

PERSPECTIVE

The eco-evolutionary importance of reproductive system variation in the macroalgae: Freshwater reds as a case study

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Abstract

The relative frequency of sexual versus asexual reproduction governs the distribution of genetic diversity within and among populations. Most studies on the consequences of reproductive variation focus on the mating system (i.e., selfing vs. outcrossing) of diploid-dominant taxa (e.g., angiosperms), often ignoring asexual reproduction. Although reproductive systems are hypothesized to be correlated with life-cycle types, variation in the relative rates of sexual and asexual reproduction remains poorly characterized across eukaryotes. This is particularly true among the three major lineages of macroalgae (green, brown, and red). The Rhodophyta are particularly interesting, as many taxa have complex haploid–diploid life cycles that influence genetic structure. Though most marine reds have separate sexes, we show that freshwater red macroalgae exhibit patterns of switching between monoicy and dioicy in sister taxa that rival those recently shown in brown macroalgae and in angiosperms. We advocate for the investigation of reproductive system evolution using freshwater reds, as this will expand the life-cycle types for which these data exist, enabling comparative analyses broadly across eukaryotes. Unlike their marine cousins, species in the Batrachospermales have macroscopic gametophytes attached to filamentous, often microscopic sporophytes. While asexual reproduction through monospores may occur in all freshwater reds, the Compsopogonales are thought to be exclusively asexual. Understanding the evolutionary consequences of selfing and asexual reproduction will aid in our understanding of the evolutionary ecology of all algae and of eukaryotic evolution generally.

KEYWORDS

asexual reproduction, haploid–diploid, life cycle, mating system, Rhodophyta, selfing, sex, streams

Abbreviation: SNPs, single nucleotide polymorphisms.

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INTRODUCTION

The evolution of sex has fueled some of the most spectacular diversification of life on Earth. All sexual eukaryotes alternate between two ploidies during their life cycle, but we often associate sex with reproduction. Whereas in mammals this is the case, the processes of syngamy (i.e., fertilization) and meiosis are spatially and temporally disassociated in most eukaryotes, leading to incredible diversity in the timing and dominance of haploid and diploid phases. Despite a rich history of theoretical and empirical work (e.g., as reviewed in Mable & Otto, 1998; Valero et al., 1992), we cannot always predict which ploidy will evolve. Moreover, common model organisms (e.g., *Drosophila* or *Arabidopsis*) used to address many hypotheses about the evolution of sex do not represent the entire spectrum of eukaryotic life-cycle diversity.

To illuminate the evolutionary and ecological consequences of life-cycle diversity, we must understand the forces that generate and maintain reproductive system variation. The prevailing reproductive mode governs the transmission of genes between generations, affecting the partitioning of genetic diversity within and among populations (Hamrick & Godt, 1996) and evolutionary responses to environmental change (Eckert et al., 2010). In contrast to angiosperms, the three lineages of macroalgae have not received the same attention despite earlier work advocating for their study to resolve outstanding questions about the evolution of sex (e.g., green macroalgae in Otto & Marks, 1996). Although a recent review of brown macroalgae by Heesch et al. (2021) demonstrated profound variation in the sexual system (i.e., separate sexes vs. hermaphroditism; see Table 1), suggesting reproductive system variability, red macroalgae have not received the same attention. This might be because the majority of marine red macroalgae have separate sexes (i.e., dioicous; Hawkes, 1990) and do not display sexual system variation comparable to that in the browns. However, we argue that an important group of red macroalgae found in freshwater ecosystems has been overlooked. We propose freshwater red macroalgae as foils to not only animals and angiosperms but also marine red, green, and brown algae (see also Haig, 2016; Heesch et al., 2021; Otto & Marks, 1996).

Unlike marine red macroalgae with independent gametophytes and tetrasporophytes, the Batrachospermales have strongly heteromorphic gametophytes and sporophytes termed the “chantransia” stage (Figure 1; Sheath, 1984). A unique type of meiosis, called vegetative meiosis, occurs at the tip of the chantransia filament in which three nuclei are lost and only one remains in the initial cell of the gametophyte. This process likely results in a loss of genetic diversity as compared to marine red macroalgae in which all four

products of meiosis—the tetraspores—are viable and can each produce a new gametophyte. In freshwater reds, gametophytes can be monoicous (i.e., hermaphroditic) or dioicous (i.e., separate sexes) and develop while remaining attached to the parental chantransia. This is distinct from mosses in which the sporophytic phase develops on and remains attached to the female gametophyte. In freshwater reds, the gametophyte develops and remains attached to the chantransia, and this physical connection between the two phases is unique among the red macroalgae. After fertilization, the diminutive carposporophyte (typically <300 μm in diameter) is retained on the gametophytic thallus (Figure 1a–c,e–h). The zygote is mitotically amplified in the carposporophyte, and the resulting diploid spores settle on the substratum, germinating to form the filamentous chantransia (Figure 1i). No studies have determined whether there are many chantransia sharing the same genotype because of this polyembryonic process, though studies in marine red macroalgae have found no evidence of repeated tetrasporophytic genotypes (Engel et al., 2004; Krueger-Hadfield et al., 2013). Moreover, chantransia can reproduce asexually through the production of monospores that recycle the sporophytic stage (Sheath, 1984). Yet, we do not know the frequency of monospore production in natural populations. In the Batrachospermales, putative monospore production by gametophytes has been reported in some taxa, but we do not know into what those gametophytically produced monospores germinate.

