

Short Note

Microsatellite locus development in the seaweed *Plocamium* sp.

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Macroalgae cover up to 80% of the benthos along the western Antarctic Peninsula (WAP; Wiencke & Amsler 2012). One of the most common and widespread members of the understory community is the red macroalga *Plocamium* sp. (Heiser *et al.* 2020). It supports among the highest amphipod and gastropod densities and is protected from predation through highly diverse chemical defences (Heiser *et al.* 2020). Haplotypic diversity, based on the mitochondrial *cox1* barcode, showed some evidence of geographical structure as well as correlation with specific chemical defences (Shilling *et al.* 2021). These coarse patterns of genetic diversity are insufficient to understand the processes structuring populations of *Plocamium* sp. along the WAP, necessitating the use of more polymorphic, nuclear loci, such as microsatellites.

Microsatellites have enabled the empirical quantification of the relative rates of selfing (i.e. self-fertilization) *vs* outcrossing (e.g. Winn *et al.* 2011) and sexual *vs* asexual reproduction (e.g. Vallejo-Marín *et al.* 2010), but studies have been restricted largely to angiosperms or animals, with far fewer investigations in macroalgae (Krueger-Hadfield *et al.* 2021). *Plocamium* sp., like many macroalgae, has a haploid-diploid life cycle, with free-living diploid tetrasporophytes and free-living haploid gametophytes, which are morphologically indistinguishable unless they are reproductive (Fig. S1; Heiser *et al.* 2020). Meiosis occurs on the tetrasporophytes, resulting in the release of haploid tetraspores. Tetraspores germinate and develop into male and female gametophytes. Gametes are mitotically produced by the gametophytes, but, following fertilization, the zygote is retained on the female gametophyte, where the carposporophyte develops. Each diploid carpospore can germinate into a tetrasporophyte. In natural populations, many thalli are vegetative, rendering it difficult to distinguish the stages. This life cycle results in unique eco-evolutionary consequences that challenge traditional understanding and the utility of common proxies to describe patterns of reproductive

system variation (Krueger-Hadfield *et al.* 2021). For example, *Plocamium* sp. has separate sexes, but this does not preclude selfing (intergametophytic selfing; see Klekowski 1969). Separate sexes, therefore, cannot be used as a proxy to deduce outcrossing in natural populations. Instead, we must use population genetic tools to empirically quantify the relative rates of selfing, outcrossing and asexual reproduction in natural populations.

We developed microsatellites to quantify patterns of genetic diversity and gene flow in *Plocamium* sp. (Heiser 2022). We chose microsatellites over other approaches for several reasons: 1) microsatellites facilitate the iterative addition of new samples to a dataset, something that is not possible in most genotyping by sequencing (GBS) approaches to identify single nucleotide polymorphisms; 2) microsatellites are an appropriate tool when existing data on ploidy and the reproductive system are absent, which may complicate downstream bioinformatics in GBS approaches; and 3) microsatellites are a powerful tool with which to quantify reproductive mode variation in macroalgae (Krueger-Hadfield *et al.* 2021). We collected *Plocamium* sp. thalli during summers between 2016 and 2018 at 'East Litchfield' and Laggard Island near Palmer Station on Anvers Island (see Supplemental Materials for details). All gametophytes had one allele and all tetrasporophytes had one or two alleles, confirming that our 10 polymorphic microsatellite loci are in single-locus genetic determinism (Table S1). There were discrepancies between the direct estimates of null allele frequencies from non-amplification in the haploid gametophytes (< 5%) and those estimated using maximum likelihood in the diploid tetrasporophytes (0–39%; Table S2). When populations are not mating at random, which is an assumption of null allele frequency estimators in diploids, discrepancies between direct and maximum likelihood estimates have been found in other haploid-diploid macroalgae (e.g. Krueger-Hadfield *et al.* 2013). As there was also no evidence for short allele dominance (Table S3), these 10 loci are promising for future population genetic analyses.

Table I. Summary statistics for 10 polymorphic microsatellite loci developed in the Antarctic *Plocamium* sp. and analysed in the gametophytic and tetrasporophytic subpopulations at two sites along the western Antarctic Peninsula. Standard errors are provided for A_E , P_A , H_E and H_O and in parentheses the variance is provided for F_{IS} .

	'East Litchfield' Proportion of tetrasporophytes = 0.571 $P_{HD} = 0.727$		Laggard Islands Proportion of tetrasporophytes = 0.447 $P_{HD} = 0.937$	
	Gametophytes ($N = 9$)	Tetrasporophytes ($N = 12$)	Gametophytes ($N = 21$)	Tetrasporophytes ($N = 17$)
R	0.75	0.818	0.75	0.875
\bar{r}_d	0.058***	0.059	0.376***	0.563***
A_E	1.8 ± 0.2	1.5 ± 0.2	2.2 ± 0.2	2.3 ± 0.1
P_A	0.4 ± 0.1	0.3 ± 0.2	0.8 ± 0.1	1.1 ± 0.1
H_E^I	0.239 ± 0.064	0.152 ± 0.065	0.338 ± 0.050	0.355 ± 0.030
H_O	-	0.133 ± 0.065	-	0.147 ± 0.033
F_{IS}	-	0.129 (0.091)	-	0.593* (0.064)

* $P < 0.0025$ (with P adjusted to 0.0025 for significance), *** $P < 0.001$.

