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## RESEARCH ARTICLE

# Tetrasporophytic bias coupled with heterozygote deficiency in Antarctic *Plocamium* sp. (Florideophyceae, Rhodophyta)

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## Abstract

Meiosis and syngamy generate an alternation between two ploidy stages, but the timing of these two processes varies widely across taxa, thereby generating life cycle diversity. One hypothesis suggests that life cycles with long-lived haploid stages are correlated with selfing, asexual reproduction, or both. Though mostly studied in angiosperms, selfing and asexual reproduction are often associated with marginal habitats. Yet, in haploid-diploid macroalgae, these two reproductive modes have subtle but unique consequences whereby predictions from angiosperms may not apply. Along the western Antarctic Peninsula, there is a thriving macroalgal community, providing an opportunity to explore reproductive system variation in haploid-diploid macroalgae at high latitudes where endemism is common. *Plocamium* sp. is a widespread and abundant red macroalga observed within this ecosystem. We sampled 12 sites during the 2017 and 2018 field seasons and used 10 microsatellite loci to describe the reproductive system. Overall genotypic richness and evenness were high, suggesting sexual reproduction. Eight sites were dominated by tetrasporophytes, but there was strong heterozygote deficiency, suggesting intergametophytic selfing. We observed slight differences in the prevailing reproductive mode among sites, possibly due to local conditions (e.g., disturbance) that may contribute to site-specific variation. It remains to be determined whether high levels of selfing are characteristic of macroalgae more generally at high latitudes, due to the haploid-diploid life cycle, or both. Further investigations of algal life cycles will likely reveal the processes underlying the maintenance of sexual reproduction more broadly across eukaryotes, but more studies of natural populations are required.

## KEYWORDS

clonal rates, gametophytes, homozygote excess, mating system, seaweed

**Abbreviations:** BSA, bovine serum albumin; dNTP, deoxynucleoside triphosphate; MLG, multilocus genotype.

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## INTRODUCTION

Sex is one of the most ubiquitous processes across eukaryotes. Meiosis (with recombination) and syngamy (or fertilization) generate an alternation between a larger and a smaller ploidy level. The timing of meiosis and syngamy and duration of each ploidy stage varies widely across eukaryotes (Mable & Otto, 1998). If outcrossing predominates, then somatic development only in the diploid stage may be favored because deleterious mutations will be masked (Otto & Marks, 1996). Somatic development in the haploid life cycle stage, on the other hand, may aid the purging of deleterious mutations if populations are mainly undergoing selfing (i.e., self-fertilization) or asexual reproduction (Otto & Marks, 1996). Such genetic hypotheses do not preclude development in both the haploid and diploid stages, such as the life cycles of many macroalgae, as the long-lived haploid stage may facilitate purging (Mable & Otto, 1998; Valero et al., 1992). Moreover, these life cycles can also be maintained by ecological differentiation, even if stages are seemingly isomorphic (Hughes & Otto, 1999). Macroalgae exhibit life cycle variation representative of eukaryotes more broadly and can therefore serve as ideal study organisms to gain insight into the ecological and genetic mechanisms underlying the alternation of haploid and diploid stages (Bell, 1994).

Macroalgae are also characterized by reproductive system variation (see Krueger-Hadfield, Guillemin, et al., 2021; Olsen et al., 2020) that is comparable to that observed in angiosperms (see Whitehead et al., 2018). The reproductive system describes the relative rates of asexual versus sexual reproduction and, in the case of sexual reproduction, the importance of selfing versus outcrossing (i.e., fertilization between two unrelated individuals; Barrett, 2011). Selfing can occur in one of two ways in species with haploid-diploid life cycles depending on whether gametophytes are monoicous (i.e., hermaphroditic; sex is determined in the haploid stage) or dioicous (separate sexes; see Beukeboom & Perrin, 2014). In monoicous taxa, intragametophytic selfing occurs when gametes are produced by the same gametophyte and results in instantaneous, genome-wide homozygosity (Klekowski, 1969). In hermaphroditic animals and monoecious plants (for which sex is determined in the diploid stage), the loss of heterozygosity occurs over successive generations during which it is slowly eroded rather than lost immediately upon fertilization as in monoicous taxa. Unlike gonochoristic animals (separate sexes) and dioecious angiosperms (separate sexes), male and female gametophytes in dioicous haploid-diploid species can also undergo selfing (i.e., intergametophytic selfing) when they share the same sporophytic parent (Klekowski, 1969). Yet, intergametophytic selfing does not provide the same reproductive assurance

as is the case with individuals that produce both egg and sperm since male and female gametophytes are separate individuals. Moreover, separate sexes also cannot be used as a proxy for outcrossing, as often done in angiosperms, and thus, the characterization of the reproductive system requires population genetic analyses. For red macroalgae, most taxa are dioicous (Hawkes, 1990), but we still lack data broadly across taxa with which to determine selfing rates in nature (see Krueger-Hadfield, Guillemin, et al., 2021). Evidence of regular sexual reproduction was seen in two species of red macroalgae; though outcrossing prevailed in the red macroalga *Gracilaria gracilis* (Engel et al., 2004), high levels of intergametophytic selfing were observed in *Chondrus crispus* (Krueger-Hadfield et al., 2015; Krueger-Hadfield, Roze, et al., 2013). Asexual reproduction, in the form of thallus fragmentation, on the other hand, was observed in soft sediment sites where tetrasporophytes of the non-native *Gracilaria vermiculophylla* dominate and their spores are unlikely to recruit, leading to gametophytes being unable to establish (Krueger-Hadfield et al., 2016). Thus, the amount of variation among species and populations is likely as common in macroalgae and warrants further investigation (Krueger-Hadfield, Guillemin, et al., 2021).

Uniparental reproduction (i.e., selfing, asexual reproduction, or both) has typically been associated with more marginal habitats (Pannell, 2015; Pannell et al., 2015; Peck et al., 1998). Asexual reproduction covers a variety of propagation of genetically identical progeny, including fragmentation and spore production (de Meeûs et al., 2007; Orive & Krueger-Hadfield, 2021). In the context of the association with marginality, we define asexual reproduction as the production of genetically identical propagules rather than clonal processes as a mode of ramification as seen in modular taxa, such as seagrasses (see discussion in Krueger-Hadfield et al., 2016). Higher latitudes are often used as proxies for “marginality,” whereby population densities are lower, growing seasons are shorter, and mates may be more difficult to find (Bierzychudek, 1985; Peck et al., 1998). Intra- and interspecific variation in macroalgal reproductive systems is likely common across latitudes and tidal gradients, hinting at similar mechanisms driving reproductive patterns across eukaryotes. For example, in the red macroalga *Chondrus crispus*, a high shore population was characterized by greater rates of intergametophytic selfing, likely due to environmental conditions generated by cyclic patterns of emergence at low tide (Krueger-Hadfield et al., 2015; Krueger-Hadfield, Roze, et al., 2013). Similarly, the frequency of asexual reproduction has been noted to increase at higher latitudes and tidal heights in the genus *Mastocarpus*, which may, in part, be driven by both abiotic factors and biotic interactions in the intertidal (Dudgeon et al., 2017; Fierst et al., 2010; Guiry & West, 1983; Krueger-Hadfield, Kübler, &

Dudgeon, 2013; Zupan & West, 1988). Understanding the proximate mechanisms that drive patterns of uniparental reproduction in taxa with haploid-diploid life cycles as compared to diploid-dominant taxa requires further investigation (Krueger-Hadfield, 2020).

