

# Sex-specific spatial variation in fitness in the highly dimorphic *Leucadendron rubrum*

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## Abstract

Sexual dimorphism in plants may emerge as a result of sex-specific selection on traits enhancing access to nutritive resources and/or to sexual partners. Here we investigated sex-specific differences in selection of sexually dimorphic traits and in the spatial distribution of effective fecundity (our fitness proxy) in a highly dimorphic dioecious wind-pollinated shrub, *Leucadendron rubrum*. In particular, we tested for the effect of density on male and female effective fecundity. We used spatial and genotypic data of parent and offspring cohorts to jointly estimate individual male and female effective fecundity on the one hand and pollen and seed dispersal kernels on the other hand. This methodology was adapted to the case of dioecious species. Explicitly modelling dispersal avoids the confounding effects of heterogeneous spatial distribution of mates and sampled seedlings on the estimation of effective fecundity. We also estimated selection gradients on plant traits while modelling sex-specific spatial autocorrelation in fecundity. Males exhibited spatial autocorrelation in effective fecundity at a smaller scale than females. A higher local density of plants was associated with lower effective fecundity in males but was not related to female effective fecundity. These results suggest sex-specific sensitivities to environmental heterogeneity in *L. rubrum*. Despite these sexual differences, we found directional selection for wider canopies and smaller leaves in both sexes, and no sexually antagonistic selection on strongly dimorphic traits in *L. rubrum*. Many empirical studies in animals similarly failed to detect sexually antagonistic selection in species expressing strong sexual dimorphism, and we discuss reasons explaining this common pattern.

## KEYWORDS

cost of reproduction, dispersal kernels, selection gradients, sexual dimorphism, sexual selection, spatial structure

## 1 | INTRODUCTION

Plant species with separate sexes are relatively uncommon (i.e., 5–6%, Renner, 2014). Separate sexes have nonetheless evolved repeatedly among flowering plants (Renner, 2014), and such transitions have often given rise to the evolution of morphological differences between sexes (Geber et al., 1999; Puixeu et al., 2019). The degree of sexual dimorphism has also switched multiple times from low to high along the evolutionary history of certain dioecious plant lineages (Tonnabel et al., 2014). Both *sex-specific costs of reproduction* and *male–male competition to access ovules* have been suggested as potential forces causing the evolution of such dimorphism. These two factors could trigger sexually antagonistic selection (Delph & Ashman, 2006; Moore & Pannell, 2011), whereby selection exerts forces in opposite directions in each sex towards sex-specific optima (Cox & Calsbeek, 2009). The goal of this study was to estimate sex-specific fitness as well as the strength and form of selection acting on morphological traits in each sex, in a highly dimorphic dioecious wind-pollinated plant species. To do so, we combine, and adapt to the case of dioecious species, recently developed statistical methods estimating *effective fecundity*, a proxy for fitness, and its dependence on morphological traits, while explicitly modelling various spatial effects that could bias such estimations.

The sex-specific cost of reproduction hypothesis posits that sexes should diverge in morphology to satisfy their respective reproductive needs (Delph & Bell, 2008; Freeman et al., 1976). Such divergence can emerge when reproduction involves a stronger cost in one sex than in the other, or when the reproductive costs of each sex imply different resource “currencies” (Freeman et al., 1976; Obeso, 2002). Several studies have shown that pollen production in males relies strongly on nitrogen, while female reproduction is limited mostly by carbon and water (e.g., Antos & Allen, 1990; van Druenen & Dorken, 2012; Harris & Pannell, 2008; McDowell et al., 2000). Males and females of dioecious plants have evolved divergent strategies of plastic allocation to resource-harvesting organs (see Tonnabel et al., 2017 for a review), probably to harvest the resources most needed for their respective reproduction. The cost of reproduction is generally higher in females than in males, at least considering the cost per reproductive structure. However, at the scale of the whole plant, this trend is often reversed in wind-pollinated plants, which produce large amounts of pollen (Harris & Pannell, 2008; Obeso, 2002; Tonnabel et al., 2017). In some dioecious species inhabiting fire-prone environments, the cost of reproduction differs markedly between sexes because females need to maintain a canopy-stored (“serotinous”) seed bank (released by fire). As water intake is necessary to maintain cones closed and prevent seed release during an unfavourable period between two fires (Martín-Sanz et al., 2017), we may expect selection for enhanced water conduction to have favoured a divergent plant architecture between sexes. Consistent with this prediction, the evolution of longer maintenance of cones in the canopy is indeed associated with the evolution of higher sexual dimorphism in the genus *Leucadendron* (Harris & Pannell, 2010).

The evolution of sex-specific differences in vegetative traits may also originate from selection for male morphologies that are better at

dispersing pollen and therefore at accessing mates (Tonnabel, David, Klein, & Pannell, 2019; Tonnabel, David, & Pannell, 2019). The male–male competition hypothesis postulates that male reproduction, by being mostly limited by mating opportunities, selects for males that exhibit traits enhancing their competitive abilities (Arnold, 1994; Bateman, 1948). Several studies have pinpointed the importance of male–male competition in shaping male reproductive and floral traits. These studies showed more extravagant floral displays in males than in females to attract pollinators (e.g., Bond & Maze, 1999; Delph & Ashman, 2006; Dorken & Perry, 2017; Elle & Meagher, 2000; Schiestl & Johnson, 2013; Waelti et al., 2009; Wright & Meagher, 2004), variation in male flowering phenology to track the female phenology (Delph & Herlihy, 2012; Forrest, 2014), increased pollen grain competitive performance in response to polyandry (Lankinen et al., 2017) and morphological evolution of structures that disperse pollen, which prevents the attachment of competing pollen to the pollinator (Cocucci et al., 2014). In wind-pollinated plants, sexual selection may also target vegetative traits such as plant size, branch length or the length of male flower peduncles, which can facilitate pollen dispersal (Eppley & Pannell, 2007; Harder & Prusinkiewicz, 2013; Klinkhamer et al., 1997; Pickup & Barrett, 2012; Tonnabel, David, Klein, et al., 2019). Wind-pollinated plants tend to evolve larger degrees of sexual dimorphism than insect-pollinated lineages, likely because pollinators require similarity of floral morphology to successfully transfer pollen (Tonnabel et al., 2014; Welsford et al., 2016). Wind-pollinated plants, which typically show large interindividual variation in fertility (Ahee et al., 2015; Schoen & Stewart, 1987), may thus be particularly subject to sexually antagonistic selection.

In the presence of genetic variation for sexual dimorphism, each sex should, in principle, ultimately reach its optimal trait value, and thereby resolve sexually antagonistic selection (Lande, 1980). Yet, a shared genetic basis of traits between sexes may temporally constrain the evolution of their morphological divergence (Lande, 1980). In constant and homogeneous environments, theory predicts that, with strong positive genetic correlations between sexes, opposite directional selection gradients between sexes should emerge early during adaptation and persist for a long time before the sexual conflict is resolved (Connallon & Hall, 2016; Lande, 1980). Consequently, one would expect evidence for antagonistic selection between sexes to be relatively common. The compilation of numerous sex-specific selection gradients in animals showed, however, a large diversity of patterns, including cases of aligned selection across sexes (Cox & Calsbeek, 2009). In plants, documented patterns of sex-specific selection provided mixed support for sexually antagonistic selection: sex-specific selection gradients have been found to have opposite signs in both insect- and wind-pollinated species (Castilla et al., 2014; Delph et al., 2011; Tonnabel, David, Klein, et al., 2019) but to have the same sign in other studies (Barrio & Teixido, 2015; Oddou-Muratorio et al., 2018). More recent theory suggests that temporal and spatial variation in selection pressures may explain the lack of signal for sexually antagonistic selection, despite differences in the optimal phenotypes of males and females (Connallon, 2015; Connallon & Hall, 2016; Zajitschek & Connallon, 2017).

