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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 20 enhancing access to nutritive resources and/or to sexual partners. We here investigated sex-21 specific differences in selection of sexually dimorphic traits and in the spatial distribution of 22 23 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 24 female effective fecundity. We used spatial and genotypic data of parent and offspring 25 cohorts to jointly estimate individual male and female effective fecundity on the one hand 26 and pollen and seed dispersal kernels on the other hand. This methodology was here adapted 27 to the case of dioecious species. Explicitly modeling dispersal avoids the confounding 28 effects of heterogeneous spatial distribution of mates and sampled seedlings on the 29 estimation of effective fecundity. We also estimated selection gradients on plant traits while 30 modeling sex-specific spatial autocorrelation in fecundity. Males exhibited spatial 31 autocorrelation in effective fecundity at a smaller scale than females. A higher local density 32 of plants was associated with lower effective fecundity in males but was not related to 33 female effective fecundity. These results suggest sex-specific sensitivities to environmental 34 heterogeneity in L. rubrum. Despite these sexual differences, we found directional selection 35 for wider canopies and smaller leaves in both sexes, and no sexually antagonistic selection 36 on strongly dimorphic traits in *L. rubrum*. Many empirical studies in animals similarly failed 37 to detect sexually antagonistic selection in species expressing strong sexual dimorphism, and 38 we discuss reasons explaining this common pattern. 39

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

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Introduction

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Plant species with separate sexes are relatively uncommon (*i.e.* 5-6%, Renner, 2014). Separate sexes 44 have nonetheless evolved repeatedly among flowering plants (Renner, 2014), and such transitions 45 often given rise to the evolution of morphological differences between sexes (Geber et al., 1999; 46 Puixeu et al., 2019). The degree of sexual dimorphism has also switched multiple times from low to 47 high along the evolutionary history of certain dioecious plant lineages (Tonnabel *et al.*, 2014). Both 48 sex-specific costs of reproduction and male-male competition to access ovules have been suggested 49 as potential forces causing the evolution of such dimorphism. These two factors could trigger 50 sexually antagonistic selection (Delph & Ashman, 2006; Moore & Pannell, 2011), whereby 51 selection exerts forces in opposite directions in each sex towards sex-specific optima (Cox & 52 Calsbeek, 2009). The goal of this study is to estimate sex-specific fitness as well as the strength and 53 form of selection acting on morphological traits in each sex, in a highly dimorphic dioecious wind-54 pollinated plant species. To do so, we combine, and adapt to the case of dioecious species, recently 55 developed statistical methods estimating *effective fecundity*, a proxy for fitness, and its dependence 56 on morphological traits, while explicitly modeling various spatial effects that could bias such 57 estimations. 58

The sex-specific cost of reproduction hypothesis posits that sexes should diverge in 59 morphology to satisfy their respective reproductive needs (Freeman et al., 1976; Delph & Bell, 60 61 2008). 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 62 et al., 1976; Obeso, 2002). Several studies have shown that pollen production in males strongly 63 64 relies on nitrogen, while female reproduction is mostly limited by carbon and water (e.g. Antos & Allen, 1990; McDowell et al., 2000; Harris & Pannell, 2008; van Drunen & Dorken, 2012). Males 65 and females of dioecious plants have evolved divergent strategies of plastic allocation to resource-66 harvesting organs (see Tonnabel et al., 2017 for a review), probably to harvest the resources most 67

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needed for their respective reproduction. The cost of reproduction is generally higher in females 68 than in males, at least considering the cost per reproductive structure. However, at the scale of the 69 whole plant, this trend is often reversed in wind-pollinated plants, which produce large amounts of 70 71 pollen (Obeso, 2002; Harris & Pannell, 2008; Tonnabel et al., 2017). In some dioecious species inhabiting fire-prone environments, the cost of reproduction markedly differs between sexes 72 because females need to maintain a canopy-stored ('serotinous') seed bank (released by fire). As 73 water intake is necessary to maintain cones closed and prevent seed release during unfavorable 74 period between two fires (Martín-Sanz et al., 2017), we may expect selection for enhanced water 75 conduction to have favored a divergent plant architecture between sexes. Consistent with this 76 prediction, the evolution of longer maintenance of cones in the canopy is indeed associated with the 77 evolution of higher sexual dimorphism in the genus *Leucadrendron* (Harris & Pannell, 2010). 78

79 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 80 (Tonnabel *et al.*, 2019a,b). The male-male competition hypothesis postulates that male reproduction, 81 by being mostly limited by mating opportunities, selects for males that exhibit traits enhancing their 82 competitive abilities (Bateman, 1948; Arnold, 1994). Several studies have pinpointed the 83 84 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 & 85 Maze, 1999; Elle & Meagher, 2000; Wright & Meagher, 2004; Delph & Ashman, 2006; Waelti et 86 al., 2009; Schiestl & Johnson, 2013; Dorken & Perry, 2017), variation in male flowering phenology 87 to track the female phenology (Delph & Herlihy, 2011; Forrest, 2014), increased pollen grain 88 competitive performance in response to polyandry (Lankinen et al., 2017) and morphological 89 90 evolution of structures that disperse pollen, which prevents the attachment of competing pollen to the pollinator (Coccuci et al., 2014). In wind-pollinated plants, sexual selection may also target 91 vegetative traits such as plant size, branch length or the length of male flower peduncles, which can 92 facilitate pollen dispersal (Klinkhamer et al., 1997; Eppley & Pannell, 2007; Pickup & Barrett, 93

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2012; Harder & Prusinkiewicz, 2013; Tonnabel *et al.*, 2019b). Wind-pollinated plants tend to evolve larger degrees of sexual dimorphism than insect-pollinated lineages, 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 inter-individual variation in fertility (Schoen & Stewart, 1987; Ahee *et al.*, 2015), may thus be particularly subject to sexually antagonistic selection.

