Extensive pollen-mediated gene flow across intensively managed landscapes in an insect-pollinated shrub native to semiarid habitats

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Abstract
Our knowledge of the impact of landscape fragmentation on gene flow patterns is mainly drawn from tropical and temperate ecosystems, where landscape features, such as the distance of a tree to the forest edge, drive connectivity and mating patterns. Yet, the structure of arid and semiarid plant communities – with open canopies and a scattered distribution of trees – differs greatly from those that are well-characterized in the literature. As a result, we ignore whether the documented consequences of landscape fragmentation on plant mating and gene flow patterns also hold for native plant communities in arid and semiarid regions. We investigated the relative contribution of plant traits, pollinator activity, and individual neighbourhood in explaining variation in mating and gene flow patterns of an insect-pollinated semiarid arborescent shrub, Ziziphus lotus, at three sites embedded in highly altered agriculture landscapes. We used 14 SSRs, seed paternity analyses, and individual mixed effect mating models (MEMMi) to estimate the individual mating variables and the pollen dispersal kernel at each site. Individual spatial location, flower density, and floral visitation rate explained most of the variation of mating variables. Unexpectedly, individual correlated paternity was very low and shrubs surrounded by the most degraded matrix exhibited an increased fraction of pollen immigration and a high effective number of pollen donors per mother shrub. Overall, our results reveal that an active pollinator assemblage ensures highly efficient mating, and maintains pollen-mediated gene flow and notable connectivity levels, even in highly altered landscapes, potentially halting genetic isolation within and between distant sites.

Keywords
agrosystems, ecological neighbourhood, habitat loss, insect pollination, mating patterns, Mediterranean scrubland
1 | INTRODUCTION

Intensive human activity has brought about massive changes in landscapes that have caused great habitat loss and degradation, two of the main threats to biodiversity worldwide (Brook et al., 2008; Foley et al., 2005). As a result, many large and continuous natural habitats have shrunk to become isolated patches of variable sizes embedded in a matrix of agricultural and urban land-uses (Fahrig, 2003) where gene flow and the mating patterns of natural populations have been modified (Lowe et al., 2005; Slatkin, 1985). Previous studies have investigated the effects of environmental and anthropogenic variables on gene flow and mating patterns in both animals (DeWoody & Avise, 2001; Harrison et al., 2012; Janečka et al., 2007; Monteiro et al., 2019; Myers & Zamudio, 2004; Uller & Olsson, 2008) and plants (Breed, Marklund, et al., 2012; Chybicki & Oleksa, 2018; Gauzere et al., 2013; Miles et al., 2019). However, individual variation in plant mating patterns has been studied mainly in dense and fragmented forests in tropical and temperate regions (Chybicki & Oleksa, 2018; Gauzere et al., 2013; Guidugli et al., 2016; Ottewell et al., 2012), but open canopy habitats as semiarid and arid environments are still poorly understood.

Semiarid and arid ecosystems have particular structural characteristics that may determine animal foraging behaviour (Llorens et al., 2012) and hence gene flow given the habitat-related particularities that affect dispersal and/or pollination patterns. These ecosystems are characterized by open habitats with low canopy density, high spatial heterogeneity, and vegetation patchiness. Typically, conspecific individuals are either sparsely distributed or form, along with other species, small aggregates shaped by positive interactions between species and fertility islands around ecosystem-engineer species (Kéfi et al., 2007; Tirado & Pugnaire, 2005). Hence, the impact of land use changes on plant mating patterns may thus differ substantially between these ecosystems and high-density continuous vegetation or forests (the typical vegetation structure in temperate and tropical ecosystems). Currently, arid/semiarid habitats cover ca. 30% of the Earth’s surface (Malamou, 2007), a proportion that is expected to raise as environmental conditions become increasingly arid. Ecosystems in arid and semiarid regions have for centuries been severely altered and transformed to intensive agriculture lands where native plant communities have been reduced to isolated patches occupying marginal sites that are unsuitable for agriculture, such as dry riverbeds (Galvin et al., 2008). Therefore, understanding the functioning of mating and pollen flow patterns in these remnant populations found in intensively managed agrosystems is of utmost importance if we are to confront the challenges of guaranteeing food security while protecting remnant biodiversity in our arid ecosystems (Kremen & Merenlender, 2018).

Animal-pollinated plants that inhabit degraded and human-shaped ecosystems frequently have high levels of genetic erosion, selfing rates, and inbreeding depression (Breed et al., 2015; Honnay & Jacquemyn, 2007; Rathcke & Jules, 1993), in part because pollinators often find it difficult to move across a highly disturbed matrix (Ghazoul, 2005; Honnay & Jacquemyn, 2007; Kleijn et al., 2019). Recent studies have underlined that, although pollinator movements and their travel paths vary according to the size/type of land-use patches and landscape context (Lander et al., 2013), an active assemblage of pollinators frequently mitigates the negative effects of shifts in mating-pattern in altered landscapes. For example, some fragmented ecosystems still host a diverse array of insect pollinators that connect distant populations via long-distance dispersal movements (Breed et al., 2015; Lander et al., 2010). Therefore, future studies should integrate variation in phenotypic traits (Vranckx et al., 2011), environmental heterogeneity, and pollinator activity as a means of evaluating the consequences of habitat loss and human disturbances on gene flow and mating patterns in native flora (Breed et al., 2015).

Because their ecological and evolutionary importance, the study of mating patterns has gained prominence in recent years, partly due to the advent of SSRs and SNPs, highly polymorphic molecular markers (García et al., 2018; Kaiser et al., 2017; Ritland, 2002). The use of these markers has advanced our knowledge of the impact of the environmental factors that shape mating and gene flow patterns at population level, and of how they vary across increasingly managed landscapes. Notably, highly polymorphic molecular markers also reveal intra-population variability in mating variables (such as male and female fecundity on an individual basis), as well as the effect of individual phenotypic traits that determine these variables (García et al., 2005; Gauzere et al., 2013). This allows us to scale our findings on intrapopulation variation in mating patterns to landscape level where ecological features that define agrosystems can be taken into account (Klein et al., 2011; Moracho et al., 2016). In contrast, most studies exploring the intrapopulation variation in mating and gene flow patterns in managed ecosystems have focused on just a single site or population (Breed, Gardner, et al., 2012; Breed, Marklund, et al., 2012; Chybicki & Oleksa, 2018; Guidugli et al., 2016; Robledo-Arnuncio & Gil, 2005). Therefore, it remains unclear whether the observed intrapopulation variation can in fact shift across from site-to-site in highly altered landscapes (Gauzere et al., 2013).