To develop freshwater reds as useful eco-evolutionary models, we briefly review the critical role reproductive systems play in influencing evolutionary trajectories and discuss the unique predictions for reproductive systems in haploid–diploid taxa. These predictions are equally applicable for all haploid–diploid taxa, including green, red, and brown algae in both freshwater and marine environments. Here, we focus on the biological characteristics that make freshwater reds useful for understanding the influence of life cycles on reproductive systems and vice versa. Finally, we conclude with a suite of questions that need to be answered in algae broadly that will add to our understanding of reproductive mode variation in natural populations, enabling more holistic syntheses across eukaryotic groups.

REPRODUCTIVE DIVERSITY

The reproductive system (sensu Barrett, 2011) describes the traits that determine the relative rates of (i) sexual versus asexual reproduction and (ii) selfing versus outcrossing. The incredible diversity in eukaryotic reproductive systems, both within and among species, has intrigued evolutionary biologists for decades. We briefly highlight the angiosperm literature to describe the two axes of reproductive mode variation—sexual

TABLE 1 A glossary of terms.

Term	Definition
Reproductive system	The traits that determine the relative rates of (i) sexual versus asexual reproduction and (ii) selfing vs. outcrossing (sensu Barrett, 2011). The second axis of variation of selfing and outcrossing is exclusively sexual as meiosis, recombination, and fertilization occur. This term encompasses asexual reproduction and for partially clonal taxa, such as algae, is preferred over the use of the term mating system (see below).
Mating system	The traits that determine the relative rates of selfing/inbreeding vs. outcrossing. This term describes the sexual axis of variation and does not include asexual processes. This term is notoriously ambiguous (see Barrett, 2014).
Monoicy	When sex is determined in the haploid stage, the occurrence of gametophytes that produce both male and female gametes (Beukeboom & Perrin, 2014).
Dioicy	When sex is determined in the haploid stage, the occurrence of gametophytes that produce only one type of gametes; separate sexes (see Intergametophytic selfing; Beukeboom & Perrin, 2014).
Trioicy	When sex is determined in the haploid stage, the occurrence of males, females, and hermaphroditic thalli; both monoicous and dioicous thalli occur in a population. We adapted the spelling for sex determined in the haploid stage. For more on trioicy, see Godin (2022).
Sexual system	The distribution of male and female functions on a thallus (see Barrett (2002) for a discussion on sexual systems in flowering plants). When sex is determined in the haploid stage, this describes whether male and female functions occur on the same thallus (monoicous or hermaphroditic), different thalli (dioicous or separate sexes), or if there are both monoicous and dioicous thalli in a population (trioicous). When sex is determined in the diploid stage, this refers to the same patterns of monoecy, dioecy, and trioecy.
Intragametophytic selfing	Origin of both gametes from a single gametophyte (Klekowski, 1969); unique to haploid–diploid taxa, in which fertilization occurs between a sperm (spermatium) and an egg (carpogonium) produced by the same gametophytic thallus. This type of selfing results in instantaneous, genome-wide homozygosity as gametes are produced by mitosis and genetically identical to the gametophyte that produced them, barring mutations. It is also only possible in monoicous thalli.
Intergametophytic selfing	Origin of each gamete from a different gametophyte (Klekowski, 1969); unique to haploid–diploid taxa, in which fertilization occurs between a sperm (spermatium) produced by one gametophyte and an egg (carpogonium) produced by a different gametophyte that share the same parental chantransia. This type of selfing can occur in monoicous or dioicous thalli if they are produced by the same chantransia. Importantly, dioicy does not prevent selfing from occurring and cannot be used as a proxy for outcrossing. It is unclear how many gametophytes a single chantransia produces or how important intergametophytic selfing may be in freshwater red algal populations. This is analogous to self-fertilization in an animal or a flowering plant.
Intergametophytic crossing	Origin of each gamete from a different gametophyte with each gametophyte originating from a different parent sporophyte (Klekowski, 1969); Fertilization that occurs between a sperm (spermatium) and an egg (carpogonium) produced by two different, unrelated gametophytes. Can occur in monoicous and dioicous thalli. This is analogous to cross-fertilization in an animal or a flowering plant.

and asexual—and provide a contrast to the unique predictions and consequences in organisms with haploid–diploid life cycles.

