2D) and nectar larcenists (rank = 171,  $P = 0.830$ ; Fig. E2E). However, the interaction with predispersal seed predators was weakly structured (rank = 968,  $P = 0.033$ ), with plants positively correlated in their interaction strength at 30–100 cm ( $P < 0.05$ ; Fig. E2F).

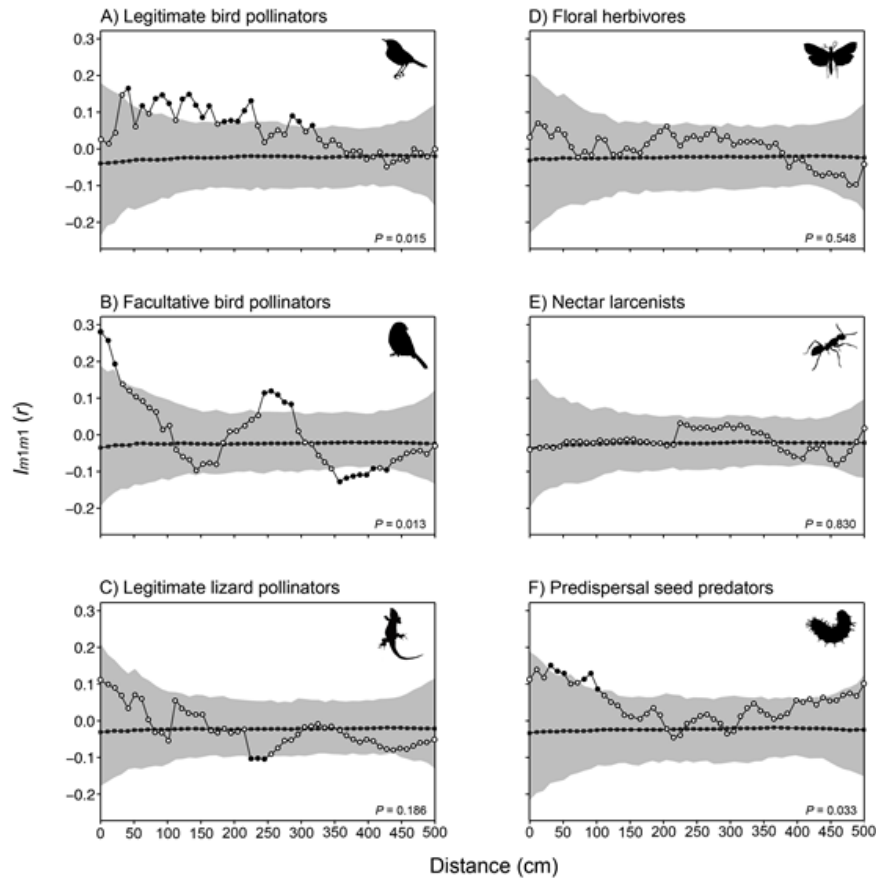


FIG. E2. Univariate mark correlation analysis using Schlather's Index  $I_{mlm}(r)$  of the interaction strength between *Isoplexis canariensis* plants and their mutualists: legitimate bird pollinators (A), facultative bird pollinators (B), and legitimate lizard pollinators (C); and between plants and their antagonists: floral herbivores (D), nectar larcenists (E) and predispersal seed predators (F). Dots, mean-weighted summary statistic of the data; black squares, expectation under the null model; and gray shading, simulation envelopes marking the 25th lowest and highest values taken from 999 simulations of the null model. Black dots indicate statistical difference from the null model ( $P < 0.05$ ), and  $P$  values indicate statistical significance of Goodness-of-Fit (GoF) test. See [Appendix A](#) for the taxonomic composition of animal guilds.

#### *Spatial analysis of plant characteristics, and their association with plant–animal interaction strengths.*

Average plant height was  $87.1 \pm 38.6$  cm (range = 26.5–220). Individuals had generally several inflorescences ( $3.8 \pm 4.3$  inflorescences, range = 1–25), with plants producing tens of floral pedicels ( $101.7 \pm 122.8$  pedicels, range = 8–828). When considering floral resources, *I. canariensis* plants had a nectar production of  $20.4 \pm 5.9$   $\mu\text{L}/24\text{h}/\text{flower}$  (range = 3.7–39.2), with  $29.6 \pm 6.2\%$  of sugar concentration (range = 17.3–50.2).