Here, we specifically investigated how individual plant traits, pollinator activity, and environmental variables affect mating and gene flow patterns within and between sites. We addressed this question for Ziziphus lotus, an engineer shrub species found in highly fragmented semiarid Mediterranean habitats. Our specific aims were to (i) characterize the mating variables for focal Ziziphus plants at three study sites; (ii) quantify the average pollen dispersal distances and estimate the proportion of immigrant pollen at each study site; and (iii) estimate the impact of individual plant traits, conspecific neighbourhood density, and pollinator visits on mating patterns across sites.

Despite the fact that land-degradation and habitat fragmentation typically increase levels of selfing in other systems, we predicted (i) low values of selfing and inbreeding given the self-incompatibility and synchronous protandrous dichogamy described for this plant species (see Section 2.1). Furthermore, in terms of phenotypic traits and pollinator activity, we predicted (ii) that, as stated in the current
literature, larger and more conspicuous shrubs, with more flowers, will attract more pollinators and thus will have more pollen donors and greater male fecundity, and lower correlated paternity (de-Lucas et al., 2008; Ghazoul, 2005; O’Connell, 2003). Likewise, according to patterns found in other degraded and fragmented habitats (mainly temperate and tropical ecosystems; see references cited above), we predicted (iii) that the most isolated adult plants or the ones surrounded by intensive agriculture activities and growing in relatively small habitat remnants will receive immigrant pollen less often and from shorter dispersal distances, and will have fewer pollen donors per mother shrub and male fecundity (i.e., less gene flow due to limited pollinator activity) (Gazoul, 2005; Santos et al., 2016; but see Dick, 2001). Lastly, according to the structural particularities of the semiarid ecosystems described above, we predicted (iv) that the neighbourhood of each individual will influence migration rates and pollen flow in semiarid ecosystems less than in temperate and tropical ecosystems, where it frequently plays a prominent role (García et al., 2005; Gauzere et al., 2013; Ottewell et al., 2012).

To achieve our goals, we used 14 specific microsatellite molecular markers (SSRs) (González-Robles et al., 2016) to obtain the multilocus genotypes of fruiting shrubs and maternal progenies, and of all nearby candidate pollen donors. Then, we applied paternity analyses using CERVUS (Kalinowski et al., 2007) and FOLDISP software (Robledo-Arnuncio et al., 2007) to identify the pollen donors for the sampled maternal progenies and to estimate male fecundities, the correlated paternity, and the effective number of sires per mother shrub. We further inferred mating variables on an individual basis jointly with the pollen dispersal kernel per site by applying a modified version of the mixed effect mating models (MEMM; Klein et al., 2008) referred to as the individual mixed effect mating models (MEMMI; Gauzere et al., 2013). Overall, our results show that a diverse and active assemblage of pollinators guarantees highly efficient mating and pollen-mediated gene flow, as well as high pollen connectivity levels, even across intensively managed landscapes, thereby potentially halting genetic isolation within and between sites.

2 | MATERIALS AND METHODS

2.1 | Study species

*Ziziphus lotus* (L.) Lam (Rhamnaceae) is a sclerophyllous shrub found in semiarid habitats throughout the Mediterranean Basin from the Iberian Peninsula, Cyprus and Sicily to the Sahara and the Arabian Peninsula (European Commission, 2013). It is a long-lived diploid (2n = 20) species, growing to up to 1–3 m in height with a 30-m canopy diameter (Rey et al., 2018). It is monoecious and produces numerous hermaphrodite and generalist flowers that are pollinated by a diverse assemblage of insects, namely bees, wasps, flies, ants, beetles, and butterflies, among others (González-Robles, Salido, et al., 2020; Pérez Latorre & Cabezudo, 2009). Its flowers are apparently self-incompatible and exhibit synchronous protandrous dichogamy, each individual matures its flowers synchronically, that is, the anthers mature first followed by the stigma without (or with little) overlap between the two sexual forms. Moreover, at a particular site, in some plants flowers begin to mature in the morning but in others in the afternoon (ca. 1:1 proportion), which also favors outcrossing events. The lifetime of each flower is less than a day (for more details, see González-Robles, 2019), but shrubs open new flowers daily from May to July and the fruiting season lasts from August to September. This shrub is distributed in the southeast of the Iberian Peninsula, typically in seasonal streams or dry riverbeds (“ramblas”) and coastal plains, and forms a semiarid European priority habitat (Habitat 5220*: Arborescent shrub with *Ziziphus*; Anon, 1992; Tirado, 2009). These habitats are often highly degraded and fragmented where remnant scrubland patches typically only contain <100 individuals (Rey et al., 2018). Anthropic activities have increased over the last 60 years in the area mainly due to urban expansion and agricultural intensification, which has led to the implantation of large areas devoted to greenhouse cultivation (Mendoza-Fernández et al., 2015; Mota et al., 1996).

2.2 | Study sites and sampling design

We chose three sites in the SE Iberian Peninsula that are representative of the environments in which this species thrives (Table S1 for further details): El Ejido (EJ, the most fragmented and degraded study area, surrounded by the largest greenhouse agricultural nucleus in southern Europe); Nijar (N, a relatively well-protected area that is one of the largest and most continuous remnant patches of this species), and La Tercia (TER, an ample area dominated by small and isolated low-density patches of *Ziziphus* (Figure 1). During the 2015 flowering season, we mapped (GARMIN GPSMAP-64s) all adult shrubs at the study sites (N = 490) and collected leaf samples, which were stored in individual envelopes in plastic zip bags at −50°C until further molecular works. For the subsequent paternity analysis, all adult shrubs were considered potential candidate pollen donors (fathers), and we randomly chose 20–25 adults per site as maternal shrubs and collected 20–25 ripe fruits (maternal progenies) from their canopies during the fruiting season that spans from August to September (Table 1).