Estimating sex-specific selection gradients requires, first, estimating male and female individual fitness, and, second, relating trait values and fitness estimates. Using genotypes of established seedlings and their potential parents, traditional methods first achieve categorical parentage assignments to then estimate individual realized reproductive successes used as fitness estimates. In next generation methods, genotypes are combined with spatial localization of sampled individuals, through spatially explicit mating models (SEMMs), to disentangle the effect of fecundity from that of the distance between mating pairs (and the distance between mothers and seedlings) on reproductive success (e.g., Oddou-Muratorio et al., 2005). To do so, dispersal is explicitly modelled and dispersal kernels are estimated for both seeds and pollen. A Bayesian method was introduced in this framework to estimate individual male and female *effective fecundities* (MEMM, Klein et al., 2008 for seed sampling on mother trees; MEMMseedlings, Oddou-Muratorio et al., 2018 for seedling sampling designs). This method considers the likelihood of genotypes conditional on the position of seedlings, so it is unaffected by any process acting on the distribution of seedlings, be it the potential parents' positions, or habitat suitability and disturbances. It analyses seedling genotypes in terms of (i) overall reproductive contribution of each potential parent as determined jointly by gamete production, gamete fertilization rates, seed maturation and germination, and seedling survival until census; (ii) dispersal events in terms of estimated dispersal kernels; and (iii) pollen or seedling competition by a mass action law. Effective fecundity refers only to relative values of the first component for each parent. It varies with, for example, male–male competitive ability through differences in overall pollen production and their subsequent ovule fertilization abilities, but not with competitive effects dependent on the composition of competitors within the pollen cloud generated by uneven spatial distribution of mates. Here we extend this methodology to dioecious species. This spatially explicit approach avoids spatial bias in effective fecundity estimation, typically generated by sampling seedlings nonuniformly with respect to the positions of their parents or by the confounding effects of the heterogeneous spatial distribution of mates (Oddou-Muratorio et al., 2018). Used as a proxy for fitness, effective fecundity thus provides the expected relative reproductive success if putative mates (for male fecundity) and regeneration sites (for female fecundity) were uniformly distributed in space, and all offspring could establish and be sampled (Klein et al., 2013). It therefore attenuates the impact of stochastic effects associated with sampling methods on fitness estimates.

Relating fitness estimates to plant traits using the selection gradients methodology proposed by Lande and Arnold (1983) can further suffer from specific statistical bias in sessile organisms living in heterogeneous environments. Indeed, small-scale spatial variation in resources fundamental to plant physiology, including sex-specific reproduction, is common across a range of habitat types (Araya et al., 2011; Silvertown et al., 1999). To disentangle the fitness effect of plant characteristics (such as their ability to harvest resources, which may be sex-specific) from that of the environment (such as the spatial distribution of resources), the spatial distribution

of individuals must be accounted for (Rauscher, 1992). Indeed, not modelling explicitly the spatial autocorrelation of unmeasured ecological variables affecting fitness can lead to detection of false-positive effects of traits on fitness, as on any other response (Guillot & Rousset, 2013). To address this problem, we fitted mixed-effect models with spatially autocorrelated random-effects, using the spaMM package (Rousset & Ferdy, 2014). To our knowledge, it is the first time that spatial effects are taken into account in the estimation of selection gradients. Moreover, spatial variation in plant density and the local sex ratio may generate spatial variation in competition for resources, which can be studied by analysing their fixed effects on plant fitness. In conclusion, our MEMMseedlings model controls for spatial confounding effects on estimations of effective fecundity relative to spatial sampling biases and to the spatial heterogeneity of plant distribution (potentially impacting competition among males), while our spaMM procedure controls for spatial environmental heterogeneity.

We applied our methodology to *Leucadendron rubrum*, a dioecious wind-pollinated serotinous shrub, endemic to the fire-prone South African fynbos. *Leucadendron rubrum* displays extreme sexual dimorphism (Figure S1), with males being typically more highly branched, having smaller leaves and taller stature than females (Harris & Pannell, 2010; Welsford et al., 2014, 2016). A single recruitment pulse typically occurs after fire, killing all adult trees and releasing seeds stored in their canopy seed bank (Cowling & Lamont, 1987). This particular life cycle allows the estimation of lifetime effective fecundity by sampling seedlings only once (i.e., after the fire event). Furthermore, because recruitment is synchronized by fire, all sampled adult individuals in the population have the same age (Bond, 1984). The strong sexual dimorphism of *L. rubrum* has been previously hypothesized to be the consequence of sex-specific resource requirements (Harris & Pannell, 2010). Indeed, the cost of reproduction in *L. rubrum* is likely to differ strongly between sexes due to the cost of maintaining the canopy-stored (“serotinous”) seed bank in females (Martín-Sanz et al., 2017). We hypothesized that, because of such maternal care, female fitness may be more sensitive to water availability than male fitness. Owing to these differences in resource requirement for reproduction, we therefore tested whether male and female effective fecundities (as defined above) display different spatial structure and whether the observed strong sexual dimorphism is associated with sex-specific selection gradients (Lande, 1980).

## 2 | MATERIALS AND METHODS

### 2.1 | Study species and site

*Leucadendron rubrum* is a dioecious wind-pollinated shrub species endemic to the Western Cape of South Africa (Rebelo, 2001) where natural fires occur every 10–15 years (Kraaij et al., 2011; van Wilgen et al., 2010). *Leucadendron rubrum* belongs to the family Proteaceae and flowers from August to September. Seed

recruitment is constrained to a short period following fires, and seeds released between fires typically fail to establish due to competition (Bond, 1984). *Leucadendron rubrum* typically starts flowering at 2–3 years, and seeds are retained in woody cones for several years (Harris & Pannell, 2010). Seeds therefore form a “serotinous” seed bank, which persists until fire kills the plants, allowing cone opening and wind dispersal of fruits via their plumed perianth (Rebelo, 2001; Williams, 1972). In serotinous species, disruption of water intake to the cone (caused by broken branch or plant death) was shown to lead to seed release, suggesting a water cost to keep the cones closed (Martín-Sanz et al., 2017; Treurnicht et al., 2016). Thus, mother death or any event leading to cone opening before the advent of fire results in seed release in poor conditions for recruitment and ultimately in the loss of progeny.

The study population was located at Bainskloof pass (33°32′21.25″S, 19°10′12.10″E) and was contained in a rectangle of 135 × 110 m (Figure S2). We studied all adult individuals of our focal population. Another population of *L. rubrum* was located at a distance of 310 m (smallest distance found between two plants from the two populations). All adults of *L. rubrum* of the focal population (i.e., 86 females and 88 males) were mapped (see Methods S1), sampled for DNA analyses and measured for several traits in 2004. In summer 2006, a fire burnt the population, killing all adults. A total of 1265 seedlings were mapped, and their leaves were sampled in the following autumn (February 2007), 4–5 months after the fire. The spatial distribution of adults and seedlings is shown in Figure S2. In one part of the study site, a ditch had been dug for construction after seeds had dispersed, so we were unable to determine the undisturbed positions of 172 seedlings located in that area, which we therefore eliminated from the data set. However, the effective fecundity of adults in this area can still be estimated without bias induced by the disturbance, thanks to the use of MEMMseedlings (see below).