Studies of macroalgal reproductive system variation have predominantly focused on taxa in the Northern Hemisphere (e.g., Couceiro et al., 2015; Engel et al., 2004; Krueger-Hadfield et al., 2016; Krueger-Hadfield, Roze, et al., 2013; Pardo et al., 2019; Sosa et al., 1998; Steen et al., 2019; van der Strate et al., 2002), with a few exceptions (e.g., Faugeron et al., 2001, 2004). In contrast to populations in the Northern Hemisphere, Antarctic macroalgae lack continuous connection to lower latitudes (e.g., South America, reviewed in Wulff et al., 2009). Questions persist about recolonization by macroalgae in Antarctica after the Last Glacial Maximum and more contemporary patterns of gene flow within the Southern Ocean and along Antarctic coastlines (reviewed in Guillemain et al., 2018; see also Parvizi et al., 2022). These questions and their answers have direct implications for understanding spatial patterns of reproductive system variation in Antarctic taxa.

The macroalgal populations that occur in subtidal habitats along the western Antarctic Peninsula have a biomass comparable to that of temperate kelp forests, in some instances covering 80% or more of the hard substratum (Amsler et al., 1995; Brouwer et al., 1995). These macroalgal forests provide living spaces for a high number and density of mesograzers and are, as such, important members of the ecosystem (Amsler et al., 1995, 2015, 2022; Huang et al., 2007). With increasing latitude along the Peninsula, there is a continuous decline in macroalgal abundance and diversity (Moe & DeLaca, 1976; Wiencke & Amsler, 2012). The haplotype diversity of six red macroalgal species examined was generally low, which was hypothesized to be the result of recolonization from small founder populations after the Last Glacial Maximum (Guillemain et al., 2018). The red macroalga *Plocamium* sp. is an abundant and widespread species which has been extensively studied near Anvers Island (western Antarctic Peninsula) for its secondary metabolite diversity (Heiser, Shilling, et al., 2023; Shilling et al., 2019, 2021; Young et al., 2013). Shilling et al. (2021) described 15 chemogroups—which are signature mixtures of secondary metabolites that differ in relative abundance among chemogroups—in *Plocamium* sp. from around the study area. The presence of cystocarpic female gametophytes (evidence of syngamy) and reproductive tetrasporophytes (evidence of meiosis) at sites near Anvers Island suggests that sexual reproduction occurs, though many thalli at the time of collection in our previous studies from there were vegetative, and we do not know the life cycle stage (Heiser, Amsler, & Krueger-Hadfield, 2023; Heiser, Shilling, et al., 2023).

Recently, we developed microsatellite loci (Heiser, Amsler, & Krueger-Hadfield, 2023), and we used the loci here to describe the prevailing reproductive system in *Plocamium* sp. at 12 sites near Anvers Island. At two sites, for which we had two consecutive years of sampling, we also used population genetic measures, such as heterozygosity and genotypic richness and evenness, and the way those measures change over two generations to quantify clonal rates and selfing across sites, expanding our understanding of the reproductive mode.

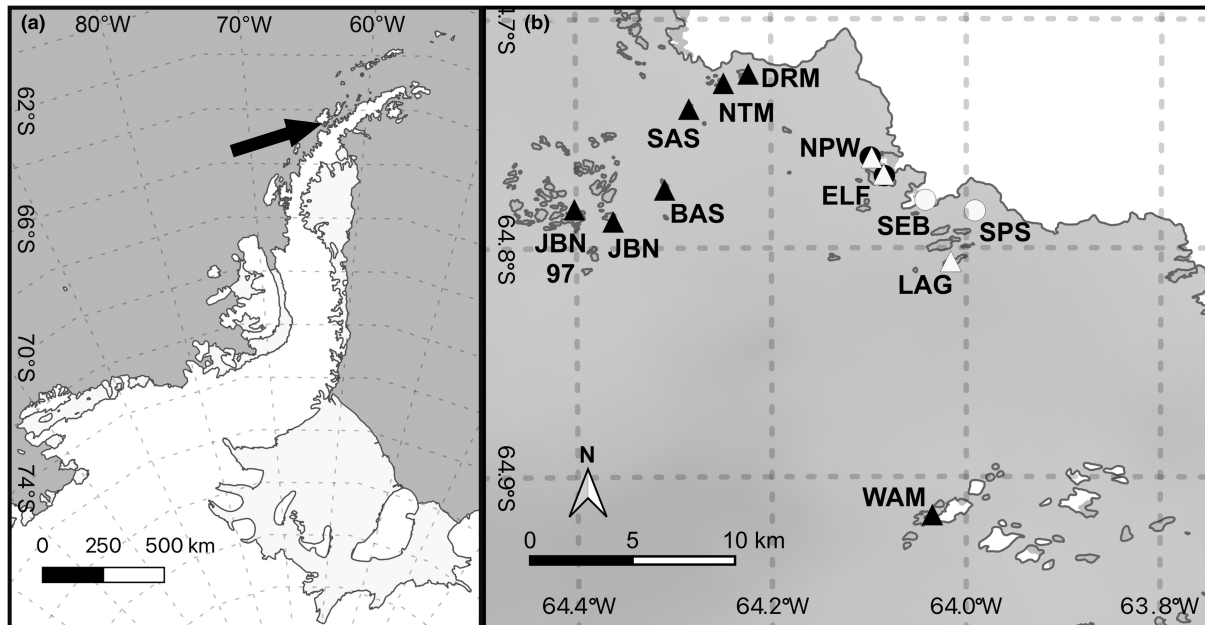
## MATERIALS AND METHODS

### Sample collection

Thalli of *Plocamium* sp. were collected from April to May 2017 and from April to June 2018 using SCUBA near the U.S. Antarctic Program's Palmer Station located on Anvers Island, Antarctica (64°46.5' S, 64°03.3' W). Thalli were sampled along transects as described in Heiser, Shilling, et al. (2023). Briefly, five thalli were collected at 3-m depth intervals between 5 and 29 m depths. At five sites, three full transects were sampled whereas at nine other sites, due to logistical constraints, only one transect was sampled. Four of the aforementioned partial transects were in addition to the sites described in Heiser, Shilling, et al. (2023); (Figure 1, Table S1 in the Supporting Information). East Litchfield and Norsel Point were sampled partially in 2017 and at all three transects in 2018 (Table S1). The partial transects at East Litchfield included three transects sampled each at three depths (17, 20, and 23 m). At Norsel Point in 2017, one full transect was sampled instead of three. All collected thalli were kept in individual mesh bags, placed in recently filled buckets of seawater, and upon return to the laboratory, transferred to flow-through tanks (1.8 m diameter, 0.9 m height) at ambient conditions (between 0.5°C and 2°C). Within 36 h, thalli were processed during which time they were inspected for reproductive structures. After photographing the thallus, a small piece was preserved in silica. If present, cystocarps were removed from female gametophytes using a razor blade prior to preservation of the gametophytic thallus for subsequent DNA extraction. Any remaining thallus (if available) was pressed and stored dry as vouchers in C.D. Amsler's herbarium collection at the University of Alabama at Birmingham.

### DNA extractions and PCR conditions

We extracted total genomic DNA using the Machery-Nagel Nucleospin® Plant II kit (Machery-Nagel) following the manufacturer's protocol except for the lysis step, which was performed at room temperature for 1 h.



**FIGURE 1** (a) Black arrow indicates the location of Palmer Station on the western Antarctic Peninsula. (b) Sites sampled for *Plocamium* sp. around Palmer Station (Anvers Island). Circles show sites sampled in 2017; triangles show sites sampled in 2018 (ELF and NPW were sampled in both years). Light colored symbols show the sites sampled with three full transects; black shows sites sampled with partial transects. BAS, Beaumont Skerries; DRM, Dream Island; ELF, East Litchfield (unofficial name for small rock east from Litchfield Island); JBN, Howard Island, part of Joubin Islands (United States Antarctic Program (USAP) Island # 1); JBN97, Joubin Islands (USAP Island #97); LAG, Laggard Island; NTM, Casey Islands; NPW, Norsel Point; SAS, Stayaway Skerries; SEB, Southeast Bonaparte (unofficial name of site near the glacier southeast of Bonaparte Point); SPS, Stepping Stones; WAM, Wauwermans Islands (USAP Island # 564). We used QGIS version 3.22 and the Quantarctica package to prepare the maps (Matsuoka et al., 2018).

