

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*)². 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

² <http://www.biodiversidadcanarias.es/atlantis/>

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_j} 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

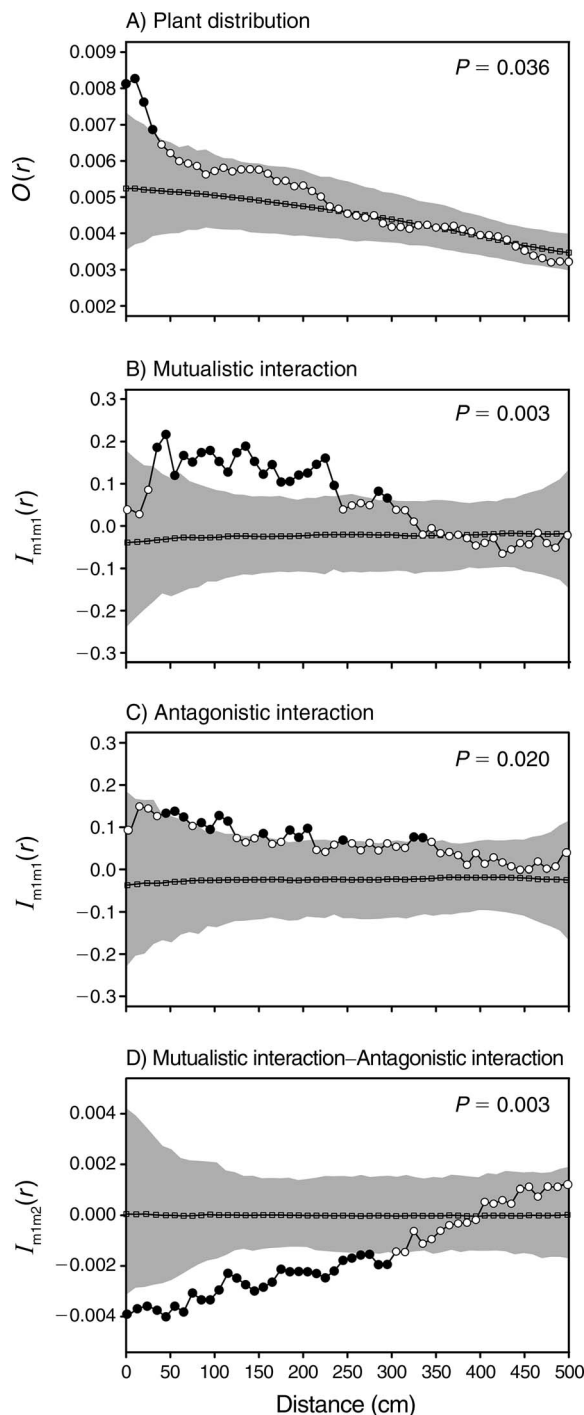


FIG. 1. Spatial analysis of *Isoplexis canariensis* (Plantaginaceae) distribution in the study population and associated quantitative marks. For mark correlation analysis, we used Schlather's I , which is analogous to the Pearson correlation coefficient of two values m_{k_i} and m_{k_j} from the same (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.