When sampling seedlings, the presence of seedlings from another closely related sympatric species (*Leucadendron salignum* P.J. Bergins) rendered the identification of *L. rubrum* juveniles difficult. To ensure that only juveniles of *L. rubrum* were included in later analyses, we genotyped juveniles (see below for details on the genotyping protocol), as well as adults from both species. This analysis aimed at assigning seedlings to either species and its results are described in Method S2 and Figure S3. We did not find evidence for the existence of hybrids between *L. rubrum* and *L. salignum*. In addition, 254 juveniles were excluded after genotyping as they belonged to *L. salignum*.

## 2.2 | Measurements of adult traits in the field

For adult shrubs, we measured in 2004 three traits describing plant architecture and three traits describing leaf morphology (available at <https://doi.org/10.5061/dryad.ngf1vhhst>). All six traits are known to be sexually dimorphic in this species (Harris & Pannell, 2010; Welsford et al., 2014, 2016; Figure S4). The three traits describing plant architecture were (i) plant height, (ii) the first diameter defined as the greatest horizontal diameter of the plant (hereafter, canopy

diameter) and (iii) the second horizontal diameter defined as the diameter perpendicular to the first diameter (hereafter, second diameter). Several leaves were collected randomly along branches of each adult, dried and photographed. Pictures were analysed to measure the three traits describing leaf morphology: (i) leaf area, (ii) length and (iii) width using IMAGEJ (Schneider et al., 2012). The number of leaves analysed per adult ranged from 10 to 23 with an average of 20.3. In females, we counted the number of cones in the last two cohorts (cones produced in the last two seasons of cone production and maintained closed since). Because older cohorts were not counted, this measure reflects cone production rather than the maintenance of the serotinous seed bank. We did not count male cones because they were too numerous.

## 2.3 | Microsatellite genotyping

We genotyped both adults and their progeny in our focal population (available at <https://doi.org/10.5061/dryad.ngf1vhhst>). For both adults and seedlings, sampled leaves were preserved in silica gel prior to DNA extraction using a modified version of the CTAB protocol (Justy et al., 2009). We designed two polymerase chain reaction (PCR) multiplexes for amplifying DNA at four microsatellite loci, each involving primers with different fluorescent labels (Multiplex 1: 4F8, 3C9, 1C7, 1C3; Multiplex 2: 3B11, 2B2, 1D7, 1B8; markers developed by Justy et al., 2009). PCRs were performed using the Qiagen Multiplex PCR Kit (Qiagen); each PCR was performed in a final volume of 10  $\mu$ M composed of 5  $\mu$ l of Multiplex Master mix (2 $\times$ ), 1  $\mu$ l of primer sequences (1  $\mu$ M), 1  $\mu$ l of DNA extracts and 3  $\mu$ l of sterile water. PCRs were performed on a Mastercycler pro thermocycler (Eppendorf, vapo.protect) with an initial denaturation step of 15 min at 95°C, 35 cycles of 30 s at 94°C, 90 s at the  $T_m$  temperature (M1: 54°C, M2: 53°C) and 1 min at 72°C, and a final step of 30 min at 60°C. Genotyping was performed on an ABI3500XL sequencer. The genotypes of all adults and offspring were scored using GeneMapper at the eight microsatellite loci, which exhibited between five and 23 alleles (see Table S1 for information per marker). After excluding individuals that did not amplify, our data set contained 82 females, 85 males and 869 juveniles, corresponding to an amplification failure of roughly 8% for both adults and juveniles. For each microsatellite marker, we used CERVUS (version 3.0.7; Kalinowski et al., 2007) to estimate the nonexclusion probabilities of the first parent, the second parent and parent pairs, to test for Hardy–Weinberg equilibrium, and to compute null allele frequencies (Table S1). Nonexclusion probabilities correspond to the probabilities that the set of loci will not exclude an unrelated candidate parent (or parent pair) of an arbitrary offspring. Finally, we used NMPI (version 1.0, Chybicki, 2018) to estimate the per-marker genotyping errors.

## 2.4 | Joint estimation of effective fecundities and both pollen and seed dispersal kernels

We used a method that uses information about the genotype and the spatial location of adults and seedlings to jointly estimate pollen and

seed dispersal kernels and the individual male and female *effective fecundities*—a proxy for fitness (see Introduction). Our method builds on the MEMM, MEMMseedlings (Oddou-Muratorio et al., 2018), which models mating and dispersal events in a hermaphroditic plant population to estimate the selfing rate, immigration rates and dispersal kernels for both pollen and seeds as well as the variance in male and female effective fecundity (i.e., using random individual effects). We modified the MEMMseedlings algorithm to produce a new version that handles separate sexes, with distinct spatial distributions of male and female plants (see Method S3, available at [https://gitlab.paca.inrae.fr/pub/tonnabel\\_mol\\_ecol](https://gitlab.paca.inrae.fr/pub/tonnabel_mol_ecol)).

MEMMseedlings takes into account both the variation in fecundity among individuals and the relative positions of putative parents and seedlings when computing the likelihood of observed genotypes conditional to dispersal parameters and individual fecundity estimations. In MEMMseedlings, putative parents that are more distant from a seedling have a lower parentage probability. This model also describes mate competition through a mass action law, that is the contribution of a given male to the pollen cloud of a given female is diluted among the contributions of all other males. For these reasons, the model can estimate variation in effective fecundities, separately from different sources of spatial variation in reproductive success, linked to (i) spatial biases in seedling sampling, (ii) the spatial distribution of mates or (iii) edge effects. Accordingly, we checked that the estimated effective fecundity of adult plants located on the border of our study population was not different from elsewhere in the study population by generalized linear mixed models (results not shown). Our model assumes the same pollen (or seed) dispersal kernels for each male (or respectively female) individual, and isotropic wind dispersal patterns. Given that anisotropy can sometimes be found in wind-pollinated plants (Austerlitz et al. 2007), we confirmed an absence of signal for anisotropy using  $\text{NM}\pi$  (see Method S3). MEMMseedlings estimates a relative measure of effective fecundity, scaled by the average effective fecundity, (see equation 1 in Methods S3), which is therefore unitless.

Given the life cycle of *L. rubrum* and the sampling of seedlings after the fire, our estimated effective fecundity integrates the effect of variation between individuals, either male or female, not only for pollen and ovule production, but also for pollen export, fertilization rate, seed maturation and dispersal, maintenance of seeds within the cones (degree of serotiny), adult survival until the fire, seed germination and juvenile survival until the seedling census. Seeds released after the fire and previously stored in the canopy were potentially fertilized in different years. Because adults of *L. rubrum* do not survive fire, the establishment of progeny after a fire thus represents their entire lifetime reproduction.

