Mates matter: Gametophyte kinship recognition and inbreeding in the giant kelp, *Macrocystis pyrifera* (Laminariales, Phaeophyceae)

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- 1 MATES MATTER: GAMETOPHYTE KINSHIP RECOGNITION AND
- 2 INBREEDING IN THE GIANT KELP, *Macrocystis pyrifera* (Linnaeus) C. Agardh¹
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30 Running title: Inbreeding in *Macrocystis pyrifera*

ABSTRACT

Inbreeding, the mating between genetically related individuals, often results in reduced survival and fecundity of offspring, relative to outcrossing. Yet, high inbreeding rates are commonly observed in seaweeds, suggesting compensatory reproductive traits may affect the costs and benefits of the mating system. We experimentally manipulated inbreeding levels in controlled crossing experiments, using gametophytes from 19 populations of *Macrocystis pyrifera* along its Eastern Pacific coastal distribution (EPC). The objective was to investigate the effects of male-female kinship on female fecundity and fertility, to estimate inbreeding depression in the F1 progeny, and to assess the variability of these effects among different regions and habitats of the EPC. Results revealed that the presence and kinship of males had a significant effect on fecundity and fertility of female gametophytes. Females left alone or in the presence of sibling males express the highest gametophyte size, number and size of oogonia, suggesting they were able to sense the presence and the identity of their mates before gamete contact. The opposite trend was observed for the production of embryos per female gametes, indicating higher costs of selfing and parthenogenesis than outcrossing on fertility. However, the increased fecundity compensated for the reduced fertility, leading to a stable overall reproductive output. Inbreeding also affected morphological traits of juvenile sporophytes, but not their heatwave tolerance. The male-female kinship effect was stronger in high latitude populations, suggesting that females from low latitude marginal populations might have evolved to mate with any male gamete to guarantee reproductive success.

Key index words: fecundity, fertility, controlled crossing experiment, parthenogenesis, reproductive assurance

Abbreviation: EPC, Easter Pacific Coast; CA-MX, California – México; PE, Perú; AT, Atacama; SC, Southern Chile; MA, Magallanes; LL, Los Lagos; P, Parthenogenesis; S, Selfing; IntraPop, crosses within population; InterPop, crosses between population; IntraHab, crosses between populations but the same habitat; InterHab, crosses between populations but different habitat.

INTRODUCTION

Reproduction is among the most studied biological processes, as it determines generational transfer of genetic material, the extent of population genetic diversity, and ultimately evolutionary trajectories of specific populations. Inbreeding, the production of offspring from the mating of individuals that are genetically more closely related than random mating, is widely reported, especially in plants. Self-fertilization in particular, i.e. mating between female and male gametes produced by the same diploid individual, is predominant in more than a third of plant species (Barrett and Harder 1996), and also occurs in animals (Jarne and Auld 2006, Charlesworth and Willis 2009). It is often associated with the negative effects of inbreeding depression (Hedrick and Garcia-Dorado 2016, Charlesworth and Willis 2009), which in turn may promote the evolution of inbreeding avoidance traits such as kin recognition (Szulkin et al. 2013), gamete incompatibility (Castric and Vekemans 2004, Billiard et al. 2012) or dispersal (Auld and Rubio de Casas 2013). Although inbreeding can have negative consequences, self-fertilization does provide reproductive assurance when cross-fertilization is uncertain (Busch and Delph 2012), may promote the purging of deleterious alleles, might enhance the effects of background selection and genetic hitchhiking by reducing the efficiency of recombination (Roze 2016), and in some cases, can actually be adaptive if it results in inclusive fitness benefits (Puurtilinen 2011).

81 Inbreeding in marine organisms is poorly understood. There is considerable diversity of
82 life cycles and mating systems in the ocean, providing a rich source of models for
83 studying the occurrence and the consequences of inbreeding. At first glance, selfing
84 might be thought to be rare in the ocean, considering the large number of species with
85 planktonic larval stages resulting in long range dispersal, which most likely reduces the
86 probability of sibling gamete encounters. However, inbreeding rates in sessile and
87 sedentary marine species can be as high as in terrestrial plants (Olsen et al. 2020).

88 Marine algae are particularly interesting models for studying mating systems because of
89 their complex life cycle, which most often includes haploid and diploid generations.
90 Meiosis in marine algae occurs on the diploid individuals, while gametes are produced
91 by haploid males and females or hermaphroditic individuals. One important aspect of
92 such a life cycle is that mating between sibling males and females (i.e. those resulting
93 from a single diploid parent) leads to the equivalent of selfing, as fertilization may occur
94 between gametes originating from the same diploid genome. Another key aspect of
95 seaweeds is that the dispersal capacity of spores and gametes can be as low as < 1 m
96 (i.e. *Postelsia palmaeformis*, Wootton and Pfister 2013), and usually less than 1 km for
97 spores (Santelices 1990, Kinlan and Gaines 2003). Reduced dispersal provides
98 opportunities for mating among relatives and between sibling gametophytes. In some
99 taxa, spores from the same parent are dispersed in mucilage or clumps and recruit
100 together (Santelices 1990), further increasing the possibility for gametophyte selfing.
101 Many species of marine algae show high levels of heterozygote deficiency within
102 populations (e.g. Benzie et al. 1997, Engel et al. 2005, Krueger- Hadfield et al. 2011,
103 Guillemin et al. 2016). Therefore, understanding whether inbreeding levels relate to
104 limited spore dispersal or a predominantly inbred mating system, such as selfing, is
105 important, yet has been explored very little. In some cases, paternity analyses reveal a

complete absence of selfing besides high inbreeding levels, which was better explained by clumped spore dispersal in the red algae *Chondrus crispus* (Krueger- Hadfield et al. 2013) and *Gracilaria gracilis* (Engel et al. 2004). Yet in other cases, selfing is inferred for the hermaphroditic brown alga *Fucus spiralis* (Perrin et al. 2007), and for the giant kelp *Macrocystis pyrifera* (Johansson et al. 2013), for which population genetic simulations suggest high mortality of homozygous juveniles accompanies selfing, implying inbreeding depression.