Results from the univariate mark-correlation analyses of plant characteristics revealed that nearby plants were more similar in height than randomly expected (rank = 1000,  $P = 0.001$ ), concretely at 0–350 cm ( $P < 0.05$ ; Fig. E3A). Relative to floral resources, we did not detect a significant spatial structure in nectar production (rank = 800,  $P = 0.201$ ; Fig. E3D), but we did in sugar concentration (rank = 1000,  $P = 0.001$ ; Fig. E3G). Concretely, plants separated by 0–260 cm were more similar in their sugar concentration than randomly expected ( $P < 0.05$ ).

In relation to the bivariate mark-correlation analyses, we detected that the mutualistic interaction strength was spatially associated with plant height (rank = 1000,  $P = 0.001$ ), showing a positive correlation at  $r > 60$  cm (Fig. E3B). The antagonistic interaction strength was also spatially associated with plant height (rank = 999,  $P = 0.002$ , Fig. E3C), and nectar production (rank = 960,  $P = 0.041$ , Fig. E3F). In the spatial association between the antagonistic interaction and plant height, we found a negative correlation at 90–340 cm ( $P < 0.05$ ). In the spatial association with nectar production, we found a positive correlation at 110–260 cm, and a negative correlation at 350–380 cm ( $P < 0.05$ ). The rest of spatial associations between animal interaction strengths and plant characteristics were not significant ( $P > 0.05$  for all GoF tests).

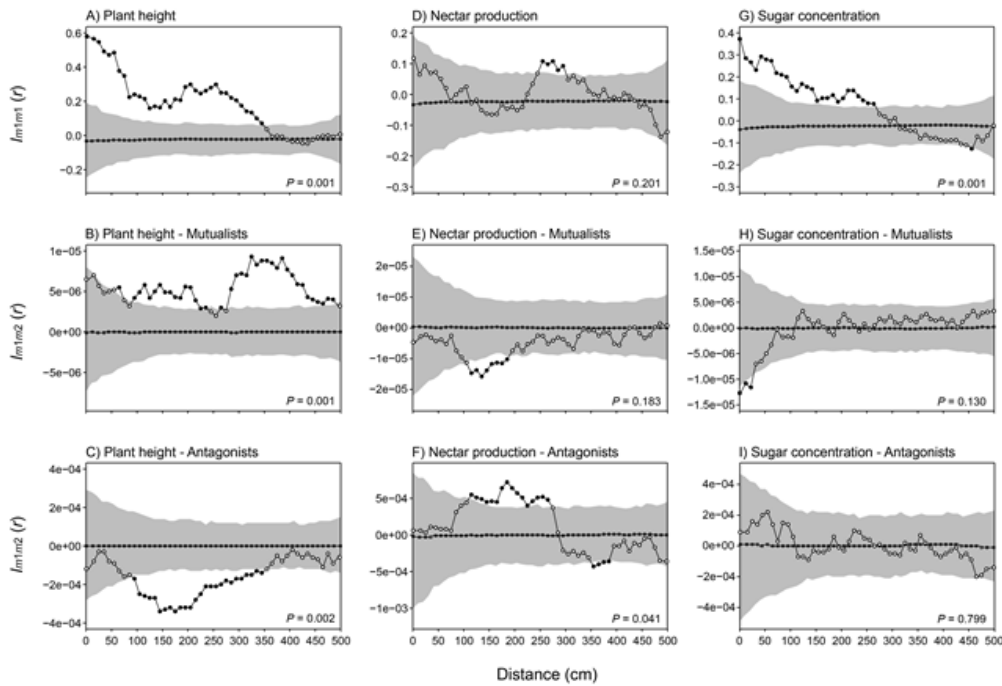


FIG. E3. Spatial analysis of plant characteristics and their association with plant–animal interaction strengths. Univariate mark correlation analysis using Schlather’s Index  $I_{m1m1}(r)$  of plant characteristics: plant height (A), floral nectar production (D), and nectar sugar concentration (G). Bivariate mark correlation analysis using Schlather’s Index  $I_{m1m2}(r)$  between plant–animal interaction strengths and plant height (B–C), nectar production (E–F), and sugar concentration (H–I). Symbol interpretation is given in the legend to Fig. E2.

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APPENDIX F. Further details on methods and results from generalized linear mixed-effects models.

