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The reproductive system of the cryptogenic alga *Chondria tumulosa* (Florideophyceae) at Manawai, Papahānaumokuākea Marine National Monument

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ABSTRACT

Determining the relative importance of sexual and asexual reproduction is critical for determining how genetic diversity is partitioned within and among populations. Macroalgae exhibit reproductive system variability, with many species capable of both sexual and asexual reproduction. Thus, rates of fragmentation (asexual) versus sporic recruitment (sexual) must be quantified. This is critical during range expansions when shifts to uniparental reproduction are common. *Chondria tumulosa* is a cryptogenic red alga that was discovered forming thick mats at Manawai, in the Northwestern Hawaiian Islands, during opportunistic sampling in 2019. It reduced the biodiversity and abundance of associated coral reef organisms. To characterize the reproductive system of *C. tumulosa* in this atoll, we visually assessed 124 thallus clumps for reproductive structures and used five microsatellite loci to genotype 41 thalli. All thalli bearing reproductive structures (20%) and all thalli that were genotyped were tetrasporophytes. No gametophytic thalli were observed using microscopy or microsatellite genotyping, though 80% of thalli observed under the microscope were vegetative. We observed *in situ* thallus fragmentation supported by the presence of 17 repeated genotypes and other genetic signatures, suggesting high rates of asexual reproduction. These results suggest that *C. tumulosa* is partially clonal in this atoll and has the potential for spreading to other nearby atolls. Rapid establishment, thallus fragmentation and tetrasporophytic dominance are consistent with other red macroalgal invasions studied to date. Understanding the reproductive system is an important component of developing management strategies for this alga and expanding our understanding of the population genetics of partially clonal taxa.

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INTRODUCTION

The reproductive system (*sensu* Barrett 2011) encompasses the axes of variation from sexual (self-fertilization, or selfing, to outcrossing) to asexual reproduction (e.g. fragmentation to spore production without meiosis or fertilization; DeMeeûs *et al.* 2007). The prevailing reproductive mode partitions genetic diversity within and among populations (Hamrick & Godt 1996) and is therefore critical to our understanding of the evolutionary ecology of a species (Barrett 2011). Outcrossing (i.e. mating between unrelated individuals) typically results in greater genetic diversity, whereas uniparental reproduction (i.e. selfing, asexual reproduction, or both) typically results in lower genetic diversity. Unlike some animals that alternate between sexual and asexual reproduction in response to environmental cues (see Halkett *et al.* 2005), sexual and asexual reproduction occur simultaneously in macroalgae (Otto & Marks 1996; see also Krueger-Hadfield *et al.* 2021).

Population genetic studies in haploid-diploid macroalgae remain generally, as well as taxonomically, limited (reviewed in Krueger-Hadfield *et al.* 2021). The complex life cycles in which haploid gametophytes alternate with diploid sporophytes leads to unique consequences for studying the ecology and evolution of populations (Krueger-Hadfield 2020). For example, the combined effects of the proportion of gametophytes and rate of clonality substantially affect the distribution of common population genetic indices (Stoeckel *et al.* 2021). In other words, predictions from diploid taxa (e.g. animals) cannot be moved wholesale to haploid-diploid algae (see also Krueger-Hadfield & Hoban 2016). In the specific case of asexual reproduction, the recovery of sexual reproduction is entirely dependent on whether the gametophytic or sporophytic generation is retained (see Drew 1955 for terminology). Since meiosis occurs at the sporophytic generation, sporophytes can start a new population with only a single thallus. In contrast, most red algae with separate sexes require both a male and a female gametophyte would be required. As an example, thallus fragmentation in *Gracilaria vermiculophylla* (Ohmi) Papenfuss has led to the dominance of

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tetrasporophytes during the invasion of soft sediment habitats, but drifting and reproductive tetrasporophytes are likely to have established new populations (e.g. Krueger-Hadfield *et al.* 2016, 2018). Such shifts to uniparental reproduction – either self-fertilization or asexual reproduction – are common during range expansions whereby individuals that are capable of self-fertilization, asexual reproduction, or both, are much more likely to become established in a novel habitat (e.g. Baker's Law; Baker 1955; Pannel *et al.* 2015). However, it is unclear whether rapid expansion and the dominance of one generation is a wider phenomenon among macroalgae. Nevertheless, the complexity of the life cycle generates challenges to studying these populations and necessitates natural history observations, such as assessing the reproductive state of thalli at the time of collection (Krueger-Hadfield & Hoban 2016; Krueger-Hadfield *et al.* 2019).