We eluted DNA in 100  $\mu$ L of autoclaved Milli-Q water (see also Krueger-Hadfield, Roze, et al., 2013).

We used the 10 microsatellite loci described in Heiser, Amsler, and Krueger-Hadfield, (2023). We amplified loci for some thalli in simplex PCRs with a final volume of 10  $\mu$ L: 2  $\mu$ L of DNA, forward and reverse primers (see Table S2 in the Supporting Information), 1X Promega GoTaq® Flexi Buffer (Promega), 2 mM of  $MgCl_2$ , 250  $\mu$ M of each dNTP (Promega), and 0.5 U of Promega GoTaq® Flexi DNA Polymerase. For some thalli, loci were amplified in duplex or multiplex PCR using the same concentrations but with adjusted forward and reverse primer concentrations (Table S2). To improve amplification for some thalli, loci, or both, we added 0.02  $\mu$ g  $\cdot$   $\mu$ L<sup>-1</sup> of bovine serum albumin (BSA) and primer concentrations as described under final simplex in Table S2.

The following PCR program was used: 95°C for 2 min, followed by 40 cycles of 95°C for 30 s,  $T_m$  for 30 s (Pc\_05 at 53°C, Pc\_09 at 59°C, all others at 56°C), and 72°C for 30 s, with a final extension at 72°C for 5 min. Fragment analysis was performed on an Applied Biosystems 3730xl at the Heflin Center for Genomic Sciences at the University of Alabama at Birmingham. One microliter of PCR product was diluted in HiDi formamide (Applied Biosystems) with one of two ladders: (1) 9.7  $\mu$ L of HiDi formamide with 0.35  $\mu$ L of GS 500 LIZ (Applied Biosystems) or (2) 9.5  $\mu$ L of HiDi

formamide with 0.5  $\mu$ L of SM594 (Mauger et al., 2012). Information on poolplexes for fragment analysis is provided in Table S2. Alleles were scored following Heiser, Amsler, and Krueger-Hadfield, (2023) using Geneious Prime (Biomatters, Ltd.). We followed Heiser, Amsler, and Krueger-Hadfield, (2023) to account for any base pair shifts between different dyes and size ladders. We used TANDEM to ensure that the rounding error for each locus was below the threshold outlined in Matschiner and Salzburger (2009); see also Krueger-Hadfield, Roze, et al. (2013).

## Data analyses

Thalli that had more than two loci with no amplification after several PCRs were excluded from subsequent analyses. We followed the protocol used in Krueger-Hadfield et al. (2016) to assign life cycle stage based on multilocus genotypes in which any thallus with at least one heterozygous locus was considered a diploid tetrasporophyte. All gametophytes identified through reproductive structures produced only one allele at the 10 microsatellite loci. Most of the reproductive tetrasporophytes had at least one heterozygous locus except for 13 reproductive tetrasporophytes (16% of all reproductive tetrasporophytes) that were fixed homozygotes. There were 146 thalli that were vegetative at the

time of collection but were homozygous at all 10 loci. For many species, including *Plocamium* spp., we lack chemical tests (e.g., resorcinol, Lazo et al., 1989) or molecular markers (e.g., sex-linked markers; Krueger-Hadfield, Flanagan, et al., 2021) with which to assign life cycle stage to vegetative thalli and to distinguish between sexes. We followed previous studies (e.g., Krueger-Hadfield et al., 2016; Krueger-Hadfield, Roze, et al., 2013) and considered all vegetative, fixed homozygotes as haploid gametophytes. Although our assignment of 146 vegetative fixed homozygotes as gametophytes may have included some tetrasporophytes, this would be at low frequency within each site, and the main pattern would not change (see Section 3). For the tetrasporophytes, we determined the number of homozygous loci for each thallus and calculated the mean ( $\pm$ SE) of homozygous loci for each site.

Using the binomial distribution, we tested whether the gametophyte to tetrasporophyte ratio differed from the predicted ratio of  $\sqrt{2}:1$ , which has been hypothesized if there were no fitness differences between life cycle stages (Destombe et al., 1989; Thornber & Gaines, 2004).

A mutation in the primer sequence may lead to non-amplification and could consequently inflate homozygosity. We calculated the frequency of null alleles after we excluded the possibility of technical errors by verifying non-amplification was not due to a PCR failure, particularly in the gametophytes. The frequency of null alleles was directly estimated based on non-amplification at a locus in gametophytes (see Krueger-Hadfield, Roze, et al., 2013). For tetrasporophytes, we used the maximum likelihood estimator implemented in ML-NullFreq (Kalinowski & Taper, 2006).

To determine the genotypic richness ( $R$ ) at a site, we first needed to assess the proportion of repeated multilocus genotypes (MLGs). We used GenClone 2.0 (Arnaud-Haond & Belkhir, 2007) to calculate  $P_{\text{sex}}$  to determine the probability that repeated MLGs originated from separate sexual events ( $p > 0.05$ ) for samples that had amplification across all loci. For  $p < 0.05$ , we concluded that repeated MLGs are ramets of the same genet. We analyzed tetrasporophytic and gametophytic datasets at sites with sufficient sample size. For Norsel Point and East Litchfield tetrasporophytic and gametophytic datasets (providing they were present), we analyzed 2017 and 2018 separately and for both years combined.

It is possible that our sampling strategy could generate spatial substructuring within sites, called the Wahlund effect (Wahlund, 1928), in which case if a site is treated as one population, then  $F_{\text{IS}}$  values may be inflated. To test for spatial substructuring, we used SPAGeDi 1.5 (Hardy & Vekemans, 2002) and calculated Ritland's kinship coefficient for each distance class (Ritland, 1996). We analyzed sites at which three full transects were sampled. Following Krueger-Hadfield,

Roze, et al. (2013), we analyzed tetrasporophytes and gametophytes together as well as separately. Not all sites had enough gametophytes to analyze them separately from tetrasporophytes. We used the following distance classes based on sampling distances among transects and depths: 5, 10, 15, 20, 30, 40, 50, 60, and 70m. We tested for significance by comparing the observed pairwise coefficients within sites with the corresponding frequency distributions of 1000 permutations.

Next, we used summary statistics to describe the reproductive system of *Plocamium* sp. in Antarctica for all tetrasporophytic data sets and for gametophytic datasets with  $n > 5$ , following recommendations by Krueger-Hadfield, Guillemin, et al. (2021). We calculated genotypic richness ( $R$ ) and the distribution of clonal membership (pareto  $\beta$ ) for each population to assess the relative proportion of repeated MLGs and importance of clonal processes at a site. Pareto  $\beta$  provides an estimate of the importance of clonal (asexual) reproduction in a population with pareto  $\beta > 2$  suggesting low rates of clonality,  $0.7 < \text{pareto } \beta < 2$  suggesting intermediate levels of partial clonality, and pareto  $\beta < 0.7$  suggesting high clonality (Krueger-Hadfield, Guillemin, et al., 2021; Stoeckel et al., 2021). We also calculated genotypic evenness ( $D^*$ , Box 3 in Arnaud-Haond et al., 2007), which is expected to increase with increasing  $R$  as populations are maintained by sexual reproduction (Baums et al., 2006; Krueger-Hadfield, Guillemin, et al., 2021). We calculated linkage disequilibrium ( $\bar{r}_D$ ) for each site following Agapow and Burt (2001). In partially clonal taxa, both clonality and selfing can lead to an increase in  $\bar{r}_D$  itself and in the variance of  $\bar{r}_D$  (Krueger-Hadfield, Guillemin, et al., 2021). The probability of identity between sibs ( $pid$ ) was calculated following Krueger-Hadfield, Guillemin, et al. (2021) and Stoeckel et al. (2021) and provides information about the resolution of the loci used to distinguish individuals. Expected heterozygosity ( $H_E$ ), observed heterozygosity ( $H_O$ ), and the inbreeding coefficient ( $F_{\text{IS}}$ ) were also calculated for each site (Krueger-Hadfield, Guillemin, et al., 2021; Stoeckel et al., 2021).