In the presence of genetic variation for sexual dimorphism, each sex should, in principle, 100 ultimately reach its optimal trait value, and thereby resolve sexually antagonistic selection (Lande, 101 102 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 103 predicts that, with strong positive genetic correlations between sexes, opposite directional selection 104 105 gradients between sexes should emerge early during adaptation and persist for a long time before the sexual conflict is resolved (Lande, 1980; Connallon & Hall, 2016). Consequently, one would expect 106 evidence for antagonistic selection between sexes to be relatively common. The compilation of 107 numerous sex-specific selection gradients in animals showed, however, a large diversity of patterns, 108 including cases of aligned selection across sexes (Cox & Calsbeek, 2009). In plants, documented 109 patterns of sex-specific selection provided mixed support for sexually antagonistic selection: sex-110 specific selection gradients have been found to have opposite signs in both insect- and wind-111 pollinated species (Delph et al., 2011; Castilla et al., 2014; Tonnabel et al., 2019b) but to have the 112 same sign in other studies (Oddou-Muratorio et al., 2018; Barrio & Teixido, 2014). More recent 113 theory suggests that temporal and spatial variation in selection pressures may explain the lack of 114 signal for sexually antagonistic selection, despite differences in the optimal phenotypes of males and 115 116 females (Connallon, 2015; Connallon & Hall, 2016; Zafitscher & 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

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parentage assignments to then estimate individual realized reproductive successes used as fitness 120 estimates. In the next generation methods, genotypes are combined with spatial localization of 121 sampled individuals, through spatially explicit mating models (SEMMs), to disentangle the effect of 122 fecundity from that of the distance between mating pairs (and the distance between mothers and 123 seedlings) on reproductive success (e.g. Oddou-Muratorio et al., 2005). To do so, dispersal is 124 explicitly modeled and dispersal kernels are estimated for both seeds and pollen. A Bayesian 125 method was introduced in this framework to estimate individual male and female effective 126 fecundities (MEMM, Klein et al., 2008 for seed sampling on mother trees; MEMMseedlings, 127 Oddou-Muratorio et al., 2018 for seedling sampling designs). This method considers the likelihood 128 of genotypes conditional on the position of seedlings, so it is unaffected by any process acting on 129 the distribution of seedlings, be it the potential parents' positions, or habitat suitability and 130 131 disturbances. It analyses seedling genotypes in terms of (1) overall reproductive contribution of each potential parent as determined jointly by gamete production, gamete fertilization rates, seed 132 maturation and germination, and seedling survival until census; (2) dispersal events in terms of 133 estimated dispersal kernels and (3) pollen or seedling competition by a mass action law. Effective 134 fecundity refers only to relative values of the first component for each parent. It varies with, e.g., 135 male-male competitive ability through differences in overall pollen production and their subsequent 136 ovule fertilization abilities, but not with competitive effects dependent on the composition of 137 competitors within the pollen cloud generated by uneven spatial distribution of mates. We here 138 extend this methodology to dioecious species. This spatially explicit approach avoids spatial bias in 139 effective fecundity estimation, typically generated by sampling seedlings non-uniformly with 140 respect to the positions of their parents or by the confounding effects of heterogeneous spatial 141 142 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 143 regeneration sites (for female fecundity) were uniformly distributed in space, and all offspring could 144

establish and be sampled (Klein et al., 2013). It therefore attenuates the impact of stochastic effects associated with sampling methods on fitness estimates. 146

Relating fitness estimates to plant traits using the selection gradients methodology proposed 147 by Lande & Arnold (1983) can further suffer from specific statistical bias in sessile organisms living 148 in heterogeneous environments. Indeed, small-scale spatial variation in resources fundamental to 149 plant physiology, including sex-specific reproduction, is common across a range of habitat types 150 (Silvertown et al., 1999; Araya et al., 2011). To disentangle the fitness effect of plant characteristics 151 (such as their ability to harvest resources, which may be sex-specific) from that of the environment 152 (such as the spatial distribution of resources), the spatial distribution of individuals must be 153 accounted for (Rausher, 1992). Indeed, not modeling explicitly the spatial autocorrelation of 154 unmeasured ecological variables affecting fitness can lead to detect false-positive effects of traits on 155 156 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 157 (Rousset & Ferdy, 2014). To our knowledge, it is the first time that spatial effects are taken into 158 account in the estimation of selection gradients. Moreover, spatial variation in plant density and the 159 local sex ratio may generate spatial variation in competition for resources, which can be studied by 160 analyzing their fixed effects on plant fitness. In conclusion, our MEMMseedlings model controls for 161 spatial confounding effects on estimations of effective fecunditity relative to spatial sampling biases 162 and to the spatial heterogeneity of plant distribution (potentially impacting competition among 163 males), while our spaMM procedure controls for spatial environmental heterogeneity. 164

We applied our methodology to Leucadendron rubrum, a dioecious wind-pollinated 165 serotinous shrub, endemic to the fire-prone South-African fynbos. L. rubrum displays extreme 166 167 sexual dimorphism (Fig. S1), with males being typically more highly branched, having smaller leaves and taller stature than females (Harris & Pannell, 2010; Welsford et al., 2014; Welsford et 168 al., 2016). A single recruitment pulse typically occurs after fire, killing all adult trees and releasing 169 seeds stored in their canopy seed bank (Cowling & Lamont, 1987). This particular life-cycle allows 170

the estimation of life-time effective fecundity by sampling seedlings only once (*i.e.* after the fire 171 event). Furthermore, because recruitment is synchronized by fire, all sampled adult individuals in 172 the population have the same age (Bond, 1984). The strong sexual dimorphism of L. rubrum has 173 174 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 175 sexes due to the cost of maintaining the canopy-stored ('serotinous') seed bank in females (Martín-176 Sanz et al., 2017). We hypothesized that, because of such maternal care, female fitness may be more 177 sensitive to water availability than male fitness. Owing to these differences in resource requirement 178 for reproduction, we therefore tested whether male and female effective fecundities (as defined 179 above) display different spatial structure and whether the observed strong sexual dimorphism is 180 associated with sex-specific selection gradients (Lande, 1980). 181

