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

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MATES MATTER: GAMETOPHYTE KINSHIP RECOGNITION AND INBREEDING IN THE GIANT KELP, *Macrocystis pyrifera* (Linnaeus) C. Agardh[1]

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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 (Puurtinen 2011).

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

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

106 complete absence of selfing besides high inbreeding levels, which was better explained
107 by clumped spore dispersal in the red algae *Chondrus crispus* (Krueger- Hadfield et al.
108 2013) and *Gracilaria gracilis* (Engel et al. 2004). Yet in other cases, selfing is inferred
109 for the hermaphroditic brown alga *Fucus spiralis* (Perrin et al. 2007), and for the giant
110 kelp *Macrocystis pyrifera* (Johansson et al. 2013), for which population genetic
111 simulations suggest high mortality of homozygous juveniles accompanies selfing,
112 implying inbreeding depression.

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

127 In this study, we investigate the effects of inbreeding in a haploid-diploid seaweed, the
128 giant kelp *Macrocystis pyrifera*. Kelp is a common name given to seaweeds with
129 distinct life cycles, yet most are large brown seaweeds of the order Laminariales.
130 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 (Westermeier 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 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 °C ± 1°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 (15.2mm^2 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

279 units, fecundity was decomposed into the length of female gametophyte, the length of
280 oogonia and the number of oogonia per gametophyte on day 12. Fertility was estimated
281 as the number of embryos per female gametophyte on day 17 and the embryogenesis
282 rate calculated as the number of embryos at day 17 over fertile females at day 12 (i.e
283 females bearing oogonia). The length of the juvenile sporophyte, the number of blades,
284 and the holdfast diameter were recorded on day 30 as proxies of F1 juvenile
285 sporophytes fitness. Data was collected using an inverted microscope (PrimoVert, Carl
286 Zeiss) with a Canon EOS REBEL T3 camera mounted. Counts were taken in 10 optical
287 fields (an area of 8,13 mm^2 each) per well at 40X magnification and digital photos were
288 processed with ImageJ software. For morphological data of juvenile sporophytes, ten
289 individuals per replicate were photographed with a scale and processed with the same
290 image software.

291 *Statistical analyses.*

292 The effect of breeding type and region (in Experiment 1) or habitat (in Experiment 2) on
293 fecundity, fertility and sporophyte growth (only in Experiment 2) were assessed by
294 mixed linear models (Searle 1987, Pinheiro and Bates 2000) using *lme4* in R (Bates et
295 al. 2015). The breeding type, region (or habitat), and their interactions were defined as
296 fixed effects. A female's identifier was defined as a random intercept, that is the
297 identifier associated with the parental sporophyte of each clone or pool of
298 gametophytes, to integrate the lack of independence of the breeding types resulting from
299 the repeated use of each gametophyte or gametophyte pool. Mortality associated with
300 the heatwave experiment was analyzed following the same procedure. Sporophytes
301 from California-Mexico and Peru were excluded from the analysis because of a much
302 lower number of individuals compared to the other regions. The response variables of
303 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,

329 respectively (Fig. 4a). Fecundity, expressed as morphological characteristics of LL
330 gametophytes revealed that the size of the vegetative parts of the female (Fig. 4b) and
331 the size of the gametes (Fig. 4c) significantly differed when exposed to different types
332 of males (ANOVA: $F_{(5,124)} = 11.55$, $p < 0.0001$; $F_{(5,127)} = 57.55$, $p < 0.0001$;
333 respectively) and among habitats (Breeding Type x Habitat, ANOVA: $F_{(5,164)} = 2.34$, $p =$
334 0.04; $F_{(5,172)} = 5.12$, $p = 0.0002$, respectively). Oogonia were bigger when exposed to
335 sib males (i.e. reaching 0.036±0.014 mm in length) than to males from different
336 sporophytes or in solitary females, with differences ranging from 11.8% in
337 parthenogenesis and 36.6% in IntraHab crosses (Fig. 4c). Female size was the largest in
338 the absence of males or with highly related males (around 0.2 mm in length), and their
339 size progressively decreased with male-female kinship (Fig. 4b). Females in
340 parthenogenesis, selfing and IntraPop1 crosses were 5x larger than females in presence
341 of males from different habitats (InterHab) (Fig. 4b).

342 Female fertility, measured as the production of embryos per female gametophyte
343 (available in LL populations only), was significantly explained by the male-female
344 kinship (Fig. 4d, ANOVA: $F_{(5,126)} = 11.94$, $p < 0.0001$) with no effect of the factor
345 Habitat or the interaction Habitat x Breeding type. Again, the highest fertility was
346 observed in parthenogenetic females, while slightly reducing with sib-male kinship in
347 intrapopulation crosses. However, between population outcrosses (i.e. intra and inter
348 habitats), fertility did not differ significantly from any of the within population crosses
349 (Fig. 4d). This pattern was explained by a higher embryogenesis rate of all but intra-
350 habitat crosses (Fig. 4e, ANOVA: $F_{(5,117)} = 24.88$, $p < 0.0001$). Embryogenesis higher
351 than 100% is explained by the continuous production of oogonia when exposed to males
352 (except for IntraHab crosses), while parthenogenetic females stopped gametogenesis
353 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

476 2010). To our knowledge, this is the first report for algae characterised by a dioecious
477 haploid-diploid life cycle.

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

495 *Inbreeding depression in the F1 diploid progeny.*

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

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

520

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

523 Consistent with previous observations (Buschmann et al. 2004), low latitude
524 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.

550 The status of low-latitude populations may be defined by low nutrients associated with
551 warmer waters and competition with warm-tolerant species (Ladah et al. 1999, Steneck
552 et al. 2002, Graham et al. 2007). Moreover, these regions are subjected to recurrent El
553 Nino-Southern Oscillations and heat waves, which commonly result in widespread
554 mortality of the giant kelp, followed by recolonizations (Soto 1985, Tegner and Dayton
555 1987, Ladah et al. 1999, Edwards 2004, Vega et al. 2005, Cavanaugh et al. 2019).
556 Populations in such habitat at range margins tend to differ systematically from those in
557 central habitats in several demographic and reproductive characteristics (Kawecki
558 2008). Modifications of the mating system, such as increased asexual reproduction, has
559 been observed in terrestrial plants as a reproductive assurance strategy in marginal
560 populations (Eckert et al. 2006). Similar trends are observed in marginal kelp
561 populations (Oppliger et al. 2011, 2014). According to Baker's law, selfing should be
562 advantageous in populations characterized by recurrent extinction-recolonization
563 (Pannel and Barret 1998) as it provides reproductive assurance when few progenitors
564 are contributing to the recolonization. In this context, a stronger compensatory effect on
565 female fecundity would be expected to maximize its fitness under a mixed mating
566 system (Zhang 2000, Gillet and Gregorius 2020). However, considering the reduced
567 fecundity and fertility observed in the marginal populations of *M. pyrifera*, it is possible
568 that females evolved to mate with any male gamete, without requiring chemical
569 recognition of kin males and differential investment in gametes.