Angiosperms display striking variability in their reproductive systems both within and among species (Whitehead et al., 2018). The manner in which we think about reproductive systems is largely based on the dichotomy between uniparental (e.g., selfing or self-fertilization) and biparental (i.e., outcrossing) reproduction. General forces likely shape the evolution of selfing and outcrossing, with important consequences for population- and species-level traits, such as gene flow or range size (Barrett, 2002). Outcrossing typically results in greater genetic diversity and the maintenance of heterozygosity (e.g., Goodwillie et al., 2010). However, outcrossing itself encompasses a range of gamete unions from biparental inbreeding to outcrossing between genetically divergent individuals, which can lead

to lowered fitness through inbreeding (Charlesworth & Charlesworth, 1987) or outbreeding depression (Waser, 1993), respectively. Selfing, on the other hand, typically results in lower genetic diversity and increased homozygosity that is often associated with declines in offspring fitness (Charlesworth & Charlesworth, 1987). We note that segregation, recombination, and fertilization occur during selfing, and it should be considered a form of asexual reproduction. Yet, selfing, as a form of uniparental reproduction, may not always be disadvantageous, such as during range expansions (Barrett, 2002) and colonization (Baker, 1955) in which it can be advantageous (Schemske & Lande, 1985). Although theoretical models have predicted when selfing and outcrossing should be favored (e.g., Lande & Schemske, 1985), recent surveys of the literature have found that species and populations often undergo mixed-mating, with both selfing and outcrossing