Inbreeding depression is largely unassessed in algae. Limited evidence comes from breeding experiments revealing heterosis in between-population crosses (Westermeyer et al. 2011, Zhao et al. 2016) or the monitoring of natural populations showing juvenile mortality associated with inbreeding levels (Teixeira et al. 2016). Inbreeding depression is not expected in haploid-diploid life cycles because the haploid stage may actually facilitate the purge of deleterious alleles. In this context, no restriction to selfing would be expected to evolve. Yet, empirical evidence is extremely scarce. For instance, synchronicity in gamete release differs among species with different selfing rates in the brown alga *Fucus* spp, with a narrower timeline of spawning in the dioic, predominantly outcrossing *F. vesiculosus* than the hermaphroditic, highly selfing *F. spiralis* and *F. guiryi* (Monteiro et al. 2012), suggesting a weaker selective constraint in selfing species. On the contrary, chemical recognition of complementary surface carbohydrates on male and female gametes has been suggested to regulate successful fertilization within, rather than between populations of *Ectocarpus siliculosus* (Schmid 1994).

In this study, we investigate the effects of inbreeding in a haploid-diploid seaweed, the giant kelp *Macrocystis pyrifera*. Kelp is a common name given to seaweeds with distinct life cycles, yet most are large brown seaweeds of the order Laminariales. Laminariales exhibit a heteromorphic haploid-diploid life cycle: the diploid individual

(i.e. the sporophyte) can be up to 60m long and form marine forests (Schiel and Foster 2015). In the giant kelp, meiosis occurs on specialized blades, the sporophylls, located at the base of the sporophyte, less than a half-meter above the substratum (Gaylord et al. 2002). This anatomical feature suggests that spores disperse very short distances from the parent sporophyte, as spores may settle within minutes after being released. Spores develop into microscopic free-living haploid individuals (i.e. gametophytes). Male gametophytes release sperm which swim to the oogonia (i.e. female gametes) sprouting on the female gametophyte. Gamete encounter is facilitated by chemical signaling from females that trigger the release and attraction of sperm (Mamer et al. 1984, Maier et al. 2001). Limited spore dispersal increases the chance of inter-gametophytic selfing (i.e. mating between female and male gametophytes produced by the same sporophyte), but also bi-parental inbreeding via outcrossing between genetically related parent sporophytes (i.e. same family members spatially clumped). Models have predicted that a significant fraction (20-40%) of fertilization events in natural populations may be through selfing (Gaylord et al. 2006, Johansson et al. 2013). Unfertilized oogonia can also induce parthenogenesis, a common feature in kelps (Druehl et al. 2005, Oppliger et al. 2007, Müller et al. 2019, Murua et al. 2020), where the nucleus of the female gamete initiates a duplication of its genetic material without cell division, leading to the restoration of diploidy and the production of a juvenile sporophyte (e.g. Oppliger et al. 2007). This reproductive system is expected to create fully homozygous individuals, therefore representing the maximum level of inbreeding. In summary, the production of sporophytes likely results from a mixed-mating system, including parthenogenesis, selfing and outcrossing.

Inbreeding depression has been seldom studied in kelp species. For the sea palm *P. palmaeformis*, a low cost of selfing has been estimated for individual fitness (Barner

et al. 2011), and inbreeding rates show no effect on population extinction risk (Wootton and Pfister 2013). On the contrary, the fitness of *M. pyrifera* can be dramatically reduced in selfed compared to outcrossed individuals (Raimondi et al. 2004): zygote production by the haploid gametophytes was reduced in inbred crosses, as well as survivorship, fecundity and fertility of the adult diploid offspring. The existence of heterosis when crossing gametophytes of distant populations further supports the idea of inbreeding depression within populations (Westermeyer et al. 2010). This cost of selfing seems spatially variable, with some populations not expressing any costs or benefits compared to outcrossing (San Miguel 2017), likely suggesting a variable efficiency of purging among populations. However, strong inbreeding coefficients have been detected in most studied populations of the species, i.e. along the coasts of California and Chile (Johansson et al. 2013 and Camus et al. 2018, respectively).

There exist apparent inconsistencies between high inbreeding rates and high inbreeding depression in some natural populations of the giant kelp. The suspected high mortality of inbred recruits in natural populations (Johansson et al. 2013) suggests strong selection for purging deleterious alleles. It may also favor strong selection against selfing if inbreeding depression is persistent. Different traits are expected to evolve in order to reduce successful fertilization among kin gametes. Life history trait evolution has been reported in response to specific conditions in kelp species, such as increased automixis (i.e. fusion of two of the four meiotic products, producing diploid gametophytes) to increase reproductive assurance at thermal range limits of *Laminaria digitata* (Oppliger et al. 2014), or an increase in asexual reproduction and an unbalanced sex ratio to avoid hybrid formation in a secondary contact zone of the *Lessonia nigrescens* species complex (Oppliger et al. 2011, 2012). These examples highlight the

capacity of kelps to adapt their life cycle and mating system in response to specific reproductive requirements or environmental conditions.

In this study, we experimentally manipulated the level of inbreeding using gametophytes of the giant kelp to investigate the effects of male-female kinship on female fecundity and fertility, as possible indicators of prezygotic barriers to selfing in the gametophytic stage, as well as the existence of inbreeding depression in the F1 diploid progeny. The study also assesses the variability of these effects among different regions and habitats of the Eastern Pacific coast.

MATERIALS AND METHODS

Field sampling and gametophyte isolation.

Fertile blades were collected from 19 populations, from Lima, Peru, to Puerto Yartau, Magellan Strait, Chile (Fig. 1), covering most of the South Eastern Pacific coastal distribution range of *Macrocystis pyrifera*. Additional samples from Santa Cruz (California, USA), Ensenada and Punta Baja (Baja California, Mexico) were collected in the northern hemisphere as replicates of the low latitude range limits of the southern hemisphere, namely Lima, Marcona and Ilo (Peru). These sampling sites represented 5 regions identified in Fig. 1, hereafter named California and Mexico (CA-MX), Peru (PE), Atacama (AT), Southern Chile (SC) and Magallanes (MA), and are used in experiment 1 (see below). Four SC localities were selected for their contrasting demography (i.e annual and perennial) and wave exposure habitats, according to Buschmann et al. (2004, 2006), and used specifically in experiment 2 (see below): Carelmapu and Pargua are perennial and wave exposed populations located nearby two annual and wave protected populations, Ilque and Metri (Buschmann et al. 2006). These

four populations defined a subgroup of SC, called hereafter Los Lagos (LL) as they were used in experiment 2.