To infer the reproductive modes that would best explain the transition of genotype frequencies between two consecutive years, we used GenAPoPop version 1.0 (Stoeckel et al., 2022) with uniform joint priors covering the mutation rate  $u \in [0.001, 0.000001, 0.00000001]$ , the clonal rate  $c \in [0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1]$ , and the selfing rate  $s \in [0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1]$ , [0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1] to cover the full spectrum of all possible quantitative reproductive modes and mutation rates. We report the spectrum of discrete posterior probabilities of the distributions of mutation, clonal, and selfing rates. We considered the inferred reproductive mode as the joint values of mutation rate, rate of clonality, and rate of selfing corresponding to the maximum posterior probability obtained

in each population following Becheler et al. (2017). At East Litchfield in 2017, 17, 20, and 23m were sampled at all three transects and only those same depths of the 2018 samples were used in this analysis. At Norsel Point in 2017, one whole transect was sampled and the same transect was resampled in 2018 and included in this analysis.

Finally, we calculated the genetic divergence ( $F_{ST}$ ) between tetrasporophytes and gametophytes within sites when there were enough gametophytes present ( $N_{\text{haploid}} > 10$ ; Engel et al., 2004; Krueger-Hadfield, Roze, et al., 2013; Stoeckel et al., 2021). Larger  $F_{ST}$  values would suggest an absence of gene flow between gametophytes and tetrasporophytes.

## Life cycle stage and chemogroup production

Thalli collected along transects had previously been assigned to chemogroups in Heiser, Shilling, et al. (2023). We plotted the proportion of gametophytes and tetrasporophytes producing each chemogroup to determine whether any chemogroup was produced by one life cycle stage.

## Data visualization

All figures were prepared in R version 3.6.3 (R Core Team, 2020) using the cowplot (Wilke, 2020), dplyr (Wickham et al., 2021), forcats (Wickham, 2021), gg-pattern (FC et al., 2022), ggplot2 (Wickham, 2016), ggpubr (Kassambara, 2020), and ggridges (Wilke, 2021) packages.

## RESULTS

### Gametophyte to tetrasporophyte ratio

We sampled 862 thalli during two field seasons from 12 different sites (two sites were sampled in both 2017 and 2018). Thirty-seven thalli did not amplify at any loci. Another 46 thalli did not amplify at three or more loci and were also excluded from the analyses. Of the 779 remaining thalli, 58 (7%) were female gametophytes as they bore cystocarps, and 79 were tetrasporophytes (10%) as they bore tetrasporangial sori (Table S1). Over 83% of thalli were vegetative at the time of collection. Of these vegetative thalli, 498 (78% of the vegetative thalli) were identified as tetrasporophytes as they had one or more heterozygous loci. The remaining 144 thalli (22% of the vegetative thalli) were homozygous at all 10 loci and were considered gametophytes.

All sites had gametophytes present except for East Litchfield in 2017 (Table 1 and Table S1; see Table S3

in the Supporting Information for results if vegetative homozygous thalli were excluded from the analyses). Joubin Island 97 and Stayaway Skerries only had two gametophytes each. The sites at Beaumont Skerries (proportion tetrasporophytes=0.44, binomial distribution,  $p=0.152$ ,  $\alpha=0.0035$ ), Dream Island (proportion tetrasporophytes=0.71, binomial distribution,  $p=0.010$ ,  $\alpha=0.0035$ ; but a small sample size of 17), Laggard Island (proportion tetrasporophytes=0.46, binomial distribution,  $p=0.043$ ,  $\alpha=0.0035$ ), and Wauwermans Islands (proportion tetrasporophytes=0.55, binomial distribution,  $p=0.041$ ,  $\alpha=0.0035$ ) all had gametophyte to tetrasporophyte ratios that did not differ from the hypothesized  $\sqrt{2}:1$ .

### Null alleles

Null alleles can lead to overestimations of homozygosity, but in haploid-diploid taxa, we can assess the null allele frequency using non-amplification in haploid gametophytes. One gametophyte did not amplify at locus Pc\_05, four at locus Pc\_16, four at locus Pc\_29, six at locus Pc\_40, and three at locus Pc\_49 after repeated attempts. We considered these null alleles, but nevertheless, null alleles were rare (at a proportion of 0.030 or less, with five loci at 0) for gametophytes. Based on a maximum likelihood estimator, null alleles were present in the tetrasporophytes (0.131 to 0.239 across loci; Table S4 in the Supporting Information), but this is likely biased due to the reproductive system (see Section 4).

### Spatial autocorrelation

We tested for spatial substructuring to evaluate whether  $F_{IS}$  values were inflated by analyzing a site as one population when it may consist of multiple smaller populations. East Litchfield (2018) was the only site with evidence of spatial structure as the slope was significant when gametophytes and tetrasporophytes were analyzed together (Table 2). At Laggard Island and Norsel Point (2018), there were significant positive kinship coefficients at 5m (Figure S1 in the Supporting Information). At East Litchfield (2018), there were significant positive kinship coefficients at 10m and related thalli were grouped together. There were no significantly positive kinship coefficients after 10m, and these continuously decreased with increasing distance (Figure S1). At Laggard Island and Norsel Point (2018), the significant positive kinship coefficient was 0.1 at 5m, suggesting thalli were related (Figure S1). After 5m, the kinship coefficients were not significantly different from zero. We did not find significant positive kinship coefficients at Southeast Bonaparte, which was relatively consistent in kinship coefficients across increasing distance (Figure S1). At Stepping Stones, the

TABLE 1 Diversity statistics based on the tetrasporophytic data sets in *Plocamium* sp. at each site.

Site	Year	Life cycle stage	N	Prop. Tetras	G	R	D*	Pareto $\beta$	$\bar{r}_D$	Pid	$H_E$	$H_O$	$F_{IS}$	Var ( $F_{IS}$ )
BAS	2018	Tetra	11	0.44*	11	1	1	2.6	0.759	0.008	0.438	0.218	0.501	1.055
		Gameto	14	–	13	0.92	0.99	2.8	0.652	0.000	0.091	–	–	–
DRM	2018	Tetra	12	0.71*	12	1	1	2.7	0.522	0.004	0.483	0.117	0.758	0.308
ELF	2017	Tetra	45	1	25	0.55	0.96	1.2	0.673	0.040	0.286	0.213	0.255	1.894
	2018	Tetra	104	0.83	66	0.63	0.98	0.8	0.716	0.008	0.434	0.209	0.520	0.986
		Gameto	22	–	19	0.86	0.98	1.8	0.571	0.000	0.109	–	–	–
JBN1	2018	Tetra	29	0.71	22	0.75	0.97	1.1	0.783	0.001	0.583	0.190	0.675	0.447
		Gameto	12	–	10	0.82	0.97	1.6	0.632	0.000	0.144	–	–	–
JBN97	2018	Tetra	13	0.87	11	0.83	0.97	1.7	0.406	0.020	0.351	0.154	0.562	1.063
LAG	2018	Tetra	49	0.46*	43	0.88	1	1.8	0.783	0.001	0.596	0.176	0.705	0.277
		Gameto	57	–	32	0.55	0.94	0.6	0.686	0.000	0.073	–	–	–
NPW	2017	Tetra	17	0.71	15	0.88	0.99	2.1	0.834	0.002	0.552	0.159	0.712	0.360
	2018	Tetra	65	0.66	59	0.91	1	2.4	0.692	0.002	0.560	0.191	0.659	0.437
		Gameto	33	–	22	0.66	0.95	0.9	0.685	0.000	0.054	–	–	–
NTM	2018	Tetra	21	0.72	19	0.9	0.99	1.8	0.674	0.003	0.504	0.181	0.641	0.680
SAS	2018	Tetra	11	0.85	10	0.9	0.98	2.5	0.205	0.049	0.271	0.191	0.296	2.109
SEB	2017	Tetra	96	0.92	36	0.37	0.94	0.4	0.470	0.045	0.276	0.266	0.038	3.650
SPS	2017	Tetra	86	0.83	64	0.74	0.99	1.3	0.239	0.064	0.245	0.221	0.100	1.775
		Gameto	17	–	12	0.69	0.94	1	–0.036	0.002	0.580	–	–	–
WAM	2018	Tetra	18	0.55*	18	1	1	3.2	0.741	0.001	0.597	0.178	0.702	0.248
		Gameto	15	–	11	0.71	0.94	0.8	0.695	0.000	0.120	–	–	–