570 In conclusion, the giant kelp, *Macrocystis pyrifera*, has the ability to reproduce
571 sexually and asexually (i.e. parthenogenesis) and with males of different levels of
572 kinship without major consequences in the fitness of females and their offspring. The
573 identified fecundity cost of selfing and parthenogenesis seems to efficiently compensate
574 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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857 gametophytes benefits kelp farming by improving blade quality and productivity
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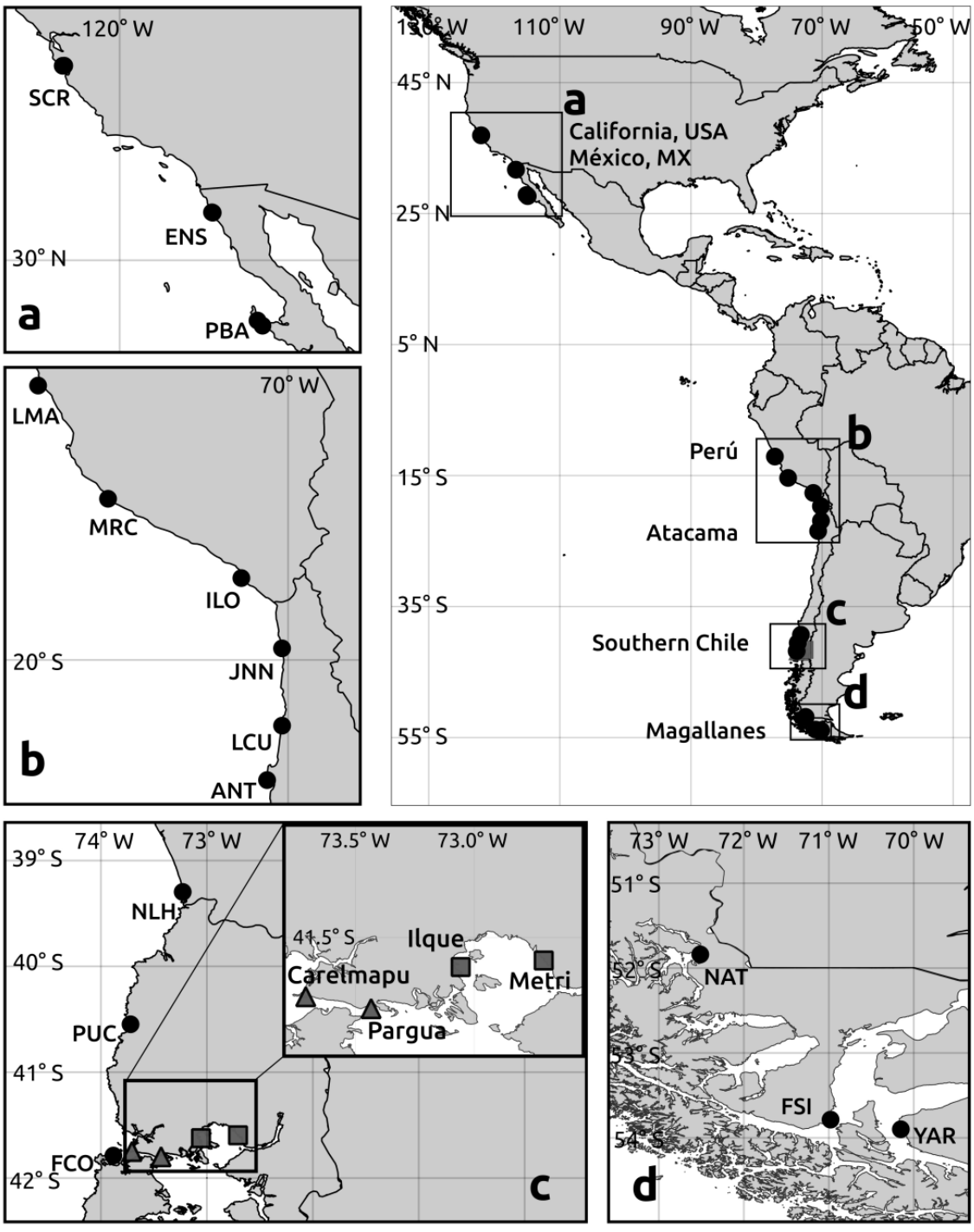
862

863 FIGURE LEGENDS

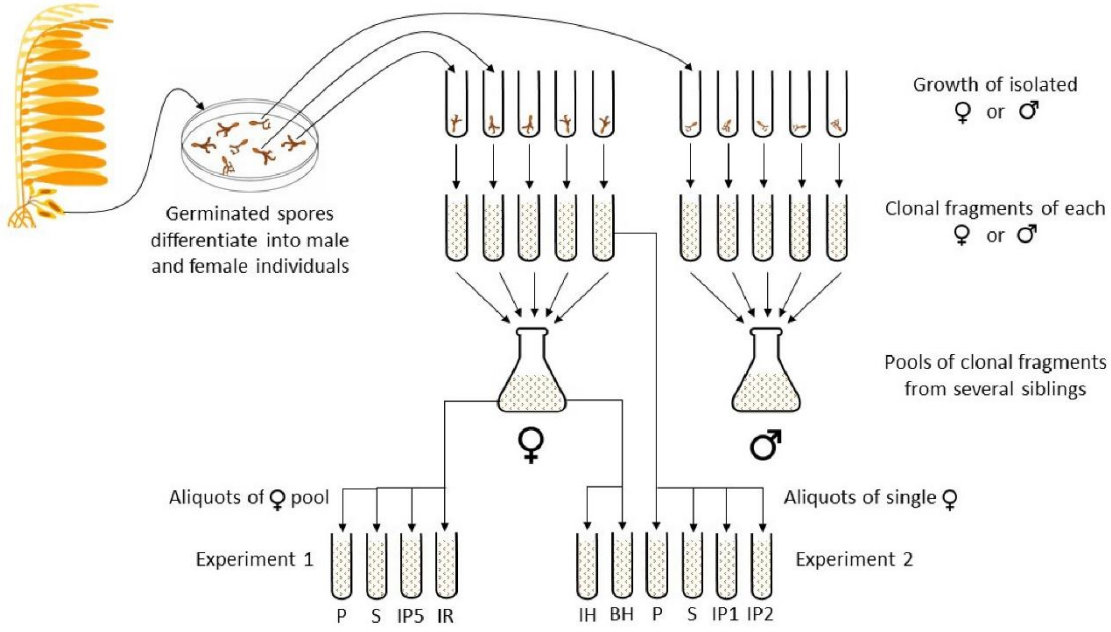
864 Fig. 1: Map of sampling sites of *Macrocystis pyrifera* (black dots), with detailed location in
865 each sampling region. (a) California: Santa Cruz (SCR); and Mexico: Ensenada (ENS) and
866 Punta Baja (PBA). (b) Perú: Lima (LMA), Marcona (MRC), Ilo (ILO); and Atacama: Junin
867 (JNN), Caleta La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

872


120° W
SCR
ENS
30° N
a
PBA
70° W
LMA
MRC
ILO
20° S
JNN
LCU
b
ANT
130° W
110° W
90° W
70° W
50° W
45° N
a
California, USA
México, MX
25° N
5° N
Perú
b
15° S
Atacama
35° S
c
Southern Chile
d
55° S
Magallanes
74° W
73° W
73.5° W
73.0° W
39° S
NLH
41.5° S
Ilque
Carelmapu
Metri
40° S
Pargua
PUC
41° S
FCO
42° S
c
73° W
72° W
71° W
70° W
51° S
52° S
NAT
53° S
FSI
YAR
54° S
d

873

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).


Growth of isolated
♀ or ♂
Clonal fragments of each
♀ or ♂
Germinated spores
differentiate into male
and female individuals
Pools of clonal fragments
from several siblings
♀
♂
Aliquots of ♀ pool
Aliquots of single ♀
Experiment 1
Experiment 2
P S IP5 IR
IH BH P S IP1 IP2