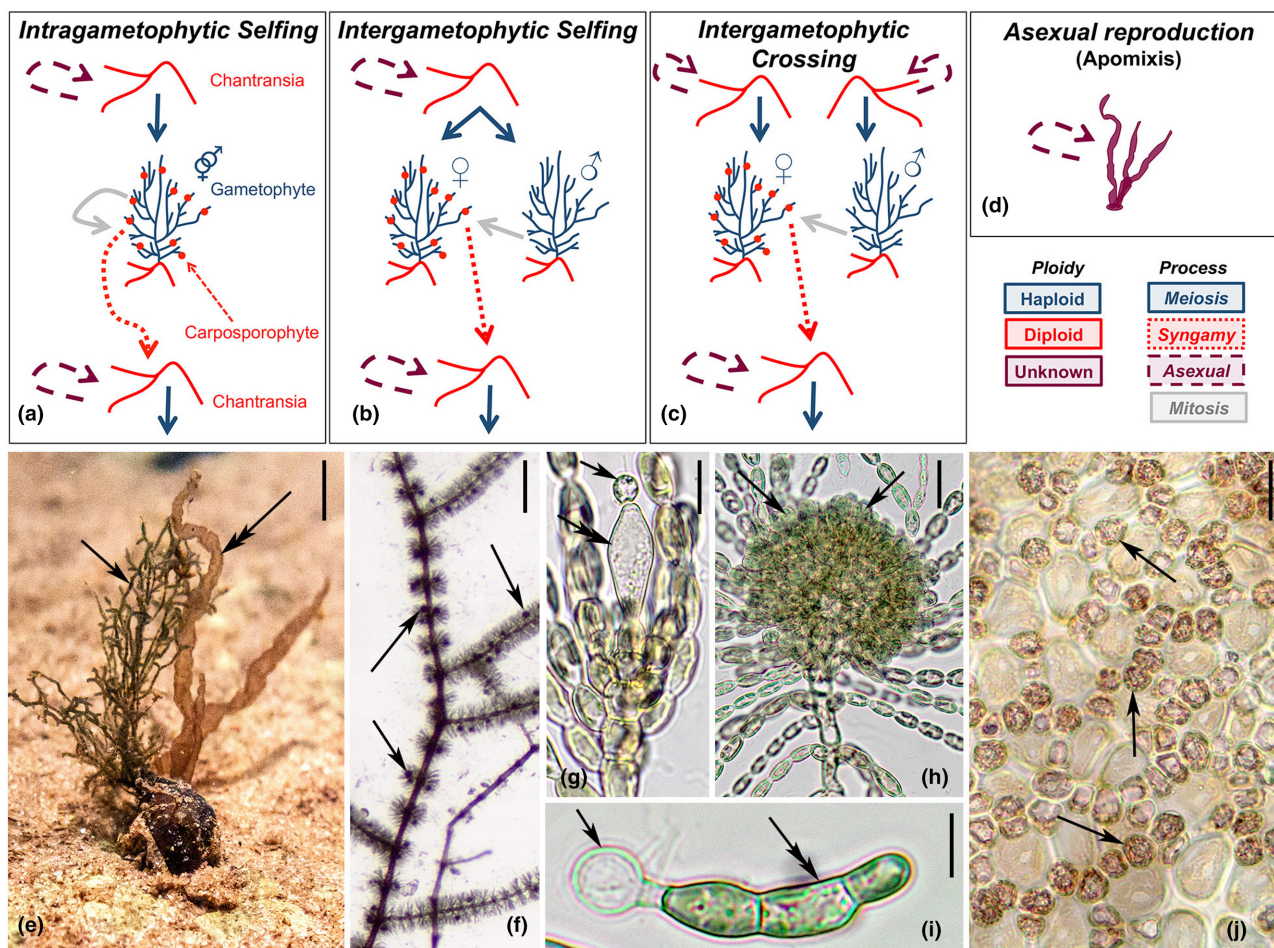


FIGURE 1 Diagrams of possible sexual and asexual reproduction by haploid–diploid freshwater red macroalgae and photos of life-cycle stages. Meiosis (darker solid line), syngamy (square dot line), asexual reproduction (long dash dot line), and mitosis (lighter solid line). Gametophytes are haploid whereas chantransia and carposporophytes are diploid. (a) Intragametophytic selfing with fertilization occurring between gametes produced by the same chantransia. (b) Intergametophytic selfing is the result of fertilization occurring between gametes produced by gametophytes that share the same parental chantransia. (c) Intergametophytic crossing occurs when males fertilize females produced by an unrelated chantransia. (d) Asexual reproduction in *Boldia erythrosiphon* via monospores recycling the upright thallus of unknown ploidy. (e) Macroscopic gametophyte of *Virescentia viride-americana* (arrow) and the macroscopic thallus of *Boldia* sp. (double arrow). (f–i) *Batrachospermum gelatinosum*. (f) Gametophyte with dark, spherical carposporophytes (arrows) attached. (g) Spermatium (arrow) attached to the inflated trichogyne of the carpogonium (double arrow) (h) Diploid carposporophyte with apical sporangia (arrows) resulting from fertilization. (i) Upon germination, all cellular contents of the carpospore (arrow) are transferred to the growing chantransia filament (double arrow). (j) thallus surface of *Boldia* showing monosporangia (arrows). Scale bars = 1 cm (e), 500 μ m (f), 10 μ m (g, i, j), 20 μ m (h). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/jpy.13407)]

(Whitehead et al., 2018), which is evolutionarily stable (Winn et al., 2011).

The evolutionary transitions between monoecy (hermaphroditic) and dioecy (separate sexes) correlate with selfing and outcrossing (Barrett, 2002). In more stable populations, dioecy, rather than monoecy, may have an advantage by enforcing outcrossing and preventing selfing, thereby maintaining genetic diversity. In disturbed habitats, such as those where frequent extinction and colonization events occur, monoecious individuals will have a selective advantage, as only one individual is necessary to (re-)establish the population (Baker, 1955). Contrary to work that has suggested trioecy (males, females, and hermaphroditic individuals) is a transitory state (Lande & Schemske, 1985),

Anderson et al. (2020) has shown that trioecy can be stable and reap the benefits of outcrossing and reproductive assurance. Yet, understanding reproductive system variation necessitates integrative ecological and genetic approaches to disentangle the forces that drive transitions in sexual system (see Table 1) and how that influences the reproductive system.

Unlike many animals that alternate between sexual and asexual reproduction in response to environmental cues (see Halkett et al., 2005), angiosperms often undergo sexual and asexual reproduction simultaneously (Vallejo-Marín et al., 2010). Although there are many forms of parthenogenesis (see de Meeûs et al., 2007; Orive & Krueger-Hadfield, 2021), for our purposes here we are referring to asexual (or clonal) reproduction that

does not include segregation, recombination, or fertilization. Asexual processes nevertheless incur similar genetic consequences as populations undergoing inbreeding or selfing (Halkett et al., 2005). Not only does the relative frequency of sexual versus asexual reproduction influence clonal diversity (Silvertown, 2008), but it can also lead to disparities in sex ratios in dioecious taxa (Yakimowski & Barrett, 2014). Partial clonality, a reproductive system in which both asexual and sexual reproduction (including varying rates of selfing to outcrossing) occur, is found across the majority of eukaryotic life on Earth, but it remains largely uncharacterized because demographic and evolutionary models are largely developed for exclusively sexual species (Arnaud-Haond et al., 2007). We need to develop better theoretical predictions and empirical studies to understand the combined influence of partial clonality with the parallel axis of variation from selfing to outcrossing (but see Stoeckel et al., 2021). It is here where macroalgae are likely to vastly expand our understanding of these processes (see also Otto & Marks, 1996).

THE EVOLUTIONARY ECOLOGY OF HAPLOID–DIPLOID LIFE CYCLES

The framework outlined above encompasses our understanding of reproductive system evolution and diversity, but it is based on the diploid life cycle of animals and the diploid stage of a subset of haploid–diploid vascular plants, mostly angiosperms. Although selection at the haploid stage is critical (e.g., Immler & Otto, 2018), somatic development only occurs in the diploid stage in animals because fertilization directly follows meiosis. Angiosperms, conifers, and ginkgoes can be viewed as “ecologically diplontic” because the haploid gametophytes are few-celled, always unisexual, and dependent on the sporophyte (but, see Delph, 2019). Changes in ploidy constitute a major genomic alteration, directly affecting phenotypic expression. Thus, it is uncertain whether predictions from “diplontic” taxa are representative of eukaryotic life-cycle diversity more broadly (see Krueger-Hadfield, 2020).

Mosses, ferns, and macroalgae (green, red, and brown algae) have distinct haploid–diploid life cycles that are subject to unique eco-evolutionary consequences compared to the more commonly studied diploid life cycles. Mosses are gametophyte-dominant (haploid) with dependent sporophytes (diploid). Ferns and kelps have independent dominant sporophytes with small gametophytes, the latter of which are challenging to work with and have often been overlooked (Nitta et al., 2017; Schoenrock et al., 2021). In all three of the macroalgal lineages, however, there are life cycles in which gametophytes and sporophytes are both macroscopic and vary along a continuum of seemingly morphologically identical to completely morphologically distinct (e.g.,

crustose and foliose morphologies, see Hughes & Otto, 1999; Krueger-Hadfield, 2020; Thornber, 2006). There are examples of life cycles in which one phase is macroscopic (e.g., kelp sporophytes) and the other microscopic (e.g., kelp gametophytes).

