1   # Evolution of life cycles and reproductive traits: insights from the brown algae

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13   ## ABSTRACT

14   Brown algae are characterized by a remarkable diversity of life cycles, sexual systems, and

15   reproductive modes, and these traits seem to be very labile across the whole group. This

16   diversity makes them ideal models to test existing theories on the evolution of alternation

17   between generations, and to examine correlations between life cycle and reproductive life

18   history traits. In this study, we investigate the dynamics of trait evolution for four life-history

19   traits: life cycle, sexual system, level of gamete dimorphism and gamete parthenogenetic

20   capacity. We assign states to up to 70 species in a multi-gene phylogeny of brown algae, and

21   use maximum likelihood and Bayesian analyses of correlated evolution, taking phylogeny into

22   account, to test for correlations between life history traits and sexual systems, and to

23   investigate the sequence of trait acquisition. Our analyses are consistent with the prediction

24   that diploid growth may evolve because it allows the complementation of deleterious

25   mutations, and that haploid sex determination is ancestral in relation to diploid sex

26   determination. However, the idea that increased zygotic and diploid growth is associated with

27   increased sexual dimorphism is not supported by our analysis. Finally, it appears that in the

28   brown algae isogamous species evolved from anisogamous ancestors.

29   ## INTRODUCTION

30   The life cycle of an organism is one of its most fundamental features and influences the

31   evolution of a variety of traits, including mode of reproduction, developmental processes,

32   mode of dispersal, adaptation to local environment and ecological success. A wide variety of

33   different life cycles are found within eukaryotes, and one of the great challenges of

34   evolutionary biology is to understand how this diversity has evolved, and how each type of life

35 cycle is stably maintained on an evolutionary timescale (Valero et al. 1992; Mable and Otto
36 1998; Otto and Gerstein 2008; Cock et al. 2014).

37 The sexual life cycle of eukaryotes involves the fusion of two gametes to form a zygote,
38 followed by meiosis. Such life cycles can be divided into three main types (Bell 1982; Valero et
39 al. 1992; Coelho et al. 2007; Otto and Gerstein 2008). In organisms with haplontic cycles,
40 mitosis only occurs during the haploid phase of the life cycle, and syngamy is followed
41 immediately by zygotic meiosis, without any mitotic division of the zygote. In diplontic life
42 cycles, mitosis occurs exclusively in the diploid phase, and meiosis takes place immediately
43 before gamete formation. Between these two 'extremes' lie diplohaplontic (or haploid-
44 diploid) life cycles, in which mitosis occurs both during the haploid and diploid phases. Both
45 phases of the cycle may remain unicellular (e.g. budding yeast) or develop into multicellular
46 organisms during either one phase (e.g. some fungi genera like *Ustilago*) or during both
47 phases (brown, green, red macroalgae). In photosynthetic organisms, multicellular haploid
48 phases are usually termed gametophytes, with gametes formed in specific organs called
49 gametangia. Diploid mitosis leads to asexual reproduction in unicellular lineages (e.g.
50 diatoms) and to somatic growth and differentiation in multicellular organisms such as vascular
51 plants. In photosynthetic organisms, multicellular diploid phases are called sporophytes, and
52 haploid meiospores are produced via meiosis. Diplohaplontic life cycles may be iso- or
53 heteromorphic. For the latter, the dominant phase may be haploid (such as in mosses) or
54 diploid (such as in vascular plants and kelps). Asymmetry in terms of the length and
55 complexity of the haploid and diploid phases can be very strong and can eventually lead to
56 transitions towards diplontic or haplontic life cycles.

57 The structure of an organism's life cycle also has important consequence for the evolution of
58 its sex determination system (Coelho et al. 2018). Haploid sex determination is common in
59 diplohaplontic lineages such as in brown algae, where either a single gametophyte produces
60 gametes of both sexes (a monoicous system, Table 1), or male and female gametes are
61 produced by different haploid gametophytes (dioicous systems, e.g. in mosses and kelps). In
62 gymnosperms and angiosperms (which also have diplohaplontic life cycles), sex is determined
63 in the diploid phase (since sexual differentiation occurs when male and female reproductive
64 organs develop), and the organism may be co-sexual (monoecious or hermaphroditic) if a
65 single sporophyte produces female and male gametes (eggs and sperm) or dioecious if male
66 and female gametes are produced by two different individuals. Correlations between the type
67 of sexual system and life history features such as spore size, antheridium number, ploidy level
68 and diversification rate are relatively well studied in angiosperms and mosses (Villarreal and

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69 Renner 2013; Goldberg et al. 2017) but studies of other eukaryotic groups are virtually
70 inexistent.