In each population, 1 to 4 sporophylls (i.e. the fertile blades) per sporophyte were collected and washed under freshwater to remove diatoms and other microorganisms from the blade surface. Small pieces of cleaned blade were then incubated in cool, sterile seawater to stimulate spore release. Blade pieces from different sporophytes were kept separate in different flasks to isolate individual sporophyte progeny. Spores settled on glass slides and germinated into male and female gametophytes. These were cultivated in Provasoli enriched seawater (McLachlan 1973) at 10 to 12 °C, under 12 h:12 h L:D photoperiod, at 25-30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of white light. After male and female differentiation, based on cell width and ramification pattern of the filamentous gametophytes, individual males and females were isolated with a Pasteur glass pipette and cultivated separately under the same laboratory conditions but using red light to avoid gametogenesis. After a period of vegetative growth, each gametophyte was mechanically fragmented at 5-10 cells per fragment, using a glass tissue grinder with teflon pestle or a plastic pestle, to expand their biomass. Clonal fragments of each gametophyte initially deposited in 6-well multiplates (Corning) were further transferred to increasing volume up to 50 mL tubes or Erlenmeyer flasks. Culture conditions were as mentioned above, but under red light until experimental crosses were conducted (see Fig. 2 and Fig. S1).

Crossing experiments.

Experiment 1 aimed to explore the regional variability of female fecundity, reproductive success and inbreeding depression. Because gametophyte fertility is variable within a progeny issued from a single parental sporophyte, vegetative fragments of 5 sister females were pooled to ensure the production of gametes and successful fertilization.

Male fragments were also pooled from 5 individual cultures originated from a single sporophyte (i.e. pool of clonal fragments of 5 sib males; Fig. 2 and Fig. S1). For CA-MX gametophytes, no sib pool was made as the identity of the parental sporophyte was not registered. Therefore, single females were fragmented and crossed with single male clonal fragments. These pools or single gametophyte clonal cultures were used as basic experimental units, and subsequently subdivided into 4 aliquots. Each aliquot was either grown alone, to stimulate parthenogenesis, or in the presence of males. Parthenogenesis occurs by nucleus endoduplication within the female gamete, leading to a fully homozygous diploid sporophyte. This represents the highest possible level of inbreeding. Selfing was achieved by mixing one female aliquot with one male pool obtained from the same parental sporophyte. Within-population crosses were performed by mixing a female aliquot with a male pool obtained from a different sporophyte of the same population. Crosses among populations but within each region were performed by mixing one female aliquot with a male super-pool obtained by mixing 4 sib male pools from two different populations of the same region. Therefore, each female pool produced F1 sporophytes either by parthenogenesis (P), selfing (S), by crosses within population (IntraPop5), or between populations within region (InterPop). Two female replicates per population followed this experimental setup (e.g. 24 female pools from Peru and Chile, and 6 single females from California-Mexico) leading to a total of 120 crosses.

The crosses were kept at a common temperature of $12\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for 36 days under white light to allow gametogenesis, during which fecundity, expressed as the number of oogonia per living female, and fertility, expressed as the embryogenesis rate determined by the number of embryos per living female, were registered every 6 days. Embryogenesis includes embryos produced by fertilized oogonia and

parthenogenetically developed embryos. Data was collected using a camera (Vi1, Nikon) mounted on an inverted microscope (Olympus, CKX53) on 5 optical fields (15.2mm² each, at 10x magnification) per replicate. Digital photos were processed with ImageJ software.

Because laboratory culture conditions might be optimal for any F1 sporophyte, the differences between inbreeding and outbreeding levels are expected to be subtle. To maximize the potential of detecting differences, the F1 sporophytes were exposed to a heatwave for 5 days at 24 °C ± 1°C under white light. The sporophyte survival rate was measured on day 5.

Experiment 2: Individual female and male gametophytes were treated independently, without pooling sibs (Fig. 2 and Fig. S1). Gametophytes were collected from 13 sporophytes per population. Each female or male clone was divided into 5 aliquots. Single female aliquots were cultured alone to stimulate parthenogenesis (P). Selfing (S) was achieved by mixing one female aliquot with one male aliquot from the same parental sporophyte. Two types of within-population crosses were performed: one female aliquot crossed with one male aliquot of a different sporophyte of the same population produced genetically identical F1 sporophytes (IntraPop1), and one female aliquot crossed with two male aliquots from different sporophytes from the same population produced the equivalent of half-sib F1 sporophytes (IntraPop2). Interpopulation crosses were achieved by mixing an aliquot of 13 females with 13 males of a different population but the same habitat (IntraHab, within annual/protected or perennial/exposed populations) or the alternative habitat (hereafter InterHab).

Each cross was repeated 3 times and data were averaged. In total, 20 female pools (i.e. 5 per population) were used to replicate the different breeding types, except for InterHab which used 6 replicates, leading to a total of 106 crosses. In each of these experimental

units, fecundity was decomposed into the length of female gametophyte, the length of oogonia and the number of oogonia per gametophyte on day 12. Fertility was estimated as the number of embryos per female gametophyte on day 17 and the embryogenesis rate calculated as the number of embryos at day 17 over fertile females at day 12 (i.e. females bearing oogonia). The length of the juvenile sporophyte, the number of blades, and the holdfast diameter were recorded on day 30 as proxies of F1 juvenile sporophytes fitness. Data was collected using an inverted microscope (PrimoVert, Carl Zeiss) with a Canon EOS REBEL T3 camera mounted. Counts were taken in 10 optical fields (an area of 8,13 mm² each) per well at 40X magnification and digital photos were processed with ImageJ software. For morphological data of juvenile sporophytes, ten individuals per replicate were photographed with a scale and processed with the same image software.

Statistical analyses.

The effect of breeding type and region (in Experiment 1) or habitat (in Experiment 2) on fecundity, fertility and sporophyte growth (only in Experiment 2) were assessed by mixed linear models (Searle 1987, Pinheiro and Bates 2000) using *lme4* in R (Bates et al. 2015). The breeding type, region (or habitat), and their interactions were defined as fixed effects. A female's identifier was defined as a random intercept, that is the identifier associated with the parental sporophyte of each clone or pool of gametophytes, to integrate the lack of independence of the breeding types resulting from the repeated use of each gametophyte or gametophyte pool. Mortality associated with the heatwave experiment was analyzed following the same procedure. Sporophytes from California-Mexico and Peru were excluded from the analysis because of a much lower number of individuals compared to the other regions. The response variables of experiments 1 and 2 were transformed with Yeo-Johnson (Yeo and Johnson 2000) and

Box-Cox (Box and Cox 1964), respectively, to fulfill the assumptions of normality and homoscedasticity. Contrast of hypotheses was performed using ANOVA Type II with the Kenward-Roger approximation to adjust degrees of freedom of the denominator (Fox 2002). Pairwise comparisons were performed with the a posteriori Tukey test using *multcomp* in R (Hothorn et al. 2008), using Bonferroni correction for false discovery rates. Pairwise comparisons were performed for breeding types only, inside the region or within the same habitat in Experiment 1 and 2, respectively.