891

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

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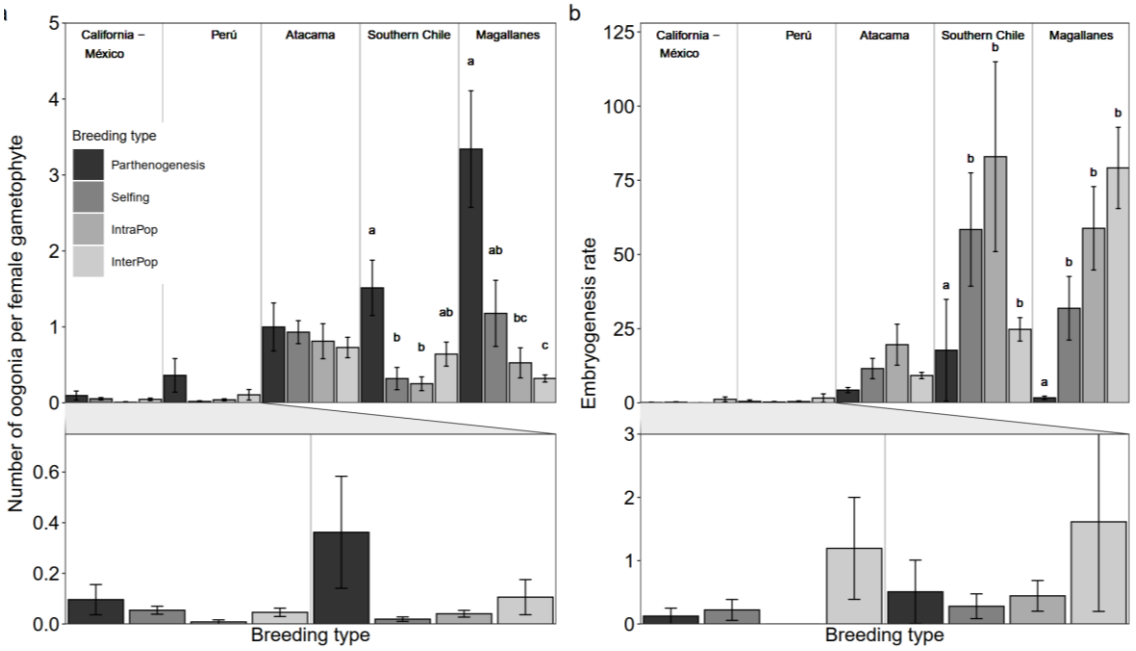

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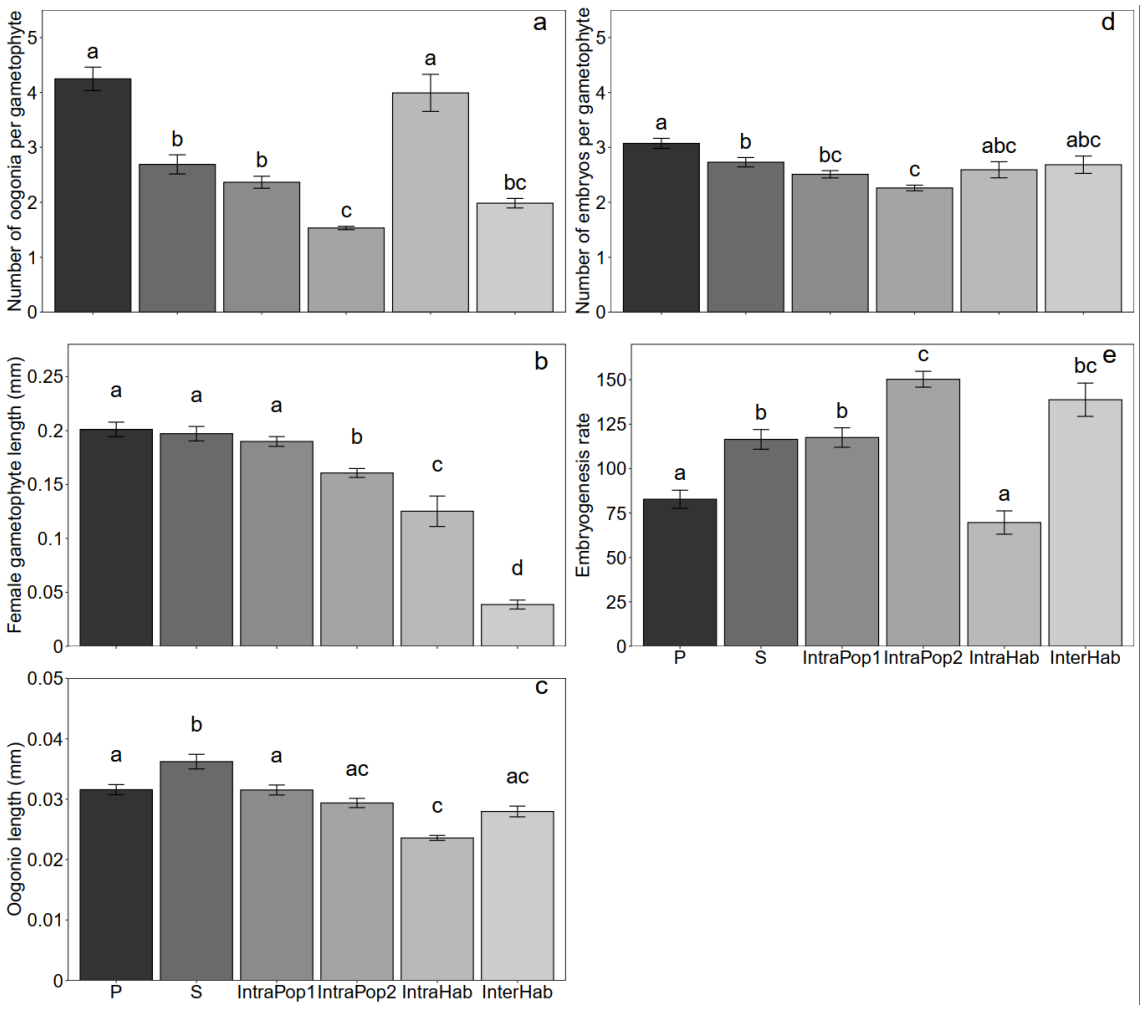
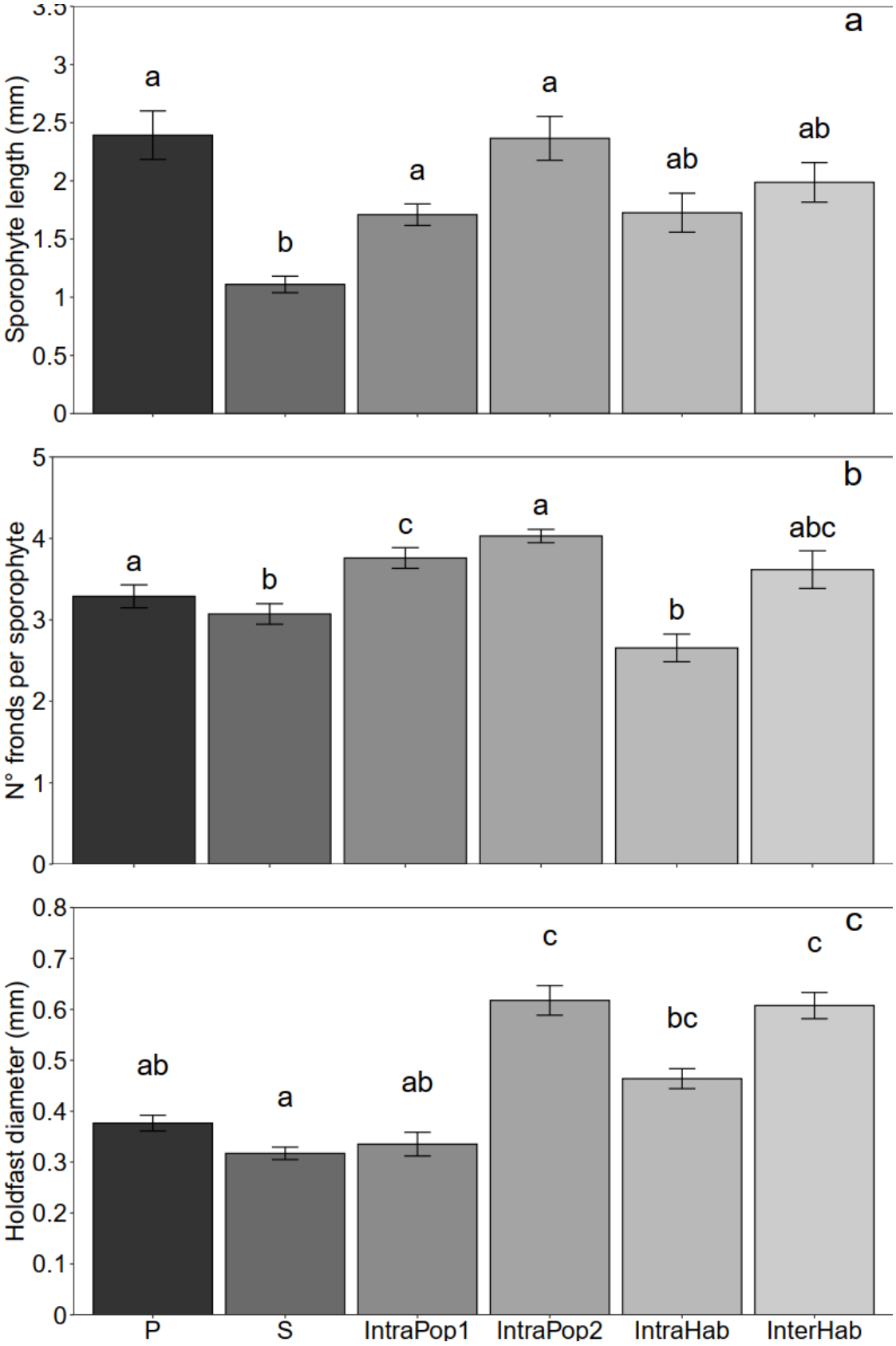
Fig. 4: Fecundity of female gametophytes from Southern Chile-Los Lagos (LL) populations, expressed as the (a) number of oogonia per female gametophyte, (b) female gametophyte length (mm) and (c) oogonia length; and fertility expressed as the (d) number of embryos per female gametophyte and (e) embryogenesis 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.