Here, we investigate the prevailing reproductive mode of the rhodophyte *Chondria tumulosa* A.R. Sherwood & Huisman. In 2019, we surveyed atolls in the Papahānaumokuākea Marine National Monument (PMNM) as part of a routine NOAA monitoring cruise. Upon arrival at Manawai (also known as Pearl and Hermes Atoll), we discovered *C. tumulosa*, a previously undescribed alga, forming dense mats and overgrowing coral reefs on the scale of thousands of square metres (Sherwood *et al.* 2020). We collected the thalli used in this study during the initial discovery of its rapid spread. Before the accumulation of *C. tumulosa* biomass, the coral reefs at Manawai were nearly pristine and had been protected from direct anthropogenic influence for centuries due to their remote location (Friedlander *et al.* 2008). The reefs were known for having an intact food web, high biodiversity and an abundance of apex predators (Maragos & Gulko 2002). Nevertheless, the PMNM has not escaped large-scale coral bleaching events (Kenyon *et al.* 2006; Couch *et al.* 2017), coral disease outbreaks (Aeby *et al.* 2011), structural damage via derelict fishing gear (Dameron *et al.* 2007) and numerous fish and invertebrate invaders (Friedlander *et al.* 2009). Although there are numerous invasive macroalgal species in the Main Hawaiian Islands (Smith *et al.* 2002), *C. tumulosa* is the first invasive-like macroalga in the PMNM. We note that we refer to this alga as cryptogenic because we do not yet know its origin and its only known distribution is within the northern reaches of the PMNM at present (Sherwood *et al.* 2020). To the best of our knowledge, this is the first species within the genus *Chondria* to display invasive behaviour of this nature and magnitude.

We observed extensive fragmentation of *C. tumulosa* thalli at Manawai as well as the formation of large mats down to 21 metres in depth. We hypothesized that the rapid increase in *C. tumulosa* biomass was due to asexual reproduction in the form of thallus fragmentation. We also predicted that tetrasporophytes would dominate the site because thallus fragmentation in other red macroalgae has led to tetrasporophyte dominance (e.g. Guillemin *et al.* 2008; Krueger-Hadfield *et al.* 2016). Our study not only expands the macroalgal taxa for which population genetic data are available but will aid in the development of best management practices for this alga by providing fundamental information regarding the reproductive mode of *C. tumulosa* at Manawai Atoll.

MATERIAL AND METHODS

Study site description

The PMNM is the largest marine protected area in the USA. It is a UNESCO World Heritage site that emphasizes the protection of the cultural, historical and ecological importance of the Northwestern Hawaiian Islands (Kikiloi *et al.* 2017), which are a 1,931-km chain of remote and nearly pristine atolls (Fig. 1). Manawai (approximately 27°49.998′N, 175° 49.998′W; Fig. 1) is one of the largest atolls in the Northwestern Hawaiian Islands, with a reef area of approximately 1,166 km^2, and several small islands and sand islets (PMNM 2020). No invasive macroalgae have been observed at Manawai to date.

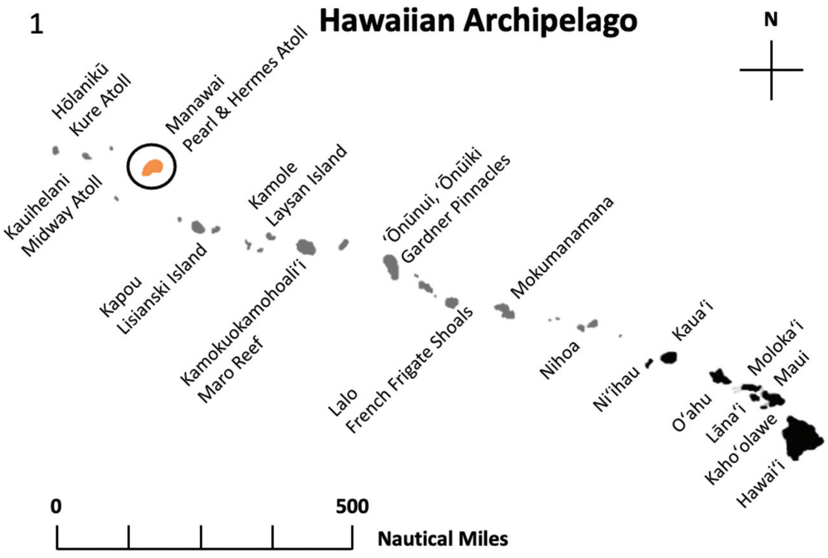


Fig. 1. Map of the Hawaiian Archipelago. Colours shown are Main Hawaiian Islands (black), Northwestern Hawaiian Islands and Atolls (grey) and Manawai (commonly Pearl and Hermes Atoll; circled). Maps data: Google, ©2021 + Canva, ©2021.

