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A novel sexual system in male gametophytes of *Laminaria pallida* (Phaeophyceae)

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ABSTRACT

Brown algae (Phaeophyceae) exhibit a wide diversity of sexual systems, reproductive modes and life histories. The evolution of this diversity has often involved transitions between sexual systems, which may influence genetic diversity and diversification, and phenotypic evolution. This study reports a novel sexual system in kelp. We identified male gametophyte strains of *Laminaria pallida* that, despite presenting morphological features typical of a male, developed both antheridia and egg-like structures, which suggest an incomplete suppression of femaleness during sexual determination of gametophytes. The sperm released by these variant male gametophytes successfully fertilize eggs produced by female gametophytes, developing into sporophytes with normal morphologies. However, morphologically abnormal sporophytic offspring were formed in isolated variant male gametophytes, indicating that the egg-like structures were not successfully self-fertilized by sperm. The causes of the unsuccessful intragametophytic selfing are unknown, but absence of pheromone production by the egg-like structures is a hypothesis suggested by available evidence. Partial co-sexuality is clearly inherited, as all male gametophyte strains descended from the same sporophyte developed egg-like structures. Further investigation into the genetics and maturation of male partheno-sporophytes will help to estimate the potential effects of this mechanism on natural populations.

HIGHLIGHTS

- Morphologically typical male gametophytes in *Laminaria pallida* developed both antheridia and egg-like structures.
- Male sexual reproductive ability was retained by partially co-sexual *L. pallida* gametophytes.
- Partial co-sexuality appears to be heritable but varies between *L. pallida* individuals.

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KEYWORDS Brown alga; development; monoicy; parthenogenesis; sexual system; sporophyte formation

Introduction

Sexual reproduction involving meiosis and gamete fusion is an almost universal feature across eukaryotes (Goodenough & Heitman, 2014), but has long been a paradox due to the high costs associated with mating and recombination (Otto, 2009). The evolutionary maintenance of sex can be explained by the evolutionary advantages of genetic recombination, shuffling alleles to rapidly generate adapted genotypes, while also limiting the accumulation of deleterious mutations (Carvalho, 2003). Sexual reproduction can be mediated by a diversity of sexual systems that have evolved along the tree of life, becoming particularly complex in some groups of multicellular eukaryotes.

Brown algae (Phaeophyceae) are a group of photoautotrophic eukaryotes that arose as a multicellular lineage independently from animals and plants c. 250 Mya (Ahmed *et al*., 2014; Coelho *et al*., 2019; Bringloe *et al*., 2020). They exhibit a wide diversity of sexual systems, reproductive modes and types of life cycles (Cock *et al*., 2014; Bringloe *et al*., 2020; Heesch *et al*., 2021). The evolution of this diversity often involved transitions between sexual systems, which may influence genetic diversity, phenotypic evolution and patterns of diversification (Barrett, 2010). Dioicy (i.e. separate sexes during the haploid phase of the life cycle) appears to have been the ancestral state (Heesch *et al*., 2021), but there are many extant monoicous (i.e. co-sexual species with haploid sexual systems) examples. While transitions to co-sexuality have occurred frequently during eukaryotic evolution (Silberfeld *et al*., 2010; Heesch *et al*., 2021), the underlying mechanisms have been largely overlooked (Käfer *et al*., 2017). In dioicous diploid species, XX/XY or ZW/ZZ systems determine sex during the diploid phase of the life cycle, but in many other eukaryotes such as in haplo-diploid brown algae, male and female sexes can be determined during the haploid (gametophyte) phase (Coelho *et al*., 2018). In these dioicous species, sex is determined by U and V sex chromosomes that are present in the female and male gametophytes, respectively (Mignerot & Coelho, 2016; Coelho *et al*., 2018).

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The ecologically and economically important brown algae of the order Laminariales, are characterized by a heteromorphic haplodiplontic life cycle alternating between macroscopic diploid sporophytes and microscopic haploid stages (meiospores, gametophytes; Lüning, 1991; Liu *et al.*, 2017). They are all dioicous with rare observations of monoicous characteristics (e.g. *Undaria pinnatifida*; Shan *et al.*, 2021); mature diploid sporophytes produce haploid meiospores that differentiate into separate male and female gametophytes. During gametogenesis, female gametophytes form oogonia that release eggs. Sperm discharge from antheridia on the male gametophyte and their attraction to the egg is triggered when the latter release the pheromone lamoxirene (Maier *et al.*, 2001). Fertilization leads to the formation of a sporophyte, closing the haplodiplontic life cycle.