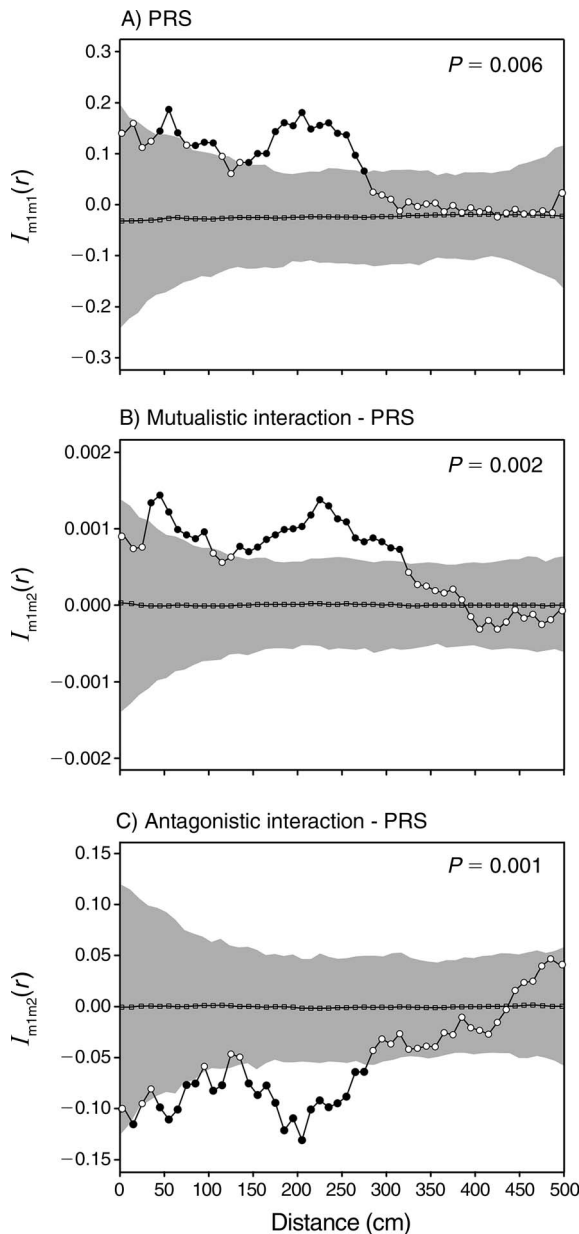


FIG. 2. (A) Univariate mark correlation analysis using Schlather's Index $I_{m1m1}(r)$ of plant reproductive success (PRS). (B, C) Bivariate mark correlation analysis using Schlather's Index $I_{m1m2}(r)$ between PRS and (B) the mutualistic interaction strength, and (C) the antagonistic interaction strength.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

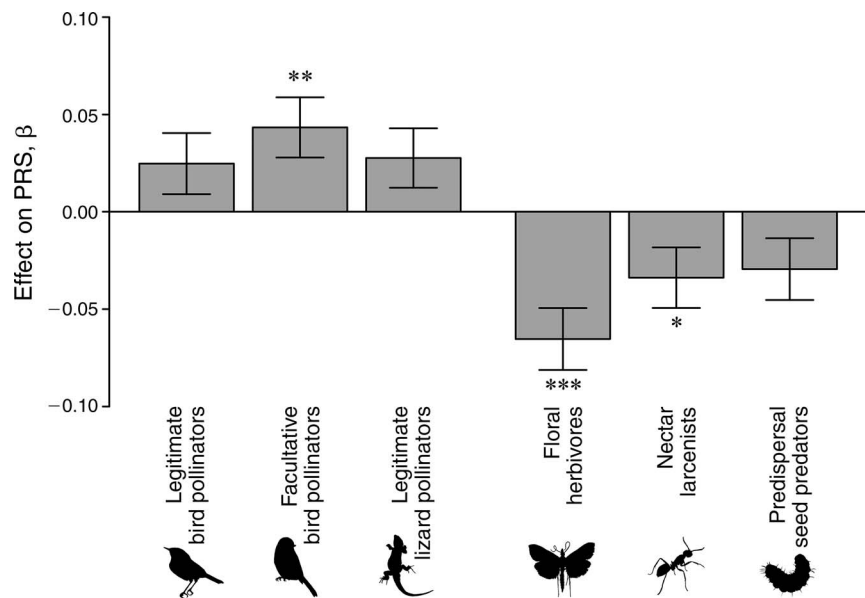


FIG. 3. Estimated effects of the interaction strengths of mutualistic and antagonistic animal guilds on plant reproductive success (PRS) of *Isoplexis canariensis*, obtained with spatially explicit, generalized linear mixed regression. Values represent the regression coefficients (β) of the individual 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>