Briefly, our MEMMseedlings model combines genotype data and spatial distribution data for both adults and offspring to estimate, in a Bayesian framework, individual male and female effective fecundities ( $F_k$  and  $R_j$ , respectively), the seed immigration rate ( $m_s$ ), the pollen immigration rate ( $m_p$ ), the rate of pollen export to the pollen cloud of nonlocal mothers ( $v$ ), the mean seed dispersal distance ( $\delta_s$ ), the mean pollen dispersal distance ( $\delta_p$ ), a parameter affecting the

shape of the seed dispersal kernel ( $b_s$ ) and that of the pollen dispersal kernel ( $b_p$ ). The estimation of pollen and seed immigration rates ( $m_s$  and  $m_p$ ) and the rate of pollen export to the pollen cloud of non-local mothers ( $v$ ) depends on the actual process of immigration, but is also affected by the fact that around 8% of the parents were excluded from the analysis due to a lack of amplification. Immigration rates therefore include the probabilities of maternity and paternity attributable to unsampled parents. Both pollen and seed immigration rates are therefore probably overestimated. Finally, when computing Mendelian transition probabilities between seedlings and putative parents, MEMMseedlings considers genotyping errors by allowing a parent-offspring genetic discrepancy at a maximum of one locus, and at each locus, the probability to mistype any allele was fixed using the per-marker genotyping error rates estimated by  $\text{NM}\pi$  (see Method S3). To describe the quality of parentage assignments associated with the data set, MEMMseedlings computes the posterior probabilities for all seedlings for which (i) both parents are known among the genotyped parents, (ii) only the mother or (iii) only the father is known, and (iv) none of the parents is known.

Estimates of dispersal kernels are based on dispersal events within the population, even if the parametric model implies dispersal in unbounded space (beyond the maximal male–female and female–seed distances found in our study population, respectively 106.4 and 139 m for pollen and seed dispersal). To describe dispersal within the study population, we computed, from the estimated kernels, the predicted proportion of seeds and pollen that dispersed within the population range and the predicted proportion that dispersed within an arbitrary short distance of 20 m.

We estimated the model parameters using two Markov chain Monte Carlo (MCMC) chains of 50,000 steps and a burn-in of 10,000 steps each. We used uniform prior distributions for the parameters  $m_s$ ,  $m_p$ ,  $v$ ,  $\delta_s$ ,  $b_s$ ,  $\delta_p$  and  $b_p$  within the intervals [0.01, 1.00], [0.01, 1.00], [0.00, 1.00], [1.00, 100], [0.01, 1.00], [1.00, 30,000] and [0.01, 10.0], respectively. For individual effective fecundity estimates ( $F_k$  or  $R_j$ ), values were sampled every 20 iterations to decrease autocorrelation and averaged after concatenating the two chains. For each of these stored iterations, we also computed the variance in effective fecundity estimates among individuals. Credibility intervals at 95% were calculated for all estimated parameters, as well as the mean value across all iterations and chains (Table 1).

## 2.5 | Sex differences in morphology, spatial distribution and analysis of number of cones

We tested for sex differences in morphology using linear mixed-effects models (LMMs) with spatially autocorrelated random effects. We analysed all measured adult traits describing either plant architecture or leaf morphology. Random individual effects can be spatially autocorrelated, for instance due to spatial variation in some ecological variables not included in the model. We fitted models with morphology as the response variable and sex as a fixed effect using the R package spaMM version 3.3.0 (Rousset & Ferdy, 2014)

**TABLE 1** Credibility intervals at 95% and mean values across MCMC iterations of the parameters of our spatially explicit model estimating variation in male and female effective fecundities ( $F_k$  and  $R_j$ ) for a natural population of *Leucadendron rubrum*

Model parameter	Credibility interval (95%)	Mean value
Mean dispersal distance of seeds (m), $\delta_s$	[8.26–13.3]	10.6
Shape of the dispersal kernel of seeds, $b_s$	[0.475–0.664]	0.567
Probability of seed immigration, $m_s$	[0.0717–0.150]	0.109
Mean dispersal distance of pollen (m), $\delta_p$	[620–28,569]	11,041
Shape of the dispersal kernel of pollen, $b_p$	[0.0973–0.203]	0.130
Probability of pollen immigration, $m_p$	[0.101–0.198]	0.148
Rate of pollen exportation to the pollen cloud of nonlocal mothers, $v$	[0.594–0.975]	0.813
Variance in female effective fecundity, $R_j$	[1.98–12.8]	5.87
Variance in male effective fecundity, $F_k$	[0.781–2.84]	1.56

Note: Credibility intervals are calculated on the concatenation of two Markov chains of 50,000 steps each (with a burn-in phase of 10,000 steps). Variance in female and male effective fecundity ( $F_k$  and  $R_j$ ) was calculated for each MCMC iteration of each chain.

in R 4.0.2 (R Core Team, 2020). We fitted models with distinct residual variances for each sex. We updated the code of the spaMM package to allow for different spatial distributions of random effects between sexes (publicly available in spaMM since version 2.6.0). The classical Matérn correlation function was used to model the spatial autocorrelation of random effects, separately for each sex, as a function of the distance between individuals. The Matérn correlation model involves two parameters: the smoothness parameter ( $\nu$ ) and the scaling parameter for distances ( $\rho$ ). We compared model fits considering (i) no spatial autocorrelation, (ii) the same spatial autocorrelation for both sexes and (iii) two sex-specific spatial autocorrelation structures. We used likelihood ratio tests (LRTs) to (i) first test for a sex-specific spatial structure in morphology, by comparing models with a different structure of random effects but the same fixed effects, and (ii) test for sexual dimorphism, by comparing the selected model in step (i) to a model fitted with a similar structure of random effects, but without sex as a fixed effect. Models were fitted either by maximum likelihood, for performing LRTs between models differing in their fixed-effects structure, or by restricted maximum likelihood, for LRTs between models differing in their random-effect structures and for computing the predictions from the model fits. We also examined whether male and female plants were spatially segregated (i.e., whether the sex ratio was spatially autocorrelated) using a binomial generalized linear mixed model (GLMM) with sex as the response variable and compared model fits with or without a

spatially autocorrelated random effect as described above. Finally, we tested whether the number of cones produced by females displayed a nonuniform spatial distribution by comparing models including cone number as the response variable, plant density (see below for its calculation) as a fixed factor, and either a spatial random effect or not.

## 2.6 | Multivariate sex-specific selection analysis

Inspired by the multivariate framework of Lande and Arnold (1983), we examined in a single full model the relationship between the relative effective fecundity as the response variable and the following explanatory variables: canopy diameter, leaf area, plant density, sex, and the interaction between each of the three former explanatory variables and sex. We fitted generalized linear mixed-effects models (Gamma GLMM with logarithm link function) to describe the variation in effective fecundity, while including spatially autocorrelated random effects. We checked that a model predicting effective fecundity using a Gamma distribution with a log link performed better than models assuming a different candidate distribution, using cAIC which is a metric similar to the traditional AIC, except that it measures prediction performance conditionally on the realization of the random effects (Vaida & Blanchard, 2005 cAIC = 18.7 for gamma with log link, 139 for gaussian with identity link, and 126 for gaussian with log link). We followed the same procedure as described for the analysis of sexual dimorphism (see previous section) to fit the models using the R package spaMM and to identify the best fitting random spatial structure (i.e., comparing a sex-specific spatial structure, a common spatial structure and a lack of it). We were thus able to compare the patterns of spatial variation of residual effective fecundity between males and females, once the effects of local density and variation in morphology were included as fixed effects in our global model. Spatial autocorrelation in effective fecundity may then reflect spatial variation in unmeasured environmental variables (such as water availability) or unmeasured plant traits. Different spatial structures between sexes therefore inform on the differential sensitivities of their effective fecundities to variation in such unmeasured variables. We took into account the variation in uncertainty of the individual effective fecundity estimates by parameterizing the residual variance as a function of the variance in MCMC estimates of individual fecundity during the fitting (see Method S4). We again fitted models with distinct residual variances for each sex. Because our MEMMseedlings model estimated large effective fecundity for several plants located in one part of the study population, we performed a sensitivity analysis testing the robustness of our results to the removal of all individuals with standardized effective fecundity greater than four standard errors (for all statistical models treating effective fecundity as the response variable). Five females and one male were removed in this sensitivity analysis, including four individuals that were particularly close to each other.