Although reported transitions to co-sexuality are extremely rare in kelps, a monoicous phenotype has been detected in some gametophyte lines of the kelp *U. pinnatifida* (Li *et al.*, 2014). Some mechanisms of asexual reproduction have been also reported in kelp species, including apomixis (formation of a new individual without meiosis or fertilization). Parthenogenesis, the development of a sporophyte from an unfertilized egg, is a common phenomenon in kelps (Lewis *et al.*, 1993; Gall *et al.*, 1996; Oppliger *et al.*, 2007; Shan *et al.*, 2013; Müller *et al.*, 2019, 2021). Unfertilized eggs develop into sporophytes of various ploidy (typically haploid, but also diploid or polyploid), which often exhibit irregular morphology and high rates of juvenile mortality (Pang & Tai, 1976). However, in some kelp species partheno-sporophytes exhibit normal morphology, reaching maturity and releasing meiospores which differentiate exclusively into female gametophytes (Lewis *et al.*, 1993). Other life-cycle variations reported in Laminariales include apogamy, where sporophytes develop from somatic cells of gametophytes (Nakahara & Nakamura, 1973; Fang, 1983; Müller *et al.*, 2019), and apospory, where a gametophyte is formed from vegetative cells of a sporophyte without meiosis or spore formation (Nakahara & Nakamura, 1973; Lewis *et al.*, 1993). Understanding the drivers and consequences of changes in sexual systems is a major question in evolutionary ecology, but also has important applied relevance for the vulnerability of species and populations to environmental change and for economic utilizations of these species. Although monoicous and parthenogenetic kelp strains in the seaweed aquaculture industry have recently emerged as a basis for crop improvement through breeding programmes producing genetically identical individuals with desired traits (reviewed in Hu *et al.*, 2021), they still represent a largely unexplored area of research.

These questions are highly relevant for the southeastern Atlantic *Laminaria pallida* (Greville), a kelp that is distributed throughout the southwestern coast of Africa from northern Namibia to southern South Africa (Assis *et al.*, 2022), and which provides a variety of ecosystem services of economic and ecological value (Critchley *et al.*, 1991; Blamey & Bolton, 2018). In this species, we have identified variant male strains derived from one sporophyte, which form egg-like structures and sporophytes under gametogenic conditions, although showing morphological features typical of a male. Since evidence of transitions from dioicy to monoicy are scarce in kelps, this study aims to (i) characterize the morphology and gametogenesis in the variant male strains, (ii) test the occurrence of pseudo-monoicy within a family line (male and female siblings gametophytes), and (iii) investigate if, in the presence of female gametophytes, variant male strains develop functional antheridia and sperm capable of fertilization, and/or form egg-like structures.

Materials and methods

Algal material

Mature sporophytes of *L. pallida* were sampled from Swakopmund, Namibia (22.672′S, 14.522′E) in July 2019. Sori were cleaned and meiospores from five sporophytes were released separately in sterile seawater. After spore germination, several male and female gametophytes were isolated from each individual to establish unisexual gametophyte stock cultures. Cultures were maintained in a vegetative state in sterile half-strength Provasoli enriched seawater (PES, Provasoli, 1968) at 12°C under 3 µmol photons m^{-2} s^{-1} of red light and 16 h:8 h light:dark (L:D) cycle in a climate-controlled chamber (Fitoclima S600, Aralab, Lisbon, Portugal). Sterile artificial seawater (Tropic Marin Sea Salt, Wartenberg, Germany) with a salinity of 34 ± 1 psu was used for maintenance and all experiments. The culture medium was changed monthly until the beginning of the experiment (c. 20 months).

Reproductive development in pseudo-monoicous gametophytes

The reproductive development of eight potentially variant male (A.1, A.2, A.3, A.7, A.9, A.10, A.12, A.13) and four female (A.6, A.10, A.11, A.12) gametophyte strains derived from sporophyte A, and nine control male (C.2, C.3, C.4, D.1, D.2, D3, F.2, F.3, F.4) gametophyte strains derived from three sporophytes C, D and F was monitored. Reproductive performance was assessed by comparing crosses performed between female gametophytes derived from independent sporophytes and both ‘variant’ and ‘normal’ male gametophytes. Specifically, the same eight potentially variant male gametophytes from sporophyte A (A.1, A.2, A.3, A.7, A.9, A.10, A.12, A.13) and three control male gametophytes derived from sporophyte C (C.2, C.3, C.4) were crossed with a pool of six female gametophyte strains derived from three individuals (D, E, F; 2 female strains per sporophyte) to avoid the possible effects of selfing.