RESULTS

Female fecundity and fertility depend on male identity.

Fecundity, expressed as the production of female gametes, was significantly affected by the presence of males (Experiment 1; ANOVA: $F_{(3,75)} = 11.36$, $p < 0.0001$. Experiment 2; ANOVA $F_{(5,108)} = 48.40$, $p < 0.0001$) but not in the same manner for all regions tested as the interaction Breeding Type x Region in Experiment 1 was statistically significant (ANOVA: $F_{(12,75)} = 3.05$, $p < 0.002$). The highest fecundity (i.e. up to 5 oogonia per female gametophyte) was observed in both experiments for females left alone (i.e. without males), when parthenogenesis was stimulated (Figs. 3a and 4a). The lowest gamete production was observed when females were exposed to males issued from different sporophytes regardless of population origin. In Experiment 2, fecundity ranged from 66.7% in Selfing to 36.6% in IntraPop2 of the parthenogenetic values. The same pattern was observed in Experiment 1 for Southern Chile and Magallanes, with InterPop fecundity being reduced to 10% of the respective parthenogenetic value in the later region. A noticeable exception was for within-habitat interpopulation crosses in Southern Chile-Los Lagos (LL), where no difference was observed with parthenogenetic females, both producing 4.0 ± 2.5 and 4.3 ± 2.0 oogonia per female,

respectively (Fig. 4a). Fecundity, expressed as morphological characteristics of LL gametophytes revealed that the size of the vegetative parts of the female (Fig. 4b) and the size of the gametes (Fig. 4c) significantly differed when exposed to different types of males (ANOVA: $F_{(5,124)} = 11.55$, $p < 0.0001$; $F_{(5,127)} = 57.55$, $p < 0.0001$; respectively) and among habitats (Breeding Type x Habitat, ANOVA: $F_{(5,164)} = 2.34$, $p = 0.04$; $F_{(5,172)} = 5.12$, $p = 0.0002$, respectively). Oogonia were bigger when exposed to sib males (i.e. reaching 0.036 ± 0.014 mm in length) than to males from different sporophytes or in solitary females, with differences ranging from 11.8% in parthenogenesis and 36.6% in IntraHab crosses (Fig. 4c). Female size was the largest in the absence of males or with highly related males (around 0.2 mm in length), and their size progressively decreased with male-female kinship (Fig. 4b). Females in parthenogenesis, selfing and IntraPop1 crosses were 5x larger than females in presence of males from different habitats (InterHab) (Fig. 4b).

Female fertility, measured as the production of embryos per female gametophyte (available in LL populations only), was significantly explained by the male-female kinship (Fig. 4d, ANOVA: $F_{(5,126)} = 11.94$, $p < 0.0001$) with no effect of the factor Habitat or the interaction Habitat x Breeding type. Again, the highest fertility was observed in parthenogenetic females, while slightly reducing with sib-male kinship in intrapopulation crosses. However, between population outcrosses (i.e. intra and inter habitats), fertility did not differ significantly from any of the within population crosses (Fig. 4d). This pattern was explained by a higher embryogenesis rate of all but intra-habitat crosses (Fig. 4e, ANOVA: $F_{(5,117)} = 24.88$, $p < 0.0001$). Embryogenesis higher than 100% is explained by the continuous production of oogonia when exposed to males (except for IntraHab crosses), while parthenogenetic females stopped gametogenesis after initial gamete production (i.e. until day 12). The same embryogenesis pattern was

observed in Experiment 1 (Fig. 3b, ANOVA: Factor Breeding type: $F_{(3,71)} = 26.79$, $p < 0.0001$).

Inbreeding depression in F1 progeny.

Male-female kinship affected different traits of the juvenile sporophytes such as sporophyte length (Fig. 5a, ANOVA: $F_{(5,82)} = 21.61$, $p < 0.0001$), number of blades (Fig. 5b, ANOVA: $F_{(5,96)} = 24.05$, $p < 0.0001$) and holdfast diameter (Fig. 5c, ANOVA: $F_{(5,92)} = 32.42$, $p < 0.0001$). Holdfast size was smaller in inbred sporophytes (i.e. parthenogenetic, selfing and intrapopulation) compared to other outcrossed progenies, and significant differences were observed between habitats (ANOVA: Habitat: $F_{(1,16)} = 5.51$, $p = 0.01$; Breeding Type x Habitat: $F_{(5,92)} = 4.21$, $p < 0.001$). However, the differences in juvenile length and blade number were largely inconsistent with parental kinship: while selfing produced the smallest values for both traits, sporophytes from intermediate levels of male-female kinship (i.e. intra-population crosses) expressed the highest values together with parthenosporophytes (Figs. 5a and 5b). Tolerance to a heat wave did not differ significantly between crosses (Fig. S2, Table S1; ANOVA: $F_{(3,45)} = 2.32$, $p = 0.09$), as mortality rates ranged between 80% to 99% at 24°C. California-Mexico and Peru were not included in the heat wave analysis because too few sporophytes were obtained from the crossing experiments.

Latitudinal variability of kinship effect on fecundity and fertility of female gametophytes.

The effects of male-female kinship on fecundity strongly varied among regions and habitats (Fig. 3a; ANOVA: Breeding Type x Region: $F_{(12,75)} = 3.05$, $p = 0.002$), with a significant effect detected only in Southern Chile and Magallanes regions. Both low latitude regions (i.e. California-Mexico and Peru) had a consistently low fecundity (i.e.

less than 1 oogonia per female gametophyte on average) compared with Chilean regions (Fig. S3, Table S2; ANOVA: Region: $F_{(4,25)} = 45.76$, $p < 0.0001$). A similar pattern was observed in fertility, with a significantly lower rate in California-Mexico and Peru (Fig. 3b; ANOVA: Region: $F_{(4,24)} = 96.62$, $p < 0.0001$) and an effect of kinship detected only in Southern Chile and Magellan regions (Fig. S4 and Table S3; ANOVA: Breeding Type x Region: $F_{(12,71)} = 5.46$, $p < 0.0001$). In the Atacama region, no effect of the male-female kinship was observed on either fecundity and fertility, even though average fecundity was similar to that detected in the Southern Chile and Magallanes regions (Fig. 3a). This pattern was explained by low fertilization rates in these regions (Fig. 3b). In Southern Chile-Los Lagos (LL), perennial and annual populations did not differ for any gametophyte trait except female length (Figs. S5, S6, S7, S8, S9 and Table S4, S5, S6, S7, S8). Comparison of morphological traits in juvenile sporophytes revealed larger blades and holdfasts in perennial populations, but no interaction with parental kinship (Figs. S10, S11, S12 and Table S9, S10, S11). The heat wave effect did not differ significantly between regions (Table S1; ANOVA: $F_{(2,15)} = 2.32$, $p = 0.13$) and no interaction was detected between kinship and region (Table S 1; ANOVA: $F_{(6,45)} = 0.54$, $p = 0.77$).