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

912


a
Sporophyte length (mm)
3.5
3
2.5
2
1.5
1
0.5
0
a
b
a
a
ab
ab
b
N° fronds per sporophyte
5
4
3
2
1
0
a
b
c
a
b
abc
c
Holdfast diameter (mm)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
ab
a
ab
c
bc
c
P
S
IntraPop1
IntraPop2
IntraHab
InterHab

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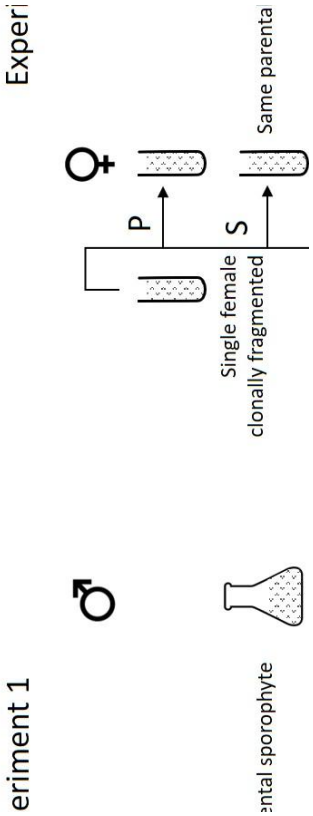

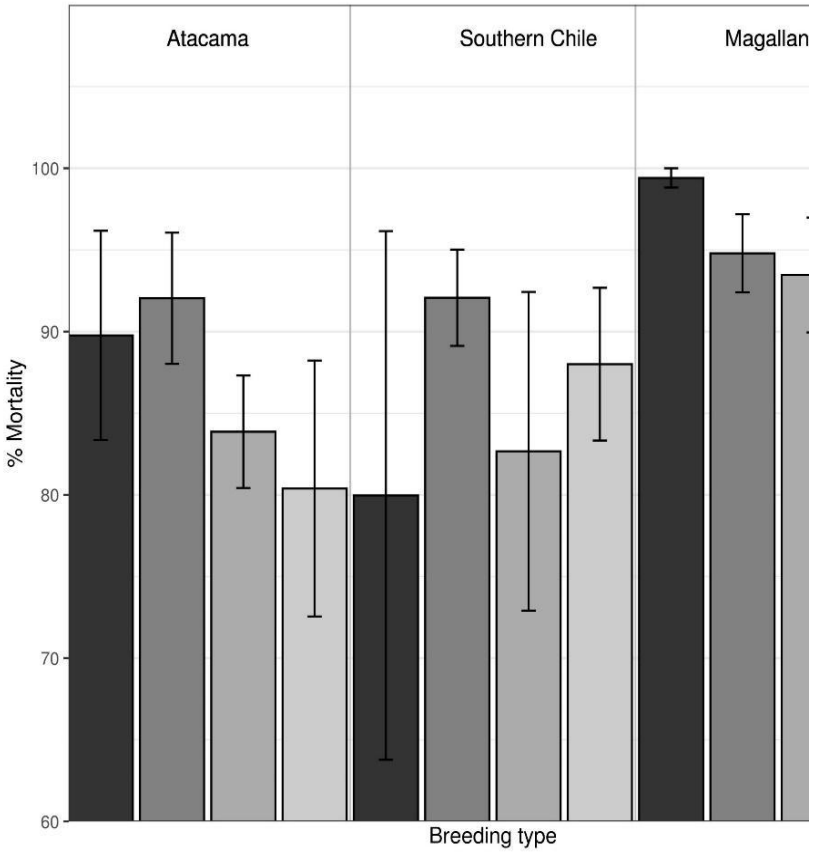

Figure S1. Diagram of the breeding types for experiment 1 and 2. P: parthenogenesis using single female pool (Experiment 1) or clone (Experiment 2), S: selfing using same parental sporophyte, IP5: intra-population cross using different parental sporophytes from the same population, IR: intra-region outcross using different parental sporophytes from different populations, IP1 and IP2: intra-pop 1 cross using different parental sporophyte from the same population, IH: outcross using different parental sporophyte from different population from same habitat and IB: outcross using different parental sporophyte from different population from different habitat.

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.

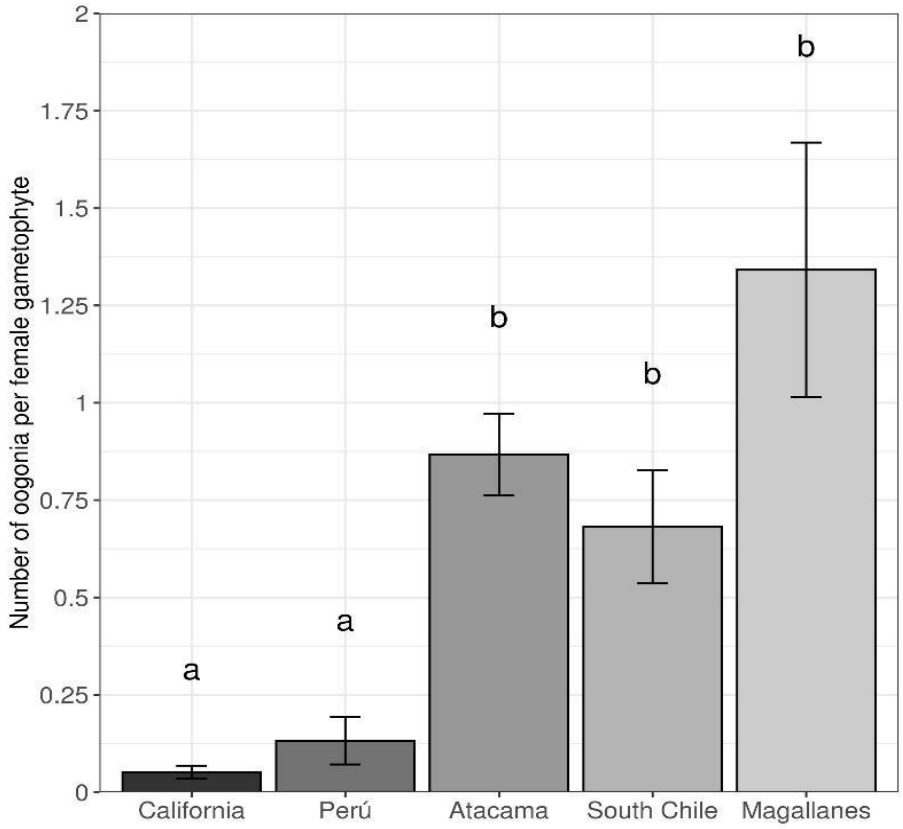

Figure S3. Fecundity expressed as the number of oogonia per female gametophyte from California, Perú, Atacama, Southern Chile and Magallanes regions. Letters represent statistical differences.