Gametophytes were gently ground using a pestle and mortar, sieved and diluted in half-strength PES to produce stock solutions of gametophytes with lengths of ≤ 100 µm. These were used to set up single-sex cultures and crosses. Crosses were prepared by combining stock solutions of each single-strain male with the same pooled mix of females (six strains). Densities from each stock solution were calculated, and the volume needed to achieve a total density of ~400 gametophytes cm^{-2} was added to Petri dishes (5.3 cm diameter, height 1.5 cm) containing 10 ml of half-strength PES. Four replicate Petri dishes were used for each male and female gametophyte strain derived from individual A (12 strains × 4 replicates = 48 Petri dishes) and per cross (11 crosses × 4 replicates = 44 Petri dishes). The gametophytes were allowed to recover from the mechanical stress induced by fragmentation for 2 days at 14°C under 3 µmol photons m^{-2} s^{-1} of red light in a 16 h:8 h L:D cycle. After this period, the gametophytes were transferred to 18 µmol photons m^{-2} s^{-1} of white light to induce gametogenesis. A temperature of 14°C was chosen as it provides optimal gametogenic conditions (tom Dieck & de Oliveira, 1993; Martins *et al*., 2019). The culture medium was refreshed every 10 days by the replacement of 5 ml of half-strength PES per Petri dish.

The reproductive development of gametophytes was followed by photographing randomly selected replicates of isolated gametophyte cultures and crosses every 2 days during the course of the experiment (26 days) using a Nikon D90 camera (Nikon, Tokyo, Japan) mounted on a Zeiss Observer D1 inverted microscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany). The diameter of eggs in female gametophytes and egg-like structures in variant male strains was measured by processing photographic data with ImageJ software (Schneider *et al*., 2012) and statistically compared with a t-test corrected for heteroscedasticity in the R Statistical Environment (R Core Team, 2021). Additionally, the relative occurrence of three ontogenetic stages (variant and control male gametophytes: vegetative, antheridia, sporophytes; female gametophytes: vegetative/oogonia, eggs released and sporophytes) was quantified after 20 days in gametogenic conditions in a minimum of 200 gametophytes per replicate using a Zeiss Observer D1 inverted microscope. The most advanced developmental stage was assigned for each gametophyte. Sporophytes were considered present as soon as the first cell division was visible in the zygote.

The reproductive success of female gametophytes cultivated in the presence of variant and 'normal' males was evaluated through the absolute number of sporophytes after 26 days. The number of sporophytes per cm^{-2} was quantified in ≥ 50 fields of view (Zeiss Observer D1 inverted microscope, 100× magnification) per replicate. Sporophyte densities were statistically compared between crosses by one-way ANOVA in the R Statistical Environment (R Core Team, 2021). Data were tested for normality within groups using the Shapiro-Wilk test and homoscedasticity using Levene's test, and post-hoc Tukey tests were conducted to determine differences between crosses ($p < 0.05$).

Results

Morphological and reproductive characterization of Laminaria pallida *variant gametophytes*

Male and female gametophytes derived from sporophyte A displayed clear sexual dimorphism in cell size, colour and shape and branching pattern (Fig. 1A,B), as expected in kelps (Müller *et al*., 1979; Luthringer *et al*., 2015; Mignerot & Coelho, 2016). Female gametophytes were characterized by large brown cells with round or ovoid shapes and filaments with few branches (Fig. 1A), whereas males were pale brown and displayed highly branched filaments formed by small cells (Fig. 1B). These morphological differences make them unambiguously identifiable and show that the variant male gametophytes exhibit phenotypic features typical of a 'normal' male (Fig. 1C).

Following the induction of gametogenesis, isolated female gametophytes underwent limited gametogenesis (per cent fertility 5–18% after 20 days), formed oogonia and released eggs that developed into sporophytes via parthenogenesis due to the absence of fertilization by males (Figs 1D, 2). Partheno-sporophytes exhibited irregular morphology (Fig. 1D), as previously reported in *L. pallida* and other kelp species (tom Dieck, 1992; Martins *et al*., 2019).