2.3 | Genotyping of maternal progenies and adult shrubs

Due to the small size of the embryos, we germinated all sampled seeds under control conditions in a Petri dish to ensure there was enough tissue for DNA extraction. We first removed by hand the fleshy tissue attached to each seed under sterile conditions (depulpation); then, we extracted the seed exocarp and mesocarp tissues and opened the endocarp to retain the embryo (1 or 2 per seed). Each embryo was seeded in a sterile Petri dish with autoclaved distilled water and maintained in a germination chamber at 18–20°C for 4–10 days. Each Petri dish was
sealed with parafilm to prevent contamination by exogenous fungi. All petri dishes were examined every 2 days to test moisture levels and to check for pathogens. When the radicles were of ca. 1–2 cm in length, the embryos were transplanted to soil in individual pots and grown in germination chambers (at 24°C with 14 h of daylight and 50% of relative humidity) for 2–3 months. These germinated seedlings served as maternal progenies and 2–3 fresh leaves per seedling were collected and stored at −50°C until DNA extractions. In all, 20–25 seeds per mother shrub were planted to obtain at least 10 seedlings per mother (a total of 700 seedlings). Nonetheless, 29.7% of seeds failed to germinate, mainly those collected from the TER site. In total, 492 germinated successfully, of which 484 were used as the sample of the maternal progenies (families with less than three offspring seedlings were eliminated from the analyses to avoid any risk of biased estimates) (Table 1).

All adults and maternal progenies were genotyped with 14 species-specific microsatellite markers for *Z. lotus*: zlo64, zlo65, zlo66, zlo67,
zlo68, zlo71, zlo73, zlo76, zlo79, zlo80, zlo86, zlo87 and zlo88; see González-Robles et al., 2016; for details on SSRs and multiplex conditions). Four fluorescent dyes (5-HEX, 56-FAM, ATTO565 and ATTO550) were used to 5-end label the forward primer of each locus (González-Robles et al., 2016). DNA extraction and PCR amplifications were performed using the methods and conditions described in González-Robles et al. (2016). Automated genotyping to analyse fluorescent-labeled PCR products was performed using an ABI 3500 Genetic Analyser sequencer (Applied Biosystems) and GeneScan 600 LIZ Size Standard (Applied Biosystems). Genemapper software version 4.1 (Applied Biosystems) was used for assigning alleles and fragment analyses. Linkage disequilibrium per locus was tested based on 10,000 permutations using Genepop v.4.2 software (Rousset, 2008); null allele frequencies were estimated in Micro-checker v.2.2.0 (van Oosterhout et al., 2004) (Table S2, González-Robles et al., 2016). Additionally, the quality of the scoring was checked independently by two people to minimize any possible genotyping errors.

2.4 | Ecological variables: Plant traits, neighbourhood, and pollinator variables

At each site, three types of variables were recorded per mother shrub to determine their phenotype, ecological neighbourhood, and the assemblage of pollinators that visit their canopy (mean values reported in Table S1). The following phenotypic traits were recorded: (i) shrub height (in m); (ii) canopy projection area (m$^2$); and (iii) average flower density per canopy (n/$225$ cm$^2$). To estimate flower density, the average number of open flowers in four replicated squares ($225$ cm$^2$ each) during the blooming period (from May to July 2015) were counted. To describe the ecological neighbourhood of each mother shrub as a proxy of local density, two circles of radii $25$ and $50$ m were defined around each mother (similar to the approximation used in Cruzan, 2001), and the number of conspecific individuals within that area (neighbourhood-$25$ and neighbourhood-$50$, respectively) were recorded by projecting the spatial location of all mapped adults using Quantum GIS 2.18.2 software (Quantum GIS Development Team, 2016). Additionally, the distance of each mother shrub to the nearest conspecific was measured. Finally, the assemblage of pollinators visiting each maternal shrub was characterized based on two 3-min censuses during the blooming period (70 mother shrub × 2 censuses/individual = 140 pollinator censuses). For each census, the following information was recorded: (i) pollination visitation rate as the mean number of insects visiting flowers; and (ii) flower visitation rate as the mean number of flowers visited by insects per census (González-Robles, 2019; González-Robles, Salido, et al., 2020).

2.5 | Estimates of mating variables and dispersal kernel parameters

Following Gauzere et al. (2013), the multilocus genotypes obtained from the maternal progenies, mother shrubs, and candidate fathers were used to estimate the proportion of migration (m) and selfing events (s) per mother shrub based on the Bayesian approach implemented in the MEMMi. Similarly, the variance of effective relative male fecundity for each candidate father ($\sigma^2$) was inferred as the amount of pollen produced by a given plant that achieves successful pollination as compared to other plants (see Oddou-Muratorio et al., 2018). MEMMi additionally estimates two parameters that define pollen dispersal kernels per site: the mean pollen dispersal distance (meters, $\delta$) and the shape (dimensionless, $b$). MEMMi was run for each study site using MCMC of $50,000$ steps and burnin of $5000$ steps. A gamma distribution was employed for the male fecundity variable and the uniform prior distributions on the intervals $[0, 1], [0, 10], [0, 100], [0, 1000]$ for $s_p, m_p, \delta, b,$ and $\sigma^2$ (total variance of quantity of pollen emitted by fathers), respectively (see Gauzere et al., 2013; for further details on the modelling approach and the parameters required). Additionally, the correlated paternity between seeds produced by a single mother shrub ($r_{gg}$; within-sibship correlated paternity) (Hardy et al., 2004) was estimated using the Poldisp software (Robledo-Arnuncio et al., 2007). The average effective number of fathers pollinating each mother per site ($N_{ep}$) was calculated based on the mean values of $r_{gg}$ per site: $N_{ep} = 1/r_{gg}$ (Gauzere et al., 2013).

Finally, a paternity analysis was performed on the maternal progenies with known mother shrubs at each site to identify the pollen donor that was most likely to have sired the maternal progenies (García et al., 2005). The number of pollen donors per mother ($N_p$) within site was also inferred. To these ends, we applied Cervus 3.0 software (Kalinowski et al., 2007) with a relaxed and strict confidence level (80% and 95%, respectively) for paternity assignment. Based on previous knowledge, the following parameters were set per site (EJ, N and TER, respectively): $n$ offsprings = $100,000$; $n$ candidate fathers = $100, 250, 110$; proportion of candidate father sampled = $0.80, 0.60, 0.80$; proportion of loci mistyped = $0.02, 0.02, 0.03$; error rate in likelihood calculations = $0.02, 0.02, 0.03$; minimum number of typed loci = $11$. Simulations also considered the inbreeding level per site (EJ = $0.1171$; N = $0.086$; TER = $0.077$) derived from previous genetic diversity analyses within our study sites (González-Robles, Manzaneda, et al., 2020) using R package Pegas (Paradis, 2010). It is important to note that, although Poldisp (with $N_{ep}$) and Cervus (with $N_p$) estimate the number of pollen donors per mother tree, they use different types of approximations: whereas Poldisp considers all possible parentage assignments within the site to estimate the $N_{ep}$ for each mother shrub, $N_p$ estimates obtained with Cervus only refer to those parentage assignments with a confidence level of 80% or 95%. Thus, $N_{ep}$ was used to refer to the average number of pollen donors per mother at site level, while $N_p$ values were used to refer to the number of pollen donors per mother at individual level.

