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1 Sexual selection in seaweed? Testing Bateman's principles in the red alga *Gracilaria gracilis*

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17 **KEY WORDS:** Bateman gradient, paternity analyses, sessile marine organisms, mating and
18 reproductive success, haploid diploid life cycle, dioicous species

19

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22

23 **ABSTRACT**

24 In anisogamous species, sexual selection is expected to be stronger in males. Bateman's principles
25 state that the variance in (i) reproductive and (ii) mating success is greater for males and (iii) the
26 relationship between reproductive success and mating success (the Bateman gradient) is also
27 stronger for males than for females. Sexual selection, based on Bateman's principles, has been
28 demonstrated in animals and some angiosperms, but never in a seaweed. Here we focus on the
29 oogamous haploid-diploid rhodophyte *Gracilaria gracilis* in which previous studies have shown
30 evidence for non-random mating, suggesting the existence of male-male competition and female
31 choice. We estimated mating and reproductive success using paternity analyses in a natural
32 population where up to 92% of fertilizations occurred between partners of that population. The
33 results show that the variance in mating success is significantly greater in males than in females and
34 that the Bateman gradient is positive only in males. Distance to female partners also explains a
35 minor part of the variance in male mating success. Although there is no evidence for sexual
36 dimorphism, our study supports the hypothesis that sexual selection occurs in *G. gracilis*, likely on
37 male traits, even if we cannot observe, characterize or quantify them yet.

38

39 **INTRODUCTION**

40 Sexual selection arises from competition among individuals for the access to mates and/or to their
41 gametes [1]. Historically developed to explain the sexual dimorphism of secondary sexual traits in
42 animal species [2], sexual selection theory postulates that anisogamy should lead to strong
43 competition among males for the access to females and ovules [3]. Therefore, under this postulate,
44 any trait that increases the successful access to female mating partners should greatly affect male
45 reproductive success [4]. This theory thus constitutes a satisfying explanation for the frequently

46 observed male-biased sexual dimorphism in several categories of traits, including ornaments and
47 armaments (but see [5]). Sexual selection in both sexes has been quantified in a large number of
48 animal species using metrics derived from Bateman's principles [6]: (i) the opportunity for selection
49 I , i.e. the variance in relative reproductive success; (ii) the opportunity for sexual selection I_s , i.e.
50 the variance relative in mating success and (iii) the Bateman gradient, i.e. the slope of the regression
51 of relative reproductive success as a function of relative mating success [7]. The sex associated with
52 the highest value of these three metrics is considered as the one under stronger sexual selection. A
53 recent meta-analysis focusing on animals indeed shows that both the Bateman gradient and the
54 opportunity for sexual selection are more often higher in males [8]. Although this trend appears
55 stronger in species with male-biased sexual dimorphism in terms of morphological or behavioural
56 traits (i.e. males developing large body, bright colours or exuberant and acrobatic courtship
57 displays), a number of studies have reported stronger sexual selection, or opportunities of sexual
58 selection, in males within species in which no sexual dimorphism has been documented [8].

59 The use of these metrics of sexual selection has been largely biased towards animals, mainly
60 vertebrates and non-sessile invertebrates [8]. Although it has been acknowledged that stronger
61 sexual selection in males should occur in any anisogamous species [9], very few studies have
62 attempted to directly verify this prediction in other anisogamous eukaryotes which typically lack
63 copulation and for which cognitive traits (such as those involved in partner choice behaviours,
64 *sensus stricto*). Therefore, these anisogamous eukaryotes, such as plants, provide stimulating study
65 models for the empirical test of sexual selection. Sexual selection in angiosperms, although once
66 fiercely debated [9,10], is now widely recognized as a possible evolutionary force, which may indeed
67 be stronger through the male function of hermaphrodites or in male individuals in dioecious species,
68 at least when pollen supply is not limiting [11–13]. Among the arguments in favour of the existence
69 of sexual selection in plants, the occurrence of male-biased sexual dimorphism in dioecious plant

70 species (e.g. males with larger or more numerous flowers than females) suggests that selection
71 through the male function to attract pollinators or to invest in reproduction may be indeed stronger
72 [14–16]. Nevertheless, Bateman’s metrics have been estimated and compared between males and
73 females in only three angiosperm species. In all three cases, sexual selection was found to be
74 stronger in males, and results were at least partly consistent with the sexual dimorphism
75 documented in these species [17–19]. Nonflowering plant species have been investigated even less
76 on the topic of sexual selection. Only one study, carried out on a non-sexually dimorphic moss,
77 estimated Bateman gradients and found evidence for a steeper relationship between reproductive
78 and mating success in male gametophytes [20]. These results suggest a potential for stronger sexual
79 selection in males of this anisogamous species, but this does not appear to have driven the evolution
80 of sexually dimorphic traits. Noteworthy, this particular study was performed in a population with
81 an extreme male-biased sex ratio, a feature that has been found to be associated with higher
82 opportunities for sexual selection and higher Bateman gradients in males [21].

83 One situation in which stronger opportunity for sexual selection in males would not be associated
84 with sexual dimorphism is when variation in mating success is not determined by heritable traits. In
85 plants, their inability to move forces pollen or male gametes to disperse in the environment via
86 pollinators or abiotic factors, so that the exact spatial location of individuals is likely to be a strong
87 determinant of mating success. Several studies have explored the impact of geographical location
88 and of local sex ratio on male and/or female reproductive success [22–26], but without explicitly
89 linking their results to sexual selection. Like plants and some animals, red algae are sessile and
90 anisogamous [27]. Male gametes are released in the water and fertilizations occur on the females,
91 which is referred as spermcast mating for sessile marine invertebrates [28]. In red algae, male
92 gametes are non-flagellated and we recently demonstrated that isopod-mediated fertilization
93 increases fertilization success [29]. In Florideophyceae, after fertilization, the diploid zygote divides

94 mitotically in a diploid structure, protected and nourished by the parental female haploid
95 gametophyte [30]. These characteristics of the reproductive life cycle suggest that fertilization
96 success in this group may be driven by different selection pressures between males and females (i.e.
97 competition between males for access to females or selective cystocarp abortion by the females)
98 [31]. In the dioicous red alga *Gracilaria gracilis*, male fertilization success shows high variance
99 [31,32], suggesting that there may be male-male competition or female choice, a sign of sexual
100 selection. However, to date in this alga, the relationship between reproductive success and mating
101 success has not been tested to determine if the Bateman gradient is steeper in males. Here, we
102 compare potentials for sexual selection among male and female gametophytes in *G. gracilis* by
103 estimating the three Bateman indexes in a natural population characterized by a balanced sex ratio.
104 We also explore the role of individual location, relative to individuals of the other sex, on the
105 variation of mating success in both males and females. The investigation of Bateman principles in
106 species outside the clades classically studied for sexual selection should help to understand how
107 and when anisogamy triggers differences in the magnitude of sexual selection between sexes, and
108 when such differences can drive the evolution of sexual dimorphism.

109 MATERIAL & METHODS

110 (a) Study species

111 Populations of the rhodophyte *Gracilaria gracilis* are patchily distributed in the intertidal zone on
112 rocky shores. Individuals occupying rock pools remain immersed at ebb tide. Its life cycle is haploid-
113 diploid, including male and female haploid gametophytes and diploid tetrasporophytes (**figure 1**).
114 Tetrasporophytes produce haploid spores via meiosis that develop into dioicous male and female
115 gametophytes. This species is characterized by a highly specialized type of oogamous reproduction
116 [26]. Spermatia (non-flagellated male gametes) are released into the environment, but female
117 gametes (carpogonium) are retained on the female gametophyte. After fertilization, the zygote

118 remains on the female gametophyte and develops into diploid sporogenous tissue (gonimoblast)
119 protected and nurtured by the parental female thallus, forming a complex, macroscopic post-
120 fertilization structure called the cystocarp. The cystocarp is fully mature approximately one month
121 after fertilization. One fertilization event gives rise to thousands of identical diploid spores that,
122 after release, can germinate into new tetrasporophyte individuals [33]. The tetrasporophyte, female
123 gametophyte and male gametophyte individuals are considered isomorphic, even though males are
124 slightly smaller than females and tetrasporophytes [34]. Both haploid and diploid individuals are
125 long-lived [35]. The thallus is formed of a few erect deciduous main axes, irregularly branched,
126 reaching up to 30 cm long, arising from a small perennial discoid holdfast, fixing the individual to
127 the bedrock. Previous studies have shown that the reproductive period occurs from March to
128 September with cystocarp production peaking in August–September [33,36]. Individuals less than 8
129 cm in length are generally non-reproductive.