Male gametophytes derived from sporophyte A developed antheridia and egg-like structures on the same fragment under gametogenic conditions, both when isolated and when crossed with a pool of female gametophytes (Figs 1E, 3), suggesting pseudo-monoicy. Male egg-like structures had diameters averaging 26.6 µm ($n = 11$), while eggs released by female gametophytes were significantly smaller ($t = -4.304$, $p = 0.001$) averaging 19.8 µm in diameter ($n = 20$). The male egg-like structures underwent cell division (Fig. 1F) developing into multicellular sporophytes with irregular and bulky morphologies (Fig. 1G), strongly resembling the partheno-sporophytes in

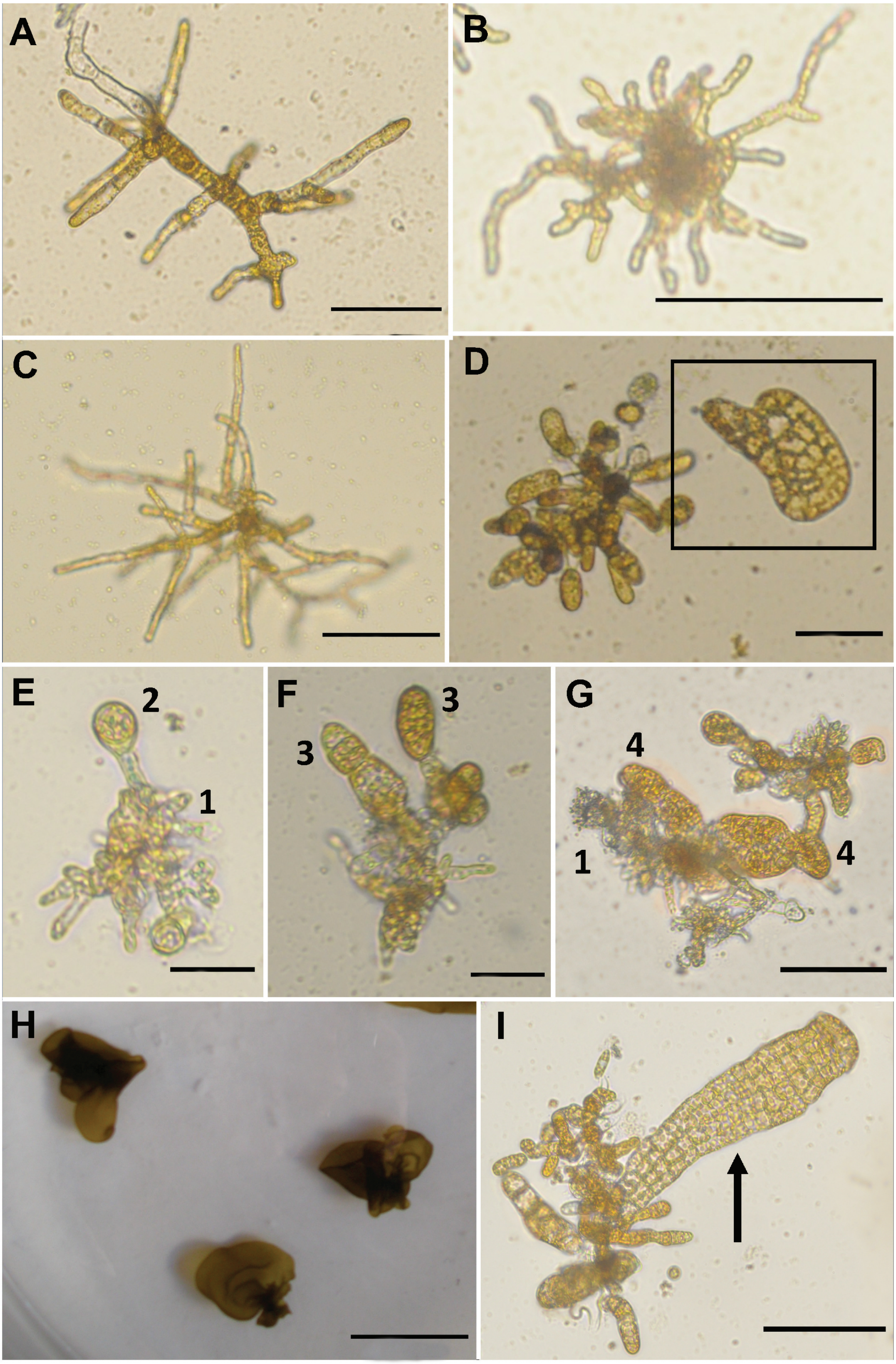

Fig. 1. Morphological characterization of female and male gametophytes of *Laminaria pallida* and the developed eggs and sporophytes. (A) Vegetative female gametophyte isolated from sporophyte A. (B) Vegetative male gametophyte isolated from sporophyte A. (C) Vegetative control male gametophyte isolated from sporophyte C. (D) Partheno-sporophyte