**Methods**

We compared non-spatial and spatial generalized linear mixed-effects models (GLMMs) maintaining the same regressors (two functional groups or six animal guilds) to test if the addition of a spatial correlation term significantly improved model fit. In the spatial GLMMs, plant coordinates were included in an exponential correlation function. This procedure allows the accommodation of plot differences in autocorrelation distances, and assumes autocorrelation only between plants within the same plot (e.g., Dormann et al. 2007). The choice of the exponential over other spatial covariance structures was based on inspection of semi-variograms of non-spatial GLMM residuals. Although the addition of the spatial correlation term did not improve model fit in any case (anova test via analysis of variance,  $P > 0.05$  for all cases), we included in the article and the present appendix the results from the spatial GLMMs.

TABLE F1. Estimated effects of the interaction strengths with mutualists and antagonists on plant reproductive success (PRS) of *Isoplexis canariensis*, analyzed with spatially explicit GLMM ( $df = 115$ ). Independent variables were standardized to mean 0 and variance 1 and the dependent variable was relative PRS. Regression coefficients ( $\beta$ ) and their associated standard errors (SE) are given. Notes: \*  $P, < 0.05$ ; \*\*  $P, < 0.01$ ; \*\*\*  $P, < 0.001$ .

Source of variation	$\beta$ coefficient	SE	P value	
Interaction strength with mutualists	0.035	0.016	0.032	*
Interaction strength with antagonists	-0.086	0.016	< 0.001	***

TABLE F2. Estimated effects of the interaction strengths with mutualistic and antagonistic animal guilds on PRS of *Isoplexis canariensis*, analyzed with spatially explicit GLMM ( $df = 111$ ). Independent variables were standardized to mean 0 and variance 1 and the dependent variable was relative PRS. Regression coefficients ( $\beta$ ) and their associated standard errors (SE) are given. Notes: \*  $P, < 0.05$ ; \*\*  $P, < 0.01$ ; \*\*\*  $P, < 0.001$ . See [Appendix A](#) for the taxonomic composition of animal guilds.

Source of variation	$\beta$ coefficient	SE	P value	
Interaction strength with legitimate bird pollinators	0.025	0.016	0.119	

Interaction strength with facultative bird pollinators	0.043	0.015	0.006	**
Interaction strength with legitimate lizard pollinators	0.028	0.015	0.074	
Interaction strength with floral herbivores	-0.065	0.016	< 0.001	***
Interaction strength with nectar larcenists	-0.034	0.016	0.032	*
Interaction strength with predispersal seed predators	-0.029	0.016	0.067	

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**María C. Rodríguez-Rodríguez, Pedro Jordano, and Alfredo Valido. 2015. Hotspots of damage by antagonists shape the spatial structure of plant–pollinator interactions. *Ecology* 96:2181–2191. <http://dx.doi.org/10.1890/14-2467.1>**

APPENDIX G. Description of the methodology used to estimate the length of interplant flights by bird pollinators, and results obtained per bird species.

### *Methods*

Simultaneously to plant monitoring, we performed focal censuses on *Phylloscopus canariensis* (Hartwig, 1886) (Phylloscopidae) and *Cyanistes teneriffae* (Lesson, 1831) (Paridae) to characterize the movement pattern of bird pollinators among individual plants of *Isoplexis canariensis* (L.) J. W. Loudon (Plantaginaceae). For this, we recorded bird movements by direct watches for a total of 257 h over 34 days in the two study patches, from 09.00 to 21.00 h. The vegetation had an open structure over most of the study patches, which facilitated bird observation with binoculars from the patch corner. Once a bird had begun feeding within the patch, we recorded with a hand held dictaphone (i) the bird species, (ii) the identity of the visited plant, and (iii) whether the bird left the patch or moved to another tagged plant to probe more flowers. In the latter case, the second plant was noted and the sequence of visited plants continued. Those plants in which the bird perched without probing at least one flower were excluded from the sequence.

Each foraging sequence was later discretized into steps (plant-to-plant movement) to estimate the linear distance between two consecutively visited plants (step length). The sequence discretization let us compute for each bird pollinator species the following values (*adehabitatLT* package in R, Calenge 2006): (1) number of steps per sequence, and (2) interplant distances (step length) measured as the Euclidean distance (cm) between two consecutive visited plants. Then, we pooled all steps per pollinator to calculate the average distance flown between plants and its frequency distribution.