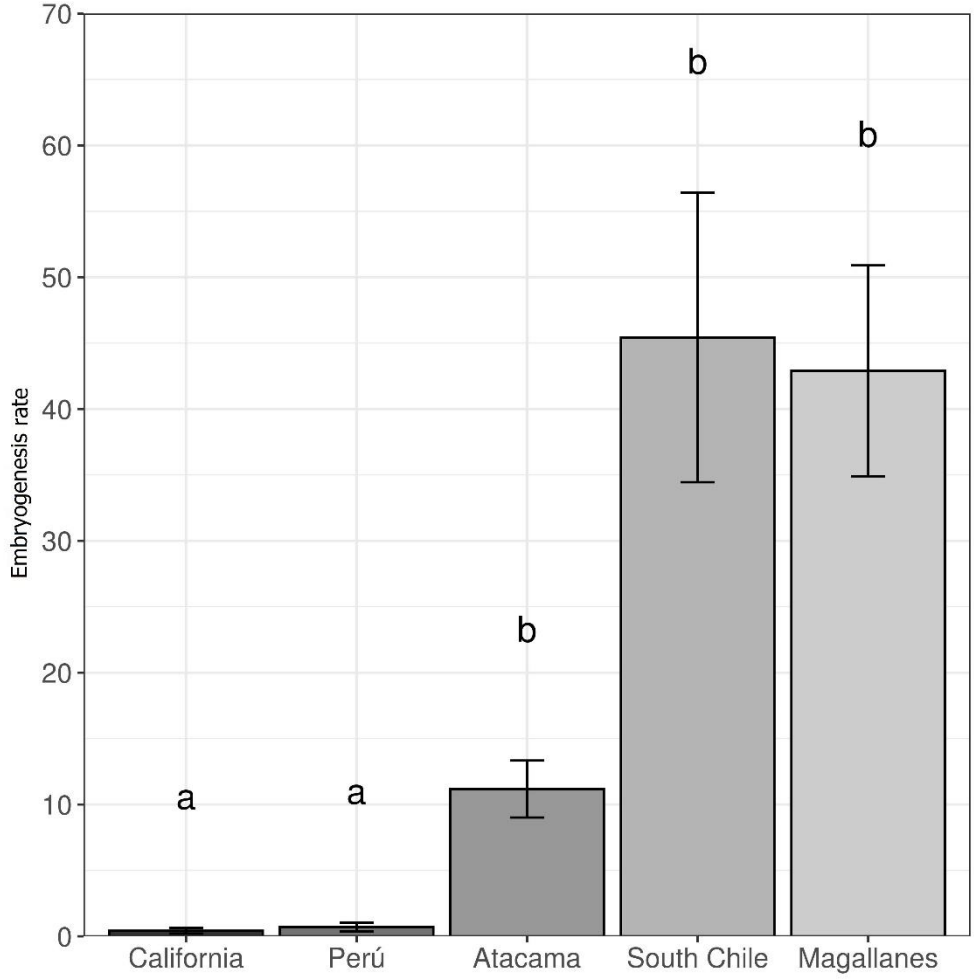

Figure S4. Embryogenesis rate of female gametophytes from California, Perú, Atacama, Southern Chile and Magallanes regions. Letters represent statistical differences.

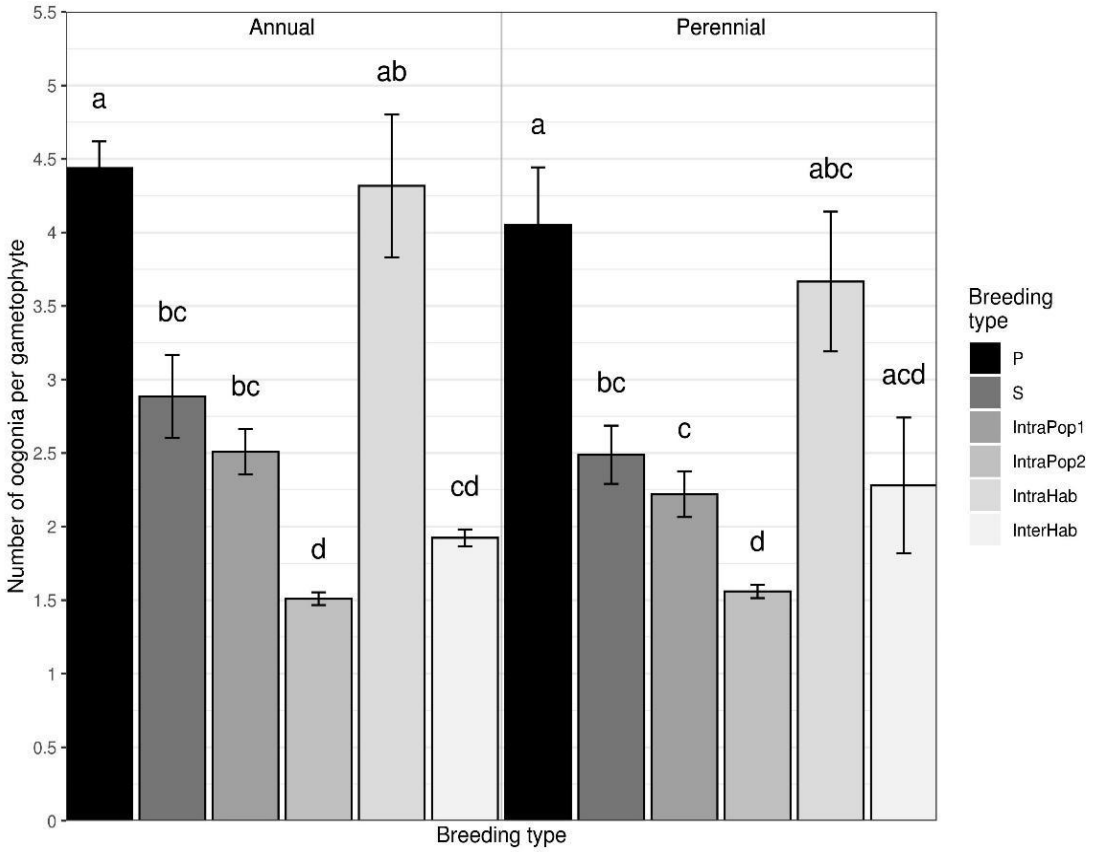

Figure S5. Number of oogonia per female gametophyte from annual and perennial Southern Chile-Los Lagos populations obtained in different breeding types: parthenogenesis (P), selfing (S), IntraPop1, IntraPop2, IntraHab and InterHab. Letters represent statistical differences.