DISCUSSION

Female fecundity and fertility are affected by male identity.

The presence of males and their kinship with females had a significant effect on different aspects of fecundity, including female size, number and size of oogonia. The phenomenon was highly consistent in showing higher values for females alone and in presence of their sib males, than with unrelated males. This effect was the result of the

presence of male gametophytes, prior to any sexual contact between gametes, and therefore strongly suggests that females can sense both the presence and the identity of males. Sex-inducing pheromones have been described in many different algae, including planktonic diatoms (Moeys et al. 2016), green algae and benthic macroalgae (see review from Frenkel et al. 2014). Most of the identified molecules act as gamete attractants, while some are considered sex inducers, as they trigger meiosis in diploids or the formation of gametes in haploids (Frenkel et al. 2014). In kelps, the only known sex pheromone is Lamoxirene, a molecule produced by the fertile female gametophyte which acts as an inducer of sperm release and attraction (Mamer et al. 1984). This single molecule is shared by all kelp species (Pohnert and Boland 2002), making it an unlikely candidate for kin recognition within species. Beyond that, there is no information about prezygotic allorecognition mechanisms in brown algae, such as those described in terrestrial plants or invertebrates. Our results suggest the existence of a more complex recognition system in *Macrocystis*, acting prior to gamete contact, allowing females to sense the presence and identity of males. It is possible that male gametophytes release a blend of molecules that stimulate the female to behave differently according to kinship. Alternatively, the chemical signaling may result from the bacterial biofilm associated with gametophytes. Growth stimulating hormones and morphogenetic compounds produced by microorganisms have been shown to be involved in the development of algae (Matsuo et al. 2005, Wichard 2015) and specific bacteria are known to induce the synthesis of algal hormones such as auxins and cytokinins (Lui et al. 2017, Dittami et al. 2014). Specific molecules released by associated bacteria are known to induce the production and posterior development of gametes in green algae (Frenkel et al. 2014). And bacteria may also be involved in pheromone degradation, therefore preventing gamete encounters due to outdated or non-

target chemical cues (Cirri et al. 2018). There is growing interest in the properties of the holobiont (i.e. the algae and their interacting microorganisms forming a single entity; Egan et al. 2012). The microorganisms associated with *Macrocystis* gametophytes could well intercede in this recognition mechanism through an endogenous signaling system and remains to be explored.

Females left alone to induce parthenogenesis were the biggest in size and produced the highest number of gametes per capita. This might suggest that males actually inhibit female fecundity. Alternatively, it can be hypothesized that, in the absence of male chemical cues, females increase their size and fecundity in order to increase their pheromone production, as a compensating mechanism potentially increasing their attractiveness. This second hypothesis is more likely, as there would be no obvious ecological or evolutionary advantage for males to inhibit female fecundity. Interestingly, the presence of sib males had a similar (yet reduced) effect on females. Considering the hypothesis of chemical recognition of males discussed above, increased female fecundity is therefore not only a consequence of the absence of males, but also of appropriate males for successful fertilization. The rate of embryo per female gamete was the lowest for parthenogenetic females (with the exception of among-population intra-habitat crosses; see discussion below), followed by selfing and within-population crosses with a single male. A low rate of transformation of oogonia into embryos is expected if females could delay the parthenogenetic production of sporophyte progeny as a way to extend the period of sexual pheromone production and male gamete attraction, saving the oogonia for the possibility of later fertilization. This is further supported by the observation that <80% of oogonia produced embryos in parthenogenetic females, while females continued to produce new oogonia in the

presence of males during the 5 days that separated the observations, and these were nearly all fertilized.

Reproductive success, although statistically different among breeding types, only varied by 2.5-3 embryos per female. Although we found strong differences in fecundity, this reduced variability in progeny number per female could be explained by a varying fertilization rate of oogonia. Indeed, fecundity progressively diminished from parthenogenetic to IntraPop2 (females with 2 different males from the same population), while the opposite trend was observed for embryogenesis rate. The reduced fertilization success associated with gametophyte kinship suggests, again, that allorecognition mechanisms are operating. In brown algae, a gamete recognition system has been proposed in *Ectocarpus siliculosus*, determined by complementary surface carbohydrates located in the cell wall of the oogonia and sperm that are required for syngamy to occur (Schmid et al. 1994). Interestingly, a preference for outcrossing was found to be linked to a locus determining the capacity to induce parthenogenesis (Mignerot et al. 2019), suggesting that inbreeding avoidance and parthenogenesis are genetically linked. Therefore, it seems that increased fecundity is a response to the lack of genetically unrelated mates for ensuring stable reproductive success, as expected under the reproductive assurance hypothesis. And contrary to expectations under an inbreeding avoidance hypothesis, the gametophyte recognition mechanism did not appear to act as a prezygotic barrier, but rather as a way to increase the chances of successfully producing new sporophytes. Compensatory investment in female gametes when male gametes are limiting appears the predominant output of theoretical models of reproductive resource allocation (Gillet and Gregorius 2020). Such a plastic response of reproductive traits to sperm/pollen limitation or the relatedness of mates has been confirmed empirically in plants and animals (e.g. Tsitrone et al. 2003, Auld and Relyea

2010). To our knowledge, this is the first report for algae characterised by a dioecious haploid-diploid life cycle.

A notable exception to the observed pattern was found in the between-populations within-habitat crosses, which behaved similar to parthenogenetic females in terms of the number of oogonia and embryogenesis rate. It is noteworthy that this pattern was evidenced in both experiments within the Southern Chile populations (InterPop - Experiment 1 and IntraHab - Experiment 2). On the contrary, female and oogonia size were significantly lower than parthenogenetic females. Following the hypothesis of compensatory reproductive investment, it is possible that males from a different population were not recognized as competent mates. This would be the case under a scenario of strong population divergence in chemical signals, which remains to be characterized. Such divergence would be expected among different habitats, either because of a divergence in the bacterial community of the holobiont, or because of a selection against admixed sporophytes due to outbreeding depression. Yet, between-habitat crosses did not follow the same pattern, by showing low fecundity and high fertilization rates. This apparent contradiction of the results from the within- and between-habitat crosses is a question that remains unanswered, but it can be hypothesized to relate to the reproductive responses of females to specific chemical signals being driven by a suite of different mechanisms.