71 One important feature of sexual life cycles in eukaryotes is the degree of similarity between
72 male and female gametes. This 'gamete dimorphism' is a continuous trait, and a number of
73 models have been proposed to explain how anisogamous organisms could evolve from an
74 isogamous ancestor (Charlesworth 1978; Hoekstra 1980; Randerson and Hurst 2001). The
75 evolution of anisogamy establishes the fundamental basis for maleness and femaleness, and
76 leads to an asymmetry in resource allocation to the offspring, leading in many cases to sexual
77 selection (Billiard et al. 2011). Anisogamy and oogamy have arisen repeatedly across the
78 eukaryotes, and these systems are thought to be derived from simpler isogamous mating
79 systems, either due to disruptive selection generated by a trade-off between the number of
80 offspring produced and offspring survival (e.g., Charlesworth 1978; Parker 1978; Bell 1982;
81 Bulmer and Parker 2002), to selection to maximize the rate of gamete encounter (e.g., Cox
82 and Sethian 1984; Hoekstra 1987; Dusenbery 2000; Togashi et al. 2012) or as a mechanism to
83 reduce cytoplasmic conflicts (e.g., Hurst & Hamilton 1992, Hutson & Law 1993, Hurst 1995).

84 Differences in gamete size in anisogamous and oogamous species may influence other
85 reproductive characteristics. In particular, gamete size may be one of the factors that
86 determines whether a gamete is capable of undergoing asexual reproduction through
87 parthenogenesis, should it fail to encounter a gamete of the opposite sex (Hoekstra 1980,
88 Billiard et al 2011). Parthenogenesis is a form of asexual reproduction, in which gametes
89 develop in the absence of fertilisation, and is commonly found in land plants, algae and
90 invertebrate organisms, as well as in a number of vertebrate species (e.g. Dawley and Bogart
91 1989). In animals and land plants, parthenogenesis has been mostly described for females
92 only, but in organisms with moderate levels of gamete dimorphism such as some brown
93 algae, development from both male and female gametes in the absence of fertilisation is
94 quite common, at least under laboratory conditions (e.g. Oppliger et al. 2007; Bothwell et al.
95 2010).

96 The different types of life cycles have evolved independently and repeatedly in different
97 eukaryotic groups, and this is also the case for the types of sexual systems. Testing
98 evolutionary hypotheses regarding the causes and consequences of life history trait diversity
99 requires data from multiple species placed in a phylogenetic context. Such comparative
100 studies have been hampered by a lack of accessible data regarding life cycles, sexual systems
101 and sex determination mechanisms across the eukaryotic tree of life, and most specifically in
102 groups outside animals and land plants. While knowledge has been recently growing on the
103 green lineage with studies extending to bryophytes and volvocine algae (Villarreal and Renner

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104   2013; Hanschen et al. 2018), we still lack views on other eukaryotic groups, that should help
105   us understand the general principles underlying the evolution of these traits.