2.6 | Statistical analyses

The values of all studied mating system variables used in the effect analyses ($m$, $\sigma^2$ and $r_{gg}$) followed a normal distribution, although $\sigma^2$
and $r_{gg}$ were previously log-transformed to meet this requirement (Figure S1). Linear mixed models (LMMs) were used to evaluate the effect of the ecological correlates on the described individual mating variables. Initially, individual height, canopy projection area, flower density, neighbourhood-25, neighbourhood-50, distance to nearest conspecific, pollination visitation rate and flower visitation rate were taken as continuous ecological covariates per individual. Prior to the model construction, the noncollinearity of ecological variables were assessed using Pearson correlations (threshold: $r \leq |.6|$) and variance inflation factors (VIF). The canopy projection area and neighbourhood-25 were removed because they showed high correlation values with height and neighbourhood-50, respectively; and furthermore their models had higher AICc values. Site was included as a nesting random factor in all models to avoid bias in the results due to habitat and landscape differences between sites. Equation (1) and (2) show the final structure of the LMMs (based on Zuur & Ieno, 2016):

$$m_{ij} = \text{Height}_{ij} + \text{Flower density}_{ij} + \text{Neighbourhood-50}_{ij} + \text{Distance to the nearest conspecific}_{ij} + \text{Pollination visitation rate}_{ij} + \text{Flower visitation rate}_{ij} + \text{Site}_{i}$$

$$\log(r_{ij}^2) \text{ or } \log(r_{ij}) = \text{Height}_{ij} + \text{Flower density}_{ij} + \text{Neighbourhood-50}_{ij} + \text{Pollination visitation rate}_{ij} + \text{Flower visitation rate}_{ij} + \text{Site}_{i}$$

(1)

(2)

where variable $y_{ij}$ is the $j$th observation in site $i$, $i = 1, ... , 3$, and Site$_i$ is the random intercept (see Table S3 for details of models' structure per each response variable).

We used the package nml4 (Pinheiro et al., 2017) and mumin (Barton, 2018) in the software r v3.4.3 (R Core Team, 2017) to fit all LMMs with maximum likelihood. Model selection was based on Akaike Information Criteria corrected for small sample sizes (AICc). Among all possible competing models ($\Delta$AICc $<$ 2 defined models equally valid), we chose the models with the least AICc and selected among them the most parsimonious one, as suggested by Burnham and Anderson (2002). Additionally, the spatial correlation of our dependent variables was controlled for by adding a correlation function to the previously selected best model, following the recommendations of Zuur et al. (2009). Thus, the effect of the spatial location was tested for by accommodating $x$, $y$ (geographic coordinates) within these correlation structures for each LMM by applying the package lme4 (Bates et al., 2015). Then, the performance of the models that accommodate spatial structure were compared with the null model that lacks spatial structure, and the best-fitted model was selected following the same criteria as explained above (lowest AICc and most parsimonious; see Table S3). When spatial autocorrelation was detected in the models, variograms were further used to check the spatial correlation and to assess the independence of residuals with the Variogram function of R-package nlme (Pinheiro et al., 2017). All selected models were validated by plotting their standardized residuals against fitted values and by testing whether they met normality and homoscedasticity (Figure S1). Although individual selfing rates ($s$) and the number of pollen donors per mother tree ($N_p$) were reported, their ecological correlates were not modelled because, in the first case, the species is typically defined as self-incompatible, as confirmed by the extremely low values of selfing found (see results), while $N_p$ is dependent on the progeny size per mother shrub.

### 3 | RESULTS

#### 3.1 | Mating patterns within and between sites and ecological correlates

The mean values per individual for each mating variable per site are shown in Table 2. Maternal shrubs showed a low proportion of selfing events ($s$), with individual values ranging from 0% to 0.71% (EJ: 0.00%–0.71%; N: 0.00%–4.82%; TER: 0.00%–0.71%; Table S4 and Figure 2a).

Paternity tests assigned 57% (TER), 34% (N), and 23% (EJ) of maternal progenies at a 95% confidence level. However, we focused on the results obtained when the 80% confidence level was applied because we were more interested in depicting an overall picture of mating patterns within and between sites than in estimating individual fitness values. In this latter case, paternity tests assigned a local candidate father for more than half of the maternal progenies surveyed, with the highest proportion found at TER (78%), followed by N (67%) and EJ (57%) (Table 3). The location of the candidate pollen donors that contributed most to sire maternal progeny within each site is given in Figure 1. Notably, most of the maternal progeny of some mother shrubs was sired by unsampled pollen donors, which suggests that there is a high frequency of immigrant pollen (Table S4). The correlated paternity per mother shrub ($r_{gg}$) ranged from −0.15 to 0.50 (EJ: −0.10 to 0.49; N: −0.06 to 0.50; TER: −0.15 to 0.45), its variation being very similar between sites (Table 2, Figure 2b and Table S4). The numbers of inferred pollen donor per mother ($N_p$) ranged from 1 to 9 (EJ: 2–9; N: 1–7; TER: 1–5) (Table S4). The average number of effective fathers pollinating each mother per site ($N_{eg}$) had higher mean values at EJ (7.81) and TER (7.35) than N (6.64) (Table 2). None of the study variables showed any significant impact on the correlated paternity ($r_{gg}$) (variance explained by fixed and random effects = 0.00; Table 4).