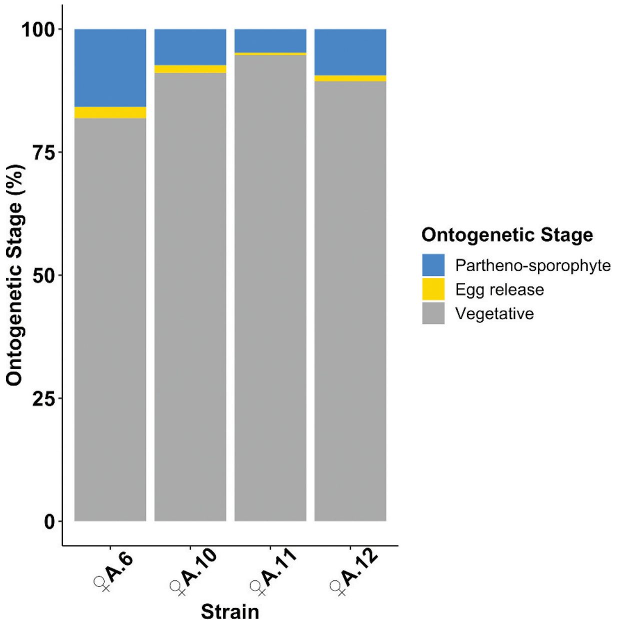

Fig. 2. Development of gametogenesis stages in different female gametophyte strains of *Laminaria pallida* derived from sporophyte A after 20 days in gametogenic conditions. 100% stacked column chart with means of each ontogenetic stage (n = 4). SE values are omitted for clarity.

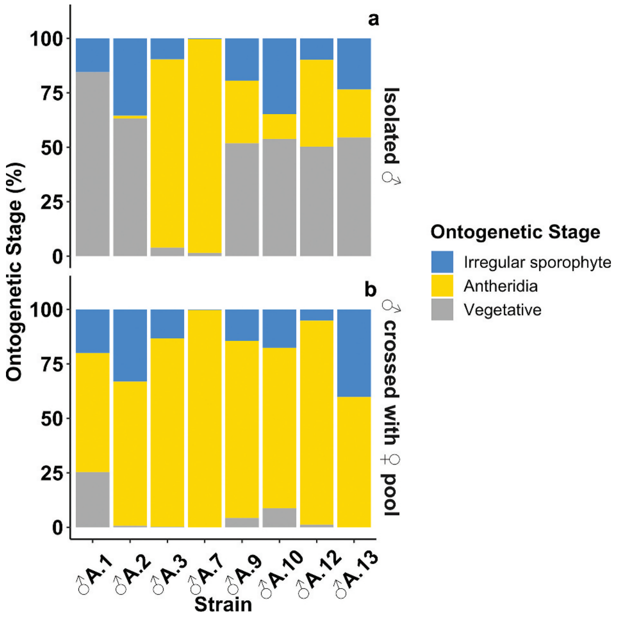

Fig. 3. Development of gametogenesis stages in different male gametophyte strains of *Laminaria pallida* derived from sporophyte A after 20 days in gametogenic conditions when isolated (A) and when crossed with a pool of female gametophytes (B). 100% stacked column charts with means of each ontogenetic stage (n = 4). SE-values are omitted for clarity.

isolated female gametophytes (Fig. 1D). These irregular sporophytes further developed to macroscopic size (Fig. 1H). Isolated control male gametophytes did not develop egg-like structures or irregular sporophytes but did develop antheridia in higher proportions (2.4-fold) than isolated males derived from sporophyte A (Supplementary figs S1, S2).