### *Results*

We recorded a total of 168 foraging sequences in the two study patches with > 1 visited plants. The passerine *P. canariensis* accounted for most of them ( $n = 153$  sequences), whereas *C. teneriffae* was a less frequent visitor ( $n = 15$  sequences). Most of plant-to-plant movements (steps) were 100–200 cm long (Fig. G1). The mean step length from the pooled data was  $266.2 \pm 250.2$  cm (median: 196.5 cm; Fig. G1), which exceeds the average distance to the nearest plant neighbor in the two study patches ( $70.3 \pm 66.4$  cm). There was a large overlap in the range of step length between *P. canariensis* (2.2–1,545.1 cm) and *C. teneriffae* (46.2–1,545.1 cm). However, *C. teneriffae* ( $375.7 \pm 387.6$  cm) tended to fly longer distances between consecutive plants than those of *P. canariensis* ( $259.1 \pm 237.7$  cm).

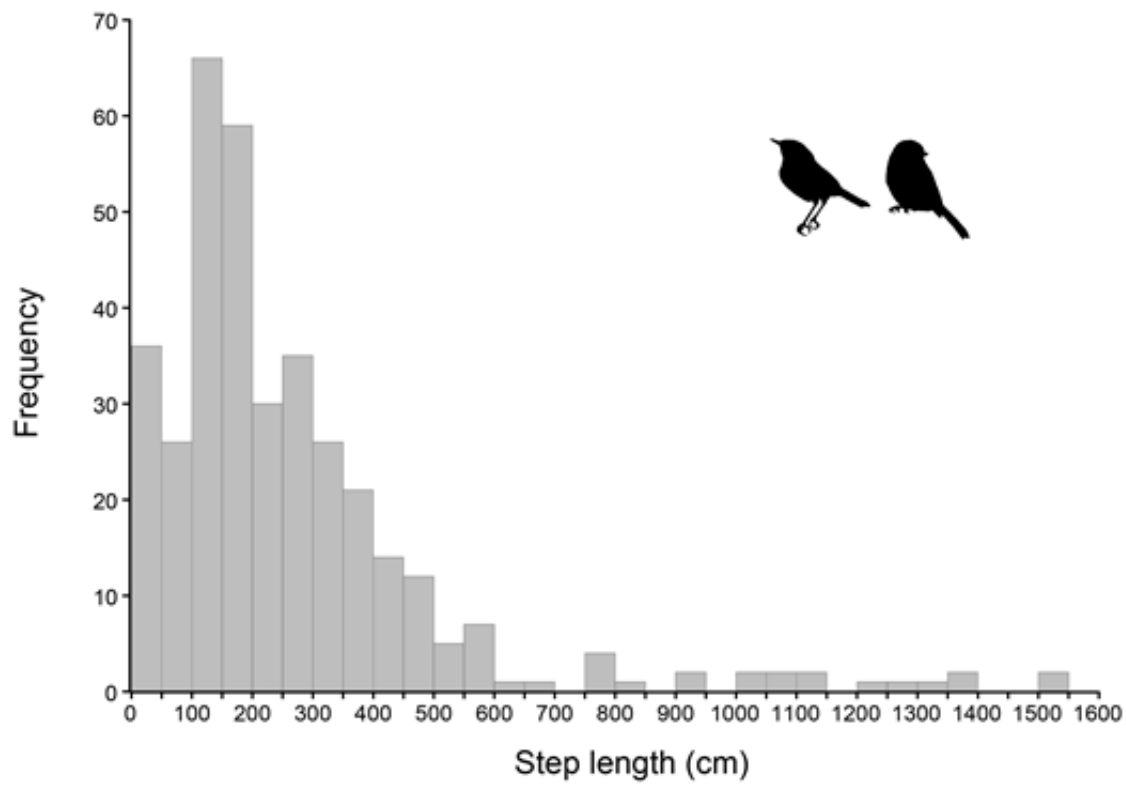


FIG. G1. Frequency distribution of plant-to-plant movements (steps) from the pooled data from *Phylloscopus canariensis* and *Cyanistes teneriffae* classified by step length (cm). The total number of recorded steps was 359 (*P. canariensis*  $n = 337$ , *C. teneriffae*  $n = 22$ ). Black silhouettes: *P. canariensis* on the left, *C. teneriffae* on the right.

#### LITERATURE CITED

Calenge, C. 2006. The package “adehabitat” for the R software: a tool for the analysis of space and habitat use by animals. *Ecological Modelling* 197:516–519.

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