106   The brown algae represent a fascinating group for studies of the evolution of life cycles and
107   reproductive traits (Bell 1997; Clayton 2009). Brown algae exhibit a remarkable range of life
108   cycle and sexual traits. Most brown algae have diplohaplontic life cycles, in which a haploid
109   gametophytic generation alternates with a diploid sporophytic generation (Figure 1). These
110   two generations show variable levels of dimorphism, ranging from isomorphic (with
111   gametophytes and sporophytes of the same size and appearance) to strongly heteromorphic
112   (where the generations are of different size and/or appearance) with either a dominant
113   gametophytic or a dominant sporophytic generation. In two orders, the Fucales and the
114   Tilopteridales, diplontic life cycles have evolved, with all members of the Fucales being
115   diplonts, but only a single species in the Tilopteridales. In diplonts, the gametophytic
116   generation is reduced to a single cell (the gamete), male and female gametes being released
117   directly from the diploid sporophyte, either from the same (monoecy/hermaphroditism) or
118   from two separate thalli (dioecy). In contrast, in all other sexual brown algal species, sexuality
119   is expressed in the haploid phase (gametophyte), with male and female gametes either
120   produced on the same thallus (monoicy) or on two separate male and female gametophytes
121   (dioicy) (Silberfeld et al. 2010; Luthringer et al. 2015). Here, we will use here the term
122   'monoecy' to describe Fucales species that are co-sexual, although the term hermaphrodite is
123   also employed in the literature, because male and female structures are produced in the
124   same conceptacle.

125   In 1997, Bell used the diversity of life cycles within the brown algae to test hypotheses on the
126   evolution of life cycles; in particular, whether evolution generally proceeds towards an
127   increase of the diploid phase at the expense of the haploid phase (Clayton, 1988), and
128   whether a positive association between a prolonged haploid phase and the rate of inbreeding
129   (as predicted by theories based on the effect of deleterious alleles, Otto and Marks 1996) is
130   observed (using gametophyte monoicy as a proxy for inbreeding by assuming that
131   gametophytic selfing may occur). However, his study was based on a phylogenetic tree
132   including only 14 species, and evolutionary relationships between brown algal orders were
133   poorly resolved, making it difficult to test his assumptions.

134   In this study, we have exploited a well-resolved phylogeny of 70 species of brown algae
135   (Silberfeld et al. 2010) to understand how life cycle and reproductive traits evolve across the
136   brown algae. We performed an extensive literature review to recover information for life
137   cycle and reproductive traits across the brown algae (Supplemental Dataset). We estimated
138   ancestral states for each of the traits, as well as the number of transitions between states and

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139 their relative timing, and assessed correlations between the life cycle and reproductive traits.
140 These analyses have allowed us to describe the evolution of life cycles and reproductive traits
141 across the brown algal phylogeny, and to assess a number of long standing hypotheses about
142 the evolution of life cycle and reproductive traits such as: 1) the possibility that diploid growth
143 evolved because it allows the complementation of deleterious mutations (Crow and Kimura
144 1978, Perrot et al. 1994, Jenkins and Kirkpatrick 1995, Otto & Goldstein 1992, Otto & Marks
145 1996), 2) that increased zygotic and diploid growth is associated with increased sexual
146 dimorphism (Bell, 1994), 3) that haploid sex determination is ancestral in relation to diploid
147 sex determination, 4) that anisogamous species evolved from isogamous ancestors (Parker et
148 al 1972, Bell 1978, Charlesworth 1978). We also test additional hypotheses, including the
149 possibility that gamete size influences the capacity for asexual reproduction through
150 parthenogenesis (Luthringer et al. 2015), and we discuss the macro-evolutionary dynamics of
151 transitions between sexual systems in the brown algae.

152 RESULTS

Ancestral state estimations and transitions between states

154 We used sequence data from 131 brown algae species to infer a phylogenetic tree (Figure 2).
155 We estimated the ancestral state of each of the four main sexual traits: type of life cycle, type
156 of sexual system, level of gamete dimorphism, and parthenogenetic capacity. Definitions of
157 the life cycle and sexual terms used in this study are provided in Table 1. Our ancestral state
158 reconstructions inferred equal rates of transition (ER model) between states for all traits,
159 except for the trait 'sexual system' where rates were different between states but
160 symmetrical (SYM model gain or loss of a trait). These patterns indicate an overall complex
161 evolutionary history for all sexual traits, involving multiple gains and losses (Figure 3, Table 2).