The individual values of the variance of effective relative male fecundity ($\sigma^2$) ranged from 0.14 to 12.75 (EJ: 0.48–2.46; N: 0.14–5.46; TER: 0.15–12.75; Table 2, Figure 2c). Taking into account only the pollen donors with offspring within each site (51 in EJ, 81 in N, and 11 in TER), a slight variation in the average number of sired embryos per pollen donor was observed (EJ: 2.7 ± 0.3, N: 1.8 ± 0.2 and TER: 1.6 ± 0.2). The maximum number of embryos sired by a single pollen donor was 12 (EJ: 10; N: 12, and TER: 2). The individual values of $\sigma^2$ were positively influenced by flower density (t-value = 3.8289, $p = .0003$) but negatively influenced by flower visitation rate (t-value = −2.9254, $p = .0047$) (variance explained by random effects = 0.00; Table 4). Thus, the shrubs with higher flower density values and/or fewer floral visits by pollinators were those that sired
more embryos. The trends of the $\sigma^2$ values between sites as a function of each explanatory variable are shown in Figure 3. The specific effects of each model within each site can be consulted in Table S5.

3.2 Migration patterns and ecological correlates

The proportion of migrant pollen donors per mother shrub ($m_i$) ranged from 25.46% to 92.33% (EJ: 50.73%–92.33%; N: 25.46%–91.67%; TER: 29.87%–59.18%) (Table S4). Table 2 and Figure 2d also show the mean values of $m_i$ per site. This high proportion of migrant pollen agreed with the fat-tailed pollen dispersal kernel ($b < 1$) observed at all study sites that ranged from 0.1644 (N) to 0.4992 (TER), and the high average pollen dispersal distances ($b$) ranging from 2055 m (EJ) to 3810 m (N) (Table 2). The best fitted model for $m_i$ accommodated a Gaussian spatial correlation structure (variance explained by random effects = 0.63; Table 4), which indicates that $m_i$ was significantly affected by the spatial location of the mother shrubs and that nearby shrubs tended to have similar $m_i$ values ($t$-value = 5.7323, $p < .0001$). Note that the range of the spatial structure reaches 50.04 m, which broadly coincides with the area covered by the neighbourhood-50 (Table 4). The specific effects of this model within each site are shown in Table S5.

4 DISCUSSION

Contrary to our expectations, we detected extensive pollen movement within and from outside each site despite the intense landscape degradation surrounding all the Z. lotus study habitats. Overall, mother shrubs had a diverse pool of pollen donors and low values of correlated paternity, which suggests that highly efficient mating and pollinator-mediated gene flow exists. Furthermore, we identified a notable effect of the study site and individual spatial location on the migration rate (all its variance was explained by random effects), suggesting that the particular ecological and degradation features of each site, or around each individual plant, also influenced the individual mating pattern within each site. In general, the study sites showed both high mean values of pollen dispersal distance and high proportions of immigrant pollen, which suggests that the habitats of this insect-pollinated shrub preserve high functional connectivity levels across the landscape despite intense habitat degradation and fragmentation. Finally, our results showed that individual phenotypic traits, most notably flower display, and pollinator attraction influence mating variables, particularly male fecundity.

4.1 The reproductive system explains uniform low levels of selfing across individuals and sites

Habitat loss and landscape changes typically alter mating patterns both within and between plant populations by decreasing gene flow between them (Honnay & Jacquemyn, 2007; Miles et al., 2019).
which lead to an increase in the frequency of selfing events and a low proportion of migrant pollen (Aguilar et al., 2008). Despite this, previous studies have found mixed trends in the effects of fragmentation on the frequency of selfing events. For example, selfing rates increase in some insect-pollinated species with increased landscape fragmentation, such as *Gomortega keule* (Lander et al., 2010) and *Eucalyptus socialis* and *E. gracilis* (Breed et al., 2015), whereas other insect-pollinated species such as *Cariniana estrellensis* (Guidugli et al., 2016) have low selfing rates. In our study, *Ziziphus lotus* mainly depends on an outcrossing pollination system, which, along with the apparent self-incompatibility of its pollen, would explain the low uniform values of selfing and inbreeding events in *Ziziphus lotus* plants.

### 4.2 Individual plant traits and pollinator activity determine variation in mating patterns and pollen-mediated gene flow

In our study, some individuals were clearly more successful in siring fruiting shrubs than others within each site (Figure 1). This variance in effective relative male fecundity was explained by the positive effect of flower density and the unexpected negative effect of the flower visitation rate. This result agrees with previous findings that demonstrate that, although a greater floral display increases the likelihood of siring progeny, it may also increase the frequency of selfing and inbreeding events (Barrett et al., 1994; Minnaar et al., 2018; Tani et al., 2009). Commonly, diverse and mobile insect pollinator assemblages increase the frequency of outcrossing events within and between populations (Breed et al., 2015). *Ziziphus lotus* benefits from a diverse assemblage of pollinators that includes 82 insect taxa (González-Robles, Manzaneda, et al., 2020). Yet, it is also known that some abundant and managed pollinators in the study area, such as *Apis mellifera*, are strongly attracted by the most abundant floral resource (i.e., *The Circe principle* formulated by Lander et al., 2011; but see also Hung et al., 2019). When it is blooming, *Ziziphus lotus* is the main flowering species in the study area, which could encourage pollinators to visit a large number of flowers on the same plant, thereby promoting geitonogamous events (i.e., pollen transport between flowers of the same individual; García et al., 2005; Karron et al., 2004; Minnaar et al., 2018) or nearby, closely related individuals.
Similarly, Valido et al. (2019) demonstrate that the introduction of beehives into a protected natural area significantly reduces the reproductive performance of endemic plants since honeybees displace most native pollinators that provide complementary pollination services.

### 4.3 Isolated individuals and small isolated populations still hold extensive pollen-mediated gene flow

Our results additionally revealed extensive extra pollen flow from outside the studied habitats that was surprisingly high in the most degraded study site which was surrounded by a highly anthropic matrix dominated by greenhouses and scarce patches of native flora (EJ). We expected that this matrix would act as a barrier to pollen flow, thereby preventing pollen immigration (Sork & Smouse, 2006). However, altered and fragmented populations are not always completely isolated (Lander et al., 2010), particularly in the case of insect-pollinated species (Breed et al., 2015). Despite the small population size of EJ, its closest *Ziziphus* habitat is only ca. 2 km away (Santa María del Águila, see more details in González-Robles, Manzaneda, et al., 2020) and some unnoticed individuals might be present in nearby habitats. Solitary individuals across agrosystems are documented to act as steppingstone structures that favour pollen flow between nearby populations (Kwak et al., 1998; Lander et al., 2010). These solitary and scattered individuals frequently show high values of long-distance pollen movement and they act as food sources and shelter for insect pollinators that connect distant vegetation patches. Thus, their presence has been associated with great genetic connectivity levels between natural remnant patches even within highly fragmented agroforest landscapes (Ismail et al., 2012; Llorens et al., 2012). Lander et al. (2010) underlined the fundamental role that these individuals play in the continued resilience of natural populations reducing their risk of inbreeding and local extinction in altered landscapes. Our models also show that the spatial location of individuals within habitat determines migration rates and that all study sites had high mean values of pollen dispersal distances (from 2055 to 3810 m), probably favored by the great diversity in the *Z. lotus* pollinator assemblage (González-Robles, Salido, et al., 2020). The great mobility of insect pollinators – especially honeybees – may ensure that pollen can be transported between habitats that lie several kilometers apart across disturbed landscapes (Dick, 2001; Steffan-Dewenter & Kuhn, 2003). The lowest mean pollen dispersal distance in our study was found at EJ, the smallest remnant habitat studied, albeit densely populated by *Z. lotus* and surrounded by a highly altered landscape. By contrast, the highest values were detected at N, a relatively well-preserved and large habitat with continuous remnant patches of this species, where adult plants are widely distributed (see Figure 1).