Inbreeding depression in the F1 diploid progeny.

The association of increased fecundity and reduced fertility represent a cost for female reproduction under inbreeding. However, the absolute number of embryos produced by parthenogenesis and selfing, under the laboratory conditions of the experiments, remained slightly higher than for outcrossing, suggesting an absence of inbreeding depression in the earliest stage of the diploid phase of *M. pyrifera*. These results differ

from previously reported inbreeding depression in Californian populations (Raimondi et al. 2004, Johansson et al. 2013, Gaylord et al. 2006). In laboratory and field experiments, Raimondi et al. (2004) found that self-fertilization had strong negative consequences on the fitness of *M. pyrifera*, as evidenced by a 40% decrease in zygote production, a five times lower frequency of sporophytes reaching sexual maturity, and a ten times reduction in reproductive tissue produced in selfed compared to outcrossed progenies. A high mortality rate (i.e. 32% to 42%) of inbred sporophytes was further inferred from population genetic modelling (Johansson et al. 2013), explaining that few highly homozygous individuals survive to adulthood. Our results on juvenile F1 sporophytes tend to be consistent with a lower early development (i.e. juvenile length, holdfast size and number of blades) when comparing selfed versus outcrossed progenies within populations. But the pattern did not strictly follow a negative correlation with the inbreeding level, as parthenogenetic sporophytes were always bigger than selfed progenies, and between-population crosses within habitat produced smaller sporophytes than IntraPop2. Here, parthenogenesis was considered the highest possible level of inbreeding, assuming the diploidization of the female gamete results in a fully homozygous sporophyte. Further analysis of the process of parthenogenesis and the developmental transition from gametophyte to sporophyte is required to better understand the early performance of parthenogenetic sporophytes.

Kinship effect on fecundity and fertility of female gametophytes among different regions and habitats of the East Pacific coast.

Consistent with previous observations (Buschmann et al. 2004), low latitude populations of both the northern and southern hemisphere had low fecundity and

fertility of female gametophytes, compared to higher latitude populations in Chile. Certainly, low latitude populations experience high temperatures, which is one of the most important factors determining the distribution range limits of marine macroalgae due to its effect on survival, growth and reproduction (Breeman 1988). The giant kelp, *M. pyrifera* is a cold temperate species, yet with a wide latitudinal range: from Alaska, USA to Baja California, Mexico and from Peru to Cape Horn (Hoffmann and Santelices 1997, Graham et al. 2007). Light, temperature, and nutrients strongly vary across such a range, and therefore influence the metabolic tradeoff between vegetative growth or survival and reproduction. The significant interaction between regions and breeding types revealed that the effects of inbreeding were stronger in the Magallanes region than at the intermediate latitude of Southern Chile, and non-significant in Atacama, Peru and California-Mexico. Therefore, low latitude females not only have a limited reproductive capacity, but also a limited capacity to sense and respond to male kinship and inbreeding. This divergence was not observed between wave exposure habitats and perennial/annual demographics at the same latitude (i.e. Southern Chile). In this region, a genetic discontinuity and a morphological divergence was observed between these two habitats (Camus et al. 2018). Sporophytes of annual populations produce more spores per sorus area than in perennial populations (Buschmann et al. 2006). This strategy is believed to allow the establishment of a dense gametophyte population that will last the winter period when all sporophytes perish. A differentiation in gametophyte reproductive traits was also expected because of the demographic differences, considering that selfing is more common in annual than perennial populations (Barrett et al. 1997) and that the evolution of resource allocation is influenced by both selfing rate and the annual-perennial habit (Zhang 2000). It is likely that the drivers of the reproductive divergence operate only at large spatial scales.

The status of low-latitude populations may be defined by low nutrients associated with warmer waters and competition with warm-tolerant species (Ladah et al. 1999, Steneck et al. 2002, Graham et al. 2007). Moreover, these regions are subjected to recurrent El Nino-Southern Oscillations and heat waves, which commonly result in widespread mortality of the giant kelp, followed by recolonizations (Soto 1985, Tegner and Dayton 1987, Ladah et al. 1999, Edwards 2004, Vega et al. 2005, Cavanaugh et al. 2019). Populations in such habitat at range margins tend to differ systematically from those in central habitats in several demographic and reproductive characteristics (Kawecki 2008). Modifications of the mating system, such as increased asexual reproduction, has been observed in terrestrial plants as a reproductive assurance strategy in marginal populations (Eckert et al. 2006). Similar trends are observed in marginal kelp populations (Oppliger et al. 2011, 2014). According to Baker's law, selfing should be advantageous in populations characterized by recurrent extinction-recolonization (Pannell and Barrett 1998) as it provides reproductive assurance when few progenitors are contributing to the recolonization. In this context, a stronger compensatory effect on female fecundity would be expected to maximize its fitness under a mixed mating system (Zhang 2000, Gillet and Gregorius 2020). However, considering the reduced fecundity and fertility observed in the marginal populations of *M. pyrifera*, it is possible that females evolved to mate with any male gamete, without requiring chemical recognition of kin males and differential investment in gametes.

In conclusion, the giant kelp, *Macrocystis pyrifera*, has the ability to reproduce sexually and asexually (i.e. parthenogenesis) and with males of different levels of kinship without major consequences in the fitness of females and their offspring. The identified fecundity cost of selfing and parthenogenesis seems to efficiently compensate for the reduced rate of embryo per oogonia, ultimately maintaining a slight but

statistically significant fitness advantage over outcrossing. Confirming the existence and identifying the nature of male chemical cues causing this differential response in high-latitude females will be a key step to understand the drivers of this compensatory resource allocation during gametophyte reproduction, and also to understand the observed latitudinal differences. The trait might be a plastic response to environmental heterogeneity. It is well-known that environmental factors, such as light quality and quantity, temperature and nutrient concentrations greatly affect fertility and fecundity of female gametophytes and embryonic sporophyte development (Lüning and Neushul 1978, Deysher and Dean 1984, 1986, Kinlan et al. 2003). What remains poorly understood is the interaction of these abiotic factors with breeding types. However, the observed latitudinal divergence while growing and reproducing in common garden conditions suggests some heritable and/or epigenetic components that may have consequences across diploid and haploid generations. In any case, questions emerge as to whether the lack of kinship effects in low-latitude populations is an optimization of the reproductive strategy under low fecundity/fertility or a maladaptation to marginal environmental conditions. This may be particularly relevant as low latitude populations of kelps are the most affected by ocean warming (Wernberg et al. 2018), and conservation strategies, including restoration plans, should consider the optimal mating strategy to secure long term demographic sustainability.