Note: For sites with sufficient gametophytic sample size, diversity statistics are provided.

Abbreviations:  $\bar{r}_D$ , multilocus estimate of linkage disequilibrium;  $D^*$ , genotypic evenness;  $F_{IS}$ , inbreeding coefficient;  $G$ , number of multilocus genotypes;  $H_E$ , expected heterozygosity;  $H_O$ , observed heterozygosity;  $N$ , total number of thalli genotyped (including those with no amplification at one or two loci);  $\beta$ ,  $\text{pareto } \beta$ ;  $\text{pid}$ , probability of identity of sibs;  $\text{Prop. Tetras}$ , proportion of tetrasporophytes at a site ( $\text{proportion is not significantly different from the expected } \chi^2_2=1$ );  $R$ , genotypic richness;  $\text{var } (F_{IS})$ , the variance of  $F_{IS}$ .

Site	Gametophytes	Tetrasporophytes	Both
ELF 2018	NA (22)	$-3.02 \times 10^{-4}$ (104)	$-7.34 \times 10^{-4}$ * (126)
LAG 2018	$-1.02 \times 10^{-3}$ (57)	$-3.70 \times 10^{-5}$ (49)	$-5.46 \times 10^{-4}$ (106)
NPW 2018	$-1.11 \times 10^{-3}$ (33)	$1.00 \times 10^{-5}$ (65)	$-5.56 \times 10^{-4}$ (98)
SEB 2017	NA (8)	$-1.37 \times 10^{-4}$ (96)	$3.42 \times 10^{-5}$ (104)
SPS 2017	NA (17)	$1.39 \times 10^{-4}$ (86)	$2.26 \times 10^{-4}$ (103)

\*Significant slopes with  $p < 0.05$ . Sample sizes are shown in parentheses. Analyses were not run for gametophytes at sites with low sample sizes.

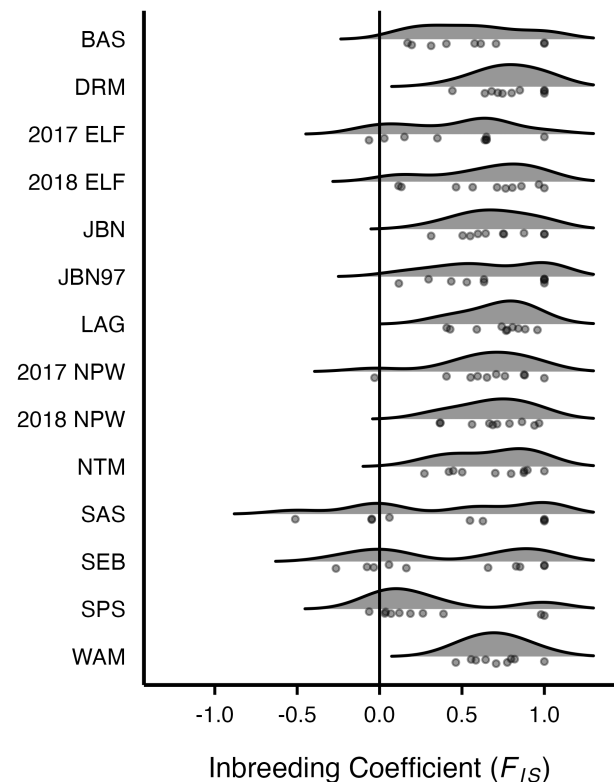
kinship coefficients were increasing with increasing distance, but the slope was not significant, such as at 40 and 60m (Figure S1). Kinship coefficients for tetrasporophytes were calculated at all sites and patterns were very similar compared to the analyses with tetrasporophytes and gametophytes together (Figure S1). Norsel Point and Laggard Island had enough gametophytes to calculate kinship coefficients for gametophytes only. Patterns at Laggard Island were very similar compared to both life cycle stages analyzed together. At Norsel Point, the kinship coefficients for gametophytes were slightly more variable but never significant. Therefore, spatial substructuring does not play a strong role in the positive  $F_{IS}$  values.

## Genotypic and genetic diversity

Three of the 12 sites we sampled were composed of all unique tetrasporophytic multilocus genotypes (MLGs; Table 1). The other sites had variable amounts of repeated MLGs. The highest numbers of repeated MLGs were observed at Stepping Stones (14), Southeast Bonaparte (12), and at East Litchfield (12 in 2017, and 15 in 2018; Table 1 and Table S5 in the Supporting Information). Often, but not always, repeated MLGs were observed at different depths and/or transects. The majority of repeated MLGs were observed twice (Table S5), but we encountered one MLG 12 times at East Litchfield (2018; eight homozygous loci), and one MLG was encountered 11 times and two MLGs 12 times at Southeast Bonaparte (each with seven homozygous loci). Most of the repeated MLGs had  $P_{sex} p < 0.05$ , and we considered them as ramets (i.e., through fragmentation, see Section 4 for more details). At East Litchfield, 14 repeated MLGs were observed in both 2017 and 2018. At Norsel Point, three repeated MLGs were observed in 2017 and 2018. For tetrasporophytes, genotypic richness ( $R$ ) varied from 0.37 to 1 (Table 1). East Litchfield and Southeast Bonaparte had the lowest  $R$ . The genotypic richness was similar between 2017 and 2018 at both East Litchfield and Norsel Point. Genotypic evenness was high across all sites ( $0.94 < D^* < 1$ ; Table 1).

Dream Island was the only site with no repeated gametophytic MLGs, but it had a low sample size of five

**TABLE 2** Spatial autocorrelation analysis for each site with three full transects using the kinship coefficient by Ritland (1996). Slopes of regression analyses from each locus and multilocus estimates.



**FIGURE 2** Inbreeding coefficient ( $F_{IS}$ ) of Antarctic *Plocamium* sp. across 12 sites (ELF and NPW were sampled in both years). Points below density plots are single-locus values, which were jittered using the rainbow function.

and was not included in analyses. At Laggard Island, we observed nine repeated MLGs. Some of the repeated MLGs were observed at the same depth and transect. The majority of repeated MLGs were observed twice (Table S5). One MLG was encountered 12 times and another seven times at Laggard Island. Most of the repeated MLGs had  $P_{sex} p < 0.05$ , and we considered them as ramets. For gametophytes,  $R$  varied from 0.55 to 0.92 (Table 1). Laggard had the lowest  $R$ . Genotypic evenness was high across sites ( $0.94 < D^* < 0.99$ , Table 1).