130 **(b) Study population and sampling**

131 The study population was located on the coast of the North Sea at Cape Gris-Nez (in the Strait of
132 Dover region, northern France, 50°53'N, 1°35'E) in a pool of ca. 5 m² isolated from other populations
133 at low tide [31,33]. In August 2020, every individual in the rock pool was sampled and spatially
134 mapped relative to a pair of fixed points. Maximum thallus length and maximum diameter,
135 measured using all the thalli emerging from the holdfast, were recorded for each individual to
136 estimate biomass [35]. Several branches of each individual were sampled and examined under a
137 dissecting microscope to establish the sexual phenotype (tetrasporophyte, female or male
138 gametophyte) of each individual based on the observed reproductive structures. Of a total of 238
139 reproductive individuals sampled, 97 tetrasporophytes, 70 females and 71 males were identified
140 (**figure 2**). There was a balanced sex ratio (1:1). For every female, cystocarps were counted on the
141 10 cm distal extremity of five branches. Thallus fragments were excised from each individual for

142 DNA extraction. An average of 32 large cystocarps (ranging from 12 to 48 cystocarps) were randomly
143 sampled and dissected from each female and stored at -20°C.

144 **(c) DNA extraction and microsatellite genotyping**

145 For individuals, DNA extraction was performed on approximately 10 mg of dried thallus tissue using
146 the NucleoSpin® 96 Plant II kit (Macherey-Nagel, GmbH & Co. KG, Düren, Germany) following the
147 manufacturer's instructions. For cystocarps, DNA extraction was performed using the Chelex
148 method (Chelex TM 100; Biorad, Hercules, California), following the protocol described in Wattier
149 et al. [37]. All male and female gametophyte individuals as well as the sampled cystocarps were
150 genotyped using 13 microsatellite loci: Gv2CT [37], Gg121, Gg155, Gg173, Gg182, Gg216 [38],
151 Ggrac_02, Ggrac_03, Ggrac_04, Ggrac_05, Ggrac_08, Ggrac_09, Ggrac_11 [39]. PCR was performed
152 in a total volume of 10 µL containing 2 µL of DNA template diluted 1:50 (gametophytes) or not
153 diluted (cystocarps), 1 X GoTaq Flexi buffer (Promega Corporation), 2 mM MgCl₂, 150 µM of each
154 dNTP (Thermo Fisher Scientific Inc., USA), 30 pmol of forward fluorescent-labelled primer, 30 pmol
155 of reverse primer (Eurofins MWG Operon, Germany), and 0.35 U GoTaq Flexi Polymerase (Promega
156 Corporation). Amplifications were carried out under the following conditions: initial denaturation at
157 95°C for 5 min, 30 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and extension
158 at 72°C for 30 s, followed by a final extension of 72°C for 10 min. Next, 2 µL of the diluted PCR
159 product 1:10 was added to 10 µL of loading buffer made up of 0.5 µL of the SM594 size standard
160 [40] and 9.5 µL of Hi-Di formamide, denatured at 95°C for 3 min, and run in an ABI 3130 XL capillary
161 sequencer (Applied Biosystems, USA). Genotypes were scored manually in Genemapper version 4.0
162 (Applied Biosystems). The gametophytes being haploid, for each cystocarp, we were able to deduce
163 the paternal genotype by subtracting the maternal genotype from the diploid cystocarp genotype
164 across all loci using the Multilocus Matches option in GenAlEx ver. 6.5 [41,42].

165 **(d) Reproductive and mating success**

166 Mating and reproductive success were estimated on the 50 females (out of the 70 initially present
167 in the population, see **figure 2**) for which there was sufficient information (i.e. estimated biomass,
168 cystocarp count per centimetre, and more than 24 cystocarps genotyped).

169 Mating success is the total number of mating partners per individual. In this study, we estimated
170 the genetic mating success, i.e. the number of individuals of the other sex with which at least one
171 zygote was produced, based on the results of the parentage assignment (**table S1**). Slightly unequal
172 numbers of cystocarps were genotyped across females, which may cause an artificial overestimation
173 of mating success for females associated with a higher sampling effort. Mating success for both
174 sexes was thus estimated using a bootstrapping procedure in which we resampled 24 genotyped
175 offspring (cystocarps) 1000 times for each female. The genetic mating success for each individual
176 was calculated from each resampling and, in further analyses, the mean "individual" mating success
177 value was used.

178 Female reproductive success is the total number of offspring a female produces. We estimated the
179 female reproductive success by multiplying the mean number of cystocarps per cm³ counted on 5
180 branches by the total volume of the individual (**table S2**) as a proxy for individual biomass [34].

181 Male reproductive success is the total number of offspring a male sires. Male reproductive success
182 can thus be estimated as the proportion of each female's cystocarps sired by that male, as
183 determined from the parental assignment (**table S1**), multiplied by female reproductive success and
184 summed over all surveyed females (**table S3**). At each sampling step of the bootstrapping procedure
185 performed for estimating mating success described above we calculated the proportion of
186 cystocarps sired by each male in all male-female pairs, to obtain an estimate of male reproductive
187 success for each male individual. The mean value of male reproductive success resulting from the
188 bootstrapping procedure was retained as an estimate of male reproductive success.

189 The relative reproductive and mating success of females and males were calculated by dividing the
190 obtained values for each individual by the respective mean for each sex (**tables S2, S3**).

191 **(e) Distance to partners**

192 To estimate the effect of distance on variation in male and female mating success, the distance of
193 each individual to all potential mating partners (i.e. individuals of the other sex) was calculated using
194 individual geographic coordinates within the population. The mean of these distances was used as
195 a proxy for the potential access to mating partners (**tables S2, S3**).

196 **(f) Statistical analyses**

197 All analyses were performed using R Statistical Software (v 4.0.3; R Core Team 2020) [43]. Function
198 `lm()` was used for all the linear regression analysis. Function `leveneTest()` was used for Levene
199 tests. The complete script of bootstrapping procedure and path analyses are available in Dryad
200 (see data availability section).

201 **Bateman's metrics**

202 We quantified the strength of sexual selection in each sex using Bateman's metrics [44–46]. First,
203 we estimated the standardized variance in reproductive success, I and in mating success I_s . I and I_s
204 were calculated by dividing respectively the variance of reproductive and mating success by the
205 square of their mean value. We tested for differences in reproductive and mating success variances
206 between sexes using Levene's test. In addition, we estimated the slope of the linear regression of
207 relative reproductive success as a function of relative mating success (Bateman gradient), on each
208 sex separately. We then analysed the whole dataset with a linear model that tested for the effect
209 of mating success and of the interaction between mating success and sex. Because uneven sampling
210 between males and females may artificially increase the estimates of variance and the statistical
211 power of the estimate of the Bateman gradient in males, a bootstrapping procedure was used to (i)

212 compare both I and I_s between males and females 50 times and (ii) test for the Bateman gradient
213 in males, based on a subsample of 50 males only.

214 **Distance to partners**

215 A linear regression of relative mating success over the mean distance to potential partners was
216 tested for males and females separately. A similar model was built, this time to test the effect of
217 distance to partners on reproductive success, again testing males and females separately. Finally,
218 we built a multiple linear regression of relative reproductive success over relative mating success
219 and distance to potential partners (i.e. the equivalent of a partial Bateman gradient), to test whether
220 any detected effect of the distance to partners on reproductive success was mediated through an
221 impact of distance on mating success.