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Materials and methods

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184 Study species and site

Leucadendron rubrum is a dioecious wind-pollinated shrub species endemic to the Western Cape of 185 South Africa (Rebelo, 2001) where natural fires occur every 10-15 years (van Wilgen *et al.*, 2010; 186 Kraaij et al., 2011). L. rubrum belongs to the Proteaceae family and flowers from August to 187 September. Seed recruitment is constrained to a short period following fires, and seeds released 188 between fires typically fail to establish due to competition (Bond, 1984). L. rubrum typically starts 189 flowering at 2-3 years, and seeds are retained in woody cones for several years (Harris & Pannell, 190 2010). Seeds therefore form a 'serotinous' seed bank, which persists until fire kills the plants, 191 allowing cone opening and wind dispersal of fruits via their plumed perianth (Williams, 1972; 192 193 Rebelo, 2001). 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 194 closed (Treurnitcht et al., 2016; Martín-Sanz et al., 2017). Thus, mother death or any event leading 195 to cone opening before the advent of fire results in seed release in poor conditions for recruitment 196 and ultimately in the loss of progeny. 197

198 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 135x110 meters (Fig. S2). We studied all adult individuals of our focal 199 population. Another population of L. rubrum was located at a distance of 310 meters (smallest 200 201 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 Supplementary Methods S1), sampled 202 for DNA analyses and measured for several traits in 2004. In summer 2006, a fire burnt the 203 204 population, killing all adults. A total of 1,265 seedlings were mapped, and their leaves were sampled in the following Fall (February 2007), four to five months after the fire. The spatial distribution of 205 adults and seedlings is shown in Fig. S2. In one part of the study site, a ditch had been dug for 206 construction after seeds had dispersed, so we were unable to determine the undisturbed positions of 207

208 172 seedlings located in that area which we therefore eliminated from the dataset. However, the 209 effective fecundity of adults in this area can still be estimated without bias induced by the 210 disturbance, thanks to the use of MEMMseedlings (see below).

When sampling seedlings, the presence of seedlings from another closely related sympatric 211 species (Leucadendron salianum P.J. Bergins) rendered the identification of L. rubrum juveniles 212 difficult. To ensure that only juveniles of L. rubrum were included in later analyses, we genotyped 213 juveniles (see below for details on the genotyping protocol), as well as adults from both species. 214 This analysis aimed at assigning seedlings to either species and its results are described in 215 Supplementary Method S2 and Fig. S3. We did not find evidence for the existence of hybrids 216 between L. rubrum and L. salianum. In addition, 254 juveniles were excluded after genotyping as 217 they belonged to L. salianum. 218

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Measurements of adult traits in the field

For adult shrubs, we measured in 2004 three traits describing plant architecture and three traits 221 describing leaf morphology (available at doi:10.5061/dryad.ngf1vhhst). All six traits are known to 222 be sexually dimorphic in this species (Harris & Pannell, 2010; Welsford et al., 2014, 2016; Fig. S4). 223 The three traits describing plant architecture were (1) plant height, (2) the first diameter defined as 224 the greatest horizontal diameter of the plant (hereafter, canopy diameter), and (3) the second 225 horizontal diameter defined as the diameter perpendicular to the first diameter (hereafter, second 226 diameter). Several leaves were collected randomly along branches of each adult, dried and 227 photographed. Pictures were analyzed to measure the three traits describing leaf morphology: (1) 228 leaf area, (2) length and (3) width using ImageJ (Schneider et al., 2012). The number of leaves 229 analyzed per adult ranged from 10 to 23 with an average of 20.3. In females, we counted the number 230 of cones in the last two cohorts (cones produced in the last two seasons of cone production and 231 maintained closed since). Because older cohorts were not counted, this measure reflects cone 232

production rather than the maintenance of the serotinous seed bank. We did not count male conesbecause they were too numerous.

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236 Microsatellite genotyping

We genotyped both adults and their progeny in our focal population (available at 237 doi:10.5061/dryad.ngf1vhhst). For both adults and seedlings, sampled leaves were preserved in 238 silica gel prior to DNA extraction using a modified version of the CTAB protocol (Justy et al., 239 2009). We designed two polymerase chain reaction (PCR) multiplexes for amplifying DNA at 4 240 microsatellite loci, each involving primers with different fluorescent labels (Multiplex 1: 4F8, 3C9, 241 1C7, 1C3; Multiplex 2: 3B11, 2B2, 1D7, 1B8; markers developed by Justy et al., 2009). PCRs were 242 performed using the Qiagen Multiplex PCR Kit (Qiagen, Hilden, Germany); each PCR reaction was 243 performed in a final volume of 10μ Composed of 5μ of Multiplex Master mix (2X), 1μ of 244 primer sequences (1µM), 1µL of DNA extracts and 3µL of sterile water. PCR reactions were 245 performed on a Mastercycler pro thermocycler (Eppendorf, vapo.protect) with an initial 246 denaturation step of 15min at 95 °C, 35 cycles of 30s at 94 °C, 1min30s at the T_m temperature (M1: 247 54°C, M2: 53°C) and 1min at 72 °C, and a final step of 30min at 60°C. Genotyping was performed 248 249 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 (Table S1 250 for information per marker). After excluding individuals that did not amplify, our dataset contained 251 252 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; 253 Kalinowski et al., 2007) to estimate the non-exclusion probabilities of the first parent, the second 254 255 parent and parent pairs, to test for Hardy-Weinberg equilibrium, and to compute null allele frequencies (Table S1). Non-exclusion probabilities correspond to the probabilities that the set of 256 loci will not exclude an unrelated candidate parent (or parent pair) of an arbitrary offspring. Finally, 257 we used NM π (Version 1.0, Chybicki, 2017) to estimate the per-marker genotyping errors. 258

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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 261 seedlings to jointly estimate pollen and seed dispersal kernels and the individual male and female 262 effective fecundities – a proxy for fitness (see the Introduction). Our method builds on the Mixed 263 Effects Mating Model, MEMMseedlings (Oddou-Muratorio et al., 2018), which models mating and 264 dispersal events in a hermaphroditic plant population to estimate the selfing rate, immigration rates 265 and dispersal kernels for both pollen and seeds as well as the variance in male and female effective 266 fecundity (i.e. using random individual effects). We modified the MEMMseedlings algorithm to 267 produce a new version that handles separate sexes, with distinct spatial distributions of male and 268 Method female plants Supplementary S3, available 269 (see at https://gitlab.paca.inrae.fr/pub/tonnabel mol ecol.). 270