162 **Life cycle**. On the basis of ancestral state reconstructions, the ancestor of all brown algae had
163 a diplohaplontic life cycle, with either isomorphic multicellular generations or with a larger
164 and morphologically more complex diploid than haploid generation (Figure 3A, Table 2).
165 Transitions between life cycles occurred most frequently from diploid-dominant to equally
166 dominant generations (i.e., involving a decrease in complexity of the sporophyte and
167 concomitant increase in the complexity of the gametophyte; Figure 3A). A change of
168 dominance from a diploid-dominant to a haploid-dominant life cycle occurred for the first
169 time at least 57.5 (±5.05) My ago (Figure 3A-B). Transitions from a diploid-dominant to a fully
170 diploid life cycle occurred twice, about 74.5 (±21.41) My ago, in the ancestor of *Tilopteris*
171 *mertensii* (Turner) Kützing and in the ancestor of the diploid order Fucales. Note however that

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172   *Tilopteris mertensii* is a rather particular case within Tilopteridales (Kuhlenkamp and Müller

173   1985) and emergence of monoecy in this species should be interpreted with caution.

174   Overall, our analysis indicated that the dominance relationship between life cycle generations

175   has been a labile trait in the brown algae, the diplontic life cycle being the only irreversible

176   state.

177   **Sexual system**. The ancestral brown alga is predicted to have exhibited haploid sex

178   determination and was most likely dioicous (separate sexes during the haploid phase) (Figure

179   3A-D, Table 2), but several independent transitions towards monoicy have occurred (Figure

180   3C-D). The transition from haploid to diploid sex determination, which involved a transition

181   from dioicy (separate sexes on different haploid gametophytes) to monoecy (both sexes on

182   the same diploid sporophyte), occurred about 74.5 My ago. This transition was simultaneous

183   with the transition from a diplohaplontic to a diplontic life cycle (Figure 3B, 3D). Separate

184   sexes during the diploid stage of the life cycle (dioecy) emerged more recently, around 17.5

185   Mya, in families of the order Fucales, with the exception of the Sargassaceae and

186   Notheiaceae, which remained monoecious. Further transitions back to monoecy occurred in

187   several genera of the Fucaceae (Xiphophora, Pelvetia and Fucus) (Table 2, Figure 3C-D).

188   Overall, our analysis suggests that the transition to diploid sex determination is irreversible

189   and concomitant with a change in type life cycle (from diplohaplontic to diplontic life cycle). In

190   contrast, transitions between separate sexes and combined sexes occurred frequently, either

191   in the haploid or in the diploid phase.

192   **Sexual dimorphism**. Regarding gamete size dimorphism, our analysis suggests that oogamy is

193   most likely the ancestral state in the brown algae (Table 2, Figure 3E-F). The oldest transition

194   took place around 114 My ago, from oogamy to isogamy in the lineage leading to the basal

195   brown algal orders Sphacelariales and Syringodermatales. Another independent transition

196   from oogamy to isogamy took place in the Ascoseirales. The Scytothamnales include both

197   isogamous and anisogamous taxa so the direction of the transition is unclear. Transitions from

198   oogamy to anisogamy are the most frequent transition. Taken together, the results indicate

199   that gamete size dimorphism level is a remarkably labile trait in the brown algae.

200   **Parthenogenesis**. The gametes of the ancestral brown algae are predicted to have been

201   unable to perform asexual reproduction through parthenogenesis (Figure 3G-H). The initial

202   transition from absence of parthenogenesis to female gamete parthenogenesis could not be

203   accurately traced in time along the early diverging branch separating the subclass

204   Fucophycideae from the earlier branching Dictyophycidae. The length of this branch renders

205   identification of the transition during 1 My time bins impossible, as most events fall in

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206 different time periods and agreement between reconstructions is very low. The oldest
207 traceable transition that could be timed, at around 85.5 My, and also the one with the highest
208 frequency, was from female-only parthenogenesis to parthenogenesis of both female and
209 male gametes. Note that parthenogenesis is the trait with the lowest sampling, as there is
210 very limited data about this trait in the literature.

Generation dominance and sexual system

212 Transitions towards the dominance of haploid phase were found to be more frequent when
213 the sexual system was monoicous (Figure 4, $q_{21} \gg q_{43}$), and overall, switches in life cycle
214 phase dominance probabilities were higher in monoicous compared to dioecious species,
215 whatever the direction (q_{21} and $q_{12} > q_{43}$ and q_{34}; Table 4, Figure 4, Figure S1). In other
216 words, monoicous species exhibit a higher turnover in terms of generation dominance.
217 Moreover, transitions from monoicous to dioicous states were slightly more frequently
218 observed than transitions from dioicous to monoicous, regardless of life cycle phase
219 dominance (Bayes Factor of 3.51 in favour of the dependent model with $q_{24} \sim q_{13} > q_{42} \sim$
220 q_{31}, Figure 4, Figure S1).