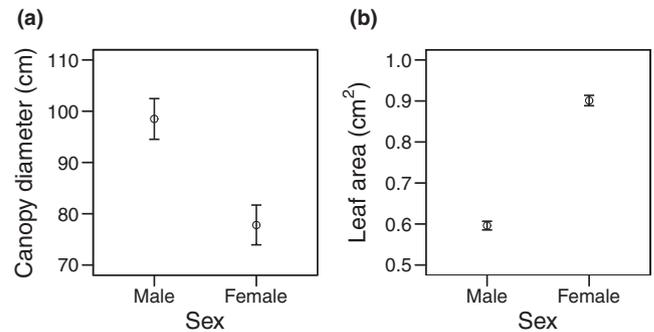
We chose to investigate the selection gradients for two morphological predictors only (canopy diameter and leaf area), because

several of the six morphological traits were highly correlated (Table S3). Notably, all three traits describing plant architecture were strongly correlated with each other (i.e., plant height, canopy diameter and second diameter, Table S3). Similarly, our three measures of leaf morphology were strongly correlated with each other (i.e., leaf area, length and width, Table S3). Estimation of multivariate selection gradients and their interpretation could be confused by such strong correlations between traits (see Chong et al., 2018 for a review). We therefore retained only two morphological traits in our selection analysis (but similar results were obtained for other combinations of traits). We standardized the two focal traits using z-scores based on the mean and variance values of the traits calculated separately for each sex. We confirmed that leaf area significantly differed between individuals by comparing, within each sex, models predicting leaf area with vs. without an individual-level random effect using an exact restricted likelihood ratio test (Crainiceanu & Ruppert, 2004) implemented in the package RLRsim 3.1-6 (Scheipl et al., 2008): for females, likelihood ratio (LR) = 1573.9,  $p < .001$ ; for males, LR = 625.1,  $p < .001$ .

We compared the performance of local plant density measured at different scales in explaining variation in male and female effective fecundity (see Method S5). We did not transform plant density into z-scores because this predictor is not an individual trait and thus its associated slope should not be considered as a selection gradient. To compare models explaining variation in effective fecundity with different scales used to compute plant density, we used cAIC. The best model included the density of plants in a quadrat of  $12 \times 12$  m around the focal individual. We therefore retained this metric to compute the values of plant density used in all subsequent analyses.

To test for the significance of selection gradients, we first compared the fit of the full model to the fit of nested models in which one of the three predictors of interest (i.e., canopy diameter, leaf area and plant density) had been removed, both as a main effect and in interactions. This revealed whether there was any effect of the focal predictor. Second, we compared the full model to models in which only the interaction between sex and one of the three variables of interest had been removed. This allowed us to test whether the effect of the predictor was different between sexes. We also built separate GLMMs for each sex, predicting effective fecundity from the three predictors of interest. We used these sex-specific models to test the effect of each predictor in each sex, if and only if the interaction between sex and a given predictor was significant. We compared the fit of our full model, which considers linear selection gradients only, to the fit of a similar model including quadratic and correlational selection terms for canopy diameter and leaf area, and to model fits including one quadratic or correlational term at a time.

We also estimated selection gradients for the same morphological traits (canopy diameter and leaf area), using as a proxy for fitness not the estimated effective fecundity but the actual number of cones empirically counted on female plants. We predicted the number of female cones from traits using a Poisson GLMM with spatially autocorrelated random effects and plant density as a covariate in



**FIGURE 1** Sexual dimorphism in canopy diameter (cm; a) and leaf area (cm<sup>2</sup>; b) of the study population *Leucadendron rubrum*. Points are prediction from models accounting for the spatial distribution of traits within each sex and error bars are 95% confidence intervals around mean predictions

addition to the two focal traits. Finally, we predicted female effective fecundity from the number of empirically counted cones, also with spatially autocorrelated random effects and plant density as a covariate.

### 3 | RESULTS

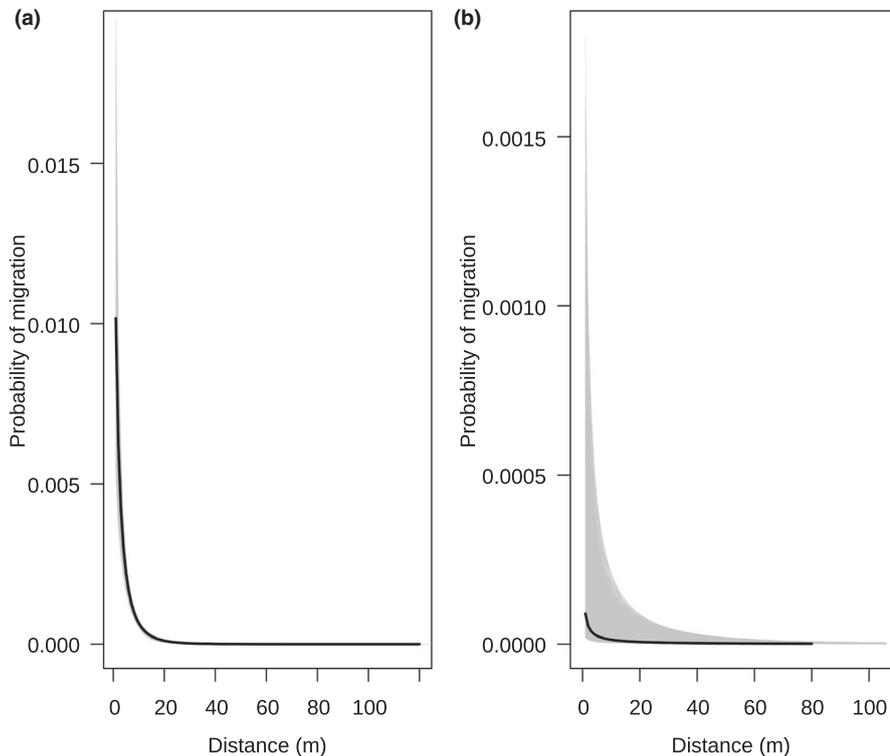
#### 3.1 | *Leucadendron rubrum* showed strong sexual dimorphism in morphology

Females had significantly smaller canopy diameters (i.e., first and second diameter measures; Figure 1a; Figure S4c, Table S4), but they were not clearly shorter (Figure S4a, Table S4). Females also had leaves with a larger area than males (Figure 1b; Table S4). Correlated measures of leaf morphology (Table S3) showed similar sexual dimorphism, as females displayed longer and wider leaves than males (Figure S4e,f, Table S4).