Otto and Marks (1996) suggested green macroalgae were an excellent group of taxa with which to test their prediction that reproductive modes should be correlated with different life cycle types. However, despite life-cycle diversity in this algal lineage, there was little data on reproductive system variation at the time (Otto & Marks, 1996), a problem that persists (Krueger-Hadfield et al., 2021). We need to generate data that describe the reproductive mode broadly across eukaryotes from which comparative analyses can be undertaken to test the correlation proposed by Otto and Marks (1996). This includes across all algal lineages regardless of their life-cycle variability.

The occurrence of monoicy or dioicy (Table 1) has distinct consequences with regard to reproductive system variation from diploid life cycles (Figure 1). For example, two forms of selfing are possible depending on whether a thallus is monoicous or dioicous (Table 1). Intragametophytic selfing occurs when fertilization is between gametes produced by the same monoicous gametophyte, resulting in *instantaneous* genome-wide homozygosity (Figure 1a; Klekowski, 1973). The evolutionary consequences of this *immediate* loss of genetic diversity across the entire genome are unclear, but is likely important for the evolution of haploid–diploid taxa (Sessa et al., 2016). Even though fertilization may occur between separate male and female gametophytes, if the pair share the same sporophytic parent, then this type of intergametophytic selfing is directly analogous to selfing in monoecious angiosperms and hermaphroditic animals (Figure 1b; Klekowski, 1969). Consequently, we cannot use the separation of sexes as a proxy for outcrossing as is often done in angiosperm and animal taxa. Instead, we must empirically quantify the reproductive mode and selfing rate (see Engel et al., 1999; Krueger-Hadfield et al., 2015). Finally, intergametophytic crossing (i.e., outcrossing) occurs when unrelated gametophytes, derived from unrelated parental sporophytes, exchange gametes (Figure 1c).

Most ferns are monoicous, and there is evidence for widespread inbreeding (Sessa et al., 2016). Crawford et al. (2009) observed syndromes in mosses in which transitions to dioicy occurred in lineages with small spores. Likewise, brown algae show many transitions, akin to angiosperms, from monoicy to dioicy, as well as from monoicy/dioicy to monoecy/dioecy (Heesch et al., 2021). Green macroalgae exhibit both inter- and intraspecific variation in monoicy and dioicy, but few studies have investigated reproductive system variation in these taxa (Krueger-Hadfield et al., 2021; Otto & Marks, 1996). Finally, most red macroalgae are dioicous (Hawkes, 1990), though there are exceptions—including

the freshwater Batrachospermales (Kumano, 2002)—and ample evidence for intraspecific variability (Krueger-Hadfield et al., 2021; Maggs, 1988).

In addition to the unique ways in which selfing can occur in mosses, ferns, and macroalgae, asexual reproduction leads to recycling of one phase and the potential loss of the other stage(s) (Figure 1d). In marine macroalgae, asexual recycling often leads to the loss of gametophytes either along environmental gradients (e.g., Gabrielson et al., 2002) or as a consequence of anthropogenic introductions (e.g., Krueger-Hadfield et al., 2016). As meiosis occurs on sporophytic thalli, it is possible to recover sexual cycling and complete the life cycle when the appropriate conditions are met. Too few studies have performed basic natural history observations in mosses, ferns, and algae such that we do not understand the long-term consequences of these phenomena nor the evolutionary costs of asexual reproduction.

FRESHWATER RED ALGAE

While most red algae inhabit marine or estuarine environments, there are freshwater species throughout the lineage: some sister to marine taxa, others freshwater species/populations of marine taxa, and lineages restricted to freshwaters, such as the Batrachospermales and Compsopogonales (Vis & Necchi, 2021). The unidirectional flow in streams could lead to dispersal of propagules downstream into unsuitable habitats. The adnate chantransia is likely perennial and maintains the population in a favorable habitat; however, the macroscopic gametophytes may be seasonal or perennial and are presumably more susceptible to seasonally variable environments (Sheath & Vis, 2015). Although the importance of chantransia has been recognized, its small size (often microscopic) makes it more difficult to investigate compared to the macroscopic gametophyte, like the microscopic stages of kelp and ferns (see Nitta et al., 2017; Schoenrock et al., 2021). With the advent of DNA sequencing, chantransia have been identified and linked with gametophytes, but genetic variation within and among populations has yet to be studied (Chiasson et al., 2005).