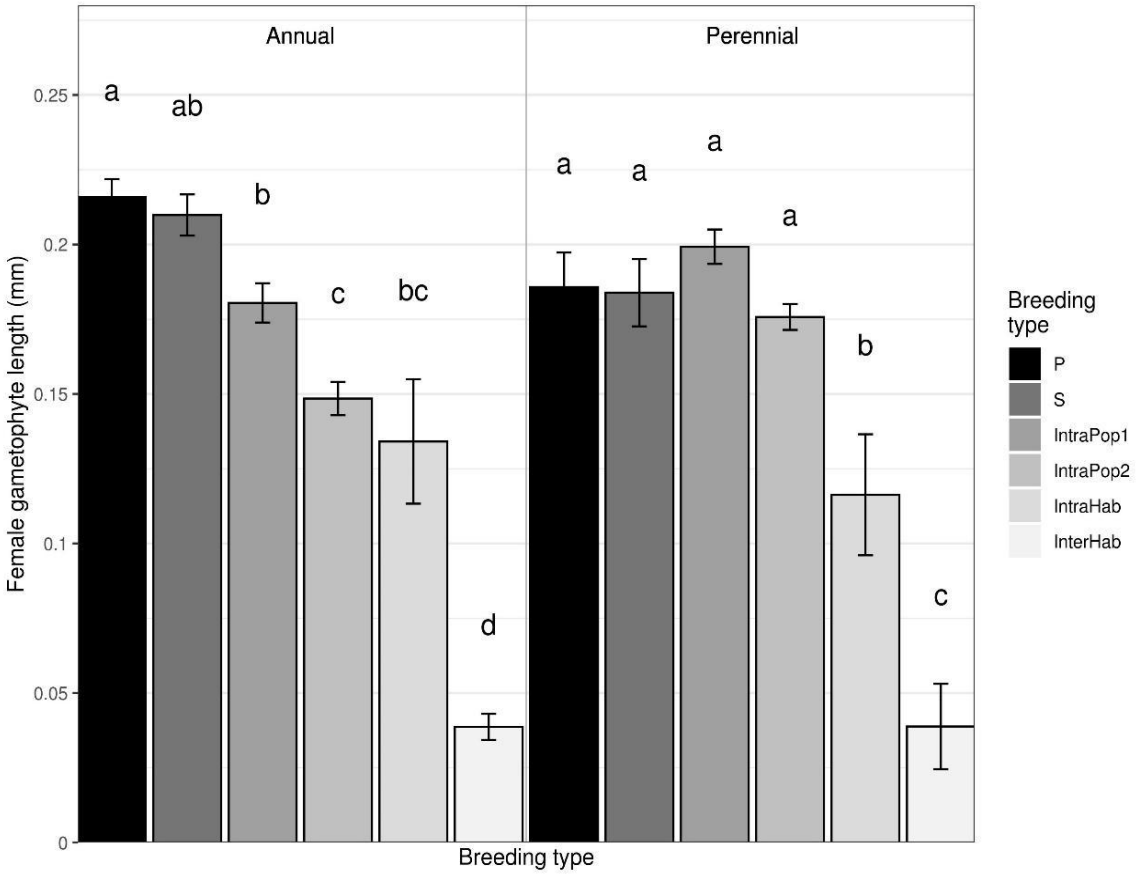

Figure S6. Female gametophyte length (mm) from annual and perennial Southern Chile-Los Lagos populations obtained in different breeding types: parthenogenesis (P), selfing (S), IntraPop1, IntraPop2, IntraHab and InterHab. Letters represent statistical differences.

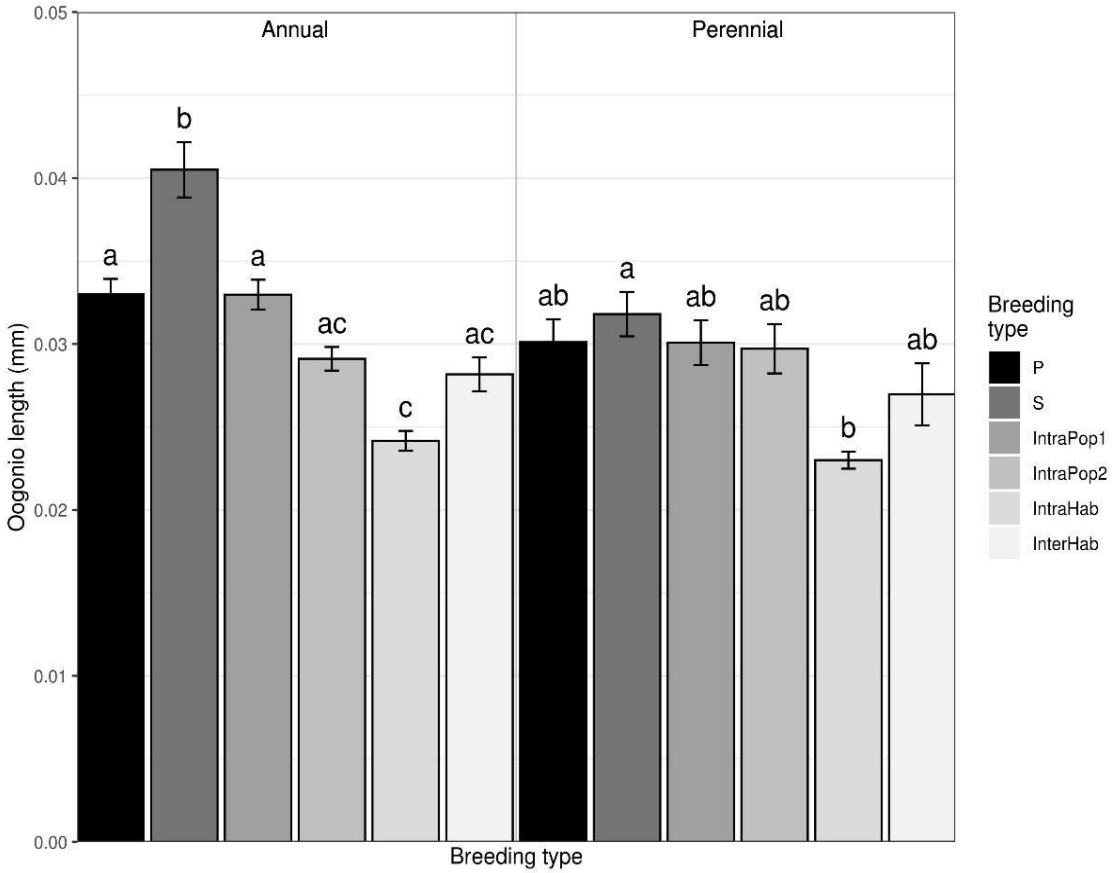

Figure S7. Oogonia length from annual and perennial Southern Chile-Los Lagos populations obtained in different breeding types: parthenogenesis (P), selfing (S), IntraPop1, IntraPop2, IntraHab and InterHab. Letters represent statistical differences.