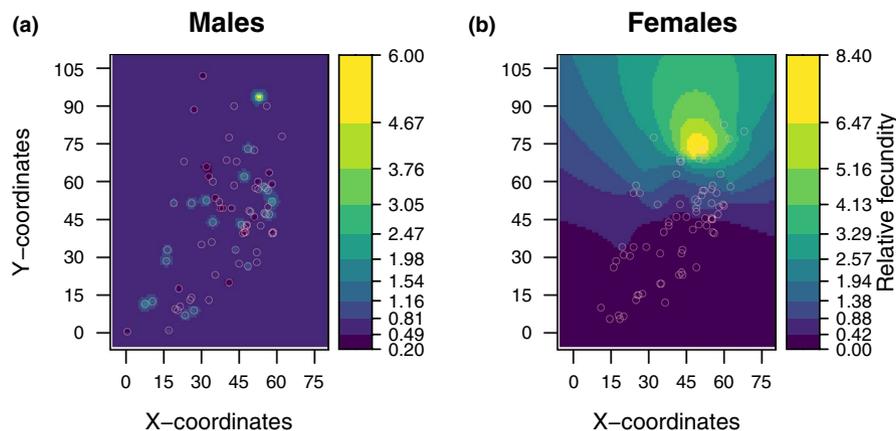
Spatial structure in the canopy diameter did not clearly differ between sexes, as the fit of a linear mixed model including sex-specific spatially autocorrelated random effects did not produce a likelihood significantly higher than the fit of the model with the same distribution of spatial random effects in both sexes (Table S4). However, a spatially structured random effect considerably improved the fit over the models without it (Table S4; Figure S5). In contrast, leaf area showed neither a sex-specific spatial structure nor a general spatial structure (Table S4). Similarly, we found no significant spatial structure for sex ratio (Table S4; Figure S6).

#### 3.2 | Dispersal occurred on a smaller spatial scale for seed than for pollen

For both pollen and seed dispersal kernels, our analysis revealed fat-tailed dispersal kernels (i.e.,  $b_s$  and  $b_p < 1$ ; Figure 2 and Table 1). Seed and pollen immigration rates were of the same order of magnitude (11% and 15% for seed and pollen respectively). Seed dispersal



**FIGURE 2** Dispersal kernels estimated under the Bayesian model for seed dispersal (a) and for pollen dispersal (b). Filled lines correspond to the posterior mean dispersal kernels obtained by averaging parameters of the concatenation of two Markov chains of 50,000 steps (burn-in phase of 10,000 steps). Grey lines illustrate the uncertainty around the averaged dispersal kernel and correspond to the kernels estimated on each iteration of the MCMC. Both dispersal kernels are represented within the minimal and maximal distances existing in our population between females and seedlings for seeds (a) and between males and females for pollen (b), that is, respectively, the maximal female-seedling and male-female distances in our population. The extrapolation of dispersal kernels beyond these limits are not represented in the plots



**FIGURE 3** Spatial prediction of the relative effective fecundity in males (a) and females (b) as predicted by a generalized linear mixed-effect model, our full model, including all fixed effects (sex, canopy diameter, leaf area, plant density, and the interaction between each of the three latter variables and sex) as well as one spatially autocorrelated random effect for each sex. Relative fecundity represents our measure of relative effective fecundity estimated by our MEMMseedlings model, and circles represent the localization of individual plants [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

occurred on a smaller spatial scale than pollen dispersal: the mean estimated dispersal distance of seeds and pollen were respectively 10.6 and 11,041 m. These estimated dispersal kernels predict that ~100% of seeds fell closer than the maximal female-seedling distance found in our population (i.e., 139 m), while only 7.56% of pollen travelled a distance shorter than the maximal male-female distance found in our population (i.e., 106.4 m). Similarly, we estimated that 86% of seeds and 1.56% of pollen was dispersed within 20 m. A fat-tailed pollen dispersal kernel accounting for distance-dependent pollen dispersal nevertheless explained our data better than modelling a uniform distribution of pollen dispersal distances (Figure S7,

see Methods S3 for a description of their comparison). Although we estimated a high probability of the pollen travelling a long distance, we estimated that about three quarters of the seedlings had a genotyped father in the population ( $1 - m_p = 0.85$  for seedlings with a known mother or  $v = 0.81$  for seedlings with an unknown mother), which is similar to the proportion found for mothers ( $1 - m_s = 0.89$ ; Table 1). Estimations of dispersal kernels with the  $NM\pi$  algorithm yielded a similar parameter estimation, yet with a notably shorter estimate of mean pollen dispersal distance (Table 1 vs. Table S2). The estimates of dispersal kernels with  $NM\pi$  were robust to the inclusion of anisotropy in dispersal events (results not shown).

### 3.3 | Autocorrelation in effective fecundity occurred on a smaller spatial scale for males than for females

Effective fecundity estimations were carried out using a set of eight microsatellite markers containing between six and 24 alleles, showing nonexclusion probabilities of parent pairs ranging between 0.12 and 0.58 and genotyping error rates ranging from 0.9% to 5.5% (Table S1). The combination of the genotype data and plant spatial distribution data provided information to assign two parents to ~88% of seedlings (figure SM3 in Methods S3).

We detected a clear sex-specific spatial structure for effective fecundity (Table S5). We found different spatial variation in effective fecundity for each sex, with coarse-grained and fine-grained spatial effects for females and males, respectively (Figure 3a vs. 3b). These results were robust to the removal of plants with standardized effective fecundity greater than four standard errors, which only slightly affected the previous conclusion (Table S5; Figure S8). Several plants with large effective fecundity were found in the disturbed area (Figure 3). The number of empirically counted cones on female plants also displayed a significant spatial autocorrelation ( $\chi^2 = 2238$ ,  $df = 3$ ,  $p < .0001$ ; Figure S9). Cone number was significantly correlated with relative effective fecundity but the effect size was small (using standardized cone number,  $\beta = 0.286$ ,  $\chi^2 = 6.02$ ,  $df = 1$ ,  $p = .0141$ ) and the two spatial distributions were not fully aligned (Figure 3a vs. Figure S9).

### 3.4 | Selection for higher leaf area and wider canopies similar in both sexes

Our spatially corrected selection gradient approach revealed that leaf area was negatively associated with effective fecundity (Table 2; Table S5; Figure 4), with similar slopes in both sexes as shown by a nonsignificant interaction between leaf area and sex (Table S5). Yet, leaf area was positively correlated to the number of counted cones on female plants ( $\beta = 0.171$ ; LRT:  $\chi^2 = 42.7$ ,  $df = 1$ ,  $p < .001$ ). Larger canopy diameter was significantly associated with higher effective fecundity (Table 2; Table S5; Figure 4). Accordingly, female plants with wider canopies displayed higher numbers of empirically counted cones ( $\beta = 0.904$ ; LRT:  $\chi^2 = 78.8$ ,  $df = 1$ ,  $p < .001$ ). The increase in effective fecundity with increasing canopy diameter was similar in both sexes, as shown by a nonsignificant interaction between sex and canopy diameter (Table S5). Our estimates of selection gradients were robust to the removal of plants with effective fecundity greater than four standard errors (Table S5; Figure S10). We found no signal for nonlinear selection, either by including all three quadratic and correlational terms at once (LRTs:  $\chi^2 = 0.709$ ,  $df = 3$ ,  $p = .871$ ), or each of them separately ((leaf area)<sup>2</sup>:  $\chi^2 = 0.129$ ,  $df = 1$ ,  $p = .719$ ; (canopy diameter)<sup>2</sup>:  $\chi^2 = 0.118$ ,  $df = 1$ ,  $p = .731$ ; leaf area  $\times$  canopy diameter:  $\chi^2 = 0.378$ ,  $df = 1$ ,  $p = .539$ ).