222 **Path analyses: indirect effects of distance and biomass on mating and reproductive success**

223 In addition, we used a pathway analysis approach (Structural Equation Modelling, see also [47]), in
224 order to dissect direct from indirect effects of distance to potential mating partners on reproductive
225 success. More precisely, we tested whether the distance to potential partners affected mating
226 success and whether this had an indirect effect on reproductive success, while testing the
227 relationship between mating and reproductive success. We also assessed the direct relationship
228 between distance to partners and reproductive success. This was done simultaneously on males and
229 females, using the `piecewiseSEM` package in R [48]. We then performed a multigroup analysis using
230 local estimation of the parameters, by first providing statistics for sex \times distance interaction using
231 type III ANOVA. This allowed us to test whether the direct or indirect effects of distance to partners
232 on reproductive success were significantly different between sexes. Testing the effect of sex \times
233 mating success interaction on reproductive success provided an additional test of a difference in the
234 Bateman gradient between sexes. In a second step, the analysis provides correlation estimates and

235 significant sex \times variable interactions are free to vary between males and females. These results
 236 were compared to results of the linear models described above. Finally, we used the same path
 237 analysis approach by additionally testing for indirect and direct effects of individual biomass on
 238 reproductive success. Because biomass was used to estimate female reproductive success, this last
 239 analysis was performed only on males.

240 RESULTS

241 Paternity analysis, performed by genotyping 1495 cystocarps from 50 females, revealed that 92% of
 242 the fertilization events (1371 cystocarps) occurred with males located in the population and only
 243 8% (124 cystocarps) with males from outside. Of the 71 reproductive males in the population, we
 244 did not detect any sired cystocarps for five of them (**tables S1, S2**) and these five were thus assigned
 245 a null reproductive and mating success.

246 (a) Bateman's metrics

247 Reproductive success, i.e. the total number of offspring, ranged from 0 to 48,112 for males and from
 248 1215 to 66,495 for females (**figure 3A-D, tables S1, S3**). Mating success, i.e. the number of sexual
 249 partners, ranged from 0 to 27 for males and from 4 to 18 for females (**figure 3 B-E, tables S1, S3**).
 250 Both the opportunity for selection I and the opportunity for sexual selection I_s were stronger for
 251 males than for females (I : 1.18 versus 0.66; I_s : 0.74 versus 0.05), although this difference was
 252 significant only for mating success in males (Levene's test: I : $F_{1,119}=0.47$, $P=0.49$; I_s : $F_{1,119}=28.75$, $P<$
 253 0.001). These results were not qualitatively affected when these analyses were performed on a
 254 subsample of 50 males to obtain equal sample sizes for males and females. Bateman gradients
 255 investigated for each sex separately provided different results in males and females: no significant
 256 relationship was found between relative mating and reproductive success in females (slope \pm s.e.:
 257 0.46 ± 0.53 , $P=0.39$), whereas it was significantly positive in males (slope \pm s.e.: 1.17 ± 0.05 , $P<0.001$)
 258 (**figure 3C-F**). This result still held when the Bateman gradient was analysed on a subsample of 50

259 males to have the same sample size as in the female dataset. However, when relative reproductive
260 success of males and females were analysed conjointly, mating success had no effect (slope \pm s.e.:
261 0.46 ± 0.39 , $P=0.25$) and the interaction between mating success and sex was non-significant but
262 with a p-value of 0.08 (slope \pm s.e.: 0.71 ± 0.40 , $P=0.08$). When using the path analysis approach, we
263 detected a significant effect of sex x mating success interaction on reproductive success, with mating
264 success positively affecting reproductive success in males only (**figure S2**).

265 One potential weakness of our approach is that we set a maximum value of female mating success
266 (i.e. the number of genotyped offspring per female), which could artificially decrease the estimated
267 female mating success and flatten the Bateman gradient in females. This is an unavoidable limit of
268 such an approach, when exhaustive genotyping is not an option, as already underlined in similar
269 studies [19,49]. However, in our case, the observed shared paternity among offspring of the same
270 female (see **table S1**) suggests that estimated female mating success would not necessarily strongly
271 increase if we had genotyped a higher number of offspring for each female.

272 **(b) Distance to partners and male biomass explained part of the variance in mating success**

273 The mean distance to potential mating partners ranged from 0.88 m to 2.18 m for females, and from
274 0.78 m to 2.59 m for males (**tables S1, S3**). In males, mating success significantly decreased when
275 their distance to potential female partners increased (slope \pm s.e.: -0.93 ± 0.21 , $P<0.001$) (**figure 4A**).
276 The same result was observed for reproductive success (slope \pm s.e.: -1.01 ± 0.28 , $P<0.001$) (**figure**
277 **S1**). However, when the distance to potential partners and mating success were simultaneously
278 included in a linear regression of reproductive success, only mating success was found to have a
279 significant effect (slope \pm s.e.: 1.21 ± 0.07 , $P<0.001$) and the distance effect was no longer significant
280 ($P=0.40$). This result suggests that the effect of distance to potential partners on male reproductive
281 success was mediated through an effect on male mating success. In contrast, distance to potential
282 male partners had no effect on either female mating success ($P=0.48$) (**figure 4B**) or reproductive

283 success ($P=0.23$) (**figure S1**). The path analysis approach provided very consistent results, with an
284 indirect effect of the distance to potential partners on reproductive success, mediated through the
285 interaction between mating and reproductive success, in males but not in females (**figure S2**).

286 Finally, when focusing on males only, we found that individual biomass positively affected mating
287 success, thus having an indirect positive effect on reproductive success. Adding the biomass in the
288 model did not qualitatively modify the effects of distance to potential female partners described
289 above (**figure S3**).

290 **DISCUSSION**

291 In this first ever test of Bateman's principles in an oogamous red alga, we found evidence in support
292 of two of Bateman's three principles, suggesting a greater potential for sexual selection in males
293 than in females in *Gracilaria gracilis*. We also detected an effect of individual location relative to
294 potential mating partners on male mating success, which indirectly affects their reproductive
295 success. Below, we discuss these different results in the light of red algal reproduction biology and
296 their possible implications for processes of sexual selection in this species.

297 The first result of our study was that the variance in mating success was significantly higher in males
298 than in females, thereby validating Bateman's second principle. This result is consistent with the
299 Darwinian sex-role hypothesis linked to anisogamy, which predicts that sexual selection is often
300 strongest in the sex that produces the smallest and most abundant gametes, i.e. males by definition
301 [5]. This expected consequence of anisogamy [3,50], even if not observed in all studies (see [8]), has
302 been detected in a large number of animal species [1,8], but also in some angiosperms [12,13,51],
303 mosses [20], fungi [52,53] and a red alga [31,32].

304 Second, our results also validate Bateman's third principle because the Bateman gradient showed a
305 significant positive relationship between reproductive and mating success in males, but not in
306 females. The stronger dependence of reproductive success on mating success in males compared

307 with females has been widely documented in animals (see for review [8]), but has only recently been
308 reported in three species of angiosperms [17–19] and one aquatic species of peat moss [20], while
309 it had never been tested in seaweeds before. Bateman’s third principle has generally been
310 interpreted as the existence of strong competition between males for access to females, whereas
311 the reproductive success of females is not strongly tied to their ability to access mates. The
312 transferability of methods used for investigating the Bateman gradient and how to interpret it in
313 animals to angiosperms was recently discussed in Tonnabel et al. [17] and Barbot et al. [19]. In
314 particular, in mating systems in which females have multiple mating partners during a mating season,
315 frequently reported in angiosperms (see for review [54]) but rare in motile animals, may bias the
316 estimation of sexual selection. Multiple mating partners should result in high values of mating
317 success, but the fact that genetic mating success is typically estimated on a subsample of offspring
318 may lead to an underestimation of the mating success at least in some individuals, and ultimately
319 to an underestimation of the variance in mating success. Such bias has been discussed in a few
320 papers based on meta-analyses of studies focused in animals and simulated data sets, suggesting
321 that the Bateman gradient may be overestimated in such a case [49,55]. In red algae, gametophytes
322 are haploid and produce gametes by mitosis, and — given that male gametophytes thus produce
323 genetically identical gametes — fertilization of multiple females by individual males can be readily
324 detected. To date, the few paternity analyses published in red algae (i.e. *G. gracilis*, [31] and
325 *Chondrus crispus*, [56]) have confirmed high levels of polyandry in natural populations, which
326 suggests that in our study the Bateman gradient, as in other plants, may be overestimated in males.