Fig. 2. Satellite image of Manawai (27°51.528′N, 175°50.898′W), Northwestern Hawaiian Islands, Hawai'i, USA. *Chondria tumulosa* sampling sites indicated with numbers (see also Table 2). Maps data: Google, ©2021.

Sample collection

Upon initial discovery of *C. tumulosa* in 2019, we sampled thalli from five forereef and backreef sites across Manawai from 2 to 19 m depths on 3–9 August 2019 (Table 1; Fig. 2). Either 24 or 25 thallus clumps were haphazardly collected at each site (N_{TOTAL} = 124). Thallus clumps were collected rather than 'individual' thalli because the mat-forming tendency of this alga makes distinguishing 'individual' thalli in the field nearly impossible (see also Krueger-Hadfield *et al.* 2013). We sampled approximately every metre to standardize sampling and prevent collecting from the same area twice. Thallus clumps were placed in prelabelled 120-ml bags (Whirl-Pak®, Madison, Wisconsin, USA) while under water and kept in a cooler on ice until processed. Small pieces of what was assumed to be a single thallus from each clump (see Results – *Chondria tumulosa* genets) were preserved in silica for subsequent DNA extractions. The remainder of the clump was drained of water, frozen at –20°C, and used to visually assess the reproductive state of a subsample of thalli.

Table 1. Manawai sampling site information. 124 samples of *Chondria tumulosa* collected from five sites around Manawai, Papahānaumokuākea Marine National Monument during August 2019.

Site	Latitude	Longitude	Depth (m)	Reef type
1	27°53.7636′N	175°56.0316′W	13	Back
2	27°57.4680′N	175°48.1248′W	2	Back
3	27°57.9672′N	175°46.467′W	19	Fore
4	27°51.0924′N	175°44.3214′W	14	Fore
5	27°47.3952′N	175°59.889′W	12	Fore

Table 2. Single locus, multilocus ± standard error, and variance values of observed heterozygosity (H_O), expected heterozygosity (H_E) and the inbreeding coefficient (F_{IS}) of *Chondria tumulosa* at Manawai.

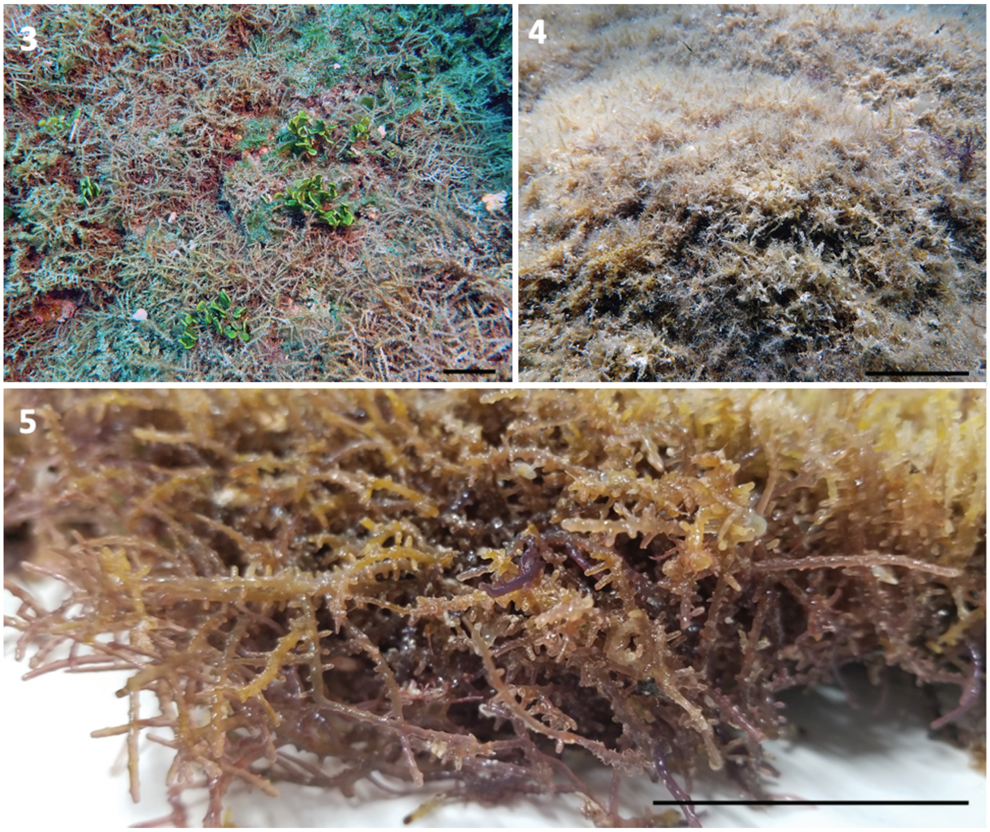
Locus	H_O	H_E	F_{IS}
Chondria_7	0.88	0.53	-0.65
Chondria_24	0.66	0.48	-0.37
Chondria_34	0.85	0.49	-0.74
Chondria_39	0.54	0.58	0.08
Chondria_75	0.90	0.52	-0.73
Multilocus	0.77 ± 0.14	0.52 ± 0.04	-0.47 ± 0.31
Variance	0.103	0.006	0.490