However, one of the first effects of habitat loss and fragmentation in natural populations is variation in individual fecundity (Oddou-Muratorio et al., 2018). Although our results suggest that extensive pollen-mediated gene flow still takes place even in isolated habitat remnants, we observed substantially lower seed germination success in some individuals, especially in one of the most isolated and disturbed sites (TER) where there were greater distances between adults. This may be indicative of delayed depression effects of biparental inbreeding that were not accounted for in our analysed

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**TABLE 3** Seed parentage assignment analyses performed using CERVUS software at the three study sites of *Ziziphus lotus* habitat

| Level     | Confidence (%) | Critical Delta | Assignments (%) |       |       |
|-----------|----------------|----------------|-----------------|------|------|
|           |                |                | Observed        | Expected |
| El Ejido  |                |                |                 |       |      |
| Strict    | 95             | 2.96           | 54 (23)         | 139 (58) |
| Relaxed   | 80             | 0.38           | 136 (57)        | 209 (87)  |
| Unassigned|                |                | 104 (43)        | 31 (13)   |
| Total     |                |                | 240 (100)       | 240 (100)  |
| Níjar     |                |                |                 |       |      |
| Strict    | 95             | 4.49           | 74 (34)         | 122 (52)  |
| Relaxed   | 80             | 1.88           | 145 (67)        | 176 (82)  |
| Unassigned|                |                | 70 (33)         | 39 (18)   |
| Total     |                |                | 215 (100)       | 215 (100)  |
| La Tercia |                |                |                 |       |      |
| Strict    | 95             | 3.17           | 13 (57)         | 14 (62)   |
| Relaxed   | 80             | 0.73           | 18 (78)         | 21 (89)   |
| Unassigned|                |                | 5 (22)          | 2 (11)    |
| Total     |                |                | 23 (100)        | 23 (100)  |
progenies, or that individuals are subjected to particularly stressful growing conditions that hamper seed germination. Analogous cases have been found in other agrosystems; for example, Ismail et al. (2012) showed how changes in population density vary the likelihood of outcrossing between individuals, while Nason et al. (1998) associated these negative reproductive effects with limitations on pollinator movements caused by habitat fragmentation. It is worth emphasizing that both the low population size and the habitat degradation are particularly intense in the TER population, two factors that are related to the intensive agricultural land-use that dominates at this site.

### 4.4 Analogies and differences with temperate and tropical forests

Overall, our results showed similar trends to those previously observed for temperate and tropical species inhabiting agrosystems, where the degree of population isolation is the most important factor determining mating patterns (see review of Sork & Smouse, 2006; but also Breed, Gardner, et al., 2012; Breed, Marklund, et al., 2012; García et al., 2005; Gauzere et al., 2013; Nason et al., 1998). As in temperate and tropical species, the factors that influenced the mating patterns in our study species include flower density and pollinator...
activity. However, we detected certain interesting differences with other ecosystems. Temperate and tropical forests are usually habitats whose closed canopies potentially prevent the arrival of pollen from distant locations or isolated trees (e.g., Prunus mahaleb García et al., 2005; or Fagus sylvatica Gauzere et al., 2013). Indeed, the typical high canopy density of these forest systems is associated with relatively short pollen dispersal distances (Robledo-Arnuncio & Gil, 2005; Sork & Smouse, 2006). Conversely, the characteristic open canopy structure and marked spatial heterogeneity and patchiness of semiarid habitats could favor long-distance pollen movement. Our results supported this idea, showing higher pollen dispersal distances and gene flow in semiarid habitats (2055–3810 m) than in other reduced or fragmented habitats of temperate and tropical areas (see Chybicki & Oleksa, 2018 [mean values = 83 m]; Duminil et al., 2016 [195, 346, and 1001 m]; García et al., 2007 [548 m]; Hanson et al., 2008 [240, 317, 343, 557 m]; Ottewell et al., 2012 [269 m]; Robledo-Arnuncio & Gil, 2005 [48 m]). These results suggest different response of mating traits to changes in land use in arid and semiarid species compared to tropical and temperate species, where the height and local density of individuals, the proximity to forest edges, and the distance to the nearest conspecific play a key role in individual mating patterns (see Breed, Marklund, et al., 2012; García et al., 2005; Gauzere et al., 2013; Ottewell et al., 2012; Santos et al., 2016). Our results also agree with findings from other fragmented or Mediterranean habitats where the vegetation has an open-canopy structure (see Llorens et al., 2012). Future meta-analysis studies should investigate whether these patterns are widespread across semiarid agrosystems and other open canopy and fragmented habitats.

4.5 | Current gaps

Despite our thorough sampling and genotyping effort, some uncertainties remain. For example, the high level of nongermination in seeds from some individual progeny, especially at the TER site, remains to be explained; likewise, it is unclear why none of the ecological variables considered in this study explained the values of individual correlated paternity. It should be noted that, although the mating parameters of some families/site (particularly TER) could have been estimated with uncertainty, this was inherent to the low progeny size of individuals from this site given its particular ecological degradation (see above). In any case, we believe that the general trends described in our work accurately describe the current situation of mating and gene flow of Z. lotus in the SE Iberian Peninsula, since our work focuses on individual mating patterns rather than on the patterns of each study site. On the other hand, Barrett and Harder (2017) underline that the mating likelihood of plant species is conditioned by complex associations between variables, in which biotic and abiotic factors could also play an important role. Thus, future studies should be performed at landscape level (sensu Fahrig, 2003) to evaluate the impact of features present in the matrix and the effect of features measured at habitat level. Moreover, given that this study only spans the early demographic stages (effective pollination), further studies should investigate the long-term consequences of observed mating patterns across successive demographic stages (fruiting, germination, survival and mortality). This will allow us to gain a comprehensive understanding of the impact of different agriculture practices on the persistence and viability of highly threatened species and allow us to address one of the most challenging goals in sustainability: meeting the demands of food security whilst preserving the remnants of our biodiversity (Kremen & Merenlender, 2018).