Generation dominance and sexual dimorphism

222 We tested if diploid dominance is correlated with an increase in sexual dimorphism. The test
223 of the dependent versus independent model showed that the difference in likelihood was not
224 significant (log BF = -0.1080, Table 3), suggesting that the evolution of the traits is not
225 correlated. Therefore, our data does not support the hypothesis that diploid growth is
226 associated with increased sexual dimorphism.

Gamete biology and sexual systems

228 Based on the idea that gamete dimorphism evolved to maximize the chances of gamete
229 encounters, one may hypothesise that separate sexes (dioecy and dioicy) would be associated
230 with small and abundant male gametes, as a mechanism to ensure that the gametes find a
231 partner of the opposite sex when gametes are release into seawater. However, we found no
232 evidence for an association between male gamete size and sexual system (sexes on same
233 versus different individuals) (Figure S2, Table 3, r=0.0909).

234 When gametes are produced by two different individuals, it may be more difficult for a
235 gamete to find a gamete of the opposite sex than if the same individual produces gametes of
236 both sexes. Accordingly, we hypothesised that parthenogenesis would be favoured in species
237 with separate sexes, as opposed to the situation where male and female gametes are
238 produced by the same individual (note that auto-incompatibility has not been described in

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239   the brown algae, with the exception of one study, Gibson 1994). However, we found no
240   evidence that parthenogenesis was more prevalent in species with separate sexes (Table 3).

241   Finally, we investigated the relationship between the size of male gametes and their
242   parthenogenetic capacity, under the hypothesis that there is a minimum threshold size for
243   male gametes, below which parthenogenesis is not possible. The phylogenetic threshold
244   model indicated that there is a positive correlation between male gamete size and
245   parthenogenetic capacity (Table 3, r=0.4242), however the highest posterior density (HPD)
246   interval of this correlation includes zero. We therefore complemented this analysis using an
247   Ornstein Uhlenbeck (OU) model (Hansen 1997; Butler and King 2004; Harmon et al. 2010).
248   This analysis concluded that the estimated optimal size for non-parthenogenetic male
249   gametes is significantly lower than that of parthenogenetic male gametes (5.49 um vs 9.30
250   um; Figure S1, Figure 5). This analysis therefore highlights a significant association between
251   male gamete size and parthenogenetic capacity.

DISCUSSION

Diploid growth evolved to complement deleterious mutations

254   Based on the analysis carried out here, the ancestral brown alga appears to have had a
255   diplohaplontic life cycle with similar diploid and haploid dominance (i.e., similar size and
256   complexity of the gametophyte and sporophyte generations). Over evolutionary time, the
257   diploid phase became dominant in some clades (and haploid dominance decreased) whereas
258   other clades evolved towards greater haploid dominance. Several theories have been
259   proposed to explain evolution towards either a dominant haploid or a dominant diploid phase
260   in the life cycle (e.g., Otto & Gerstein 2008). Hypotheses based on the effect of deleterious
261   alleles have proposed that being diploid generally increases mean fitness due to the masking
262   of deleterious alleles (due to complementation of these alleles by non-mutant alleles), while
263   developing as a haploid allows more efficient purging of deleterious alleles because they are
264   exposed to selection (Otto & Goldstein 1992, Rescan et al 2016, Scott & Rescan 2017). The
265   balance between these two forces determines whether evolution proceeds towards an
266   increase of the haploid or the diploid phase, and depends critically on the importance of
267   sexual exchanges within populations. Indeed, under higher rates of inbreeding or asexual
268   reproduction, the benefit of purging deleterious alleles remains associated with alleles
269   increasing the haploid phase, therefore haploidy is favoured. In contrast, outcrossing and/or
270   more frequent sex tend to favour diploidy (Otto & Marks 1996). Very few estimates of
271   inbreeding coefficients or rates of asexual reproduction are available for brown algae but this
272   idea was tested by Bell (1997) by looking at the correlation between the sexual system of a