MEMMseedlings takes into account both the variation in fecundity among individuals and 271 the relative positions of putative parents and seedlings when computing the likelihood of observed 272 conditional to dispersal parameter and individual fecundity genotypes estimations. In 273 MEMMseedlings, putative parents that are more distant from a seedling have a lower parentage 274 275 probability. This model also describes mate competition through a mass action law, i.e. the contribution of a given male to the pollen cloud of a given female is diluted among the contributions 276 of all other males. For these reasons, the model can estimate variation in effective fecundities, 277 separately from different sources of spatial variation in reproductive success, linked to (i) spatial 278 biases in seedling sampling, (ii) the spatial distribution of mates, or (iii) edge effects. Accordingly, 279 we checked that the estimated effective fecundity of adult plants located on the border of our study 280 281 population was not different than 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 282 male (or respectively female) individual, and isotropic wind dispersal patterns. Given that 283 anisotropy can sometimes be found in wind-pollinated plants (Austerlitz *et al.* 2007), we confirmed 284

an absence of signal for anisotropy using NM π (see Supplementary Method S3). MEMMseedlings estimates a relative measure of effective fecundity, scaled by the average effective fecundity, (see equation (1) in Supplementary methods S3), which is therefore unitless.

Given the life cycle of *L. rubrum* and the sampling of seedlings after the fire, our estimated 288 effective fecundity integrates the effect of variation between individuals, either male or female, not 289 only for pollen and ovule production, but also for pollen export, fertilization rate, seed maturation 290 and dispersal, maintenance of seeds within the cones (degree of serotiny), adult survival until the 291 fire, seed germination and juvenile survival until the seedling census. Seeds released after the fire 292 and previously stored in the canopy were potentially fertilized in different years. Because adults of 293 L. rubrum do not survive fire, the establishment of progeny after a fire thus represents their entire 294 lifetime reproduction. 295

Briefly, our MEMMseedlings model combines genotypes data and spatial distribution data 296 for both adults and offspring to estimate, in a Bayesian framework, individual male and female 297 effective fecundities (F_k and R_i , respectively), the seed immigration rate (m_s), the pollen immigration 298 rate (m_p) , the rate of pollen export to the pollen cloud of non-local mothers (v), the mean seed 299 dispersal distance (δ_s), the mean pollen dispersal distance (δ_p), a parameter affecting the shape of the 300 seed dispersal kernel (b_s) and that of the pollen dispersal kernel (b_p). The estimation of pollen and 301 seed immigration rates (m_s and m_p) and, the rate of pollen export to the pollen cloud of non-local 302 mothers, (v) depends on the actual process of immigration, but is also affected by the fact that 303 around 8% of the parents were excluded from the analysis due to a lack of amplification. 304 Immigration rates therefore include the probabilities of maternity and paternity attributable to 305 unsampled parents. Both pollen and seed immigration rates are therefore likely overestimated. 306 Finally, when computing Mendelian transition probabilities between seedlings and putative parents, 307 MEMMseedlings considers genotyping errors by allowing a parent-offspring genetic discrepancy at 308 a maximum of one locus, and at each locus, the probability to mistype any allele was fixed using the 309 per-marker genotyping error rates estimated by NM π (see Supplementary Method S3). To describe 310

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the quality of parentage assignments associated with the dataset, 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) 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 meters for pollen and seeds 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 20m.

We estimated the model parameters using two MCMC chains of 50,000 steps and a burn-in 320 of 10,000 steps each. We used uniform prior distributions for the parameters m_s , m_p , v, δ_s , b_s , δ_p and 321 *b_p* within the intervals [0.01,1.00], [0.01,1.00], [0.00,1.00], [1.00,100], [0.01,1.00], [1.00,30000] and 322 [0.01,10.0], respectively. For individual effective fecundity estimates (F_k or R_i), values were 323 sampled every 20 iterations to decrease autocorrelation and averaged after concatenating the two 324 325 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 326 parameters, as well as the mean value across all iterations and chains (Table 1). 327

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329 Sex differences in morphology, spatial distribution and analysis of number of cones

We tested for sex differences in morphology using Linear Mixed-effects Models (LMM) with spatially autocorrelated random effects. We analyzed 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) 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

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for different spatial distributions of random effects between sexes (publicly available in spaMM 337 since version 2.6.0). The classical Matérn correlation function was used to model the spatial 338 autocorrelation of random effects, separately for each sex, as a function of the distance between 339 individuals. The Matérn correlation model involves two parameters: the smoothness parameter (*v*), 340 and the scaling parameter for distances (ρ). We compared models fits considering (1) no spatial 341 autocorrelation, (2) the same spatial autocorrelation for both sexes and (3) two sex-specific spatial 342 autocorrelation structures. We used likelihood ratio tests (LRTs) to (i) first test for a sex-specific 343 spatial structure in morphology, by comparing models with a different structure of random effects 344 but the same fixed effects, and (ii) to test for sexual dimorphism, by comparing the selected model 345 in step (i) to a model fitted with a similar structure of random effects, but without sex as a fixed 346 effect. Models were fitted either by maximum likelihood, for performing LRTs between models 347 differing in their fixed-effects structure, or by restricted maximum likelihood, for LRTs between 348 models differing in their random-effect structures and for computing the predictions from the model 349 fits. We also examined whether male and female plants were spatially segregated (*i.e.* whether the 350 sex ratio was spatially autocorrelated) using a binomial generalized linear mixed model (GLMM) 351 with sex as the response variable and compared model fits with or without a spatially autocorrelated 352 random effect as described above. Finally, we tested whether the number of cones produced by 353 females displayed a non-uniform spatial distribution by comparing models including cone number 354 as the response variable, plant density (see below for its calculation) as a fixed factor, and either a 355 spatial random effect or not. 356