327 Nevertheless, in the handful of investigated plant species, the positive Bateman gradient found in
328 males, but not in females, has been interpreted as the direct result of female reproductive success
329 not being limited by pollen supply, at least in the experimental conditions in which these studies
330 were conducted [17,19]. Pollen limitation is another common feature in angiosperms [11,57] that

331 should, when it occurs, decrease the difference in magnitude of sexual selection between females
332 and males because in pollen-limiting situations, female reproductive success becomes limited by
333 access to male gametes. Although this relationship has not been directly tested yet, in situations of
334 low plant and/or pollinator densities, despite the much higher production of male gametes
335 compared with female gametes at the population level, female Bateman gradients are expected to
336 become significant [3,19]. In sessile marine organisms, female fertility has been assumed to be
337 limited due to short sperm lifespans and their dilution in turbulent flow [58,59], but several
338 mechanisms such as synchronous release of motile gametes and chemotaxis have evolved in a range
339 of invertebrates and brown seaweed, to ensure fertilization rates of up to 80–100% (for reviews,
340 see [60,61]). In red algae, it has long been hypothesized that fertilization success should be
341 particularly low due to the characteristics of their life cycle [62,63]. Male gametes are not
342 flagellated, have a short lifespan and fertilization takes place on a remote female organ. The absence
343 of any documented mechanism of synchronous gamete release or chemotaxis has raised questions
344 on the efficiency of gamete encounters in rhodophytes [63]. Searles [62] even hypothesized that
345 the evolution of cystocarps in which the female nurtures, amplifies and protects the zygote is
346 probably a response to low rates of fertilization. Rare gamete encounters should lead to a positive
347 Bateman gradient in female *G. gracilis*, a result that was not found in the current study. Therefore,
348 in the studied population, dispersal appears to be efficient enough to ensure the sufficient access
349 of female gametophytes to reproduction and thus the production of cystocarps. This argument is
350 further supported by our evidence that all measured females had multiple fertilization partners.
351 Although data on natural fertilization success are still sparse in red algae, Engel et al. [31] and Maggs
352 et al. [64] reported that male gametes did not appear to be limiting because the majority of
353 fertilization takes place during low tide when the spermatium concentration is high [65]. In addition,
354 isopod-mediated transport of male gametes, a phenomenon similar to pollination, recently

355 demonstrated in a red alga [29] may compensate for the lack of flagella, increasing fertilization rates
356 and thus explaining any absence of gamete limitation. Further work is needed to investigate
357 whether the Bateman gradient can become significant for females under spermatium-limiting
358 conditions, i.e. without “pollinators” or in a more open environment.

359 Here, the variance in mating success was greater in males and strongly correlated with their
360 reproductive success. This relationship seems to suggest that variance in reproductive success is
361 higher in males than in females. However, even if the opportunity for selection (I) tended to be
362 stronger in males, the difference between sexes was not significant, and therefore did not confirm
363 Bateman’s first principle. These results differ from what has been found in other animal and plant
364 species [7,8]. One possible explanation for this discrepancy is that female reproductive success
365 depends on other factors not related to their access to reproduction but, for example, to their ability
366 to mobilize resources. Interestingly, a trade-off between sexual reproduction and growth has been
367 demonstrated in females for two species of red algae (*G. chilensis*, [66] and *G. domingensis*, [67]).

368 Our results thus suggest that sexual selection may be stronger in males in *G. gracilis*. Consequently,
369 any heritable trait increasing individual access to mating partners may be under sexual selection in
370 males, leading to some sort of sexual dimorphism. In animals, numerous studies have focused on
371 dimorphic traits that allow males to increase the number of partners, such as ornaments or
372 armaments [4]. In angiosperms, some sexually dimorphic traits such as different metrics of flower
373 size and phenology have been shown to be under stronger selection [68,69] or sexual selection [19]
374 in males. In the current study, some constraints in our data collection prevented us from
375 investigating the impact of biomass on female reproductive success. When focusing on males, we
376 found that biomass increased mating success, suggesting that this trait may thus be under sexual
377 selection, although nothing is currently known about its heritability in *G. gracilis*.

378 If the variance in male mating success largely depends on the geographical location of individuals, a
379 typically non-heritable trait, a distance effect may explain why sexual selection seems stronger in
380 males in a species in which no obvious sexual dimorphism seems to occur. A distance effect on
381 reproductive success has been commonly reported in plants [22–26] and also in *G. gracilis* [30]. In
382 the current study, our tests indeed revealed that only male (and not female) reproductive success
383 depends on the distance to potential mates. This effect arises from the dependence of mating
384 success on distance. However, distance explains only a small part of the variance in male mating
385 success, and specific male traits, not yet identified, may nevertheless be involved in this variable
386 access to mates.

387 Given that red algae are cryptogams (i.e. without visible sexual organs) and have a simple
388 morphology (i.e. thallus), the identification of dimorphic sexual characters remains a real challenge.
389 In *G. gracilis* there is no marked sexual dimorphism [33,34]. Nevertheless, some male traits may
390 help increase access to females by improving gamete quality and/or efficiency, such as the
391 phenology of gamete production, or the level of production of mucilage strands in spermatia that
392 may aid dispersal and animal transport [29,70]. These traits may be subject to sexual selection in
393 males, and offer exciting avenues for future research.

394 By investigating the Bateman gradient, the current study mainly focused on the equivalent of pre-
395 copulation sexual selection mechanisms [8], i.e. those involved in the transport of gametes from
396 male to female reproductive structures. However, other processes, can occur after mating such as
397 the selection of some particular male gametes by female tissues, a mechanism often referred to as
398 cryptic female choice in animals and which may also occur in plants [55]. It is thus possible that our
399 estimate of the Bateman gradient in *G. gracilis* also captured the consequences of such cryptic
400 choice. In red algae, female choice could occur within a female structure called the trichogyne [71].
401 Several spermatia may adhere to a trichogyne [72], but only one male nucleus goes on to fuse with

402 the female gamete nucleus [73]. Due to the long and costly development of cystocarps, female
403 choice may play an important role after fertilization in *G. gracilis*. Through this process, females can
404 improve offspring quality by selectively aborting zygotes, on the basis of their paternity. This
405 phenomenon has been widely described in plants where abortions are frequent [74–77] and also in
406 fungi [78]. Post-mating abortions have also been described after controlled crosses in Gracilariaceae
407 [79–81], including individuals of *G. gracilis* sampled from the same population as that studied here
408 [32].

409 In conclusion, our study suggests that sexual selection occurs with a higher intensity in males than
410 in females in *G. gracilis*, a species that shows no clear sexual dimorphism. Our results pave the way
411 for future research to examine particular traits and their susceptibility to sex-specific sexual
412 selection. Finally, our investigation on a non-obvious candidate species shed light on sexual selection
413 being a possible evolutionary driver in a hitherto little-studied phylum of the tree of life.