Reproductive state

Each of the 124 clumps consisted of many interwoven thalli (Figs 3–5) and these can be more than one genet (or genetic individual, see Harper 1980). We selected three representative thallus pieces from each clump for the microscopic examination of reproductive structures. The thalli selected from each clump encompassed the range in morphological variation found in each clump ($N_{MICROSCOPY}$ = 372). Although cystocarps and spermatangia have not previously been observed in *C. tumulosa* (Sherwood *et al.* 2020), studies in closely related *Chondria* species suggest that reproductive structures are visible via surface scanning (Sutti 2018). We presume that this alga follows the typical red algal life cycle with an alternation of gametophytes and tetrasporophytes, with the cystocarp visible on the female thallus. At the time of sampling, we had yet to encounter any gametophytes in this species. Additionally, tetraspores were observed in *C. tumulosa* during surface scans (Fig. S1; see also Sherwood *et al.* 2020). Therefore, each thallus was scanned at 40× using a compound microscope (Leica DM500, Deerfield, Illinois, USA) and then described as a tetrasporophyte (presence of tetrasporangial sori), male gametophyte (presence of spermatangial sori), female gametophyte (presence of cystocarps), or as vegetative (no observable reproductive structures). These data serve as an atoll-wide estimate of the proportion of observable vegetative vs reproductive thalli at the time of collection.

DNA extraction, microsatellite development and PCR conditions

Total genomic DNA was isolated using either a NucleoSpin® Plant II Kit or NucleoSpin® 96 Plant Kit (Macherey-Nagel,



Figs 3–5. *Chondria tumulosa in situ* at Manawai. Scale bars = 3 cm.
Figs 3, **4**. *Chondria tumulosa* displaying mat-forming morphology.
Fig. 5. Close-up view of intertwined thalli.

Düren, Germany). The manufacturer's instructions were followed for all steps except the cell lysis step, in which the lysate was held at room temperature for one hour and elution was performed with 100 µl of molecular grade water.

To develop microsatellite loci, we pooled 8 µl of DNA from each of 10 thalli and generated single sequence repeat (SSR)-enriched sequences commercially at Microsynth ECOGENICS GmbH (Balgach, Switzerland). Di-, tri- and tetranucleotide motifs were identified and primer sequences were designed using MSATCOMMANDER v1.0.8 (Faircloth 2008). A set of quality filters were applied using R v4.0.2 (R Development Core Team 2020) following a protocol modified from Schoebel *et al.* (2013; see also Ryan *et al.* 2021; Heiser *et al.* 2023). Loci were removed if they had primer pairs that possessed a high pair penalty, or if melting temperatures were too dissimilar. We also removed primer pairs that matched more than one region to minimize the likelihood of amplifying multiple regions of the genome. We selected primer pairs with the longest consecutive repeat region to avoid repetitive regions interfering with primer binding. After this filtering, 152 primer pairs remained.