For tetrasporophytes, the probability of identity ( $pid$ ) varied from 0.001 to 0.064 across sites. At East Litchfield,  $pid$  was slightly higher in 2017 (0.040) as compared to 2018 (0.008), but it was not different

between years at Norsel Point (0.002). For gametophytes,  $pid$  was  $<0.002$  at all sites.

Pareto  $\beta$  (importance of clonal reproduction in a population) varied from 0.4 to 3.2 in the tetrasporophytes and from 0.8 to 2.8 in the gametophytes (Table 1). In 2017 and 2018, tetrasporophytic pareto  $\beta$  at East Litchfield (1.2 and 0.8, respectively) and Norsel Point (2.1 and 2.4, respectively) was similar. In tetrasporophytes, linkage disequilibrium ( $\bar{r}_D$ ) varied from 0.205 to 0.834 among sites. It was similar between years at East Litchfield (0.673 and 0.716) and decreased slightly at Norsel Point (0.834 and 0.692). In gametophytes,  $\bar{r}_D$  varied from  $-0.036$  to 0.695 among sites.

Overall, mean tetrasporophytic expected heterozygosity ( $H_E$ ) varied from 0.245 to 0.597, and mean observed heterozygosity ( $H_O$ ) varied between 0.154 and 0.266 (Table 1, Figure S2 in the Supporting Information). In gametophytes,  $H_E$  varied from 0.054 to 0.580.

Multilocus  $F_{IS}$  (inbreeding coefficient) was overwhelmingly positive and varied from 0.038 to 0.758 among sites (Table 1). Single locus  $F_{IS}$  varied greatly within sites (Figure 2). Overall, single locus  $F_{IS}$  values were generally positive (130 out of 140; Table S6 in the Supporting Information). For example, Southeast Bonaparte had the highest variance (3.650), and Wauwermans Islands had the lowest (0.248). However, there were no patterns with depth (Table S7 in the Supporting Information).

For the tetrasporophytes that were genotyped over two successive years, we observed low rates of clonality but high selfing rates at Norsel Point ( $c \sim 0.1$ ,  $s \sim 0.9$ ) and vice versa at East Litchfield ( $c = 0.8-0.9$ ,  $s = 0.2-0.1$ ; Table S8 and Figure S3 in the Supporting Information). This resolution suggests that our loci were sufficiently polymorphic to resolve MLGs.

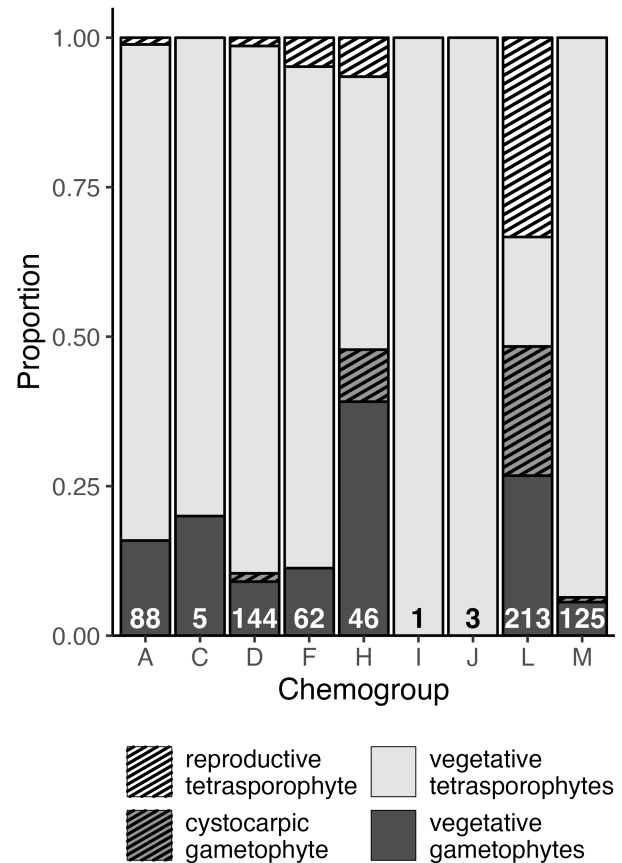
Genetic differentiation as measured by  $F_{ST}$  between life cycle stages at Beaumont Skerries, East Litchfield (2018), Howard Island, Laggard Island, Norsel Point (2018), Stepping Stones, and Wauwermans Islands never exceeded 0.009.

## Life cycle stage and chemogroup production

No chemogroup was exclusively produced by gametophytes (Figure 3). Chemogroups I and J were only seen in tetrasporophytes, but sample size for these chemogroups was very small ( $\leq 3$ ). Similarly, we did not observe any patterns between chemogroup production and whether thalli were vegetative or reproductive.

## DISCUSSION

In *Plocamium* sp., most of the sites we sampled near Palmer Station were tetrasporophyte biased



**FIGURE 3** *Plocamium* sp. produces a variety of chemogroups which are “signature mixtures” called chemogroups of defensive secondary metabolites. Fifteen chemogroups, named A through O, are described in Shilling et al. (2021) and chemogroup determination for the transects is described in Heiser, Shilling, et al. (2023). The stacked barplot shows the proportion of thalli producing each chemogroup with the proportion of tetrasporophytes (white and light gray background) and gametophytes (two shades of darker gray background). Stripes indicate life cycle stages of thalli identified through reproductive structures upon collection. No stripes indicate life cycle stages identified through multilocus genotypes.

accompanied by strong heterozygote deficiency, an uncommon combination compared to previous population genetic studies of haploid-diploid macroalgae (reviewed in Krueger-Hadfield, Guillemin, et al., 2021). Overall genotypic richness and evenness were high across sites, suggesting asexual reproduction in the tetrasporophyte stage is not solely or largely responsible for the ploidy bias as observed in other macroalgal taxa (e.g., Krueger-Hadfield et al., 2016). The exceptions were mainly East Litchfield and Southeast Bonaparte with lower genotypic richness but, nevertheless, high genotypic evenness. The sites that did have lower genotypic richness were also sites with pareto  $\beta$  values suggesting some background level of asexual reproduction, likely through thallus fragmentation and reattachment (see below). We discuss the drivers and consequences of tetrasporophyte bias, homozygosity, and reproductive system variation observed in

*Plocamium* sp. around Anvers Island on the western Antarctic Peninsula.

## Ploidy bias

Ploidy biases are well documented in macroalgae (as examples, see reviews by Fierst et al., 2005; Krueger-Hadfield et al., 2019; Scrosati & DeWreede, 1999; Thornber & Gaines, 2004). In the red macroalgae *Chondrus crispus* (reviewed in Collén et al., 2014) and *C. verrucosus* (Bellgrove & Aoki, 2008), many intertidal populations are gametophyte biased. In contrast, ploidy dominance varies among species but sporophytes are often observed at greater frequencies in many populations in the Gracilariales (reviewed in Kain & Destombe, 1995, but also see Vieira et al., 2022), the Ceramiales (Cecere et al., 2000), some species of the Gigartinales (e.g., *Hypnea musciformis*; Caires et al., 2013), as well as in other red macroalgae, such as *Pterocladia capillacea* (Patarra et al., 2020) and green macroalgae, such as two *Ulva* spp. (Du et al., 2019). In the genus *Plocamium*, and more broadly across other red macroalgal taxa, we know remarkably little about the spatiotemporal patterning of life cycle stages, especially outside of peak reproductive periods. In our study, which was conducted between April and June, only 17% of thalli bore reproductive structures, which may be due to phenology of the reproductive cycle. Subsequent observations in early to mid-January 2023 revealed that most thalli bore tetrasporangial stichidia, whereas by the end of January, the proportion of vegetative thalli was already increasing (C.D. Amsler, personal observations).