^I For gametophytes, H_E^A , unbiased expected heterozygosity was adjusted by a factor of $(2N - 1)/(2N - 2)$; see Engel *et al.* (2004).

P_{HD} = ploidy diversity; N = number of thalli; R = genotypic richness; \bar{r}_d = linkage disequilibrium; A_E and P_A = mean and private allelic richness, respectively (using rarefaction and the smallest sample size in gametophytes, $N = 9$); H_E = unbiased expected heterozygosity; H_O = observed heterozygosity calculated in tetrasporophytes only; F_{IS} = inbreeding coefficient calculated in tetrasporophytes only (single-locus values are provided in Table S5).

We performed some preliminary analyses from thalli sampled at 'East Litchfield' and Laggard Island (see Supplemental Materials). Thalli at 'East Litchfield' appear to be slightly tetrasporophyte biased (Table I). We did encounter several repeated multilocus genotypes (MLGs) in the gametophytes and tetrasporophytes at each site. Most of the repeated MLGs were considered as distinct individuals (or genets) based on P_{sex} (see discussion in Arnaud-Haond *et al.* 2007). The one exception was a pair of repeated tetrasporophytic MLGs at Laggard Island that we considered as ramets of the same genet. The subtidal environment is highly dynamic, with regular iceberg scour that removes benthic organisms. *Plocamium* sp. can form secondary attachments (Heiser 2022), making potential fragmentation and reattachment after removal possible. It may also be that we detected a genotypic signature of the carposporophyte in which morphologically distinct, tetrasporophytic MLGs share the same genotype because they originate from carpospores produced in the same carposporophyte or from carpospores that are produced by different fertilization events by the same male-female pair. Engel *et al.* (2004) and Krueger-Hadfield *et al.* (2011, 2013) did not find these genotypic signatures of the carposporophyte in natural populations of *Gracilaria gracilis* or *Chondrus crispus*, respectively, suggesting that carpospores might be dispersed in clumps or the genotypic signature of the carposporophyte is localized to a few centimetres. As all carpospores generated from each zygote are genetically identical (barring mutation), we would be unable to detect whether an adult tetrasporophyte originated from one carpospore or hundreds of carpospores.

Future explorations of *Plocamium* sp. populations along the WAP will enable us to determine whether the trend of tetrasporophytic bias with strong heterozygote deficiency, such as that seen at Laggard Island, is common. In other red macroalgae, heterozygote deficiency (e.g. inbreeding coefficient (F_{IS}) > 0; Table I, and see Table S5 for single-locus values) and high selfing rates have been associated with gametophytic bias (Krueger-Hadfield *et al.* 2013). By contrast, tetrasporophytic bias has been associated with outcrossing ($F_{IS} = 0$; Engel *et al.* 2004) or with heterozygote excess and clonality (e.g. $F_{IS} < 0$; Krueger-Hadfield *et al.* 2016). We did detect two 'fixed' homozygous tetrasporophytes (thalli were reproductive at the time of sampling) at 'East Litchfield' and four at Laggard Island, suggesting high selfing rates (see Krueger-Hadfield *et al.* 2013). However, we will need to use spatially explicit sampling to determine whether the observed heterozygote deficiency is due to the reproductive system and/or a Wahlund effect (see also Heiser 2022).

Haploid-diploid life cycles are predicted to be correlated with selfing, asexual reproduction or both (Otto & Marks 1996). Reduced inbreeding depression is thought to ease transitions to selfing in angiosperms (Barrett 2002) and has been shown to occur at range edges where selfing may be common (Pujol *et al.* 2009). Similar reductions in inbreeding depression may occur in haploid-diploid algae, but the long-lived gametophytes may enable efficient purging of deleterious mutations. The purging of deleterious alleles may further reduce the negative consequences of selfing in natural populations (see Charlesworth & Charlesworth 1987). To date, very

little work has explored the effects of inbreeding depression in macroalgae (but see Barner *et al.* 2011). Future work using these loci in *Plocamium* sp. will allow us to quantify selfing rates in populations along the WAP, setting the stage for empirical work on the role of inbreeding depression and its evolutionary consequences in haploid-diploid taxa. As the reproductive system partitions genetic diversity within and among populations, understanding patterns of reproductive system variation is critical to deciphering the processes that structure algal-dominated communities.

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Author contributions

SH. and CDA collected the samples. SH and SAK-H performed the marker development, calculated summary statistics, analysed the data and wrote the paper. All authors contributed to the final version of the manuscript.

Supplemental material

A supplemental methods and results section including two supplemental figures and five supplemental tables will be found at <https://doi.org/10.1017/S0954102022000475>.

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