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273 species (monoicous or dioicous) and the relative dominance (i.e., size) of the haploid and
274 diploid phases of the life cycle, assuming that monoicous species will tend to be more inbred
275 due to selfing. At the time, Bell concluded that monoicous species did not tend to have more
276 dominant haploid phases (Bell 1997). In contrast to Bell's analysis, which was based on a small
277 number of brown algal species, our results do appear to support Otto and Marks' ideas, at
278 least to some extent, because transitions towards dominance of the haploid phase were
279 found to be more frequent when the sexual system was monoicous, consistent with the idea
280 that monoicy is correlated with haploid growth. Estimates of inbreeding coefficients within
281 natural populations of monoicous species would be extremely valuable to shed further light
282 into these phenomena.

283 Somatic mutations have been proposed as another possible source of selection for diploidy,
284 as these mutations should have a lower impact on the fitness of diploid organisms (e.g., Otto
285 & Gerstein 2008). This idea is consistent with the general observation that larger organisms
286 tend to be diploid rather than haploid. Indeed, this pattern holds true for the brown algae,
287 since all the largest brown algae (e.g., Laminariaceae, Sargassaceae, Fucaceae) have a
288 dominant diploid phase.

289 Bell (1997) has also proposed that the different biology of spores and gametes should select
290 for larger sporophytes (allowing efficient dispersal of spores) and smaller gametophytes (so
291 that gametes are released close to the substratum, in order to facilitate gamete encounters).
292 While this type of constraint may explain why many brown algal species have a smaller
293 gametophyte, it does not explain the evolution towards larger gametophytes that occurred in
294 some clades, nor the transitions towards diploid cycles in which gametes are released by large
295 diploid individuals.

Diploid growth is associated with increased gamete dimorphism

297 The theory for the evolution of gamete dimorphism based on the trade-off between gamete
298 number and offspring fitness predicts that dimorphism may evolve when zygote size has a
299 strong effect on fitness (i.e. when offspring fitness increases more than linearly in relation to
300 zygote size). Accordingly, one may predict that if a larger zygote size is needed for larger
301 diploid development, increased diploid growth would favour higher levels of gamete
302 dimorphism (Parker et al. 1972). Bell (1994) proposed an alternative theory that also predicts
303 a correlation between diploid growth and gamete dimorphism, in which the direction of
304 causality is reversed, i.e., sexual selection caused by gamete dimorphism favouring diplontic
305 cycles in order to increase genetic differences between gametes produced by the same
306 organism. Although our results show that diplontic brown algal species are exclusively

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307 oogamous, suggesting a link between strong sexual dimorphism and diploidy, those
308 associations may not be relevant because they are based on only one group, the Fucales.
309 Therefore, overall, our analyses have not allowed to validate the idea that diploid growth is
310 associated with increased sexual dimorphism.

Evolution of sexual systems in the brown algae

312 The ancestral sexual system of brown algae corresponds to haploid sex determination and
313 dioicy, with several transitions towards monoicy having occurred independently over
314 evolutionary time. Transition towards a diplontic life cycle in the Fucales involved a
315 monoecious/hermaphrodite intermediate state, with subsequent independent re-emergence
316 of dioecy in some lineages. It is interesting to note that transitions from separate sexes to co-
317 sexuality are relatively frequent in haploid sexual systems, which contrasts to what is the
318 most commonly accepted direction of evolution in clades with diploid sex determination (i.e.,
319 monoecy to dioecy). Note, however, that although dioecy was considered to be an
320 evolutionary dead end in angiosperms, more recent phylogenetic analysis are challenging this
321 conclusion, and the idea that reversals to monoecy in angiosperms may be more frequent
322 than thought before is increasingly becoming accepted (Kafer et al. 2017; Pannell 2017).