The Batrachospermales house two-thirds of the freshwater red algal species diversity and display considerable variation in sexual system with inter- and intra-specific variation in monoicy and dioicy. We performed a preliminary analysis of transitions from monoicy to dioicy using sequence data and species descriptions (Figure 2). The monospecific genera are all either monoicous or with an unknown the sexual system (Figure 2). Ten genera exhibit variation within and among species that is comparable to mosses (Crawford et al., 2009) and brown algae (Heesch et al., 2021). As an example, the species rich genus *Sheathia* has both dioicous and

monoicous species, as well as species with both monoicous and dioicous populations (Figure 2). In addition, there are several species only known as chantransia; whether the gametophyte phase has been permanently lost or the conditions under which gametophytes are produced have not been met is an open question. This diversity within and among species provides an opportunity to study the consequences of reproductive mode variability on the genetic variation among species as well as within and among populations.

In contrast to the life cycle exemplified by the Batrachospermales, the life cycle in other freshwater red algae, such as the Compsopogonales, is assumed to be asexual (Figure 1d,e). The two monospecific genera in this order, *Compsopogon* and *Boldia*, each have a disc of cells that attach a macroscopic thallus to the substratum. They reproduce via monospores obliquely cut off the vegetative cells on the thallus surface (Figure 1j), but we do not know their ploidy level. Although both taxa appear to reproduce via monospores only, the genus *Boldia* is restricted in its distribution to eastern North America, whereas the genus *Compsopogon* is common worldwide in tropical and subtropical locations (Necchi et al., 2013; Vis & Necchi, 2021). Empirical quantification of the reproductive mode is necessary in *Boldia* and *Compsopogon* as is contrasting this mode to that of monospores produced by the chantransia in the Batrachospermales.

FUTURE PERSPECTIVES

Few studies have investigated genetic structure in both the haploid and diploid stages for haploid–diploid taxa (Krueger-Hadfield et al., 2021). Nevertheless, understanding the evolutionary consequences of selfing and asexual reproduction is critical for our understanding of not only algal evolutionary ecology but also eukaryotic evolution more broadly. Detailed studies of green, brown, and red macroalgae will be key to understanding these processes. Specifically, freshwater red algae exhibit intra- and interspecific variation in monoicy and dioicy as well as the propensity for prolific clonal spore production that enables the exploration of evolutionary consequences of selfing and asexuality in haploid–diploid taxa. To develop a predictive tool, we can visualize the axes of reproductive variation as a triangle in which species or populations can be located depending on their reproductive mode(s) (Figure 3a; adapted from Barrett, 2011; Fryxell, 1957). For taxa in the Batrachospermales, we predict populations or species will be found in the middle to bottom of the triangle when both gametophytes and chantransia are present. The rate of selfing or outcrossing will likely depend on whether the population or species is monoicous or dioicous. We expect natural populations to undergo mixed mating with varying rates of intra- and intergametophytic