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AUTHOR CONTRIBUTIONS

Pedro J. Rey designed the research. Ana González-Robles, Pedro J. Rey and Antonio J. Manzaneda conducted field samplings. Ana González-Robles performed germination experiments and genotyped maternal progenies and adult individuals. Teresa Salido processed GIS data to obtain individual neighbourhood metrics. Ana González-Robles and Cristina García analysed the data. Ana González-Robles, Cristina García and Pedro J. Rey led the writing of the manuscript. All authors contributed critically to the final version and gave their final approval for publication.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study have been made available in the Mendeleve repository at: http://dx.doi.org/10.17632/ynjzrw6f5x.1

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Ismail, S. A., Ghazoul, J., Ravikanth, G., Shaanker, R. U., Kushalappa, C. G., & Kettle, C. J. (2012). Does long-distance pollen dispersal preclude inbreeding in tropical trees? Fragmentation genetics of {\em Dyssoxylum malabaricum} in an agro-forest landscape. Molecular Ecology, 21, 5484–5496.

Janečka, J. E., Blankenship, T. L., Hirth, D. H., Kilpatrick, C. W., Tewes, M. E., & Grassman, L. I. (2007). Evidence for male-biased dispersal in bobcats {\em Lynx rufus} using relatedness analysis. Wildlife Biology, 13, 38–47.

Kaiser, S. A., Taylor, S. A., Chen, N., Sillett, T. S., Bondra, E. R., & Webster, M. S. (2017). A comparative assessment of SNP and microsatellite markers for assigning parentage in a socially monogamous bird. Molecular Ecology Resources, 17, 183–193.

Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology, 16, 1099–1106.

Karron, J. D., Mitchell, R. J., Holmquist, K. G., Bell, J. M., & Funk, B. (2004). The influence of floral display size on selfing rates in {\em Mimulus ringens}. Heredity, 92, 242–248.

Kéfi, S., Rietkerk, M., Alados, C. L., Pueyo, Y., Papanastasis, V. P., El-Aich, A., & de Ruiter, P. C. (2007). Spatial vegetation patterns and imminent desertification in Mediterranean arid ecosystems. Nature, 449, 213–217.

Klein, D., Bommarco, R., Figen, T. P. M., Garibaldi, L. A., Potts, S. G., & van der Putten, W. H. (2019). Ecological intensification: Bridging the gap between science and practice. Trends in Ecology & Evolution, 35, 154–166.

Klein, E. K., Carpentier, F. H., & Oddou-Muratorio, S. (2011). Estimating the variance of male fecundity from genotypes of progeny arrays: Evaluation of the Bayesian forward approach. Methods in Ecology and Evolution, 2, 349–361.

Klein, E. K., Desassins, N., & Oddou-Muratorio, S. (2008). Pollen flow in the wildservice tree, {\em Sorbus torminalis} (L.) Crantz. IV. Whole interindividual variance of male fecundity estimated jointly with the dispersal kernel. Molecular Ecology, 17, 3323–3336.

Kremen, C., & Merenlender, A. M. (2018). Landscapes that work for biodiversity and people. Science, 362, eaau6020. https://doi.org/10.1126/science.aau6020

Kwak, M. M., Velterop, O., & van Andel, J. (1998). Pollen and gene flow in fragmented habitats. Applied Vegetation Science, 1, 37–54.

Lander, T. A., Bebb, D. P., Choy, C. T. L., Harris, S. A., & Boshier, D. H. (2011). The Circe principle explains how resource-rich land can waylay pollinators in fragmented landscapes. Current Biology, 21(15), 1302–1307.

Lander, T. A., Boshier, D. H., & Harris, S. A. (2010). Fragmented but not isolated: Contribution of single trees, small patches and long-distance pollen flow to genetic connectivity for {\em Gomortega keule}, an endangered Chilean tree. Biological Conservation, 143, 2583–2590.

Lander, T. A., Klein, E. K., Stoeckel, S., Mariette, S., Musch, B., & Oddou-Muratorio, S. (2013). Interpreting realized pollen flow in terms of pollinator travel paths and land-use resistance in heterogeneous landscapes. Landscape Ecology, 28(9), 1769–1783.

Llorens, T. M., Byrne, M., Yates, C. J., Nistelberger, H. M., & Coates, D. J. (2012). Evaluating the influence of different aspects of habitat fragmentation on mating patterns and pollen dispersal in the bird-pollinated {\em Banksia sphaerocarpa} var. caesia. Molecular Ecology, 21, 314–328.

Lowe, A. J., Boshier, D., Ward, M., Bacles, C. F. E., & Navarro, C. (2005). Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. Heredity, 95, 255–273.

Malagnoux, M. (2007). Arid land forest of the World: Global environmental perspectives. Forestry Department (FAO). Paper presented at the International Conference on Afforestation and Sustainable Forests as a Means to Combat Desertification. Jerusalem, Israel. http://www.fao.org/3/a-ah836e.pdf

Mendoza-Fernández, A. J., Martínez-Hernández, F., Pérez-García, F. J., Garrido-Becerra, J. A., Benito, B. M., Salmerón-Sánchez, E., Guirado, J., Merlo, M. E., & Mota, J. F. (2015). Extreme habitat loss in a Mediterranean habitat: {\em Maytenus senegalensis} subsp. europaea. Plant Biosystems, 149, 503–511.

Miles, L. S., Rivkin, L. R., Johnson, M. T. J., Munshi-South, J., & Verrelli, B. C. (2019). Gene flow and genetic drift in urban environments. Molecular Ecology, 28, 4138–4151.

Minnar, C., Anderson, B., de Jager, M. L., & Karron, J. D. (2018). Plant-pollinator interactions along the pathway to paternity. Annals of Botany, 123, 225–245.