323 In diploid sexual systems, two main selective effects have been proposed to explain
324 transitions from co-sexuality to dioecy: inbreeding depression (selfing is less likely to occur
325 when male and female gametes are produced by separate individuals) and the effect of trade-
326 offs between male and female fitness (Charlesworth & Charlesworth 1978, Charnov 1982). In
327 haploid sexual systems, the opposite transition (from separate sexes towards co-sexuality)
328 could also in principle be caused by a change in the shape of the trade-off between male and
329 female reproductive success (leading to a higher fitness of gametophytes producing both
330 types of gametes) or by selection for inbreeding (selfing), either through the automatic
331 transmission advantage associated with selfing (Fisher 1941) or for reproductive assurance
332 when population density is low. Note, however, that parthenogenesis occurs in all monoicous
333 species, and this process may represent an alternative way of dealing with mate limitation
334 and reproductive assurance. Assuming that selfing occurs following transitions to monoicy,
335 such transitions should occur more easily when inbreeding depression is low. One would
336 therefore expect more transitions to monoicy in taxa with a prolonged haploid phase
337 (assuming that at least a proportion of the deleterious alleles affecting the fitness of diploids
338 will be purged during the haploid phase of the life cycle), but this is not what we observe here
339 ($q31<q42$, Fig. 4). Examining the proximate mechanisms involved in the transitions between
340 separate sexes and co-sexuality in both haploid and diploid systems and more natural
341 population data, for example in populations with different densities, would be valuable to

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342 shed light on the mechanisms and evolutionary forces driving the shifts among sexual systems
343 in the brown algae.

Anisogamy is ancestral in the brown algae

345 In agreement with the conclusion obtained by Silberfeld et al (2010), our analysis points
346 towards an oogamous ancestor of brown algae, with several independent transitions towards
347 anisogamous and isogamous clades. This stands in contrasts with theoretical scenarios
348 representing the evolution of gamete dimorphism from an isogamous ancestor (e.g.,
349 Sanderson & Hurst 2001, Parker et al 1972; Charlesworth 1978, Lehtonen and Koko 2011;
350 Lehtonen et al 2016), in which isogamy is ancestral and anisogamy represents an
351 intermediate step during the process of increased gametic differentiation. To our knowledge,
352 the possible selective forces favouring reduced gamete dimorphism have not been explored
353 by theoretical models. Theories based on disruptive selection caused by a trade-off between
354 the number of gametes produced and zygote size (e.g., Parker et al 1972, Bulmer & Parker
355 2002) have shown that the shape of the relation between zygote size and fitness is critical for
356 the evolution of gamete dimorphism. It would be interesting to explore whether a change in
357 the relation between zygote size and fitness (for example, due to a decrease in size of the
358 diploid organism) may favour transitions from oogamy to anisogamy or isogamy. This type of
359 evolutionary mechanism may generate a positive correlation between the degree of gamete
360 dimorphism and the relative importance of the diploid phase, leading to an inversion of the
361 causal relationship in Bell's (1994, 1997) hypothesis mentioned above, i.e., decrease in the
362 size of the diploid organism would drive a decrease in gamete dimorphism.

363 Lehtonen and Kokko showed that evolution of anisogamy requires some level of gametic
364 competition and limitation (Lehtonen and Kokko 2011). Therefore, it is likely that in specific
365 conditions the system may return to isogamy or near isogamy, for instance if there is a low
366 level of gamete competition or if there is no gamete limitation.

Evolution of gamete size and parthenogenetic capacity

368 There are marked differences between the relative parthenogenetic capacities of male and
369 female gametes in isogamous, anisogamous and oogamous brown algal species (Luthringer et
370 al. 2015). Overall, both male and female gametes of isogamous species are capable of
371 parthenogenesis, whereas only the female gametes of anisogamous species are
372 parthenogenetic. In many oogamous species neither the male nor the female gametes
373 undergo parthenogenesis. It has been suggested that increased gamete size leads to
374 increased parthenogenetic capacity, up to a point, but that in oogamous species, the large

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375 female gamete loses its flagella becoming specialised for zygote production and is no longer

376 capable of initiating parthenogenetic development (Luthringer et al. 2015).