In general, all the male gametophyte strains derived from sporophyte A had attached sporophytes developed from egg-like structures (Fig. 3). Only the male strain A.7 showed extremely limited sporophyte formation (derived from egg-like structures). Proportions of male gametophytes with attached sporophytes differed between strains but were generally similar for each strain whether isolated or crossed with a pool of female gametophytes. Overall, the male gametophyte strains developed more antheridia in the presence of female gametophytes than when isolated (Fig. 3), suggesting a female influence on male antheridia formation.

Variant male gametophytes produce functional sperm

When a pool of female gametophytes was crossed with male gametophytes isolated from sporophyte A, healthy sporophytes with normal morphologies were produced (Figs 1I, 4), indicating the successful fertilization of eggs by sperm released from pseudo-monoicous males. Sporophyte density differed significantly between the crosses (Fig. 4; $F = 47.52$, $p < 0.001$). In general, most of the variant male gametophyte strains led to the production of similar sporophyte densities compared with ‘normal’ male gametophyte strains, except for variant male strains A.1 and A.13. Interestingly, the greatest sexual (i.e. from crossing) sporophyte densities were produced by variant male strains A.3 and A.7 (Fig. 4), which showed the lowest numbers of egg-like structures and irregular sporophytes (Fig. 3A), suggesting a negative correlation between the ability to form egg-like structures and the release of functional sperm.

Discussion

This study identified vegetatively typical male gametophyte strains derived from a *L. pallida* sporophyte that produced both antheridia and egg-like structures under gametogenic conditions, suggesting monoicous variant lines. The sperm released by the variant male

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produced by a female gametophyte. (E, F, G) Fertile variant male gametophytes isolated from sporophyte A showing antheridia (1), egg-like structure (2), sporophytes with cell division (3) and dimorphous sporophytes attached (4). (H) Partheno-sporophytes from variant male gametophytes isolated from sporophyte A, showing irregular morphology. (I) Juvenile sporophyte (arrow) with normal morphology derived from the cross between a variant male gametophyte isolated from sporophyte A and a pool of female gametophytes. Scale bars: A–C, I: 100 µm; D: 60 µm; E-F: 30 µm; G: 70 µm; H: 20 mm.

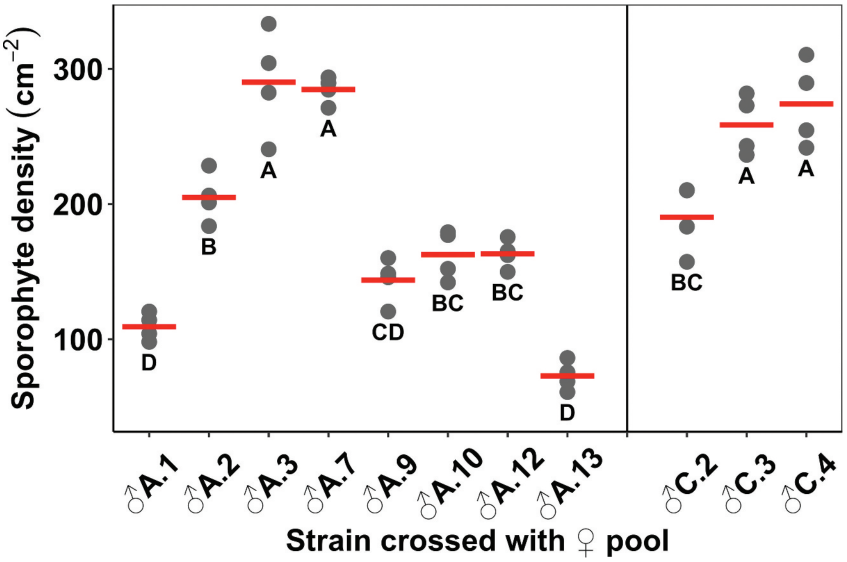

Fig. 4. Reproductive success of different male strains of *Laminaria pallida* crossed with a female pool, after 26 days in gametogenic conditions. Dots representing each datapoint, with horizontal bar showing the mean for each group (n = 4). Panels separate variant male gametophytes isolated from sporophyte A from control male gametophytes derived from sporophyte C. Different letters indicate significant differences between crosses ($p < 0.05$).

gametophytes successfully fertilized eggs produced by female gametophytes, developing into sporophytes with normal morphologies. Similarly, a monoicous phenotype has been previously detected in some morphologically male gametophyte lines of *U. pinnatifida* cultivars (Li *et al.*, 2014), with genetic analysis indicating the absence of a female genome (Li *et al.*, 2014, 2017). Under gametogenic conditions these genetically male gametophytes developed both male and female reproductive structures producing sperm and eggs, respectively.