TABLE 2 Spatially corrected selection gradients on plant architecture and leaf morphology estimated in a natural population of *Leucadendron rubrum*

	$\beta$	SE
Intercept	-1.27	1.48
Sex (ref = female)	1.62	1.49
Plant density	0.0424	0.0338
Canopy diameter	0.427	0.107
Leaf area	-0.0864	0.0997
Sex $\times$ Plant density	-0.109	0.0377
Sex $\times$ Canopy diameter	0.0411	0.138
Sex $\times$ Leaf area	-0.199	0.130

Note: Relative effective fecundities were predicted from canopy diameter, leaf area, plant density and the interaction between each of these three predictors and sex (with females as reference). The estimates ( $\beta$ ) and their associated conditional standard error (SE) obtained for these fixed effects are reported. Since the model is a Gamma GLMM with a log link function, all estimates are provided on the scale of the linear predictor (i.e., log). We also estimated in this model one spatially autocorrelated random effect for each sex. The variance of the spatial random effect was 0.438 for males, with Matérn parameters  $\nu = 47.2$  and  $\rho = 7.74$ . The variance of the spatial random effect was 3.41 for females, with Matérn parameters  $\nu = 0.423$  and  $\rho = 0.0105$ . The residual variance parameter  $\Phi$  was 0.282 and 0.676 for males and females, respectively.

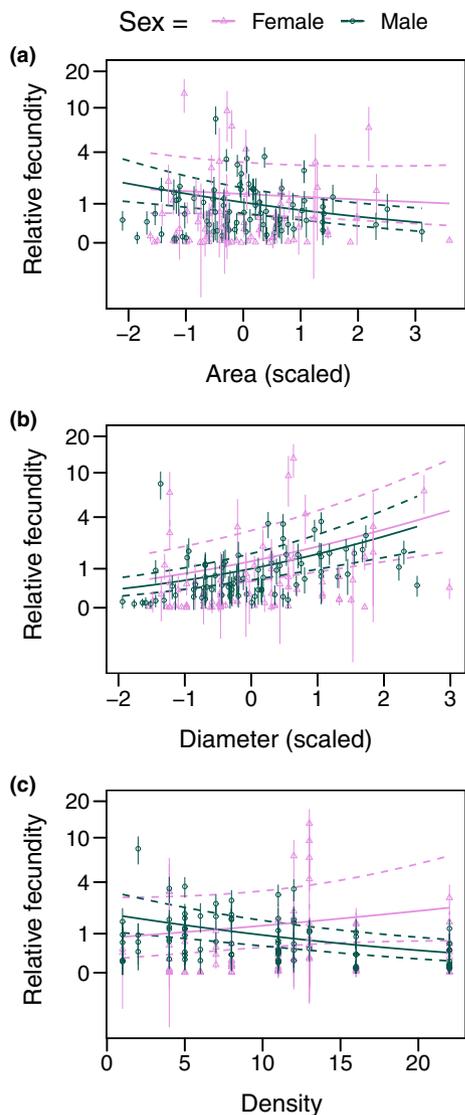
### 3.5 | The effect of plant density on effective fecundity is sex-specific

The effect of plant density on effective fecundity (Figure S6) differed between sexes, as revealed by a significant interaction between sex and plant density (Table S5; Figure 4c). Plant density was negatively associated with male effective fecundity, but female effective fecundity showed no association with plant density (Table S5). The effect of the interaction between sex and plant density, however, was only marginally significant when the plants with the highest effective fecundity were removed from the robustness analysis (Table S5; Figure S10).

## 4 | DISCUSSION

### 4.1 | Novel methods for dealing with spatial bias affecting selection estimates in plants

Technical and methodological improvements in parentage assignments now allow for estimation of plant fitness in natural populations from genetic data, and provide the link between fitness and plant traits through selection gradients analyses (e.g., Burczyk et al., 2006; Burczyk & Prat, 1997; van Kleunen & Burczyk, 2008; Meagher & Thompson, 1986). We have developed a methodology that estimates effective fecundity in dioecious plants while accounting for biases associated with their spatial distribution.



**FIGURE 4** Partial-dependence effect plots of leaf area (a), canopy diameter (b) and plant density (c) on the relative effective fecundity as predicted by our full model. Points indicate observed trait values as well as the relative effective fecundity  $\pm$ SD stemming from MCMC estimations. Curves indicate model predictions computed as partial-dependence effects  $\pm$ 95% confidence intervals. Relative fecundity designates our measure of relative effective fecundity [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

Beyond the interest in documenting spatial patterns of seed and pollen dispersal, the addition of this spatially explicit component to classical parentage methods (Jones et al., 2010) improves the estimation of effective fecundity in the presence of confounding effects, such as spatial bias in sampling descendants, spatial variation in the intensity of mate competition triggered by a nonuniform distribution of mates, or border effects (Oddou-Muratorio et al., 2018). We investigated effects of traits on effective fecundity by classical selection gradient methods (Lande & Arnold, 1983), in which we explicitly modelled the effect of spatially correlated unmeasured environmental factors on effective

fecundity. This newly developed framework will be particularly suited to estimating selection in natural populations, given that spatial biases are typically difficult to avoid regarding both sampling and uncontrolled factors.

#### 4.2 | No contemporary sexually antagonistic selection despite strong sexual dimorphism

The signal we found for selection of larger canopy diameters in both sexes may indicate a “budget effect” of plant size, where larger plants acquire more resources that can be reallocated to gamete production (Delph & Ashman, 2006). In females, the number of counted cones was positively related to canopy diameter. However, cone number only poorly explained effective fecundity. Moreover, the spatial distribution of cone number and female effective fecundity did not fully match, suggesting that processes occurring after cone production, such as cone maintenance or mother plant survival, affect female effective fecundity. In males, selection for wider canopies could be linked to flower production if both sexes are subject to similar ontogenetic constraints. We found evidence for similar selection for smaller leaf area in both sexes. Smaller leaves were previously shown to be correlated in *L. rubrum* with thinner, and more numerous, branches and less efficient water transport from roots to branch apex (Harris, 2007). Selection for smaller leaves in males may reflect selection for a greater number of inflorescences held on more flexible branches, a trait long hypothesized to enhance pollen dispersal (Klinkhamer et al., 1997). Smaller leaves may also represent a decreased mechanical hindrance to pollen dispersal. Selection for smaller leaves in females, however, contradicts our expectation of selection for enhanced water transport to the cones. The number of cones counted on females was furthermore positively related to leaf area. The fact that leaf area relates to the number of cones, and to effective fecundity in opposite ways, suggests that any positive effects of leaf area on fecundity through female cone production may have been masked by trade-offs with other key life history components for serotinous plants (e.g., adult survival until the fire). In the absence of positive genetic correlation between sexes, sexual conflicts may be resolved once each sex reaches its respective optimum. However, we did not find evidence for stabilizing selection in the study population, which one could expect if each sex was at its optimum with sufficient genetic variation in the studied traits.