Of the remaining 152 primer pairs, 74 loci with the greatest number of uninterrupted motif repeats were tested across a panel of seven thalli and a negative control (Table S1). Simplex PCRs were performed to amplify each locus using a Biometra thermocycler (AnalytikJena, Centennial, Colorado, USA) and the following program: 2 min at 95°C, followed by 30 cycles of 95°C for 30s, 56°C for 30s, and 72°C for 30s, with a final extension of 5 min at 72°C. The PCR contained a final volume of 15 µl: 2 µl DNA template, 1X Promega buffer (Madison, Wisconsin, USA), 250 µM of each dNTP, 2 mM $MgCl_2$, 1 mg ml^{-1} bovine serum albumin (BSA), 1 U Promega Go*Taq* polymerase, and 250 nM of each primer. Amplification was confirmed by visually inspecting bands for the expected size on a 2% 1X TAE gel pre-stained with GelRed (Biotium, Fremont, California, USA).

Thirteen loci (Table S1) were amplified in simplex using a SimpliAmp™ thermocycler (ThermoFisher Scientific, Waltham, Massachusetts, USA) with the same program as above and a final volume of 15 µl: 2 µl DNA template, 1X Promega buffer, 250 µM of each dNTP, 2 mM $MgCl_2$, 1 mg ml^{-1} BSA, 1 U Promega GoFlexi *Taq* polymerase, 250 nM fluorescent-labeled forward primer (using 6-FAM, VIC, NED, or PET; Table S1), 150 nM unlabeled-forward primer, 400 nM unlabeled-reverse primer.

One µl of PCR product was diluted in 9.7 µl Hi-Di Formamide (ThermoFisher Scientific) and 0.35 µl size ladder for fragment analysis at the University of Alabama at Birmingham Heflin Center for Genomic Sciences (Birmingham, Alabama, USA).

From these initial fragment analyses, we had five loci that produced reliable patterns and we moved forward with these five loci. Alleles were scored manually and binned using TANDEM (Matschiner & Salzburger 2009) to ensure that all loci had an average rounding error below the recommended error threshold for each locus (Matschiner & Salzburger 2009).

Ploidy assignment and ratio

Ploidy was assigned using the multilocus genotype (MLGs) for each thallus following Krueger-Hadfield *et al.* (2013). If one or more loci were heterozygous, then the thallus was considered a diploid tetrasporophyte. A thallus with only one allele at each locus would be considered a haploid gametophyte.

Once ploidy had been assigned, the binomial distribution was used to detect deviations from the hypothesized ploidy ratio of $\sqrt{2}$:1 (0.41 tetrasporophyte to 0.59 gametophytes; Destombe *et al.* 1989; Thornber & Gaines 2004). We chose to focus on this hypothesis for expected ploidy ratios in haploid-diploid macroalgae to facilitate comparison to other studies with strong biases in life cycle stage for which population genetic data exist (e.g. for *Chondrus*: Krueger-Hadfield *et al.* 2013; for *Gracilaria*: Krueger-Hadfield *et al.* 2016). We also calculated the new diversity metric: ploidy diversity (P_{HD}; Krueger-Hadfield *et al.* 2019).

Data analyses

Conducting population genetic work in haploid-diploid taxa requires special consideration regarding not only sampling strategy, but also data analyses. Most population genetic software is based on diploid-specific assumptions, rendering the use of these programs and tools challenging in haploid-diploid taxa. Thus, we followed the recommendations for analysing population genetic data in partially clonal taxa outlined by Krueger-Hadfield & Hoban (2016), Krueger-Hadfield *et al.* (2021) and Stoeckel *et al.* (2021), and outlined below.

Null allele frequencies were calculated using ML-Null Freq (Kalinowski & Taper 2006; Table S2).

Repeated MLGs can be the result of asexual reproduction (likely fragmentation in *C. tumulosa*) or distinct sexual events. To assess the likelihood that two identical multilocus genotypes were the result of sex, we calculated P_{sex} in GenClone 2.0 (Arnaud-Haond & Belkhir 2007). Repeated MLGs were considered ramets (i.e. clones) if P_{sex} *p*-values were < 0.05 and genets (i.e. 'individuals') if P_{sex} *p*-values were > 0.05.