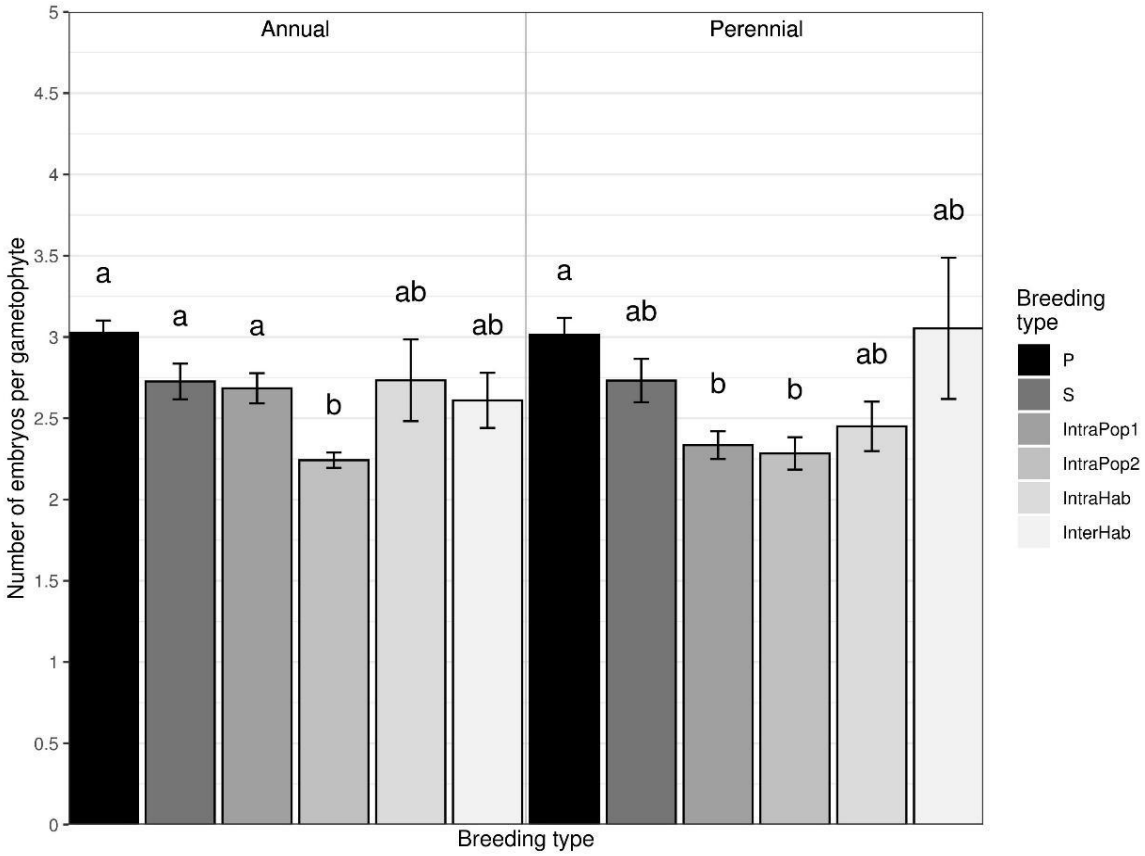

Figure S8. Number of embryos per female gametophyte from annual and perennial Southern Chile-Los Lagos populations obtained in different breeding types: parthenogenesis (P), selfing (S), IntraPop1, IntraPop2, IntraHab and InterHab. Letters represent statistical differences.

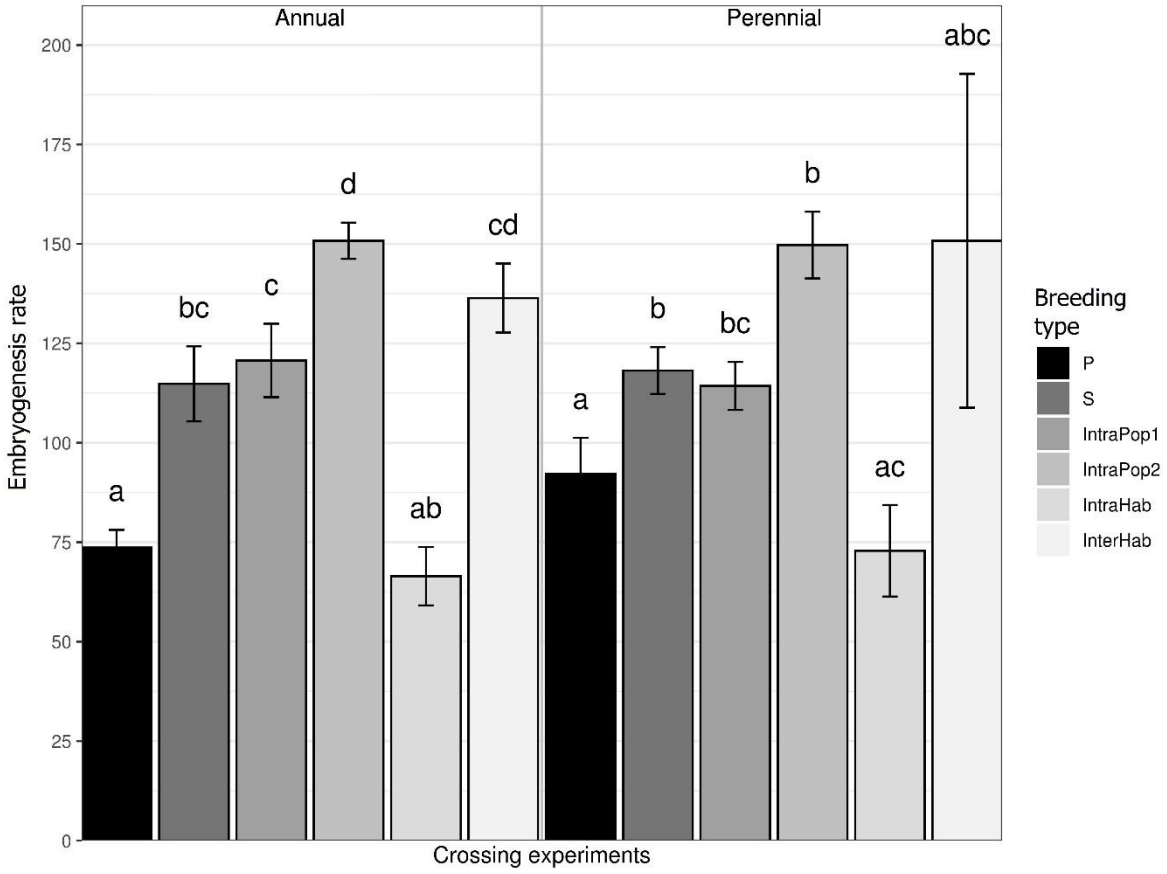

Figure S9. Embryogenesis rate from annual and perennial Southern Chile-Los Lagos populations obtained in different breeding types: parthenogenesis (P), selfing (S), IntraPop1, IntraPop2, IntraHab and InterHab. Letters represent statistical differences.

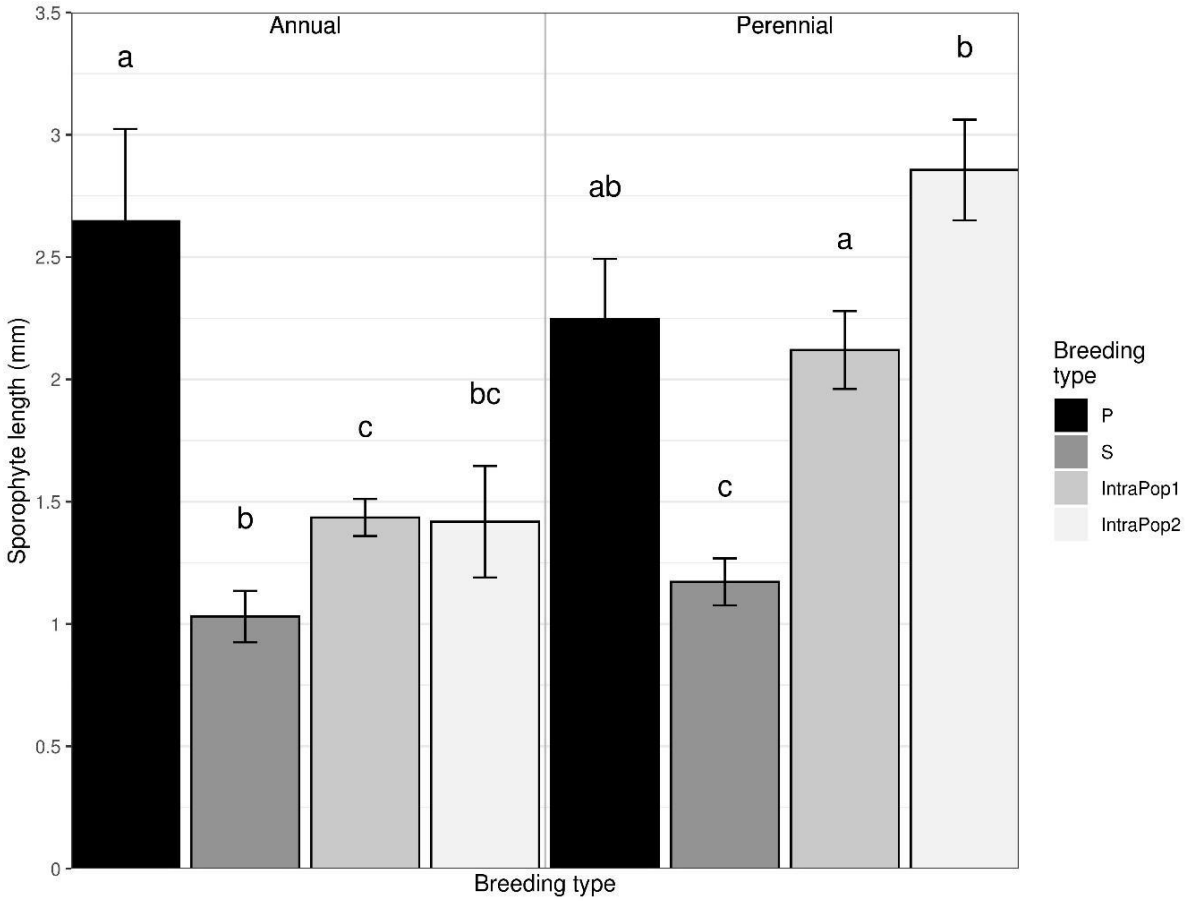

Figure S10. Sporophyte length (mm) from annual and perennial Southern Chile-Los Lagos populations obtained in different breeding types: parthenogenesis (P), selfing (S), IntraPop1 and IntraPop2. Letters represent statistical differences.