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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 363 fecundity, while including spatially autocorrelated random effects. We checked that a model 364 predicting effective fecundity using a Gamma distribution with a log link performed better than 365 models assuming a different candidate distribution (compare gamma log link: cAIC=18.7 with 366 gaussian with a link identity: cAIC=139; or gaussian with a log link: cAIC=126 – cAIC is a metric 367 similar to the traditional AIC, except that it measures prediction performance conditionally on the 368 realization of the random effects, Vaida & Blanchard, 2005). We followed the same procedure as 369 described for the analysis of sexual dimorphism (see previous section) to fit the models using the R 370 package spaMM and to identify the best fitting random spatial structure (*i.e.* comparing a sex-371 specific spatial structure, a common spatial structure and a lack of it). We were thus able to compare 372 the patterns of spatial variation of residual effective fecundity between males and females, once the 373 374 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 375 unmeasured environmental variables (such as water availability) or unmeasured plant traits. 376 Different spatial structures between sexes therefore inform on the differential sensitivities of their 377 effective fecundities to variation in such unmeasured variables. We took into account the variation 378 in uncertainty of the individual effective fecundity estimates by parameterizing the residual variance 379 as a function of the variance in MCMC estimates of individual fecundity during the fitting (see 380 Supplementary Method S4). We again fitted models with distinct residual variances for each sex. 381 Because our MEMMseedlings model estimated large effective fecundity for several plants located 382 in one part of the study population, we performed a sensitivity analysis testing the robustness of our 383 results to the removal of all individuals with standardized effective fecundity greater than four 384 385 standard errors (for all statistical models treating effective fecundity as response variable). Five females and one male were removed in this sensitivity analysis, including four individuals that were 386 particularly close from each other. 387

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We chose to investigate the selection gradients for two morphological predictors only 388 (canopy diameter and leaf area), because several of the six morphological traits were highly 389 correlated (Table S3). Notably, all three traits describing plant architecture were strongly correlated 390 with each other (*i.e.* plant height, canopy diameter and second diameter, Table S3). Similarly, our 391 three measures of leaf morphology were strongly correlated with each other (*i.e.* leaf area, length 392 and width, Table S3). Estimation of multivariate selection gradients and their interpretation could be 393 confused by such strong correlations between traits (see Chong et al., 2018 for a review). We 394 therefore retained only two morphological traits in our selection analysis (but similar results were 395 obtained for other combinations of traits). We standardized the two focal traits using z-scores based 396 on the mean and variance values of the traits calculated separately for each sex. We confirmed that 397 leaf area significantly differed between individuals by comparing, within each sex, models 398 predicting leaf area with vs. without an individual-level random effect using an exact restricted 399 likelihood ratio test (Crainiceanu and Ruppert, 2004) implemented in package RLRsim 3.1-6 400 (Scheipl et al., 2008): for females, likelihood ratio (LR)=1573.9, p<0.001; for males, LR=625.1, 401 p<0.001. 402

We compared the performance of local plant density measured at different scales in 403 explaining variation in male and female effective fecundity (see Supplementary Method S5). We did 404 not transform plant density into z-scores since this predictor is not an individual trait and thus its 405 associated slope should not be considered as a selection gradient. To compare models explaining 406 variation in effective fecundity with different scales used to compute plant density, we used *cAIC*. 407 The best model included the density of plants in a quadrat of 12x12m around the focal individual. 408 We therefore retained this metric to compute the values of plant density used in our all subsequent 409 410 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

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whether there was any effect of the focal predictor. Second, we compared the full model to models 414 in which only the interaction between sex and one of the three variables of interest had been 415 removed. This allowed us to test whether the effect of the predictor was different between sexes. We 416 also built separate GLMMs for each sex, predicting effective fecundity from the three predictors of 417 interest. We used these sex-specific models to test the effect of each predictor in each sex, if and 418 only if, the interaction between sex and a given predictor was significant. We compared the fit of 419 our full model, which considers linear selection gradients only, to the fit of a similar model 420 including quadratic and correlational selection terms for canopy diameter and leaf area, and to 421 422 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 addition to the two focal traits. Finally, we predicted the female effective fecundity from the number of empirically counted cones, also with spatially autocorrelated random effects and plant density as a covariate.

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L. rubrum showed strong sexual dimorphism in morphology

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

Results

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; Fig. 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; Fig. S6).

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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 447 and b_p lower than one; Fig. 2 and Table 1). Seed and pollen immigration rates were of the same 448 449 order of magnitude (11% and 15% for seed and pollen respectively). Seed dispersal occurred on a smaller spatial scale than pollen dispersal: the mean estimated dispersal distance of seeds and pollen 450 were respectively of 10.6 and 11,041 meters. These estimated dispersal kernels predict that ca. 451 100% of seeds fell closer than the maximal female-seedling distance found in our population (*i.e.* 452 139 meters), while only 7.56% of pollen traveled a distance shorter than the maximal male-female 453 distance found in our population (i.e. 106.4 meters). Similarly, we estimated that 86% of seeds and 454 1.56% of pollen was dispersed within 20 meters. A fat-tailed pollen dispersal kernel accounting for 455

distance-dependent pollen dispersal nevertheless explained our data better than modeling a uniform 456 distribution of pollen dispersal distances (Fig. S7, see Supplementary Methods S3 for a description 457 of their comparison). Although we estimated a high probability for the pollen to travel long distance, 458 we estimated that about three quarters of the seedlings had a genotyped father in the population (1-459 m_{ν} =0.85 for seedlings with a known mother or ν =0.81 for seedlings with an unknown mother), 460 which is similar to the proportion found for mothers $(1-m_s=0.89; Table 1)$. Estimations of dispersal 461 kernels with the NM π algorithm yielded similar parameter estimation, yet with a notable shorter 462 estimate of mean pollen dispersal distance (Table 1 vs. S2). The estimates of dispersal kernels with 463 $NM\pi$ were robust to the inclusion of anisotropy in dispersal events (results not shown). 464

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466 Autocorrelation in effective fecundity occurred on a smaller spatial scale for males 467 than females

Effective fecundity estimations were carried out using a set of 8 microsatellite markers containing between 6 and 24 alleles, showing non-exclusion probabilities of parent pairs ranging between 0.12 to 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 Supplementary Methods S3).