The Antarctic is one of several locations where tetrasporophyte bias in *Plocamium* sp. has been documented (Downing, 1995; Kain, 1982, 1986), suggesting that these patterns may be representative of the genus. Kain (1982, 1984, 1986) tested several hypotheses that may be driving tetrasporophyte bias, including differential depth distributions and substrate preferences (i.e., animals or other macroalgae). In our study, we sampled across the depth distribution of *Plocamium* sp. and did not observe a pattern with life cycle stage (Figure S4 in the Supporting Information). Even if some of the 146 vegetative fixed homozygous thalli were in fact tetrasporophytes, this would only strengthen the pattern of tetrasporophytic dominance we observed within and among sites.

Diploid stages have been hypothesized to be favored when nutrients are not limiting (Lewis, 1985), though this hypothesis was based on unicellular taxa, which raised questions as to whether it would apply for multicellular species (Bell, 1994). Vieira et al. (2021) demonstrated that resource limitation more broadly may have differential effects on haploid and diploid

stages of unicellular and multicellular taxa. Krueger-Hadfield and Ryan (2020) observed an increase in growth rates of adult *Gracilaria vermiculophylla* thalli under nutrient-enriched conditions but no differences among life cycle stages. Gametophytes did tend to suffer greater mortality regardless of nutrient conditions in the laboratory. In contrast, the *G. gracilis* (as *G. verrucosa*) gametophytic holdfasts performed better under nutrient-limiting conditions whereas tetrasporophytic holdfasts performed better under nutrient-enriched conditions as well as under higher intensities of UV radiation (Destombe et al., 1993). Antarctic seas are nutrient rich (Ducklow et al., 2007); hence, nutrients would only rarely, if ever, be expected to be limiting for macroalgal growth along the western Antarctic Peninsula (Wiencke & Amsler, 2012). Vieira et al. (2021) expanded the nutrient limitation hypothesis to include resources in general and showed that *G. chilensis* haploids would perform better under resource limiting conditions. As such, nutrient availability in the Antarctic environment may contribute to the relative dominance of tetrasporophytes over gametophytes in *Plocamium* sp., which remains to be empirically tested.

An alternative explanation for a higher abundance of tetrasporophytes may be a difference in the number of spores produced by the carposporophytes (retained on the female gametophytic thallus) versus tetrasporophytes (Fierst et al., 2005). Large numbers of carpospores are produced in each carposporophyte through mitotic amplification prior to release. However, this was not supported by Kain (1982, 1984), who observed no difference in spore production of *Plocamium cartilagineum*. Instead, carpospores were hypothesized to have a higher survival rate. In general, carpospores are larger than tetraspores, and carpospores have faster sinking rates that are thought to result in shorter dispersal distances (Destombe et al., 1992; Okuda & Neushul, 1981). In *Sarcopeltis antarctica* (formerly *Gigartina skottsbergii*, but see Hughey et al., 2020), which also is tetrasporophyte biased, DNA damage after exposure to ultraviolet radiation was higher in tetraspores compared to carpospores leading to higher spore mortality and, consequently, fewer gametophytic germlings (Roleda et al., 2008). Not only spores can be affected: the growth of *Galaxias gracilis* holdfasts was more negatively affected by ultraviolet radiation in haploids than it was in diploids (Destombe et al., 1993).

Ploidy bias may also be the result of juvenile and adult survival. For example, Engel et al. (2001) observed that the survival of adult thalli was the most important factor determining ploidy ratios in *Gracilaria gracilis*. Juvenile *G. gracilis* (Destombe et al., 1989) and adult *G. vermiculophylla* (Krueger-Hadfield & Ryan, 2020) gametophytes have higher mortality compared to tetrasporophytes. In *G. chilensis*, female gametophytes had higher survival (Vieira

et al., 2018a) and growth rates (Vieira et al., 2021), but tetrasporophytic germlings outperformed gametophytic germlings at very low and very high densities of adult fronds (Vieira et al., 2018b). In contrast, carpospore survival in *G. chilensis* was higher compared to tetraspores (Vieira et al., 2018b). Kamiya and Kawai (2002) showed evidence that maternal care provides germlings with additional nutrients leading to increased growth rates as compared to carpospores released from cystocarps which had been excised from the female gametophyte shortly after fertilization. Based on the extensive investigation of each life cycle stage in *G. chilensis*, Vieira et al. (2022) observed that varying environmental factors throughout the year favored different stages. Whether similar mechanisms maintain both life cycle stages in Antarctic *Plocamium* sp. and what drives ploidy biases in macroalgae more generally warrants further investigation.

### Tetrasporophytic bias coupled with high homozygosity

Previous studies that have documented a tetrasporophytic bias in red macroalgae have also documented heterozygote excess (e.g., Krueger-Hadfield et al., 2016; see also Krueger-Hadfield, Guillemain, et al., 2021). However, Steen et al. (2019) observed sporophyte bias in the brown macroalga *Dictyota dichotoma* that was sometimes, but not always, associated with homozygote excess. In *Plocamium* sp., we determined tetrasporophyte bias accompanied by strongly positive  $F_{IS}$  values, suggesting an excess of homozygotes. Excess homozygosity in a population may be due to the following three phenomena: (i) null alleles, (ii) spatial substructuring (i.e., Wahlund effect; Wahlund, 1928), or (iii) inbreeding (or selfing).

We observed little evidence of null alleles. While null alleles might positively inflate the  $F_{IS}$  values at some loci, they do not explain these patterns more generally in *Plocamium* sp. (see also Engel et al., 2004; Krueger-Hadfield, Roze, et al., 2013; Krueger-Hadfield et al., 2016; and the discussion of  $F_{IS}$  in marine taxa by Olsen et al., 2020). We also did not find strong evidence for spatial substructuring across sites for which we could conduct spatial autocorrelation analyses. We interpret the lack of substructuring as evidence of a sampling strategy that did not artificially conflate populations (e.g., Wahlund effect). On average, 7.3–8.8 loci were homozygous, including fixed homozygous tetrasporophytes, though there was no pattern with depth. Thus, we hypothesize that a high degree of intergametophytic selfing, an extreme form of inbreeding, likely occurs at sites like this, thereby generating positive  $F_{IS}$  values. Future work will also need to include paternity analyses to determine the levels of relatedness among

male and female pairs (see Engel et al., 1999; Krueger-Hadfield et al., 2015).

### Variation in reproductive system across populations

We observed that the prevailing reproduction mode differed across sites in which there were varying levels of clonality. The dynamic polar environment is subject to varying degrees of iceberg disturbance resulting in scouring of benthic organisms from the sea floor (Smale et al., 2007, 2008). The frequency of iceberg scouring decreases with depth and is influenced by both prevailing wind conditions pushing icebergs against the shore and variation in annual sea ice cover, which can protect the benthos from scouring if present (Smale et al., 2007). Macroalgal thalli may get dislodged and fragmented in this process, leading to drifting thalli. If species subsequently form secondary attachments in a different location, such as has been observed in *Plocamium* sp. (Downing, 1995; Heiser, Shilling, et al. 2023; Kain, 1989; Shepherd, 1981), iceberg scouring may be a mechanism for fragmentation and dispersal. Although we did not carry out a quantitative assessment of site-specific levels of iceberg scour, we suspect that differences in exposure to icebergs among sites may contribute to varying proportions of repeated MLGs. Even in locations without iceberg disturbance, for example, in California, *Plocamium cartilagineum* thalli showed evidence of breakage (Downing, 1995). Breakage was frequently observed in the understory and was hypothesized to be caused by the large brown canopy species, such as *Pterygophora californica*, causing abrasion (Downing, 1995). *Plocamium* sp. in Antarctica can be observed in open areas as well as covered by brown canopy-forming macroalgae draping over the benthos (authors' personal observations). Abrasion and subsequent distribution of fragments through iceberg disturbance as well as overstory macroalgae is, therefore, a possibility and warrants further investigation.