Although male function appears intact, there is reason to suspect that a full transition to monoicy may not have occurred. Morphologically abnormal sporophytic offspring were formed in isolated variant male gametophytes, indicating parthenogenesis and therefore that egg-like structures were not successfully self-fertilized by spermatozoids. Although sex-specific markers have been developed for kelp species (Lipinska *et al.*, 2015) to genetically discriminate between diploid sporophytes generated from sexual reproduction and partheno-sporophytes, no amplification of sex-specific bands was successful in *L. pallida* gametophytes and sporophytes using these PCR-based markers. Sexual reproduction generates normal diploid sporophytes, while parthenogenesis might produce haploid, diploid or polyploid sporophytes with abnormal morphology and high mortality in early stages (Lewis *et al.*, 1993; Gall *et al.*, 1996). The results suggest partial pseudo-monoicy in *L. pallida* with a reproductive system closer to female parthenogenesis.

The absence or low levels of sex pheromone production by the egg-like structures might be a potential explanation for the unsuccessful intragametophytic selfing in our study, as male gamete attraction is vital for effective sexual reproduction (Frenkel *et al.*, 2014). It was recently shown in the giant kelp *Macrocystis pyrifera* that although female morphological features can be expressed in a genetically male strain in the absence of the U chromosome, the U-specific region may be required to fully express the female developmental programme as the gametes of feminized males are unable to produce the sperm-attracting pheromone lamoxirene and developed into partheno-sporophytes (Müller *et al.*, 2021). These gametes were also smaller in size than female eggs until they started parthenogenetic development. In contrast, our results suggest that male egg-like structures are larger than female eggs. Most of the measured structures appeared bloated, with internal structures similar to early stages of parthenogenetic development. The large size of these structures may also be a sign of abnormal processes hindering sexual reproductivity, or that there is little to no delay before parthenogenesis is triggered in these egg-like structures. The spontaneous loss of sex pheromone production triggered the production of parthenogens in two lineages of the brown alga *Scytosiphon lomentaria* from the Sea of Japan coast and from the Pacific coast (Hoshino *et al.*, 2021). Other processes that could potentially also cause unsuccessful development of embryos seem unlikely due to successful development of the fertilized eggs from the female gametophytes in the same conditions.

All the male gametophytes derived from sporophyte A exhibited a pseudo-monoicous phenotype, suggesting that this trait is inherited. Pseudo-monoicy was also found to be heritable in the kelp

U. pinnatifida (Shan & Pang, 2021). The pseudo-monoicous phenotype may be caused by a mutation or lesion in a gene or regulatory region of the V chromosome, and the pseudo-monoicous phenotype is therefore likely to be genetically associated with the male-determining system (Coelho *et al*., 2019). The sporophyte generating the pseudo-monoicous gametophytes in this study was sampled from Namibia in the northern distributional range of the species, while male gametophyte strains with a similar phenotype were previously reported from a South African population (I. Maier pers. comm.; Coelho *et al*., 2019). The limited gene flow between these regions (Assis *et al*., 2022) and the occurrence of pseudo-monoicy in two widely distant populations suggests that such variants are widespread and may be selectively maintained in local populations. Brown algal U/V haploid systems are characterized to date by relatively small non-recombining sex-determining chromosomal regions, with symmetrical evolution and low degeneration of the U and V chromosomes (Ahmed *et al*., 2014; Immler & Otto, 2015). In the brown algal model species *Ectocarpus*, genetic analysis has shown dominance of the V (male) chromosome over the U (female), suggesting that the default gender of gametophytes is female, which is switched off by male expression (Ahmed *et al*., 2014). Incomplete suppression of femaleness may lead to the expression of both male and female phenotypes in the same gametophyte, as has been recorded in *U. pinnatifida* (Li *et al*., 2014). The monoicous gametophytes of *U. pinnatifida* developed both oogonia and antheridia and were able to undergo reproduction via intragametophytic selfing, producing healthy and fertile sporophytes that successfully mature and release spores that developed only in male gametophytes (Li *et al*., 2014).