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CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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FIGURE LEGENDS

Fig. 1: Map of sampling sites of *Macrocystis pyrifera* (black dots), with detailed location in each sampling region. (a) California: Santa Cruz (SCR); and Mexico: Ensenada (ENS) and Punta Baja (PBA). (b) Perú: Lima (LMA), Marcona (MRC), Ilo (ILO); and Atacama: Junin (JNN), Caleta La Cuchara (LCU) and Antofagasta (ANT). (c) Southern Chile: Nihue (NLH), Pucatrihue (PUC) and Faro Corona (FCO). In the box, Southern Chile-Los Lagos (LL), where triangles are perennial (Carelmapu and Pargua) and squares are annual (Ilque and Metri) populations, respectively. (d) Magallanes: Puerto Natales (NAT), Faro San Isidro (FSI) and Yartuo (YAR).

Fig. 2: Diagram of the workflow for the isolation of female and male gametophytes used for crossing experiments. The progeny of each sampled sporophyte (top left) was handled independently. Spores released from collected sporophylls were cultivated until germination and differentiation into ♂ and ♀ gametophytes. These were isolated and cultivated separately and fragmented into clonal filaments of 5 to 15 cells. For experiment 1, pools of clonal fragments from 5 ♀ gametophytes were established for each progeny. The same protocol was applied to ♂. Each pool was aliquoted into 4 units, to be used in the different breeding treatments: P: parthenogenesis; S: selfing, exposing the ♀ pool to a ♂ pool issued from the same sporophyte; IP5: intra-population outcross using a pool of 5 sib ♂ issued from a different sporophyte of the same population as the ♀ pool; IR: intra-region outcross using a ♂ pool issued from a sporophyte from a different population but the same region. For experiment 2, single ♀ clones were aliquoted into 4 units: IP1 and IP2 are intra-population outcross using a 1 or 2 cloned ♂, respectively, issued from different sporophytes of the same population. Additionally, interpopulation crosses were performed on ♀ pools issued from 13 different sporophytes, crossed with ♂ pools issued from 13 different sporophytes of a different population and the same habitat for intra-habitat crosses (IH) or the alternative for between habitat crosses (BH).

Fig. 3: Fecundity expressed as the number of oogonia per female gametophyte (a) and
embryogenesis rate (b) of females from California-Mexico, Perú, Atacama, Southern Chile and
Magallanes regions obtained in the different breeding types: parthenogenesis (P), selfing (S),
outcross within population (IntraPop5) and between populations of a same region (InterPop).
Letters represent statistical differences between treatments within each population.

Fig. 4: Fecundity of female gametophytes from Southern Chile-Los Lagos (LL) populations, expressed as the (a) number of oogonia per female gametophyte, (b) female gametophyte length (mm) and (c) oogonia length; and fertility expressed as the (d) number of embryos per female gametophyte and (e) embryogenesis rate obtained in the different breeding types: parthenogenesis (P), selfing (S), outcross within population using 1 or 2 males (IntraPop1 and IntraPop2, respectively), between populations of the same habitat (IntraHab) and between habitats (InterHab). Letters represent statistical differences.

Fig. 5: Morphological characters evaluated on juvenile sporophytes from Southern Chile-Los Lagos populations obtained in the different breeding types (see Fig. 4). (a) Sporophyte length (mm). (b) number of blades per sporophyte. (c) Holdfast diameter (mm). Letters represent statistical differences.

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Figure S2. % mortality of juvenile sporophytes from Atacama, Southern Chile and Magallanes regions after exposure for 5 days to a heat wave of 24°C obtained in the different breeding types: parthenogenesis (P), selfing (S), IntraPop5, and InterPop.

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931 **Figure S3.** Fecundity expressed as the number of oogonia per female gametophyte from
932 California, Perú, Atacama, Southern Chile and Magallanes regions. Letters represent
933 statistical differences.

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938 **Figure S4.** Embryogenesis rate of female gametophytes from California, Perú,
939 Atacama, Southern Chile and Magallanes regions. Letters represent statistical
940 differences.

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945 **Figure S5.** Number of oogonia per female gametophyte from annual and perennial
946 Southern Chile-Los Lagos populations obtained in different breeding types:
947 parthenogenesis (P), selfing (S), IntraPop1, IntraPop2, IntraHab and InterHab. Letters
948 represent statistical differences.

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953 **Figure S6.** Female gametophyte length (mm) from annual and perennial Southern
954 Chile-Los Lagos populations obtained in different breeding types: parthenogenesis (P),
955 selfing (S), IntraPop1, IntraPop2, IntraHab and InterHab. Letters represent statistical
956 differences.

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961 **Figure S7.** Oogonia length from annual and perennial Southern Chile-Los Lagos
962 populations obtained in different breeding types: parthenogenesis (P), selfing (S),
963 IntraPop1, IntraPop2, IntraHab and InterHab. Letters represent statistical differences.

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968 **Figure S8.** Number of embryos per female gametophyte from annual and perennial
969 Southern Chile-Los Lagos populations obtained in different breeding types:
970 parthenogenesis (P), selfing (S), IntraPop1, IntraPop2, IntraHab and InterHab. Letters
971 represent statistical differences.

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976 **Figure S9.** Embryogenesis rate from annual and perennial Southern Chile-Los Lagos
977 populations obtained in different breeding types: parthenogenesis (P), selfing (S),
978 IntraPop1, IntraPop2, IntraHab and InterHab. Letters represent statistical differences.

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Figure S10. Sporophyte length (mm) from annual and perennial Southern Chile-Los Lagos populations obtained in different breeding types: parthenogenesis (P), selfing (S), IntraPop1 and IntraPop2. Letters represent statistical differences.

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990 **Figure S11.** Number of fronds per sporophyte from annual and perennial Southern
991 Chile-Los Lagos populations obtained in different breeding types: parthenogenesis (P),
992 selfing (S), IntraPop1, IntraPop2, IntraHab and InterHab. Letters represent statistical
993 differences.

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998 **Figure S12.** Holdfast diameter of sporophytes from annual and perennial Southern
999 Chile-Los Lagos populations obtained in different breeding types: parthenogenesis (P),
1000 selfing (S), IntraPop1, IntraPop2, IntraHab and InterHab. Letters represent statistical
1001 differences.