Monteiro, W. P., Veiga, J. C., Silva, A. R., Carvalho, C. S., Lanes, E. C. M., Rico, Y., & Jaffé, R. (2019). Everything you always wanted to know about gene flow in tropical landscapes (but were afraid to ask). PeerJ, 7, e6446. https://doi.org/10.7717/peerj.6446

Moracho, E., Moreno, G., Jordano, P., & Hampe, A. (2016). Unusually limited pollen dispersal and connectivity of Pedunculate oak (Quercus robur) refugial populations at the species’ southern range margin. Molecular Ecology, 25, 3319–3331.

Mota, J. F., Peñas, J., Castro, H., & Cabello, J. (1996). Agricultural development vs biodiversity conservation: The Mediterranean semiarid vegetation in El Ejido (Almeria, southeastern Spain). Biodiversity and Conservation, 5, 1597–1617.

Myers, E. M., & Zamudio, K. R. (2004). Multiple paternity in an aggregate breeding amphibian: The effect of reproductive skew on estimates of male reproductive success. Molecular Ecology, 13, 1951–1963.

Nason, J. D., Herre, E. A., & Hamrick, J. L. (1998). Breeding structure of a tropical keystone plant resource. Nature, 391, 385–387.

O’Connell, L. M. (2003). The evolution of inbreeding in western redcedar (Thuja plicata: Cupressaceae) (PhD Thesis). University of British Columbia, Vancouver, BC, Canada.

Oddou-Muratorio, S., Gauzere, J., Bontemps, A., Rey, J. F., & Klein, E. K. (2018). Tree, sex and size: Ecological determinants of male vs. female fecundity in three {\em Fagus sylvatica} stands. Molecular Ecology, 27, 3131–3145.

Ottewell, K., Grey, E., Castillo, F., & Karubian, J. (2012). The pollen dispersal kernel and mating system of an insect-pollinated tropical palm, {\em Oenocarpus bataua}. Heredity, 109, 332–339.

Paradis, E. (2010). PEGAS: An R package for population genetics with an integrated-modular approach. Bioinformatics, 26, 419–420.

Pérez Latorre, A. V., & Cabezudo, B. (2009). {\em Ziziphus Miller}. In G. Blanca, B. Cabezudo, M. Cueto, C. Fernández López, & C. Morales Torres (Eds.), {\em Flora vascular de andalucía oriental} (Vol. 3, pp. 46–50). Consejería de Medio Ambiente, Junta de Andalucía.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team. (2017). nlme: Linear and nonlinear mixed effects models. R package version 3.1-131. https://CRAN.R-project.org/package=nlme

Quantum GIS Development Team. (2016). Quantum GIS Geographic Information System. Open source geospatial foundation project. http://qgis.osgeo.org

R Core Team. (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-project.org/

Rathcke, B. J., & Jules, E. S. (1993). Habitat fragmentation and plant-pollinator interactions. Current Science, 65, 273–277.

Rey, P. J., Cancio, I., Manzaneda, A. J., González-Robles, A., Valera, F., Salido, T., & Alcántara, J. M. (2018). Regeneration of a keystone semiarid shrub over its range in Spain: Habitat degradation over- rides the positive effects of plant-animal mutualisms. Plant Biology, 20, 1083–1092.

Ritland, K. (2002). Extensions of models for the estimation of mating systems using n independent loci. Heredity, 88, 221–228.
Robledo-Arnuncio, J. J., Austerlitz, F., & Smouse, P. E. (2007). Poldisp: A software package for indirect estimation of contemporary pollen dispersal. *Molecular Ecology Notes*, 7, 763–766.

Robledo-Arnuncio, J. J., & Gil, L. (2005). Patterns of pollen dispersal in a small population of *Pinus sylvestris* L. revealed by total-exclusion paternity analysis. *Heredity*, 94, 13–22.

Rousset, F. (2008). GENEPOP’007: A complete reimplementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, 8, 103–106.

Santos, A. S., Cazetta, E., Dodonov, P., Faria, D., & Gaiotto, F. A. (2016). Landscape-scale deforestation decreases gene flow distance of a keystone tropical palm, *Euterpe edulis* Mart (Arecalesae). *Ecology and Evolution*, 6, 6586–6598.

Slatkin, M. (1985). Gene flow in natural populations. *Annual Review of Ecology, Evolution, and Systematics*, 16, 393–430.

Sork, V. L., & Smouse, P. E. (2006). Genetic analysis of landscape connectivity in tree populations. *Landscape Ecology*, 21, 821–836.

Steffan-Dewenter, I., & Kuhn, A. (2003). Honeybee foraging in differentially structured landscapes. *Proceedings of the Royal Society B: Biological Sciences*, 270, 569–575.

Tani, N., Tsumura, Y., Kado, T., Taguchi, Y., Lee, S. L., Muhammad, N., Ng, K. K. S., Numata, S., Nishimura, S., Konuma, A., & Okuda, T. (2009). Paternity analysis-based inference of pollen dispersal patterns, male fecundity variation, and influence of flowering tree density and general flowering magnitude in two dipterocarp species. *Annals of Botany*, 104, 1421–1434.

Tirado, R. (2009). 5220 Matorrales arborescentes con *Ziziphus* (*). En: VV.AA., *Bases ecológicas preliminares para la conservación de los tipos de hábitat de interés comunitario en España* (pp. 68). Ministerio de Medio Ambiente, y Medio Rural y Marino.

Tirado, R., & Pugnaire, F. I. (2005). Community structure and positive interactions in constraining environments. *Oikos*, 111, 437–444.

Uller, T., & Olsson, M. (2008). Multiple paternity in reptiles: Patterns and processes. *Molecular Ecology*, 17, 2566–2580.

Valido, A., Rodríguez-Rodríguez, M. C., & Jordano, P. (2019). Honeybees disrupt the structure and functionality of plant-pollinator networks. *Scientific Reports*, 91(9), 4711. https://doi.org/10.1038/s41598-019-41271-5

van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., & Shipley, P. (2004). MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Resources*, 4, 535–538.

Vranckx, G., Jacquemyn, H., Muys, B., & Honnay, O. (2011). Meta-analysis of susceptibility of woody plants to loss of genetic diversity through habitat fragmentation. *Conservation Biology*, 26, 228–237.

Zuur, A. F., & Ieno, E. N. (2016). A protocol for conducting and presenting results of regression-type analyses. *Methods in Ecology and Evolution*, 7, 636–645.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. Springer.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.