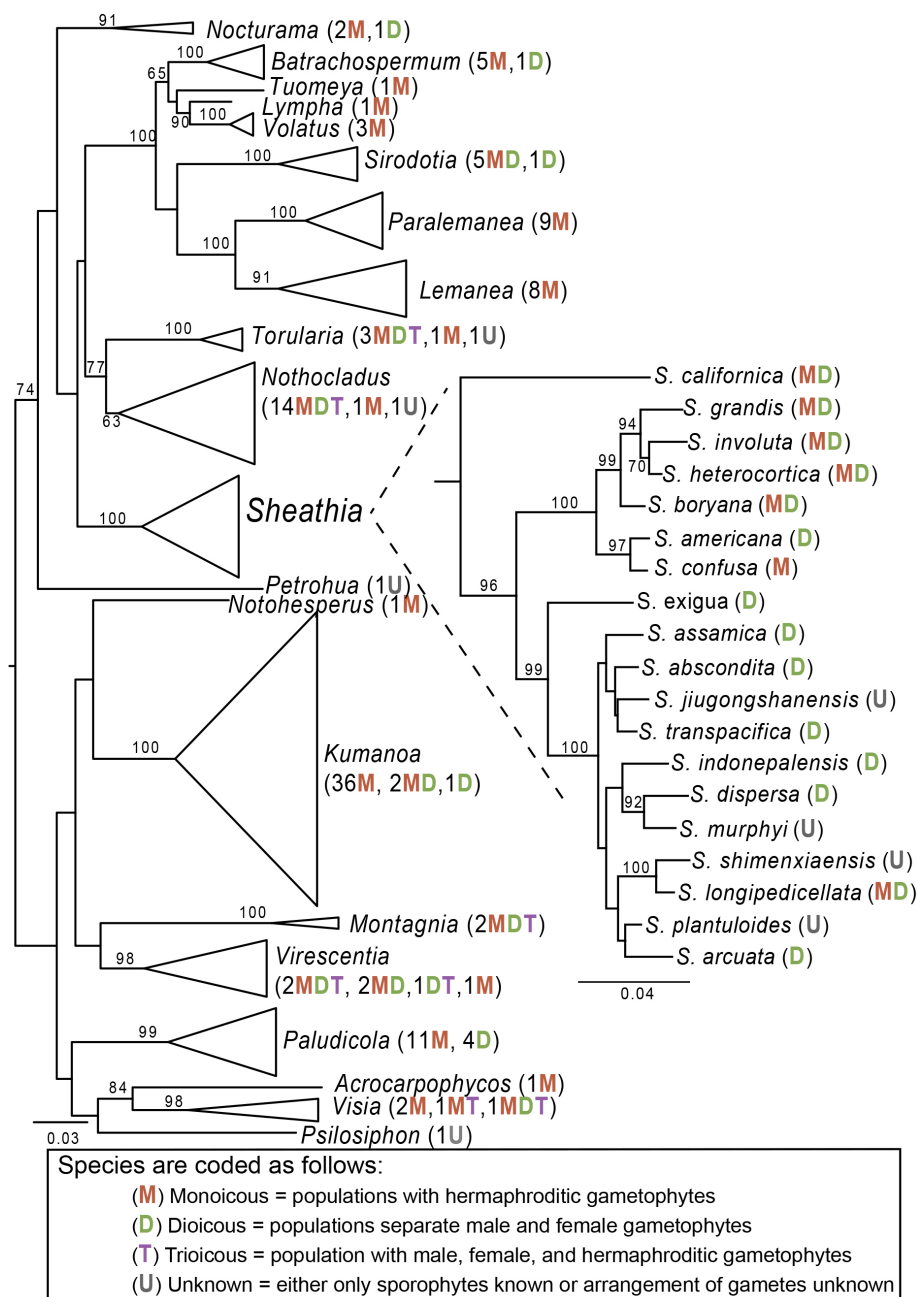


FIGURE 2 Phylogenetic relationships among genera within the Batrachospermales and species within the genus *Sheathia* based on the maximum likelihood analysis of the *rbcl* gene. Numbers on branches are bootstrap support from 1,000 replicates. Numbers in parentheses correspond to the number of species within the genus. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/jpy.13407)]

selfing versus outcrossing in monoicous taxa, whereas mixed mating will vary along a continuum of intergametophytic selfing and outcrossing in dioicous taxa. When chantransia reproduce through both vegetative meiosis and monospores, we expect to detect signatures of partial clonality (i.e., genetic signatures of sexual and asexual reproduction) in the populations. Chantransia-only populations should be exclusively asexual. Finally, as *Compsopogon* and *Boldia* are assumed to be exclusively asexual, they should occupy the apex of the triangle (G in Figure 3a, not shown in Figure 3b). However, recent work in obligately asexual taxa, such as bdelloid

rotifers (Laine et al., 2022), has suggested that sexual reproduction has occurred and does occur, and if this is the case in *Compsopogon* and *Boldia*, then their position within the triangle would shift.

We can fill in these gaps in population genetic data by using polymorphic nuclear markers, such as microsatellites or single nucleotide polymorphisms (SNPs). Recent work has also shown that the effective population size of haplids is critical to the underlying diversity in haploid–diploid populations (Stoeckel et al., 2021). Moreover, as many macroalgae are partially clonal (Otto & Marks, 1996), we need to be sure