We detected a clear sex-specific spatial structure for effective fecundity (Table S5). We 473 found different spatial variation in effective fecundity for each sex, with coarse-grained and fine-474 grained spatial effects for females and males, respectively (Fig. 3a vs. 3b). These results were robust 475 to the removal of plants with standardized effective fecundity greater than four standard errors, 476 which only slightly affected the previous conclusion (Table S5; Fig. S8). Several plants with large 477 effective fecundity were found in the disturbed area (Fig. 3). The number of empirically counted 478 cones on female plants also displayed a significant spatial autocorrelation (χ^2 =2238, *df*=3, *p*<0.0001; 479 Fig. S9). Cone number was significantly correlated with relative effective fecundity but the effect 480 size was small (using standardized cone number, β =0.286, χ ²=6.02, *df*=1, *p*=0.0141) and the two 481 spatial distributions were not fully aligned (Fig. 3a vs. S9). 482

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

Our spatially corrected selection gradient approach revealed that the leaf area was negatively 485 associated with effective fecundity (Table 2 and S5; Fig. 4), with similar slopes in both sexes as 486 shown by a non-significant interaction between leaf area and sex (Table S5). Yet, the leaf area was 487 positively correlated to the number of counted cones on female plants (β =0.171; LRT: χ^2 =42.7, 488 *df*=1, *p*<0.001). Larger canopy diameter was significantly associated with higher effective fecundity 489 (Table 2 and S5; Fig. 4). Accordingly, female plants with wider canopies displayed higher numbers 490 of empirically counted cones (β =0.904; LRT: χ^2 =78.8, *df*=1, *p*<0.001). The increase in effective 491 fecundity with increasing canopy diameter was similar in both sexes, as shown by a non-significant 492 interaction between sex and canopy diameter (Table S5). Our estimates of selection gradients were 493 robust to the removal of plants with effective fecundity greater than four standard errors (Table S5; 494 Fig. S10). We found no signal for non-linear selection, either by including all three quadratic and 495 correlational terms at once (LRTs: χ^2 =0.709, df=3, *p*=0.871), or each of them separately ((leaf 496 area)²: $\chi^2=0.129$, df=1, p=0.719; (canopy diameter)²: $\chi^2=0.118$, df=1, p=0.731; leaf area x canopy 497 diameter: χ^2 =0.378, df=1, p=0.539). 498

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

The effect of plant density on effective fecundity (Fig. S6) differed between sexes, as revealed by a significant interaction between sex and plant density (Table S5; Fig. 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 was however only marginally significant when the plants with the highest effective fecundity were removed in the robustness analysis (Table S5; Fig. S10).

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Discussion

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

Technical and methodological improvements in parentage assignations now allow for estimation of 510 plant fitness in natural populations from genetic data, and provide the link between fitness and plant 511 traits through selection gradients analyses (e.g. Meagher & Thompson, 1986; Burczyk & Prat, 1997; 512 Burczyk et al., 2006; van Kleunen & Burczyk, 2008). We have developed a methodology that 513 estimates effective fecundity in dioecious plants while accounting for biases associated with their 514 spatial distribution. Beyond the interest of documenting spatial patterns of seed and pollen dispersal, 515 the addition of this spatially explicit component to classical parentage methods (Jones, 2010) 516 improves the estimation of effective fecundity in the presence of confounding effects, such as spatial 517 518 bias in sampling descendants, spatial variation in the intensity of mate competition triggered by a non-uniform distribution of mates, or border effects (Oddou-Muratorio et al., 2018). We 519 investigated effects of traits on effective fecundity by classical selection gradient methods (Lande & 520 Arnold, 1983), in which we explicitly modeled the effect of spatially correlated unmeasured 521 environmental factors on effective fecundity. This newly developed framework will be particularly 522 523 suited to estimating selection in natural populations, given that spatial biases are typically difficult to avoid regarding both sampling and uncontrolled factors. 524

No contemporary sexually antagonistic selection despite strong sexual dimorphism 525 The signal we found for selection of larger canopy diameters in both sexes may indicate a 'budget 526 effect' of plant size, where larger plants acquire more resources that can be reallocated to gamete 527 production (Delph & Ashman, 2006). In females, the number of counted cones was positively 528 529 related to canopy diameter. However, cone number only poorly explained effective fecundity. The spatial distribution of cone number and female effective fecundity were moreover not fully 530 matching, suggesting that processes occurring after cone production, such as cone maintenance or 531 mother plant survival affect the female effective fecundity. In males, selection for wider canopies 532

could be linked to flower production if both sexes are subject to similar ontogenetic constraints. We 533 found evidence for similar selection for smaller leaf area in both sexes. Smaller leaves were 534 previously shown to be correlated in *L. rubrum* with thinner, and more numerous, branches and less 535 efficient water transport from roots to branch apex (Harris, 2007). Selection for smaller leaves in 536 males may reflect selection for greater number of inflorescences held on more flexible branches, a 537 trait long hypothesized to enhance pollen dispersal (Klinkhamer et al., 1997). Smaller leaves may 538 also represent a decreased mechanical hindrance to pollen dispersal. Selection for smaller leaves in 539 females, however, contradicts our expectation of selection for enhanced water transport to the cones. 540 The number of cones counted on females was furthermore positively related to leaf area. The fact 541 that leaf area relates to the number of cones, and to effective fecundity in opposite ways, suggests 542 that any positive effects of leaf area on fecundity through female cone production may have been 543 masked by trade-offs with other key life history components for serotinous plants (e.g. adult 544 survival until the fire). In the absence of positive genetic correlation between sexes, sexual conflicts 545 may be resolved once each sex reaches its respective optimum. We however did not find evidence 546 for stabilizing selection in the study population, which one could expect if each sex was at its 547 optimum with sufficient genetic variation in the studied traits. 548

Only 17% of studies estimating selection gradients in animals identified sexually 549 antagonistic selection (Cox & Calsbeek, 2009). The paucity of evidence for sexually antagonistic 550 selection, with which our study concurs, is inconsistent with the idea that genetic correlations 551 between sexes should maintain sexually antagonistic selection over long periods of time (Lande, 552 1980). To explain this inconsistency, both theory and experiments have suggested that temporal or 553 spatial ecological changes may result in variable pattern of selection acting on males and females, 554 555 with both sexes displaying trait values remaining far away from their ever changing ecological optimum (Kokko & Rankin, 2006; Delph et al., 2011; Sheridan & Bickford, 2011; Long et al., 556 2012; Berger et al., 2014; Connallon, 2015; Connallon & Hall, 2016). Recent theoretical 557

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developments showed that positive inter-sexual covariance for resource acquisition traits could also impede the identification of sexually antagonistic selection (Zafitschek & Connallon, 2017).