We calculated genotypic richness, or the number of unique genotypes per sampling effort, using $R = \frac{G-1}{N-1}$, with *G*, the number of distinct genotypes, and *N*, the total number of genotyped samples, following Dorken & Eckert (2001). Genotypic evenness (*E.5*), or the distribution of genotypes across sampling effort, was also calculated. The probability of identity (*pid*) was calculated following Krueger-Hadfield *et al.* (2021) and Stoeckel *et al.* (2021), based on recommendations by Arnaud-Haond *et al.* (2007). The *pid* has mostly been used in exclusively sexual populations to determine the resolution of the markers used to distinguish among MLGs (e.g. Waits *et al.* 2001), though some studies have used this to rank populations by levels of clonality (Stoeckel *et al.* 2006, 2021).

We calculated *Pareto* β, an estimate of clonal membership (Arnaud-Haond *et al.* 2007). *Pareto* β decreases with increasing clonality: *Pareto* β < 0.7 suggests high clonal rates, between 0.7 and 2.0 suggests partial clonality, and >2.0 suggests low rates of clonality (Krueger-Hadfield *et al.* 2021). Observed heterozygosity (H_O), expected heterozygosity (H_E), the inbreeding coefficient (F_{IS}), and linkage disequilibrium ($\bar{r}_D$) were all calculated following Krueger-Hadfield *et al.* (2021). Deviations from zero suggest non-random mating and large F_{IS} variance is typically expected in partially clonal populations (Stoeckel *et al.* 2021).

RESULTS

Chondria tumulosa genets

We initially extracted 10 mg of dried material from each thallus clump (N = 124). The thallus material we preserved in silica gel was not the same material as we observed for reproductive structures under the microscope. We had preserved thalli in the field in silica gel, whereas we haphazardly selected thalli for observation under the microscope in the laboratory in Charleston. However, clumps were composed of tightly woven thalli and we likely preserved multiple genotypes in silica. We observed multiple alleles for many of our 124 putative 'individuals'. Thus, we re-extracted DNA from a single thallus piece to ensure a single genet was extracted. This reduced our genetic data set from 124 thalli to 41 thalli but reflected a more conservative approach to ensure we genotyped a single thallus as a putative genet.

Microsatellite loci

All five loci were polymorphic with either two or three alleles (Table S1). All loci had an average rounding error below the recommended error threshold as assessed by TANDEM (Matschiner & Salzburger 2009) except for locus *Chondria*_39 (Table S1). Poorly binned alleles for *Chondria*_39 were checked manually (see also Krueger-Hadfield *et al.* 2013). Null allele frequencies were very small ranging from 0%–4% (Table S2).

Proportion of reproductive thalli

Approximately 80% of thalli were vegetative (lacking visually identifiable reproductive structures) at the time of collection (302 out of 372 thalli; *c.* 81%). Approximately 20% of thalli were reproductive tetrasporophytes (70 out of 372 thalli; *c.*

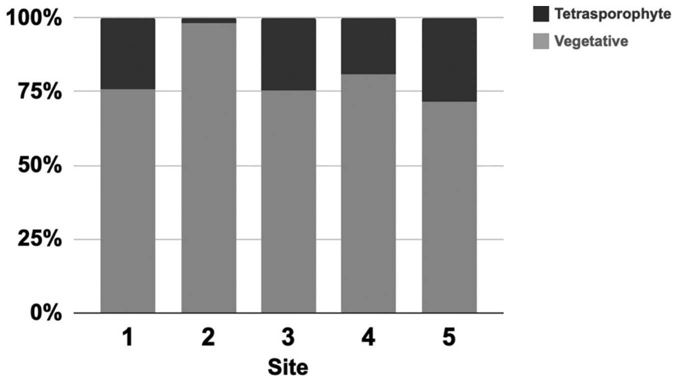


Fig. 6. Proportion of *Chondria tumulosa* tetrasporophytic to vegetative thalli by site across Manawai (N_{TOTAL} = 372 samples, 74–75 thalli per site).

19%). No reproductive gametophytes (zero out of 372) were observed during surface scans (Fig. 6).

Ploidy assignment and ratio

All 41 genotyped thalli were heterozygous at one or more loci and were considered tetrasporophytes. All sites sampled at Manawai, therefore, deviated from the predicted ploidy ratio of $\sqrt{2}$:1 and P_{HD} was 0.

Genotypic and genetic diversity

Twenty seven distinct MLGs were observed across the 41 genotyped thalli (R = 0.475, $E.5$ = 0.342; Table 3). Six genotypes were re-encountered at least once, and one genotype was re-encountered eight times within a single site (Table S3). Several thalli were found to share the same MLG within each site. Although MLGs were also shared between sites, these were always considered distinct genets based on P_{sex} p-values (Table S3).