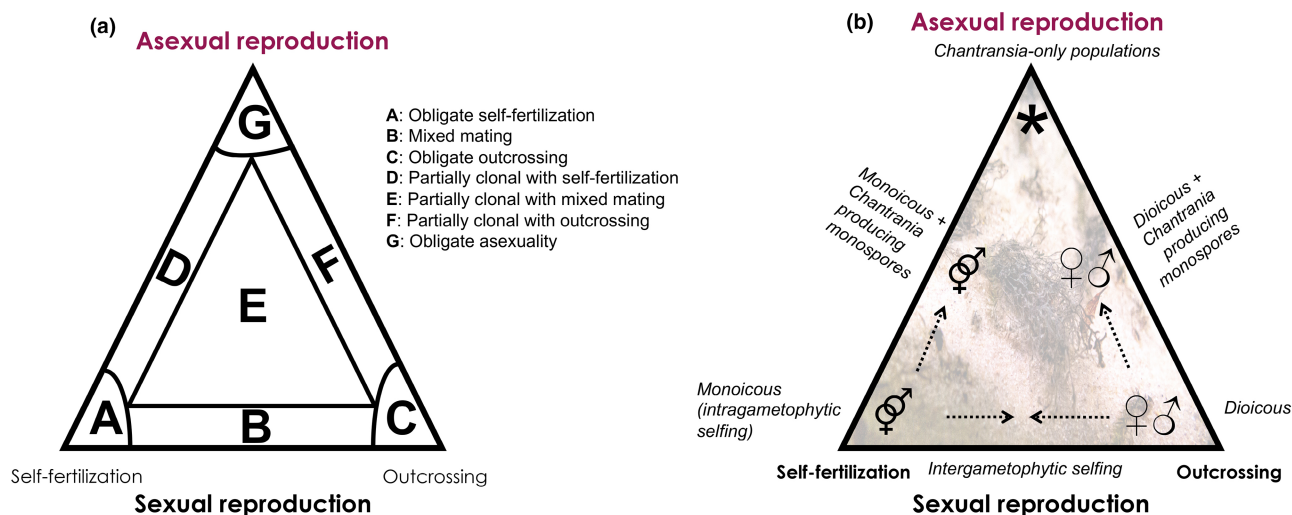


FIGURE 3 Variation in the reproductive system in freshwater red macroalgae adapted from Fryxell (1957) and Barrett (2011). (a) Each letter represents a specific reproductive system: A—obligate self-fertilization (or selfing); B—mixed mating with varying rates of self-fertilization to outcrossing; C—obligate outcrossing; D—partially clonal with varying rates of self-fertilization; E—partially clonal with mixed mating; F—partially clonal with varying rates of outcrossing; and G—obligately asexual. (b) In the Batrachospermales, we expect populations or species to be in the middle to bottom of the triangle when both gametophytes and chantransia are present. The rates of selfing or outcrossing will depend on whether the population or species is dioicous or monoicous, respectively. We expect natural populations to undergo mixed mating with varying rates of intergametophytic selfing, though we note that only monoicous gametophytes are capable of undergoing both intra- and intergametophytic selfing. Chantransia likely undergo vegetative meiosis (sexual) as well as producing monospores (asexual), and therefore we expect most freshwater red algae to be partially clonal, though rates of sexual vs. asexual reproduction will vary at a population level. For chantransia-only populations, we predict many signatures of asexual reproduction, such as many repeated genotypes. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/jpy.13407)]

that approaches enable the genotyping of every locus for each thallus to accurately assess clonal lineages. For microsatellites, fragment analysis on capillary sequencers are still efficient methods for genotyping thalli. Recent protocols, such as Lepais et al. (2020), have applied high throughput sequencing to 20–40 highly multiplexed microsatellites (e.g., SSRseq). For SNPs, Delord et al. (2018) developed a method that enables the genotyping of hundreds of loci, with estimate of genotyping error essential for accurately describing sexual versus asexual reproductive modes. Using either microsatellites or SNPs will enable analyses as reviewed in Krueger-Hadfield et al. (2021), in which indirect methods can describe the reproductive system. For example, Stoeckel et al. (2021) recommend calculating β , though no studies in algae have to date reported this measure of clonal distribution as previously advocated by Arnaud-Haond et al. (2007). Not only can this metric assess rates of clonality, but it can also assess the proportion of haploids. Based on available data from mosses and algae, Krueger-Hadfield et al. (2021) considered β values greater than 2 as indicative of low rates of clonality, values between 0.7 and 2 as partially clonal, and values less than 0.7 as highly clonal. Indirect methods, such as calculating β , can be complemented by paternity analyses (i.e., direct methods) to characterize the genotype of offspring (i.e., do offspring have the same genotype as the maternal thallus?), the number of sires per female, and

the level of relatedness among pairs of males and mating pairs (e.g., Engel et al., 1999; Krueger-Hadfield et al., 2015). Finally, temporal genotyping has been shown to improve assessments of the reproductive system, especially in partially clonal taxa. Although clonal rates can be approximated by estimating genotypic richness (i.e., R , the ratio of different genotypes to the total genotyped sample size), this approach is highly dependent on sampling strategy and sample size (Arnaud-Haond et al., 2007). Likewise, although population genetic indices, such as F_{IS} and linkage disequilibrium, may be used as a proxy, they are inaccurate for low to moderate clonal rates and do not disentangle the effects of selfing from clonality (Becheler et al., 2017). Instead, repeated genotyping is required to calculate genotype transitions from one generation to the next (Becheler et al., 2017). Gathering these types of data will allow us to answer questions such as the following:

Characterizing the reproductive system

- What are the relative rates of sexual versus asexual reproduction at the population and species levels?
- Do we observe evidence of repeated sporophyte (or chantransia) genotypes, and if yes, then can we distinguish between monospore production and cystocarpic reproduction (sensu Krueger-Hadfield et al., 2015)?

- To what extent are sporophytes heterozygous at the population and species levels?

Characterizing the mating system

- What are the relative rates of selfing versus outcrossing at the population and species levels?

Characterizing the influence of the sexual system on the mating system

- How do the rates of selfing versus outcrossing vary across species and populations depending on monoecy, dioecy, and trioecy?

Moreover, we can correlate phylogeographic patterns with the reproductive system of a given taxon, such as the role of geographic parthenogenesis (e.g., Baker's law, Baker, 1955) in understanding the discrepancy in the distribution between the geographically restricted *Boldia* and the more widespread *Compsopogon*. The ideas proposed here are nevertheless equally applicable to other haploid–diploid taxa, including green, brown, and red macroalgae. Investigating the sexual lives of these algae will enhance our understanding of evolution by encompassing eukaryotic diversity.

AUTHOR CONTRIBUTIONS

Stacy A. Krueger-Hadfield: Conceptualization (lead); funding acquisition (lead); supervision (equal); visualization (equal); writing – original draft (lead); writing – review and editing (equal). **Sarah J. Shainker-Connelly:** Data curation (equal); funding acquisition (supporting); visualization (supporting); writing – original draft (equal); writing – review and editing (equal). **Roseanna M. Crowell:** Data curation (equal); formal analysis (equal); visualization (supporting); writing – original draft (equal); writing – review and editing (equal). **Morgan L. Vis:** Conceptualization (equal); data curation (equal); formal analysis (lead); supervision (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal).

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