414

415 **References:**

- 416 1. Hosken DJ, House CM. 2011 Sexual selection. *Curr. Biol.* **21**, R62–R65. (doi:10.1016/j.cub.2010.11.053)
- 417 2. Darwin C. 1872 The descent of man, and selection in relation to sex. *New York: Appleton & Co.*
418 (doi:10.5962/bhl.title.70891)
- 419 3. Lehtonen J. 2022 Bateman gradients from first principles. *Nat. Commun.* **13**, 3591.
420 (doi.org/10.1038/s41467-022-30534-x)
- 421 4. Dewsbury DA. 2005 The Darwin-Bateman paradigm in historical context. *Integr. Comp. Biol.* **45**, 831–837
422 (doi:10.1093/icb/45.5.831)
- 423 5. Kokko H, Jennions MD. 2008 Parental investment, sexual selection and sex ratios. *J. Evol. Biol.* **21**, 919–
424 948. (doi:10.1111/j.1420-9101.2008.01540.x)
- 425 6. Bateman AJ. 1948 Intra-sexual selection in *Drosophila*. *Heredity* **2**, 349–368. (doi:10.1038/hdy.1948.21)
- 426 7. Jones AG. 2009 On the Opportunity for sexual selection, the Bateman gradient and the maximum Intensity
427 of sexual selection. *Evolution* **63**, 1673–1684. (doi:10.1111/j.1558-5646.2009.00664.x)
- 428 8. Janicke T, Häderer IK, Lajeunesse MJ, Anthes N. 2016 Darwinian sex roles confirmed across the animal
429 kingdom. *Sci. Adv.* **2**, e1500983. (doi:10.1126/sciadv.1500983)
- 430 9. Arnold SJ. 1994 Is there a unifying concept of sexual selection that applies to both plants and animals?
431 *Am. Nat.* **144**, S1–S12.
- 432 10. Grant V. 1995 Sexual selection in plants: Pros and Cons. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 1247–1250.
- 433 11. Burd M. 1994 Bateman's principle and plant reproduction: The role of pollen limitation in fruit and seed
434 set. *Bot. Rev.* **60**, 83–139. (doi:10.1007/BF02856594)
- 435 12. Delph LF, Ashman T-L. 2006 Trait selection in flowering plants: How does sexual selection contribute?
436 *Integr. Comp. Biol.* **46**, 465–472. (doi:10.1093/icb/icj038)
- 437 13. Moore JC, Pannell JR. 2011 Sexual selection in plants. *Curr. Biol.* **21**, R176–R182.
438 (doi:10.1016/j.cub.2010.12.035)
- 439 14. Hodgins KA, Barrett SCH. 2008 Natural selection on floral traits through male and female function in wild
440 populations of the heterostylous *Daffodil Narcissus Triandrus*. *Evolution* **62**, 1751–1763.
441 (doi:10.1111/j.1558-5646.2008.00404.x)
- 442 15. Briscoe Runquist RD, Geber MA, Pickett-Leonard M, Moeller DA. 2017 Mating system evolution under
443 strong pollen limitation: Evidence of disruptive selection through male and female fitness in *Clarkia*
444 *xantiana*. *Am. Nat.* **189**, 549–563. (doi:10.1086/691192)
- 445 16. Paterno GB, Silveira CL, Kollmann J, Westoby M, Fonseca CR. 2020 The maleness of larger angiosperm
446 flowers. *Proc. Natl. Acad. Sci.* **117**, 10921–10926. (doi:10.1073/pnas.1910631117)
- 447 17. Tonnabel J, David P, Pannell JR. 2019 Do metrics of sexual selection conform to Bateman's principles in
448 a wind-pollinated plant? *Proc. R. Soc. B Biol. Sci.* **286**, 20190532. (doi:10.1098/rspb.2019.0532)
- 449 18. Kwok A, Dorken ME. 2022 Sexual selection on male but not female function in monoecious and dioecious
450 populations of broadleaf arrowhead (*Sagittaria latifolia*). *Proc. Biol. Sci.* **289**, 20220919.
451 (doi:10.1098/rspb.2022.0919)

- 452 19. Barbot E, Dufaÿ M, De Cauwer I. 2023 Sex specific selection patterns in a dioecious insect-pollinated
453 plant. *Evolution*, in press
- 454 20. Johnson MG, Shaw AJ. 2016 The effects of quantitative fecundity in the haploid stage on reproductive
455 success and diploid fitness in the aquatic peat moss *Sphagnum macrophyllum*. *Heredity* **116**, 523–530.
456 (doi:10.1038/hdy.2016.13)
- 457 21. Janicke T, Morrow EH. 2018 Operational sex ratio predicts the opportunity and direction of sexual
458 selection across animals. *Ecol. Lett.* **21**, 384–391. (doi:10.1111/ele.12907)
- 459 22. Graff A. 1999 Population Sex structure and reproductive fitness in gynodioecious *Sidalcea malviflora*
460 (malvaceae). *Evolution* **53**, 1714–1722. (doi:10.1111/j.1558-5646.1999.tb04556.x)
- 461 23. Oddou-Muratorio S, Klein EK, Demesure-Musch B, Austerlitz F. 2006 Real-time patterns of pollen flow
462 in the wild-service tree, *Sorbus torminalis* (Rosaceae). III. Mating patterns and the ecological maternal
463 neighborhood. *Am. J. Bot.* **93**, 1650–1659. (doi:10.3732/ajb.93.11.1650)
- 464 24. Isagi Y, Saito D, Kawaguchi H, Tateno R, Watanabe S. 2007 Effective pollen dispersal is enhanced by the
465 genetic structure of an *Aesculus turbinata* population. *J. Ecol.* **95**, 983–990. (doi:10.1111/j.1365-
466 2745.2007.01272.x)
- 467 25. De Cauwer I, Dufay M, Cuguen J, Arnaud J-F. 2010 Effects of fine-scale genetic structure on male mating
468 success in gynodioecious *Beta vulgaris* ssp. *maritima*. *Mol. Ecol.* **19**, 1540–1558. (doi:10.1111/j.1365-
469 294X.2010.04586.x)
- 470 26. De Cauwer I de, Arnaud J-F, Schmitt E, Dufaÿ M. 2010 Pollen limitation of female reproductive success
471 at fine spatial scale in a gynodioecious and wind-pollinated species, *Beta vulgaris* ssp. *maritima*. *J. Evol.*
472 *Biol.* **23**, 2636. (doi:10.1111/j.1420-9101.2010.02119.x)
- 473 27. Dixon PS. 1973 Biology of the Rhodophyta. *University reviews in botany*. Edinburgh: Oliver & Boyd.
- 474 28. Bishop JDD, Pemberton AJ. 2006 The third way: spermcast mating in sessile marine invertebrates. *Integr.*
475 *Comp. Biol.* **46**, 398–406. (doi:10.1093/icb/icj037)
- 476 29. Lavaut E, Guillemin M-L, Colin S, Faure A, Coudret J, Destombe C, Valero M. 2022 Pollinators of the sea:
477 A discovery of animal-mediated fertilization in seaweed. *Science* **377**, 528–530.
478 (doi:10.1126/science.abo6661)
- 479 30. Kamiya M, Kawai H. 2002 Dependence of the carposporophyte on the maternal gametophyte in three
480 ceramiacean algae (Rhodophyta), with respect to carposporophyte development, spore production and
481 germination success. *Phycologia* **41**, 107–115. (doi:10.2216/i0031-8884-41-2-107.1)
- 482 31. Engel CR, Wattier R, Destombe C, Valero M. 1999 Performance of non-motile male gametes in the sea:
483 Analysis of paternity and fertilization success in a natural population of a red seaweed, *Gracilaria gracilis*.
484 *Proc. R. Soc. Lond. B Biol. Sci.* **266**, 1879–1886. (doi:10.1098/rspb.1999.0861)
- 485 32. Engel CR, Valero M, Lagadeuc Y, Destombe C. 2002 Non-random mating in controlled multiple-donor
486 crosses in *Gracilaria gracilis* (Gracilariaceae, Rhodophyta). *Eur. J. Phycol.* **37**, 179–190.
487 (doi:10.1017/S096702620200358X)
- 488 33. Destombe C, Valero M, Vernet P, Couvet D. 1989 What controls haploid—diploid ratio in the red alga,
489 *Gracilaria verrucosa* ? *J. Evol. Biol.* **2**, 317–338. (doi:10.1046/j.1420-9101.1989.2050317.x)