Average expected heterozygosity (H_E) at Manawai was 0.52 and observed heterozygosity (H_O) was 0.77 (Table 2). Heterozygote excess was observed with single locus F_{IS} values ranging from −0.37 to 0.08 with a variance of 0.490 (Table 2). The *Pareto* β index was small but >0.7 (*Pareto* β = 0.729). There was little evidence of linkage disequilibrium ($\bar{r}_D$= 0.062; Table 3) and low probability of identity between sibs (*pid* = 0.003; Table 3).

Table 3. Population genetic summary statistics calculated for *Chondria tumulosa* as recommended for haploid-diploid taxa (Krueger-Hadfield *et al.* 2021; Stoeckel *et al.* 2021).

Statistic	*Chondria tumulosa*
Proportion diploid	1.0
Ploidy diversity (P_{HD})	0.0
Probability of identity between sibs (*pid*)	0.003
Genotypic richness (R)	0.48
Genotypic evenness ($E.5$)	0.34
Pareto β	0.729
Linkage disequilibrium ($\bar{r}_D$)	0.062

DISCUSSION

We used *in situ* observations, microscopy and population genetic analyses to characterize the prevailing reproductive mode of the macroalga *C. tumulosa* at Manawai. Thallus size was variable from qualitative observations, in which thalli were found detached from the reef and drifting in the water column, suggesting a propensity for fragmentation (see also Sherwood *et al.* 2020). The presence of reproductive tetrasporophytes suggest that meiosis occurs at Manawai, but the fate of the tetraspores remains unknown as no gametophytes were observed. The combination of largely negative F_{IS} values, high F_{IS} variance, and a low *Pareto* β value suggest partial clonality (see also Krueger-Hadfield *et al.* 2021). Whenever a thallus could be identified by either microscopy (via the presence of reproductive structures) or genotyping (haploid vs diploid), it was always a tetrasporophyte. We discuss the eco-evolutionary consequences of these results for *C. tumulosa* in the PMNM below.

Tetrasporophytic bias

Large differences in mortality, fecundity, or both can drive shifts in expected ploidy ratios (Thornber & Gaines 2004). Survival differences among spores (Destombe *et al.* 1992; Roleda *et al.* 2004, 2008; Vieira *et al.* 2018b), juveniles (Destombe *et al.* 1993; Guillemin *et al.* 2013; Vieira *et al.* 2018a) and adults (Vieira *et al.* 2018a) have been found to influence ploidy ratios in red algae. Similarly, the fertilization rate and the number of carpospores produced can lead to ploidy bias (Fierst *et al.* 2005). While we predict that the 'isomorphic' *C. tumulosa* gametophytes and tetrasporophytes are phenotypically distinct (see Hughes & Otto 1999), we doubt that these differences alone maintain the dominance of tetrasporophytes observed at Manawai.

There is limited oceanographic data from within the PMNM, but the Hawaiian Archipelago is well known for experiencing strong northern winter swells (Stopa *et al.* 2011). Large-scale mat removal as a product of high wave energy likely leads to increased levels of fragmentation throughout the winter season. In *G. vermiculophylla*, Lees *et al.* (2018) found thallus strength differed among tetrasporohytes and gametophytes, in which the former required more force to dislodge a secondary branch as compared to the latter. The authors concluded that mechanical differences of these thalli may contribute to tetrasporophytic dominance in soft sediment habitats (see also Krueger-Hadfield & Ryan 2020). Yet, Carrington *et al.* (2001) found no differences in mechanical strength of *Chondrus crispus* Stackhouse gametophytes and tetrasporophytes, suggesting equal susceptibility to dislodgement. In contrast, in *Chondrus retortus* Kaoru Matsumoto & S. Shimada, tetrasporophytes exhibited weaker tissue strength and attachment (Bellgrove & Aoki 2019). If gametophytes are found in future sampling, it will be important to determine if there are differences in the biomechanical properties of *C. tumulosa* life cycle stages that contribute to ploidy bias because of fragmentation or dislodgement.

Colonization events can also generate strong ploidy bias if only one stage is introduced. For example, in Peru, a single male clone of *G. chilensis* was introduced leading to a fixed population of male gametophytes (Robitzch *et al.* 2019). Given the unknown origin of *C. tumulosa*, we cannot exclude the possibility that only tetrasporophytes were introduced to Manawai. However, the observation of tetraspores suggests that gametophytes may be found in the future if tetraspores are able to settle and germinate. It is unclear at present whether the life cycle can be completed in the PMNM, but the combination of more in depth and seasonal sampling is necessary to fill the knowledge gaps of this alga's life cycle in the Northwestern Hawaiian Islands.