An alternative explanation to the lack of detected antagonistic selection is that antagonistic 560 selection is present but masked by a positive correlation between a locally varying unmeasured 561 ecological factor and both effective fecundity and a focal morphological trait (Price *et al.*, 1988). A 562 large body of theoretical work also suggests that the combination of a strong sexual dimorphism 563 with a lack of sexually antagonistic selection found in L. rubrum may result from adaptation to 564 changing ecological conditions, which cause patterns of selection between sexes to align. A longer 565 fire return interval, or low resource availability, are predicted to select for increased resource 566 allocation to plant survival and a lower allocation to cone maintenance (Tonnabel *et al.*, 2012). That 567 is because serotinous plants need to survive until fire to reproduce and can therefore afford to 568 maintain cones over long periods of time only if it does not come at the expense of their own 569 survival up until the fire. These particular ecological conditions may have weakened selection in 570 females for traits improving current water conductance and cone maintenance, and favored instead 571 traits improving cone production and adult survival. Understanding the emergence of sexual 572 dimorphism through sex-specific selection estimations will therefore require either experimental 573 protocols controlling environmental conditions or extensive estimations of spatio-temporal variation 574 in sex-specific selection in natural populations. Long-term studies in animals have indeed 575 commonly found large temporal variation in selection pressures acting on various traits (e.g. Grant 576 & Grant, 2002; Reimchen & Nosil, 2002; Reed et al., 2013; Acker et al., 2015), including both 577 temporal and spatial variation in the direction of sexually antagonist selection pressure on sexually 578 dimorphic traits (Fargevieille, 2016). 579

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Sex-specific spatial distribution of effective fecundities

The observation that reproductive costs differ between males and females (e.g. Antos & Allen, 1990; McDowell *et al.*, 2000; Harris & Pannell, 2008; van Drunen & Dorken, 2012) has been pivotal to discussions on the evolution of sexual dimorphism in plants (Freeman *et al.*, 1976; Delph 84 Bell, 2008). Resources key to male and female reproduction commonly display small-scale 855 variation in the wild (Silvertown *et al.*, 1999; Araya *et al.*, 2011). Therefore, it is a simple corollary 856 of the sex-specific cost of reproduction hypothesis that male and female fitness should often exhibit 857 different spatial patterns in natural populations, as found in our study population. These sex-specific 858 spatial patterns of fitness variation call for future studies relating small-scale variation in key 859 resource types (Silvertown *et al.*, 1999; Araya *et al.*, 2011) and plant fitness in both males and 850 females.

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Only male effective fecundity was affected by density

Male effective fecundity was negatively associated with plant density while no association was 592 found for females. The negative effect of plant density on male effective fecundity might be 593 triggered by increased competition over nutritive resources affecting pollen production, by negative 594 effects of a closed canopy on pollen dispersal, or by competition effects at the seedling stage 595 affecting their offspring. The lack of an effect of plant density on female reproduction suggests that, 596 either they are less affected than males by competition with other plants, or that the negative effects 597 of competition are counter-balanced by positive effects of reproducing in a high density patch. In 598 both cases, it suggests different reproductive needs and ecology in males and females. Plant density 599 600 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 601 herb (Tonnabel et al., 2019b). We note however that this sex-specific effect of density on effective 602 density was only marginally significant when removing individuals with large fecundity. 603

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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 fattailed 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; 610 Oddou-Muratorio et al., 2005; but see Matter et al., 2013) and wind-pollinated species (Austerlitz et 611 al., 2004; Goto et al., 2006; Gaüzere et al., 2013; Geber et al., 2014 but see Ahee et al., 2015). Our 612 estimated pollen dispersal kernel showed a markedly fat-tailed distribution, whereby a large 613 proportion of pollen was able to disperse over large distances; similar large distance pollen dispersal 614 was reported in both wind-pollinated and animal-pollinated species (e.g. Devaux *et al.*, 2005; 615 O'Connell et al., 2007). Given the large estimates of pollen dispersal distances and the short 616 distance to the nearest population, the low estimates of pollen immigration are unexpected, 617 especially given the same order of magnitude as seed immigration rate. This discrepancy is 618 nevertheless consistent with other studies showing that the amount of long distance dispersal 619 inferred by spatially explicit parentage modeling is not always congruent with the amount predicted 620 621 by dispersal kernels inferred from local dispersal events (Chybicki & Oleksa, 2018; Hardy et al., 2019). Such inconsistencies may emerge when extrapolation of dispersal kernels does not properly 622 account for an increased probability of encountering obstacles between populations. 623

In conclusion, we found sex-specific variation in fitness in a natural population of a highly 624 dimorphic plant species, despite similar directional selection in both sexes. Plant density impacted 625 males and females differently, suggesting that sexes may display different sensitivities to 626 competition over resources, regardless of competition for mates. The fixed life-form of plants might 627 often generate spatial structure in fitness, as displayed in our study population. We therefore 628 advocate for the generalization of spatial methods for estimating selection gradients, combined with 629 spatially explicit fitness estimation methods for estimating selection acting on plants in the wild. In 630 the long run, such methods should also account for the sex-specific temporal variation in plant 631 632 phenology and the relatedness between potential mates as both can also influence fitness (Ismail & Kokko, 2019) and are likely to show spatial patterns. 633

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We dedicate this manuscript to IO who passed away before the completion of this study, which she 636 637 initiated. She inspired and is still inspiring our research in many ways. We are grateful to Nicolas Bierne and Pierre-Alexandre Gagnaire for their helpful advice on population genetic structure 638 analysis, to John Pannell for helpful discussions and English editing, to Jeremy Midgley for help in 639 the field and attracting our interest in this system. Elodie Flaven-Noguier, Fabienne Justy and 640 Clémence Hatt helped with the DNA extractions, genotyping and molecular biology protocols. This 641 work was supported by a grant from the FRB to IO and AM, from the ANR "Evorange", ANR-09-642 PEXT-011 to OR and from the ANR "MeCC", ANR-13-ADAP-006 to OR, EKK and SOM. JT was 643 supported by a grant to John Pannell from the FNS, 31003A_163384. OR acknowledges support 644 645 from the Peter Wall Institute of Advanced Studies, UBC, and from CNRS.