- 490 34. Kain(Jones) JM, Destombe C. 1995.A review of the life history, reproduction and phenology of *Gracilaria*.
491 *J. Appl. Phycol.* **7**, 269–281. (doi:10.1007/BF00004001)
- 492 35. Engel CR, Åberg P, Gaggiotti OE, Destombe C, Valero M. 2001 Population dynamics and stage structure
493 in a haploid-diploid red seaweed, *Gracilaria gracilis*. *J. Ecol.* **89**, 436–450. (doi:10.1046/j.1365-
494 2745.2001.00567.x)
- 495 36. Destombe C, Godin J, Bodard M. 1988 The decay phase in the life history of *Gracilaria verrucosa*: the
496 consequences in intensive cultivation. *Algal Biotechnol. Ed. T Stadler Al*
- 497 37. Wattier R, Dallas JF, Destombe C, Saumitou-Laprade P, Valero M. 1997 Single locus microsatellites in
498 *Gracilariales* (Rhodophyta): high level of genetic variability within *Gracilaria gracilis* and conservation in
499 related species. *J. Phycol.* **33**, 868–880. (doi:10.1111/j.0022-3646.1997.00868.x)
- 500 38. Luo H, Möorchen M, Engel CR, Destombe C, Epplen JT, Epplen C, Saumitou-Laprade P, Valero M. 1999
501 Characterization of microsatellite markers in the red alga *Gracilaria gracilis*. *Mol. Ecol.* **8**, 700–702.
502 (doi:10.1046/j.1365-294x.1999.00879.x)
- 503 39. Mauger S, Baud A, Le Corguillé G, Tanguy G, Legeay E, Creis E, Valero M, Destombe C, Potin P. submitted.
504 Genetic resources of macroalgae: development of an efficient method using microsatellite markers in non-
505 model organisms. *Algal Res.*
- 506 40. Mauger S, Couceiro L, Valero M. 2012 A simple and cost-effective method to synthesize an internal size
507 standard amenable to use with a 5-dye system. *Prime Res. Biotechnol. PRB* **2**, 2315–5299.
- 508 41. Peakall R, Smouse PE. 2006 Genalex 6: Genetic analysis in Excel. Population genetic software for teaching
509 and research. *Mol. Ecol. Notes* **6**, 288–295. (doi:10.1111/j.1471-8286.2005.01155.x)
- 510 42. Peakall R, Smouse PE. 2012 GenAEx 6.5: Genetic analysis in Excel. Population genetic software for
511 teaching and research—an update. *Bioinformatics* **28**, 2537–2539. (doi:10.1093/bioinformatics/bts460)
- 512 43. R Core Team. 2020 R: A language and environment for statistical computing. R Foundation for Statistical
513 Computing, Vienna, Austria. <https://www.R-project.org/>.
- 514 44. Wade MJ. 1979 Sexual selection and variance in reproductive success. *Am. Nat.* **114**, 742–747.
- 515 45. Wade MJ, Arnold SJ. 1980 The intensity of sexual selection in relation to male sexual behaviour, female
516 choice, and sperm precedence. *Anim. Behav.* **28**, 446–461. (doi:10.1016/S0003-3472(80)80052-2)
- 517 46. Arnold SJ, Wade MJ. 1984 On the measurement of natural and sexual selection: theory. *Evolution* **38**,
518 709–719
- 519 47. Henshaw JM, Jennions MD, Kruuk LEB. 2018 How to quantify (the response to) sexual selection on traits.
520 *Evolution* **72**, 1904–1917. (doi.org/10.1111/evo.13554)
- 521 48. Lefcheck JS. 2016 PiecewiseSEM: piecewise structural equation modelling in R for ecology, evolution and
522 systematics. *Methods Ecol. Evol.* **7**, 573–579. (doi:10.1111/2041-210X.12512)
- 523 49. Anthes N, Häderer IK, Michiels NK, Janicke T. 2017 Measuring and interpreting sexual selection metrics:
524 evaluation and guidelines. *Methods Ecol. Evol.* **8**, 918–931. (doi.org/10.1111/2041-210X.12707)
- 525 50. Schärer L, Rowe L, Arnqvist G. 2012 Anisogamy, chance and the evolution of sex roles. *Trends Ecol. Evol.*
526 **27**, 260–264. (doi:10.1016/j.tree.2011.12.006)

- 527 51. Murphy CG. 1998 Interaction-Independent sexual selection and the mechanisms of sexual selection.
528 *Evolution* **52**, 8–18. (doi:10.1111/j.1558-5646.1998.tb05133.x)
- 529 52. Nieuwenhuis BPS, Aanen DK. 2012 Sexual selection in fungi. *J. Evol. Biol.* **25**, 2397–2411.
530 (doi:10.1111/jeb.12017)
- 531 53. Beekman M, Nieuwenhuis B, Ortiz-Barrientos D, Evans JP. 2016 Sexual selection in hermaphrodites,
532 sperm and broadcast spawners, plants and fungi. *Philos. Trans. R. Soc. B Biol. Sci.* **371**, 20150541.
533 (doi:10.1098/rstb.2015.0541)
- 534 54. Pannell JR, Labouche A-M. 2013 The incidence and selection of multiple mating in plants. *Philos. Trans.*
535 *R. Soc. Lond. B. Biol. Sci.* **368**, 20120051. (doi:10.1098/rstb.2012.0051)
- 536 55. Tonnabel J, David P, Janicke T, Lehner A, Mollet J-C, Pannell JR, Dufay M. 2021 The scope for postmating
537 sexual selection in plants. *Trends Ecol. Evol.* **36**, 556–567. (doi:10.1016/j.tree.2021.02.013)
- 538 56. Krueger-Hadfield SA, Roze D, Correa JA, Destombe C, Valero M. 2015 O father where art thou? Paternity
539 analyses in a natural population of the haploid–diploid seaweed *Chondrus crispus*. *Heredity* **114**, 185–194.
540 (doi:10.1038/hdy.2014.82)
- 541 57. Knight TM *et al.* 2005 Pollen limitation of plant reproduction: pattern and process. *Annu. Rev. Ecol. Evol.*
542 *Syst.* **36**, 467–497. (doi:10.1146/annurev.ecolsys.36.102403.115320)
- 543 58. Levitan DR, Sewell MA. 1998 Fertilization success in free-spawning marine invertebrates: review of the
544 evidence and fisheries implications. *Can. Spec. Publ. Fish. Aquat. Sci.* **125**, 159–164.
- 545 59. Yund PO. 2000 How severe is sperm limitation in natural populations of marine free-spawners? *Trends*
546 *Ecol. Evol.* **15**, 10–13. (doi:10.1016/S0169-5347(99)01744-9)
- 547 60. Berndt M-L, Callow JA, Brawley SH. 2002 Gamete concentrations and timing and success of fertilization
548 in a rocky shore seaweed. *Mar. Ecol. Prog. Ser.* **226**, 273–285.
- 549 61. Serrão EA, Havenhand J. 2009 Fertilization strategies. In *Marine Hard Bottom Communities*. 149–164.
550 (doi:10.1007/b76710_10)
- 551 62. Searles RB. 1980 The strategy of the red algal life history. *Am. Nat.* **115**, 113–120.
- 552 63. Santelices B. 2002 Recent advances in fertilization ecology of macroalgae. *J. Phycol.* **38**, 4–10.
553 (doi:10.1046/j.1529-8817.2002.00193.x)
- 554 64. Maggs CA, Fletcher HL, Fewer D, Loade L, Mineur F, Johnson MP. 2011 Speciation in red algae: members
555 of the Ceramiales as model organisms. *Integr. Comp. Biol.* **51**, 492–504. (doi:10.1093/icb/icr075)
- 556 65. Engel CR, Destombe C. 2002 Reproductive ecology of an intertidal red seaweed, *Gracilaria gracilis*:
557 influence of high and low tides on fertilization success. *J. Mar. Biol. Assoc. U. K.* **82**, 189–192.
558 (doi:10.1017/S0025315402005349)
- 559 66. Guillemain ML, Valenzuela P, Gaitán-Espitia JD, Destombe C. 2014 Evidence of reproductive cost in the
560 triphasic life history of the red alga *Gracilaria chilensis* (Gracilariales, Rhodophyta). *J. Appl. Phycol.* **26**,
561 569–575. (doi:10.1007/s10811-013-0072-x)
- 562 67. Guimarães M, Plastino EM, Oliveira EC. 1999 Life history, reproduction and growth of *Gracilaria*
563 *domingensis* (Gracilariales, Rhodophyta) from Brazil. *Botanica Marina* **42**, 481–486.
564 (doi:10.1515/BOT.1999.055)