Only 17% of studies estimating selection gradients in animals identified sexually antagonistic selection (Cox & Calsbeek, 2009). The paucity of evidence for sexually antagonistic selection, with which our study concurs, is inconsistent with the idea that genetic correlations between sexes should maintain sexually antagonistic selection over long periods of time (Lande, 1980). To explain this inconsistency, both theory and experiments have suggested that temporal or spatial ecological changes may result in variable patterns of selection acting on males and females, with both sexes

displaying trait values remaining far away from their ever changing ecological optimum (Berger et al., 2014; Connallon, 2015; Connallon & Hall, 2016; Delph et al., 2011; Kokko & Rankin, 2006; Long et al., 2012; Sheridan & Bickford, 2011). Recent theoretical developments showed that positive intersexual covariance for resource acquisition traits could also impede the identification of sexually antagonistic selection (Zajitschek & Connallon, 2017).

An alternative explanation to the lack of detected antagonistic selection is that antagonistic selection is present but masked by a positive correlation between a locally varying unmeasured ecological factor and both effective fecundity and a focal morphological trait (Price et al., 1988). A large body of theoretical work also suggests that the combination of a strong sexual dimorphism with a lack of sexually antagonistic selection found in *Leucadendron rubrum* may result from adaptation to changing ecological conditions, which cause patterns of selection between sexes to align. A longer fire return interval, or low resource availability, are predicted to select for increased resource allocation to plant survival and a lower allocation to cone maintenance (Tonnabel et al., 2012). That is because serotinous plants need to survive until fire to reproduce and can therefore afford to maintain cones over long periods of time only if it does not come at the expense of their own survival up until the fire. These particular ecological conditions may have weakened selection in females for traits improving current water conductance and cone maintenance, and favoured instead traits improving cone production and adult survival. Understanding the emergence of sexual dimorphism through sex-specific selection estimations will therefore require either experimental protocols controlling environmental conditions or extensive estimations of spatiotemporal variation in sex-specific selection in natural populations. Long-term studies in animals have indeed commonly found large temporal variation in selection pressures acting on various traits (e.g., Acker et al., 2015; Grant & Grant, 2002; Reed et al., 2013; Reimchen & Nosil, 2002), including both temporal and spatial variation in the direction of sexually antagonist selection pressure on sexually dimorphic traits (Fargevieille, 2016).

### 4.3 | Sex-specific spatial distribution of effective fecundities

The observation that reproductive costs differ between males and females (e.g., Antos & Allen, 1990; van Drunen & Dorken, 2012; Harris & Pannell, 2008; McDowell et al., 2000) has been pivotal to discussions on the evolution of sexual dimorphism in plants (Delph & Bell, 2008; Freeman et al., 1976). Resources key to male and female reproduction commonly display small-scale variation in the wild (Araya et al., 2011; Silvertown et al., 1999). Therefore, it is a simple corollary of the sex-specific cost of reproduction hypothesis that male and female fitness should often exhibit different spatial patterns in natural populations, as found in our study population. These sex-specific spatial patterns of fitness variation call for future studies relating small-scale variation in key resource types (Araya

et al., 2011; Silvertown et al., 1999) and plant fitness in both males and females.

### 4.4 | Only male effective fecundity was affected by density

Male effective fecundity was negatively associated with plant density while no association was found for females. The negative effect of plant density on male effective fecundity might be triggered by increased competition over nutritive resources affecting pollen production, by negative effects of a closed canopy on pollen dispersal, or by competition effects at the seedling stage affecting their offspring. The lack of an effect of plant density on female reproduction suggests that either they are less affected than males by competition with other plants, or that the negative effects of competition are counter-balanced by positive effects of reproducing in a high-density patch. In both cases, it suggests different reproductive needs and ecology in males and females. Plant density was shown to negatively affect both male and female effective fecundity in a wind-pollinated tree (Oddou-Muratorio et al., 2018), and was also shown to impede pollen dispersal in a wind-pollinated herb (Tonnabel, David, Klein, et al., 2019). However, we note that this sex-specific effect of density on effective fecundity was only marginally significant when removing individuals with large fecundity.

### 4.5 | Pollen and seed dispersal kernels typical of plant dispersal behaviour

Our spatially explicit method allowed the estimation of dispersal kernels, which revealed a fat-tailed seed dispersal kernel in the anemochorous *L. rubrum*. Most seeds dispersed close to the mother plant, but some fraction dispersed much further. Similarly, a meta-analysis including species from various plant families, continents, vegetation types and growth forms found a predominance of fat-tailed seed dispersal kernels (Bullock et al., 2017). Investigations of pollen dispersal kernels are scarce, but they typically also indicate fat-tailed kernels in both insect- (Austerlitz et al., 2004; Oddou-Muratorio et al., 2005; but see Matter et al., 2013) and wind-pollinated species (Austerlitz et al., 2004; Goto et al., 2006; Gauzère et al., 2013; Geber et al., 2014 but see Ahee et al., 2015). Our estimated pollen dispersal kernel showed a markedly fat-tailed distribution, whereby a large proportion of pollen was able to disperse over large distances; similar large-distance pollen dispersal has been reported in both wind-pollinated and animal-pollinated species (e.g., Devaux et al., 2005; O'Connell et al., 2007). Given the large estimates of pollen dispersal distances and the short distance to the nearest population, the low estimates of pollen immigration are unexpected, especially given the same order of magnitude as seed immigration rate. This discrepancy is nevertheless consistent with other studies showing that the amount

of long-distance dispersal inferred by spatially explicit parentage modelling is not always congruent with the amount predicted by dispersal kernels inferred from local dispersal events (Chybicki & Oleska, 2018; Hardy et al., 2019). Such inconsistencies may emerge when extrapolation of dispersal kernels does not properly account for an increased probability of encountering obstacles between populations.

In conclusion, we found sex-specific variation in fitness in a natural population of a highly dimorphic plant species, despite similar directional selection in both sexes. Plant density impacted males and females differently, suggesting that sexes may display different sensitivities to competition over resources, regardless of competition for mates. The fixed life-form of plants might often generate spatial structure in fitness, as displayed in our study population. We therefore advocate for the generalization of spatial methods for estimating selection gradients, combined with spatially explicit fitness estimation methods for estimating selection acting on plants in the wild. In the long run, such methods should also account for the sex-specific temporal variation in plant phenology and the relatedness between potential mates as both can also influence fitness (Ismail & Kokko, 2020) and are likely to show spatial patterns.

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#### AUTHOR CONTRIBUTIONS

I.O., A.M. and A.C. designed the study, collected data and mapped the plants; E.K.K. produced the mating model; F.R. implemented methods allowing fitting of sex-specific spatial random effects in spaMM; F.R. and A.C. designed the statistical procedures for measuring selection gradients in the presence of spatial autocorrelation; J.T. and A.C. performed the analyses; J.T. produced the genotyping data set and drafted the manuscript; all authors discussed the results and edited the manuscript.

#### DATA AVAILABILITY STATEMENT

All genotype and morphological trait data are available in Dryad at <https://doi.org/10.5061/dryad.ngf1vhhst>. The new MEMMseedlings source code for dioecious species is available at [https://gitlab.paca.inrae.fr/pub/tonnabel\\_mol\\_ecol](https://gitlab.paca.inrae.fr/pub/tonnabel_mol_ecol). The new version of spaMM including the possibility of different spatial distributions of random effects between sexes is publicly <https://CRAN.R-project.org/package=spaMM>.

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#### SUPPORTING INFORMATION

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