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Data accessibility

All genotype and morphological trait data are available in Dryad at doi:10.5061/dryad.ngf1vhhst. 894 The for **MEMMseedlings** source code dioecious species is available 895 new at https://gitlab.paca.inrae.fr/pub/tonnabel_mol_ecol. The new version of spaMM is publicly available 896 in spaMM since version 2.6.0 at https://CRAN.R-project.org/package=spaMM. 897

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Author contributions

900 IO, AM and AC designed the study, collected data, and mapped the plants; EKK produced the 901 mating model; FR implemented methods allowing fitting of sex-specific spatial random effects in 902 spaMM; FR and AC designed the statistical procedures for measuring selection gradients in the 903 presence of spatial autocorrelation; JT and AC performed the analyses; JT produced the genotyping 904 dataset and drafted the manuscript; all authors discussed the results and edited the manuscript.

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Figure captions

Figure 1: Sexual dimorphism in canopy diameter in cm (A.) and leaf area in cm² (B.) of the study 907 population *L. rubrum*. Points are prediction from models accounting for the spatial distribution of 908 traits within each sex and error bars are 95% confidence intervals around mean predictions. 909

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Figure 2: Dispersal kernels estimated under the Bayesian model for seed dispersal (A.) and for 911 pollen dispersal (B.). Filled lines correspond to the posterior mean dispersal kernels obtained by 912 averaging parameters of the concatenation of two Markov chains of 50,000 steps (burn-in phase of 913 10,000 steps). Grey lines illustrate the uncertainty around the averaged dispersal kernel and 914 correspond to the kernels estimated on each iteration of the MCMC. Both dispersal kernels are 915 represented within the minimal and maximal distances existing in our population between females 916 and seedlings for seeds (A.) and between males and females for pollen (B.), *i.e.*, respectively, the 917 maximal female-seedling and male-female distances in our population. The extrapolation of 918 dispersal kernels beyond these limits are not represented in the plots. 919

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921 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, 922 canopy diameter, leaf area, plant density, and the interaction between each of the three latter 923 924 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 925 model, and circles represent the localization of individual plants. 926 927

Figure 4: Partial-dependence effect plots of leaf area (A.), canopy diameter (B.) and plant density 928 (C.) on the relative effective fecundity as predicted by our full model. Points indicate observed trait 929 930 values as well as the relative effective fecundity ± SD stemming from MCMC estimations. Curves indicate model predictions computed as partial-dependence effects \pm 95% confidence intervals. 931 Relative fecundity designates our measure of relative effective fecundity. 932

933 934 Figure S1: Pictures of females and males Leucadendron rubrum plants (A.), and of male (B.) and female (C.) inflorescences magnification. Credit picture: Isabelle Olivieri. 935

937 Figure S2: Map of the study site showing distribution of females (red circles), males (blue triangles), juveniles (green crosses), non-genotyped females (red full circles) and non-genotyped 938 males (blue full circles). One non-genotyped male that was located nearby the population is not 939 represented in the map. 940

Figure S3: Results of a genetic analysis of the sampled individuals using the software 942 STRUCTURE (Pritchard et al., 2000). Four types of samples were tested: juvenile plants of the 943 third undetermined morphological group and, adult plants of L. salignum and of L. rubrum and 944 juvenile plants of L. rubrum. Four genetic groups were selected by this analysis and are represented 945 here by four different colors (*i.e.* blue, orange, pink and yellow). Individuals are displayed on two 946 different panels (A. and B.) only for the sake of readability but all individuals belong to the same 947 dataset described in Supplementary Methods S2. 948

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Figure S4: Sexual dimorphism in plant height in cm (A.), in canopy first and second diameters in 950 cm (B. & C.), in leaf area in cm² (D.), in leaf length in cm (E.) and in leaf width in cm (F.). Raw 951 data have been jittered around their x-axis value for visualization purposes and overlaid with both a 952 953 violin plot and a boxplot using default settings in the R package ggplot2 version 3.1.0 (Wickham, 2016). 954 955

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- **Figure S5:** Prediction of canopy diameter in males (A.) and females (B.) as predicted by a linear mixed-effect models including sex as a fixed effect as well as one spatially autocorrelated random effect for each sex.
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Figure S6: Density for all individuals (A.), for males (B.) and for females (C.) as numbers of individuals in quadrats of 12x12 meters.

Figure S7: Comparisons of conditional likelihood between the model presented in the main text accounting for spatial structure of pollen dispersal (in green) and a model neglecting such a spatial structure of pollen dispersal (in brown) as a function of Bayesian iterations which were run for 50,000 steps. These simulations were run using uniform prior distributions with the following intervals [0.01,1.00], [0.01,1.00], [0.00,1.00], [1.00,100], [0.01,10], [1.00,30000], [0.01,1.10] for the parameters m_s , m_p , v, δ_s , b_s , δ_p and b_p , except for the second model in which δ_p and b_p were not modeled.

- **Figure S8**: Replicate of Fig. 3 after the removal of individuals with effective fecundity greater than four standard errors (*i.e.* five females and one male). See Fig. 3 for legend details. Red crosses correspond to the locations of plants removed in the sensitivity analysis.
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Figure S9: Spatial prediction of the number of empirically counted cones as predicted by a
generalized linear mixed-effect model including all fixed effects (canopy diameter, leaf area, plant
density) as well as one spatially autocorrelated random effect.

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Figure S10: Replicate of Fig. 4 after the removal of individuals with effective fecundity greater thanfour standard errors (*i.e.* five females and one male). See Fig. 4 for legend details.