The thalli collected at East Litchfield, Howard Island, Joubin Island 97, Southeast Bonaparte, and Stepping Stones exhibited some genetic signatures of asexual reproduction. We observed the most repeated MLGs from the same depth and transect at East Litchfield (2018) and Southeast Bonaparte. Repeated tetrasporophytic MLGs could be produced by the settlement of genetically identical carpospores (Searles, 1980). Since carpospores are not flagellated, the distance they travel is likely dependent on water movement. Overall calmer conditions may lead to spores settling closer to each other, leading to repeated MLGs observed in proximity to one another. In the intertidal, few studies have noted such patterns to date. In the studies wherein repeated tetrasporophytes

were observed within close proximity to each other, these were thought to be the result of sampling the same genet twice in the case of *Chondrus crispus* (Krueger-Hadfield et al., 2011; Krueger-Hadfield, Roze, et al., 2013). In *Gracilaria vermiculophylla*, however, repeated MLGs were likely the result of thallus fragmentation in which many ramets spread across soft sediment sites (Krueger-Hadfield et al., 2016). This is also possible in *Plocamium* sp., especially for repeated MLGs observed further apart, because we have seen evidence that it easily forms secondary attachments (Heiser, Shilling, et al. 2023). *P. cartilagineum* is also known to have creeping stolons with multiple uprights (Downing, 1995; Kain, 1989; Shepherd, 1981). Secondary attachments may also in part explain some of the re-encountered gametophytic MLGs at some sites in *Plocamium* sp. In a previous study by Lees et al. (2018), the observed repeated gametophytic MLGs in *G. vermiculophylla* populations may have been due to low polymorphism of the microsatellites. Gametophytes have a single allele at each locus, which decreases the ability to resolve unique genotypes. In Antarctic *Plocamium* sp., our low *pid* values suggest our microsatellite loci were sufficient to distinguish among individuals (Waits et al., 2001). Future studies should investigate whether there are differences between gametophytes and tetrasporophytes in attachment strength and in the capacity to re-attach following dislodgement (see also Carrington et al., 2001).

Our comparison of samples collected in 2017 and 2018 at Norsel Point and East Litchfield allowed us to further explore the relative rates of selfing and clonality at two sites. At Norsel Point, we observed very high selfing rates. In contrast, when we analyzed samples collected at East Litchfield in 2017 and 2018, we detected high rates of clonality with some background level of selfing, which is further supported by lower genotypic richness than most sites. These two sites were also quite different based on qualitative observations (see Table S9 in the Supporting Information). Norsel Point was dominated mostly by the branching brown macroalga *Desmarestia anceps*, with higher macroalgal cover overall. By contrast, East Litchfield was dominated mostly by the bladed brown macroalga *Himantothallus grandifolius*, which drapes over the sea floor. East Litchfield was a little less steep and started to bottom out at about 29 m whereas Norsel Point had a mostly near vertical wall down to about 35 m after which it continued deeper on a gradual slope. In addition, at East Litchfield, we observed more drift algae, including *Plocamium* sp., though we note that we avoided sampling drifting thalli. *Plocamium* sp. was also more abundant and larger at this site (authors' personal observations). The absence of drift *Plocamium* sp., lack of more readily available benthic habitat, and the steeper slope at Norsel Point may prevent fragmented thalli from

reattaching. Natural history observations like these are critical for understanding patterns of variation among sites, particularly for interpreting genetic patterns.

## Drivers of the reproductive system in Antarctic *Plocamium* sp.

Uniparental reproduction is thought to benefit populations in marginal habitats, including high latitudes, where the scarcity of mates can make fertilization difficult (Pannell et al., 2015; Peck et al., 1998). In macroalgae, intergametophytic selfing does not provide the same benefits as selfing in monoecious angiosperms or hermaphroditic animals since separate male and female gametophytes are required for syngamy. Signatures of intergametophytic selfing rather appear to be a result of limited spore dispersal in red macroalgae due to the lack of flagellated spores or gametes (Krueger-Hadfield, Roze, et al., 2013). If spores disperse in clumps, such as suggested in *Chondrus crispus* (Krueger-Hadfield et al., 2015; Krueger-Hadfield, Roze, et al., 2013), those originating from the same tetrasporophytic parent may settle and grow close to each other. Clumped spore dispersal would be beneficial under conditions of low gametophytic abundance, such as observed in *Plocamium* species. Care was taken during collections to only sample a single thallus from a holdfast. If spores disperse and settle in clumps, identifying and sampling a single genet may be challenging, especially with the thick gloves required for Antarctic diving gear. Genetic material was preserved from one single branch ensuring that only one "individual" was sampled. Since we observed signatures of intergametophytic selfing across sites, it appears to be representative for the reproductive system of *Plocamium* sp. in the area.

## Life cycle stage and chemogroup production

In several red macroalgal species, gametophytes and tetrasporophytes have been determined to differ in secondary metabolite production (see Payo et al., 2011; Thornber et al., 2006; Vergés et al., 2008; Wright et al., 2000). In *Plocamium* sp., no chemogroup was produced solely by gametophytes. Even though two chemogroups were only observed in tetrasporophytes, the sample sizes were too small to conclude that these chemical signatures are stage specific. This does not exclude the possibility that life cycle stages may differ in total secondary metabolite concentration as was observed in the red macroalga *Delisea pulchra* (Wright et al., 2000). It is possible that secondary metabolite concentrations in tetrasporophytes could be contributing to the dominance of this life cycle stage in *Plocamium* sp., but more work on this is necessary.

Overall, we determined that Antarctic *Plocamium* sp. populations are tetrasporophyte biased, but the thalli are highly homozygous, a pattern that to the best of our knowledge has not been observed in other red macroalgae. We observed variation in population genetic indices among sites that warrants future investigation to determine underlying environmental drivers (e.g., disturbance levels) of propagule dispersal and how those may be linked to shaping evolution of the reproductive system. Intergametophytic selfing may either be due to the high latitude location of this species, or it may be a more general signature of macroalgae with haploid-diploid life cycles (see Otto & Marks, 1996).

## AUTHOR CONTRIBUTIONS

**Sabrina Heiser:** Conceptualization (equal); data curation (lead); formal analysis (equal); funding acquisition (supporting); investigation (lead); methodology (equal); visualization (lead); writing – original draft (equal); writing – review and editing (equal). **Charles Amsler:** Conceptualization (equal); funding acquisition (equal); investigation (supporting); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal). **Solenn Stoeckel:** Formal analysis (supporting); software (equal); writing – review and editing (equal). **James B McClintock:** Funding acquisition (equal); writing – review and editing (equal). **Bill Baker:** Funding acquisition (equal); writing – review and editing (equal). **Stacy Annette Krueger-Hadfield:** Conceptualization (equal); formal analysis (equal); funding acquisition (supporting); investigation (supporting); methodology (equal); software (equal); visualization (supporting); writing – original draft (equal); writing – review and editing (equal).

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## DATA AVAILABILITY STATEMENT

Genetic data and code are provided on Zenodo (10.5281/zenodo.7608044) and the United States Antarctic Program Data Center (<https://www.usap-dc.org/view/project/p0010016>).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**FIGURE S1**

**FIGURE S2**

**FIGURE S3**

**FIGURE S4**

**TABLE S1**

**TABLE S2**

**TABLE S3**

**TABLE S4**

**TABLE S5**

**TABLE S6**

**TABLE S7**

**TABLE S8**

**TABLE S9**

**DATA S1**

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