- 565 68. Campbell DR. 1989 Measurements of selection in a hermaphroditic plant: variation in male and female
566 pollination success. *Evolution* **43**, 318–334. (doi:10.1111/j.1558-5646.1989.tb04230.x)
- 567 69. O’Connell LM, Johnston MO. 1998 Male and female pollination success in a deceptive orchid, a selection
568 study. *Ecology* **79**, 1246. (doi:10.2307/176740)
- 569 70. Brawley SH, Johnson LE. 1992 Gametogenesis, gametes and zygotes: An ecological perspective on sexual
570 reproduction in the algae. *Br. Phycol. J.* **27**, 233–252. (doi:10.1080/00071619200650241)
- 571 71. Pickett-Heaps JD, West J. 1998 Time-lapse video observations on sexual plasmogamy in the red alga
572 *Bostrychia*. *Eur. J. Phycol.* **33**, 43–56. (doi:10.1080/09670269810001736523)
- 573 72. Hommersand MH, Fredericq S. 1990 Sexual reproduction and cystocarp development. Pp. 305-45.
574 *Cambridge University Press*. 305–345.
- 575 73. Fredericq S, Hommersand MH. 1989 Proposal of the Gracilariales ord. Nov. (Rhodophyta) based on an
576 analysis of the reproductive development of *Gracilaria verrucosa*. *J. Phycol.* **25**, 213–227.
577 (doi:10.1111/j.1529-8817.1989.tb00116.x)
- 578 74. Hossaert M, Valero M. 1988 Effect of ovule position in the pod on patterns of seed formation in two
579 species of *Lathyrus* (Leguminosae: papilionoideae). *Am. J. Bot.* **75**, 1714–1731. (doi:10.1002/j.1537-
580 2197.1988.tb11248.x)
- 581 75. Willson MF, Burley N. 1983 *Mate choice in plants: tactics, mechanisms, and consequences*. Princeton
582 University Press.
- 583 76. Rigney LP. 1995 Postfertilization causes of differential success of pollen donors in *Erythronium*
584 *grandiflorum* (Liliaceae): nonrandom ovule abortion. *Am. J. Bot.* **82**, 578–584. (doi:10.1002/j.1537-
585 2197.1995.tb11502.x)
- 586 77. Havens K, Delph LF. 1996 Differential seed maturation uncouples fertilization and siring success in
587 *Oenothera organensis* (Onagraceae). *Heredity* **76**, 623–632. (doi:10.1038/hdy.1996.89)
- 588 78. Bruggeman J, Debets AJM, Hoekstra RF. 2004 Selection arena in *Aspergillus nidulans*. *Fungal Genet. Biol.*
589 **41**, 181–188. (doi:10.1016/j.fgb.2003.10.007)
- 590 79. van der Meer JP. 1986 Genetics of *Gracilaria tikvahiae* (Rhodophyceae). Xi. Further characterization of
591 a bisexual mutant. *J. Phycol.* **22**, 151–158. (doi:10.1111/j.1529-8817.1986.tb04158.x)
- 592 80. Richerd S, Destombe C, Cuguen J, Valero M. 1993 Variation of reproductive success in a haplo-diploid
593 Red Alga, *Gracilaria verrucosa* : Effects of parental identities and crossing distance. *Am. J. Bot.* **80**, 1379–
594 1391. (doi:10.1002/j.1537-2197.1993.tb15382.x)
- 595 81. Gurgel CFD, Fredericq S, Norris JN, Yoneshigue-Valentin Y. 2008 Two new flat species of *Gracilaria*
596 (Gracilariales, Rhodophyta) from Brazil. *Phycologia* **47**, 249–264. (doi:10.2216/PH06-59.1)

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609

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611

612 **Data availability:** Genotypic data, complete script of bootstrapping procedure and path analyses
613 were deposited and are available in Dryad doi.org/10.5061/dryad.t4b8gtj6c. All other data used in
614 the analyses are available in the paper or the supplementary materials (**tables S1-S3** and **figure S1-**
615 **S3**)

616 **Figure Captions**

617 **Figure 1** - Life cycle of *Gracilaria gracilis*. The individuals are fixed to the rocky substrate via a
618 perennial holdfast. The deciduous erect thalli decay after reproduction in late autumn and grow
619 back from the holdfast in spring. Diploid tetrasporophytes produce haploid spores from meiosis that

620 develop into dioicous male and female haploid gametophytes. Spermata (non-flagellated male
621 gametes) are released into the water column. After fertilization, the zygote is retained on the female
622 and develops into a complex, macroscopic post-fertilization structure: the cystocarp. One
623 fertilization gives rise to thousands of identical diploid carpospores that, after release and
624 germination, grow into new tetrasporophyte individuals.

625

626 **Figure 2** - Map of the Cape Gris-Nez intertidal rock pool showing the locations of the 71 male
627 (triangles) and the 70 female (circles) gametophyte individuals sampled within the population (the
628 tetrasporophyte individuals are not shown). Mating success, i.e. the numbers of sexual partners, is
629 proportional to (filled) symbol size. Females with missing information that were not included in
630 Bateman analyses are indicated with open circles. The rock pool edges are shown in grey. The grey
631 polygons within the rock pool indicate the presence of rocks.

632

633 **Figure 3** - Relationship between reproductive and mating success and their distribution in males
634 (dark blue) and females (green). Distribution of reproductive success (A,D), mating success (B, E)
635 and the Bateman gradient (C,F) for males and females, respectively. The significance of the Bateman
636 gradient, estimated by the slope of the linear regression, is indicated on the graph (C, F).

637

638 **Figure 4** - Relationship between mating success and mean distance to partners (A) in males and (B)
639 in females. The significance of the relationship, estimated by the slope of the linear regression, is
640 shown on each graph.

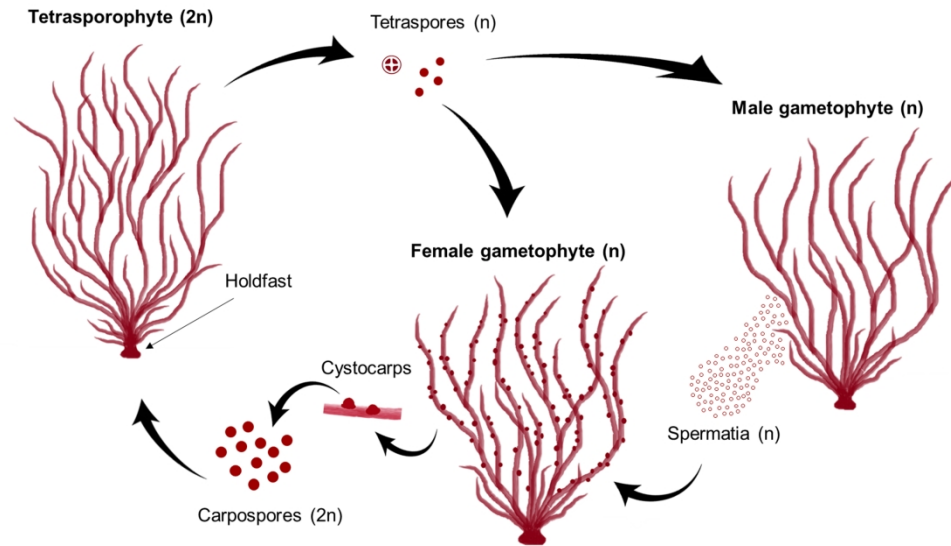


Figure 1 - Life cycle of *Gracilaria gracilis*. The individuals are fixed to the rocky substrate via a perennial holdfast. The deciduous erect thalli decay after reproduction in late autumn and grow back from the holdfast in spring. Diploid tetrasporophytes produce haploid spores from meiosis that develop into dioicous male and female haploid gametophytes. Spermata (non-flagellated male gametes) are released into the water column. After fertilization, the zygote is retained on the female and develops into a complex, macroscopic post-fertilization structure: the cystocarp. One fertilization gives rise to thousands of identical diploid carpospores that, after release and germination, grow into new tetrasporophyte individuals.