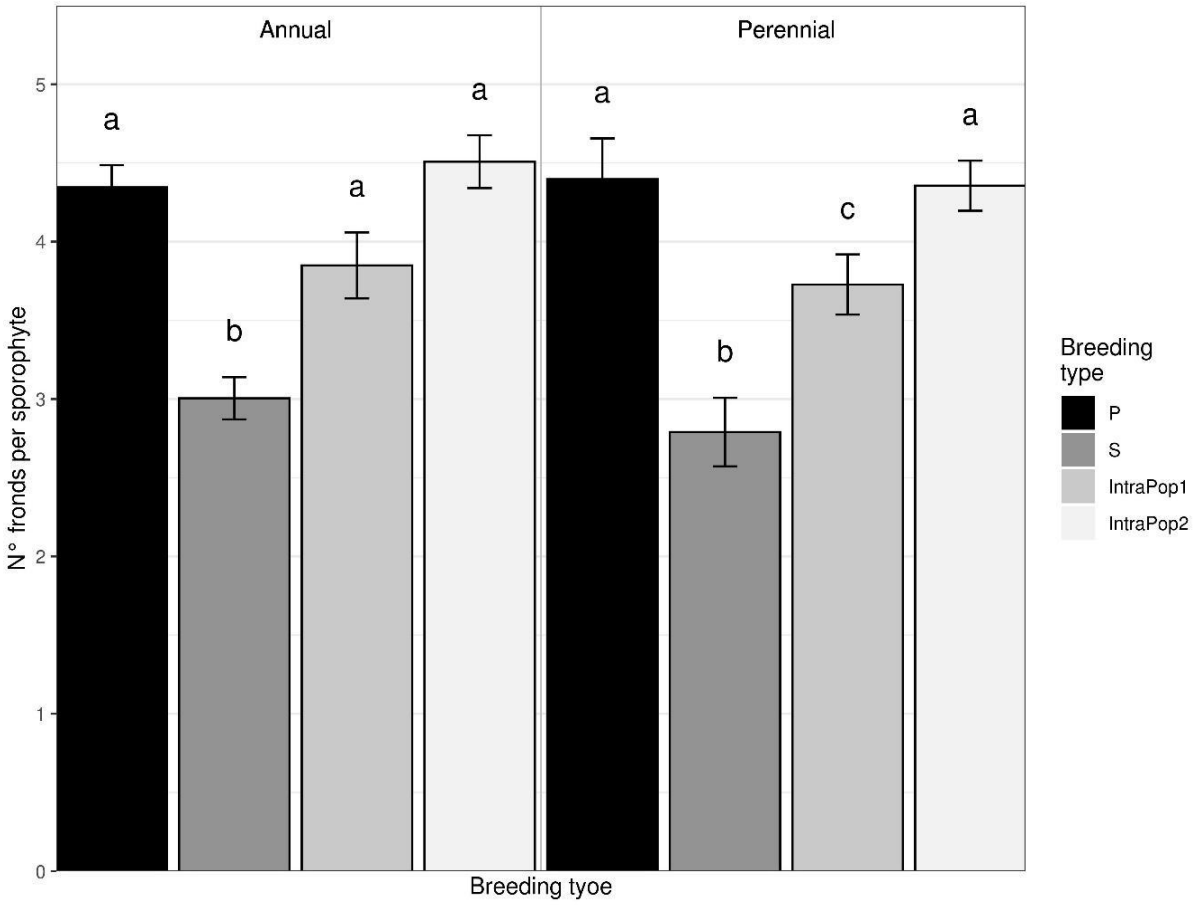

Figure S11. Number of fronds per sporophyte from annual and perennial Southern Chile-Los Lagos populations obtained in different breeding types: parthenogenesis (P), selfing (S), IntraPop1, IntraPop2, IntraHab and InterHab. Letters represent statistical differences.

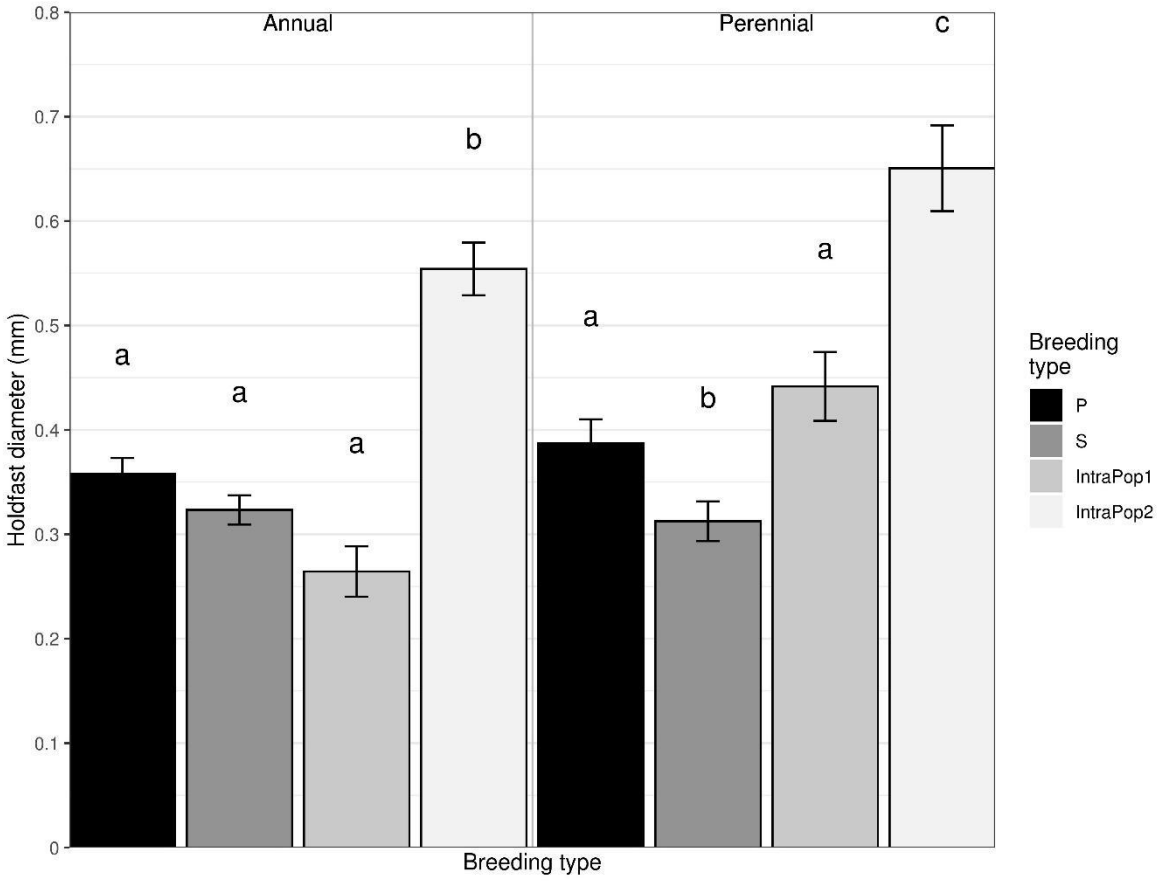

Figure S12. Holdfast diameter of sporophytes from annual and perennial Southern Chile-Los Lagos populations obtained in different breeding types: parthenogenesis (P), selfing (S), IntraPop1, IntraPop2, IntraHab and InterHab. Letters represent statistical differences.

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 sporphyte.

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