Population genetics in partially clonal macroalgae

As predicted for haploid-diploid taxa (Otto & Marks 1996), there were signatures of asexual reproduction in *C. tumulosa* at Manawai. Partial clonality has been found in other macroalgae, such as the green alga *Cladophoropsis membranacea* (Bang *ex* C. Agardh) Børgesen, in which there was frequent clonal propagation (van der Strate *et al.* 2002). Likewise, Couceiro *et al.* (2015) described partial clonality in two different species of *Ectocarpus* Lyngbye, in which predominantly asexual reproduction led to the dominance of sporophytes. Moreover, sporophytic dominance has also been observed in the brown alga *Dictyota dichotoma* (Hudson) J. V. Lamouroux as a result of asexual reproduction (Steen *et al.* 2019), suggesting that this phenomenon is not limited to rhodophytes.

In *C. tumulosa*, we found an excess of heterozygotes, substantial variation in F_{IS}, and low *Pareto β*. With many unique genotypes, low linkage disequilibrium is expected. High linkage disequilibrium values only occur in partially clonal populations when one or few large clones skew the genetic association between loci. These patterns are predicted for growing populations that have not yet reached the environmental carrying capacity or in populations with low intraspecific competition (Navascués *et al.* 2010). We have collected samples in 2021 at Manawai that will allow us to incorporate temporal analyses (e.g. Becheler *et al.* 2017) to better understand reproductive mode variation and the relative rates of clonality.

The role of the reproductive system in habitat conservation

The PMNM is one of the most remote island archipelagos in the world and has historically been reported as a pristine and intact ecosystem (Maragos & Gulko 2002; Wilkinson 2004). However, in the last one or two decades, the PMNM has faced countless environmental stressors (Kenyon *et al.* 2006; Dameron *et al.* 2007; Friedlander *et al.* 2009; Aeby *et al.* 2011; Couch *et al.* 2017), with overgrowth of *C. tumulosa* being just the most recent. Management efforts for invasively behaving macroalgae are often challenging and these challenges are amplified when working in the PMNM due to the remote nature of this region. However, understanding how a species reproduces is the first step to developing both practical and proactive management strategies (Barrett *et al.* 2008; Barrett 2011).

These data suggest that *C. tumulosa* has the capacity for rapid spread to other nearby atolls and beyond via thallus fragmentation. Additionally, the presence of reproductive tetrasporophytes suggests that this alga can also reproduce via spore recruitment – a more cryptic form of dispersal. Although the origin of *C. tumulosa* is still unknown, it is having extensive ecological impact and is altering the benthic landscape of Manawai. Thus, it is critical to prevent further spread throughout the PMNM by developing management strategies that consider both this alga's propensity for thallus fragmentation and its ability for spore recruitment.

Future directions

When discrete individuals are not easily distinguishable, sampling for population genetics is challenging as each 'sample' is assumed to be a distinct genet in downstream analyses. This is largely because many population genetic assumptions and sampling strategies are based on unitary organisms in which distinguishing between 'individuals' is easier (see also Arnaud-Haond *et al.* 2007). *Chondria tumulosa* has intertwined thalli, forming tight mats that render it challenging to preserve a single, putative genet in sufficient biomass for subsequent DNA extraction. This is further complicated by challenges inherent to the complexity of the haploid-diploid life cycle (Krueger-Hadfield & Hoban 2016) and the challenges of remote field work. For example, the samples included in this study were collected during a routine monitoring cruise that did not anticipate conducting large-scale population genetic collections (Fig. S2). However, because of the remote location of the PMNM, days on site at any given atoll are extremely limited. Additional studies on the biology of this alga, such as the fate of tetraspores through culturing, will be challenging due to the remote nature of the PMNM and its understandably strict regulations for *C. tumulosa* (no live material may leave the atoll). Extensive spatio-temporal characterizations of *C. tumulosa*'s genetic diversity are critical for predicting the potential spread throughout the PMNM, while simultaneously expanding our understanding of the population genetics of partially clonal, haploid-diploid taxa (Otto & Marks 1996; Stoeckel *et al.* 2021).

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