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Table S1. Table of ANOVA results for the effect of breeding type (parthenogenesis, selfing, IntraPop5 and InterPop), region (Atacama, Southern Chile and Magallanes) and the interaction for the % mortality of juvenile sporophytes exposed for 5 days to a heat wave of 25°C.

Fixed effects	SumSq	MeanSq	DF _{Num}	DF _{Den}	Fvalue	Pr(>F)
Breeding type	4.10	1.37	3	45	2.32	0.09
Region	2.74	1.37	2	15	2.32	0.13
Breeding type:region	1.93	0.32	6	45	0.54	0.77

Table S2. Table of ANOVA results for the effect of breeding type (Parthenogenesis, selfing, IntraPop5 and InterPop), region (California, Perú, Atacama, Southern Chile and Magallanes) and the interaction for fecundity express as the number of oogonia per female gametophyte.

Fixed effects	SumSq	MeanSq	DF _{Num}	DF _{Den}	Fvalue	Pr(>F)
Breeding type	10.19	3.40	3	75	11.36	< 0.0001
Region	54.71	13.68	4	25	45.76	< 0.0001
Breeding type:Region	10.95	0.91	12	75	3.05	0.002

Table S3. Table of ANOVA results for the effect of breeding type (Parthenogenesis, selfing, IntraPop5 and InterPop), region (California, Perú, Atacama, Southern Chile and Magallanes) and the interaction for fertilization rate.

Fixed effects	SumSq	MeanSq	DF _{Num}	DF _{Den}	Fvalue	Pr(>F)
Breeding type	14.23	4.74	3	71	26.79	< 0.0001
Region	68.44	17.11	4	24	96.62	< 0.0001
Breeding type:Region	11.59	0.97	12	71	5.46	< 0.0001

Table S4. Table of ANOVA results for the effect of breeding type (Parthenogenesis, selfing, IntraPop1, IntraPop2, IntraHab and InterHab), habitat (annual: Ilque and Metri, perennial: Carelmapu and Pargua,) and the interaction for fecundity express as the number of oogonia per female gametophyte.

Fixed effects	SumSq	MeanSq	DF _{Num}	DF _{Den}	Fvalue	Pr(>F)
Breeding type	108.27	21.65	5	108	48.40	< 0.0001
Habitat	0.81	0.81	1	19	1.82	0.19
Breeding type:Habitat	2.661	0.53	5	128	0.32	0.32

Table S5. Table of ANOVA results for the effect of breeding type (Parthenogenesis, selfing, IntraPop1, IntraPop2, IntraHab and InterHab), habitat (annual: Ilque and Metri, perennial: Carelmapu and Pargua,) and the interaction for fecundity express as female gametophyte length.

Fixed effects	SumSq	MeanSq	DF _{Num}	DF _{Den}	Fvalue	Pr(>F)
Breeding type	41.48	8.30	5	124	11.55	< 0.0001
Habitat	8.92	8.92	1	19	12.53	0.002
Breeding type:Habitat	8.36	1.67	5	164	2.34	0.04

Table S6. Table of ANOVA results for the effect of breeding type (Parthenogenesis, selfing, IntraPop1, IntraPop2, IntraHab and InterHab), habitat (annual: Ilque and Metri, perennial: Carelmapu and Pargua,) and the interaction for fecundity express as oogonia length.

Fixed effects	SumSq	MeanSq	DF _{Num}	DF _{Den}	Fvalue	Pr(>F)
Breeding type	105.60	21.12	5	127	57.55	< 0.0001
Habitat	0.15	0.15	1	19	0.42	0.53
Breeding type:Habitat	9.36	1.87	5	172	5.12	0.0002

Table S7. Table of ANOVA results for the effect of breeding type (Parthenogenesis, selfing, IntraPop1, IntraPop2, IntraHab and InterHab), habitat (annual: Ilque and Metri, perennial: Carelmapu and Pargua,) and the interaction for fertility express as the number of embryos per gametophyte.

Fixed effects	SumSq	MeanSq	DF _{Num}	DF _{Den}	Fvalue	Pr(>F)
Breeding type	46.78	9.36	5	126	11.94	< 0.0001
Habitat	1.08	1.08	1	19	1.39	0.25
Breeding type:Habitat	5.80	1.16	5	169	1.49	0.20

Table S8. Table of ANOVA results for the effect of breeding type (Parthenogenesis, selfing, IntraPop1, IntraPop2, IntraHab and InterHab), habitat (annual: Ilque and Metri, perennial: Carelmapu and Pargua,) and the interaction for fertilization rate.

Fixed effects	SumSq	MeanSq	DF _{Num}	DF _{Den}	Fvalue	Pr(>F)
Breeding type	75.78	15.16	5	117	24.88	< 0.0001
Habitat	0.49	0.45	1	19	0.73	0.40
Breeding type:Habitat	2.39	0.48	5	146	0.78	0.57

Table S9. Table of ANOVA results for the effect of breeding type (Parthenogenesis, selfing, IntraPop1 and IntraPop2), habitat (annual: Ilque and Metri, perennial: Carelmapu and Pargua,) and the interaction for sporophyte length.

Fixed effects	SumSq	MeanSq	DF _{Num}	DF _{Den}	Fvalue	Pr(>F)
Breeding type	41.93	13.98	3	145	34.61	<0.0001
Habitat	2.09	2.09	1	15	5.17	0.04
Breeding type:habitat	10.56	3.52	3	145	8.71	<0.0001

Table S10. Table of ANOVA results for the effect of breeding type (Parthenogenesis, selfing, IntraPop1, IntraPop2, IntraHab and InterHab), habitat (annual: Ilque and Metri, perennial: Carelmapu and Pargua,) and the interaction for number of fronds per sporophyte.

Fixed effects	SumSq	MeanSq	DF _{Num}	DF _{Den}	Fvalue	Pr(>F)
Breeding type	56.40	18.80	3	147	33.85	<0.0001
Habitat	0.17	0.17	1	15	0.31	0.58
Breeding type:habitat	0.93	0.31	3	147	0.56	0.65

Table S11. Table of ANOVA results for the effect of breeding type (Parthenogenesis, selfing, IntraPop1, IntraPop2, IntraHab and InterHab), habitat (annual: Ilque and Metri, perennial: Carelmapu and Pargua,) and the interaction for holdfast diameter of sporophytes.

Fixed effects	SumSq	MeanSq	DF _{Num}	DF _{Den}	Fvalue	Pr(>F)
Breeding type	64.93	26.23	3	114	41.23	<0.0001
Habitat	1.82	1.82	1	16	4.65	0.05
Breeding type:habitat	7.86	1.96	3	114	4.99	0.0009