Variation between variant male strains in both development of pseudo-parthenosporophytes and reproductive success points to genetic variation for pseudo-monoicous phenotype and suggests that pseudo-monoicy is a polygenic trait. Of note, the two male strains exhibiting the lowest percentages of partheno-sporophytes derived from egg-like structures lead to the highest production of normal diploid sporophytes when crossed with female gametophytes, suggesting that female expression might negatively affect the male gamete production. While most strains presented high antheridia development when crossed with females, the energy expense of female expression may lead to lower sperm count or quality. It appears that potential deactivation of female suppression comes at a limited cost to male gametogenesis.

Brown algae exhibit a remarkable degree of reproductive plasticity during their life history (Bothwell *et al*., 2010; Coelho *et al*., 2011). This might be related to their capacity to adapt to new environmental conditions, which is particularly important in regions with few individuals and/or scattered populations, where the relative energetic costs of mating are high. Changes in the reproductive system are often associated with range expansions (e.g. Krueger-Hadfield *et al*., 2016), marginal habitats (Tatarenkov *et al*., 2005), limits of distribution (Oppliger *et al*., 2014) and environmental changes (Coleman & Wernberg, 2018). In the brown alga *Fucus radicans*, asexual populations maintained by clonal reproduction of adventitious branches tend to be distributed in lower salinity areas than sexual populations (Ardehed *et al*., 2015). In Japan, parthenogenetic populations of *Scytosiphon lomentaria* were biased to colder waters and more wave-exposed areas compared with sexual populations inhabiting the coast along warm ocean currents (Hoshino *et al*., 2021). A southern-limit population of *L. digitata* showed a high propensity for producing unreduced spores, a consequence of life in a marginal habitat (Oppliger *et al*., 2014). Monoicous and parthenogenetic species have the advantage of expanding populations from a single founding individual. Therefore, it would be interesting to understand whether the pseudo-monoicous *L. pallida* strains found near the northern distributional edge play an adaptive role in marginal ecological niches, contributing to the geographic distribution of the species over evolutionary time. Genetic studies (e.g. PCR-based sex markers) covering the entire *L. pallida* distributional area will help to clarify how common pseudo-monoicous specimens are in the field and if they are geographically limited to marginal habitats.

The discovery of new sexual systems may present opportunities in the development of aquaculture of *L. pallida*. This kelp is harvested from wild stocks or beach cast collected for abalone feed, alginate extraction and the production of growth stimulants for agriculture (Rothman *et al*., 2020; Msuya *et al*., 2022). There is increasing commercial interest in pursuing kelp aquaculture in the African region (Msuya *et al*., 2022) and previously a novel breeding method using monoicy and parthenogenesis for crossbreeding was developed for the kelp *U. pinnatifida* (Li *et al*., 2017; Shan & Pang, 2021). Parthenogenesis-derived female spores were crossed with male spores obtained from a monoicous-derived male sporophyte generating genetically identical sporophytic offspring. This novel method employing spores from unisexual sporophytes is an important tool in modern kelp aquaculture, since it stabilizes desirable phenotypes such as high productivity and increased stress resistance (Shan *et al*., 2019; Shan & Pang, 2021). Thus, future work cultivating female and male partheno-sporophytes of *L. pallida* into maturity are clearly needed to check the possibility of employing this new breeding method in this important African kelp species. Additionally, further studies should also focus on proving insight into the driving forces and the potential ecological benefits or consequences of male asexual reproduction in *L. pallida* to accurately design policies for management.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Supplementary information

The following supplementary material is accessible via the Supplementary Content tab on the article's online page at https://doi.org/10.1080/09670262.2024.2314487

Supplementary figure S1. Development of gametogenesis stages in different control male gametophyte strains of *Laminaria pallida* derived from sporophytes C, D and F after 20 days in gametogenic conditions when isolated. 100% stacked column charts with means of each ontogenetic stage (n = 4). SE values are omitted for clarity.

Supplementary figure S2. Morphological characterization of control male gametophytes of *Laminaria pallida* derived from sporophytes C (picture A), F (B) and D (C). All gametophytes show antheridia (arrows), but no egg-like structure or partheno-sporophytes. Scale bars: 100 µm.

Author contributions

P. Liboureau: original concept, experimental design and work, data analysis, drafting and editing manuscript; G.A. Pearson: original concept, supervision and editing manuscript; E.A. Serrão: sampling and editing manuscript; N. Martins: original concept, experimental design, supervision and editing manuscript.

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