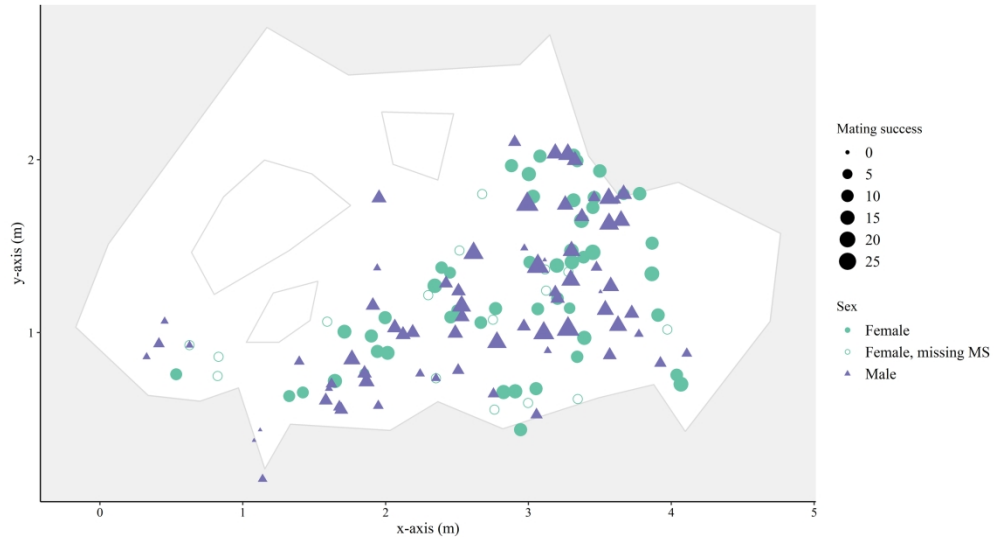


Figure 2 - Map of the Cape Gris-Nez intertidal rock pool showing the locations of the 71 male (triangles) and the 70 female (circles) gametophyte individuals sampled within the population (the tetrasporophyte individuals are not shown). Mating success, i.e. the numbers of sexual partners, is proportional to (filled) symbol size. Females with missing information that were not included in Bateman analyses are indicated with open circles. The rock pool edges are shown in grey. The grey polygons within the rock pool indicate the presence of rocks.

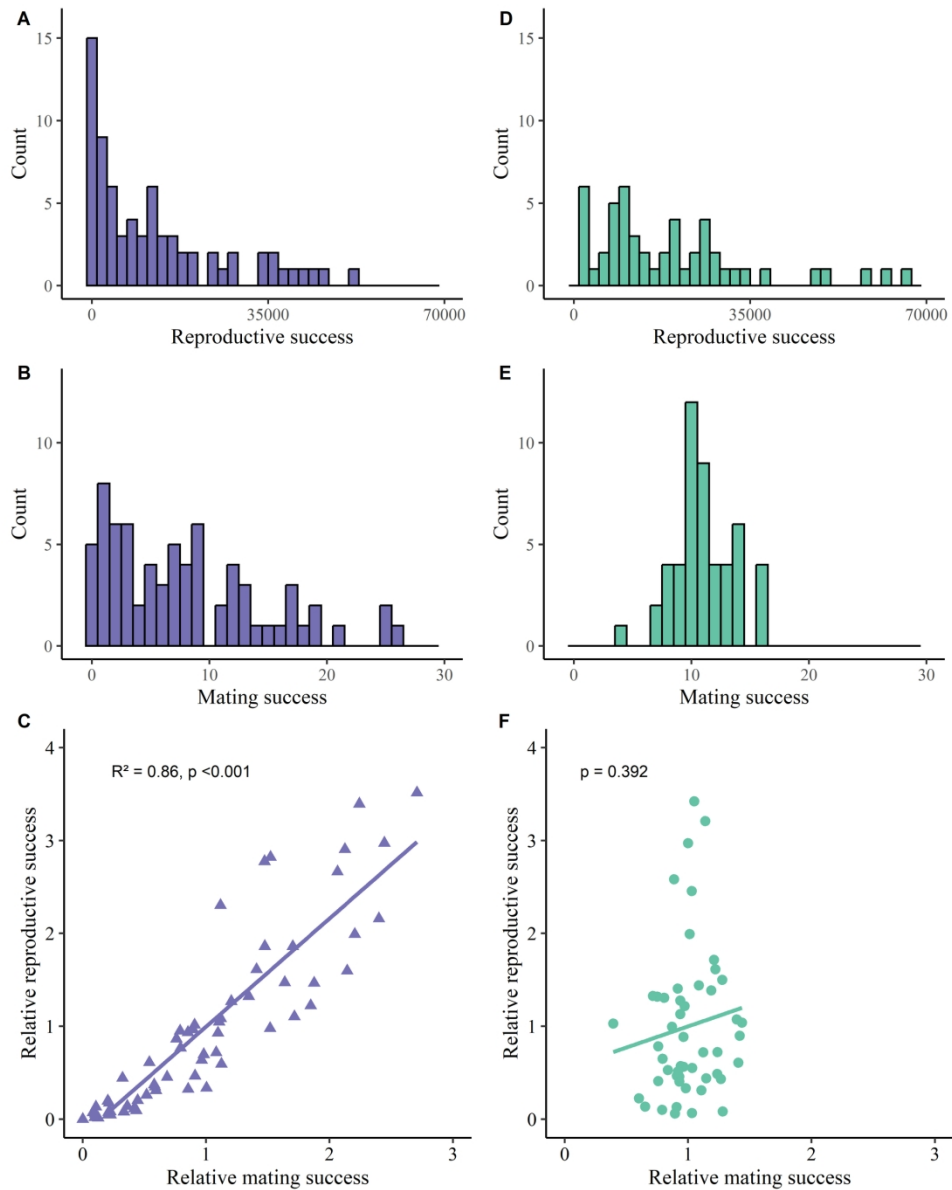


Figure 3 - Relationship between reproductive and mating success and their distribution in males (dark blue) and females (green). Distribution of reproductive success (A,D), mating success (B, E) and the Bateman gradient (C,F) for males and females, respectively. The significance of the Bateman gradient, estimated by the slope of the linear regression, is indicated on the graph (C, F).

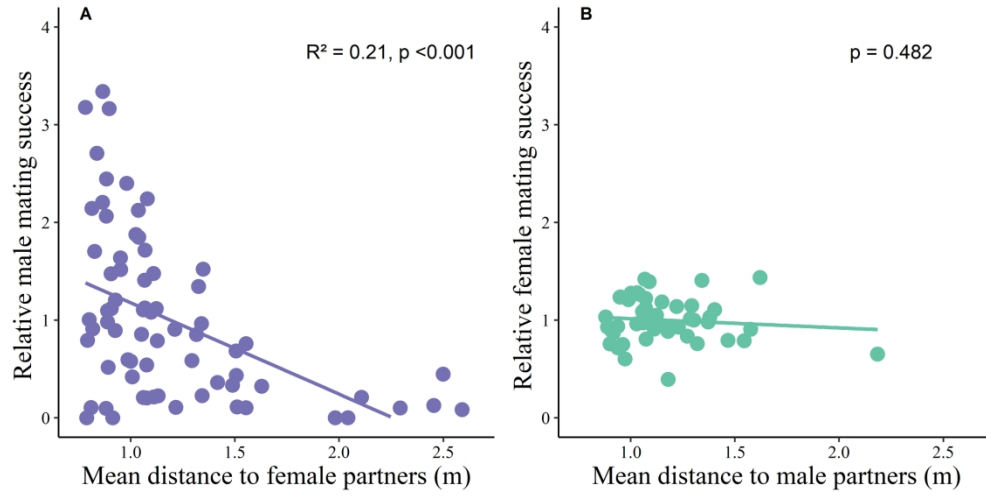


Figure 4 - Relationship between mating success and mean distance to partners (A) in males and (B) in females. The significance of the relationship, estimated by the slope of the linear regression, is shown on each graph.