377 The gametes of the ancestral brown alga seem to have been unable to perform asexual

378 reproduction through parthenogenesis, suggesting that the emergence of gamete

379 parthenogenetic capacity was a derived, and perhaps adaptive trait for instance in marginal

380 populations or other situations where mates are limited (Stalker 1956; Bierzychudek 1987;

381 Oppliger et al. 2014). Data from field populations of a range of species would be needed to

382 further understand whether parthenogenesis is adaptive. It is noteworthy that

383 parthenogenetic capacity is assessed under laboratory conditions, and that the contribution

384 of parthenogenesis to recruitment in natural populations would be worth exploring further

385 (Oppliger et al. 2007, 2014). Note that a recent study in field populations of the Ectocarpus

386 showed no evidence that parthenogenesis plays a significant role under field conditions

387 (Couceiro et al. 2015). In contrast, studies in field populations of another brown algal species,

388 *S. lomentaria* suggested that parthenogenesis is prevalent in field populations (Hoshino et al.

389 2018). Interestingly, female-only parthenogenetic populations have larger gamete sizes

390 relative to 'sexual' populations of the same species, consistent with a link between gamete

391 size and parthenogenetic capacity, and opening the possibility that parthenogenesis may be

392 an adaptive trait.

393 Methods

394 *Molecular data*

395 Alignments were based on the data published by Silberfeld and colleagues (Silberfeld et al.

396 2010) that included five mitochondrial genes (*atp9:* mitochondrial ATP synthase subunit 9

397 gene*, cox1* and *cox4:* Cytochrome c oxidase subunit 1 and 3 genes, *nad1* and *nad4:* NADH

398 dehydrogenase subunit 1 and 4)*, four plastid genes (*rbcL:* large subunit of plastid encoded

399 ribulose-1,5-biphosphate carboxylase oxygenase gene*, psaA:* photosystem I P700 chlorophyll

400 a apoprotein A1 gene*, psbA:* photosystem II protein D1 gene*, and *atpB:* ATP synthase subunit

401 b gene), and a nuclear gene (*LSU:* large subunit of 28S rRNA gene). Our final tree contained

402 131 species. To attribute trait states to the species in this tree we replaced some entities,

403 depending on the availability of life-history information (i.e., kept the sequence data used to

404 build the tree but used the data on life-history from another close relative) and added

405 sequences from Genebank for species of the genera *Padina, Sargassum, Alaria* and

406 *Ectocarpus* (Table S1). No information was available about the life histories of the closest

407 relatives of the Phaeophyceae, e.g. Phaeothamniophyceae, so we used *Vaucheria*, a

408 siphonous genus in the heterokont class Xanthophyceae for which life cycle and reproductive

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409   trait information are available, as an outgroup. The final species list used for the trait analysis,

410   for which we had life cycle and reproductive trait information, was comprised of 77 species,

411   including the outgroup.

412   *Phylogenetic reconstruction*

413   All sequences were aligned using MAFFT (Katoh et al. 2009), and the best substitution model

414   was estimated using the phymltest function in the *ape* R package (Paradis et al. 2004). The

415   concatenated alignment was used for Bayesian Inference with Beast v1.8.2 (Drummond et al.

416   2012) with three different gene partitions, for nuclear, plastid and mitochondrial genes. Each

417   partition was unlinked for the substitution model. We used birth-death with incomplete

418   sampling as tree prior, and four calibration nodes as described in (Silberfeld et al. 2010) (see

419   nodes A to D, Figure 2). We used log-normal priors for two of the calibrations: *Padina*-like

420   clade A, lognormal distribution (mean 5 Ma, sd 1, and lower boundary at 99.6 Ma);

421   *Nereocystis-Pelagophycus* clade B: lognormal distribution (mean 20 Ma, sd 1, and lower

422   boundary at 13 Ma), and normal priors for the root (Phaeophyceae root age D: normal

423   distribution (u=155, sd=30 Ma), and the Sargassaceae node C to a normal distribution (u=60,

424   sd=15, with lower boundary 13 Ma). Finally, the MCMC was set to 50 million generations with

425   a sampling every 1,000. The posterior distribution was summarized using Treeannotator

426   v1.7.0 (Drummond et al. 2012) to obtain a Common Ancestor Tree (Heled and Bouckaert

427   2013). For the macroevolutionary analyses (see below), a set of 100 trees were sampled from

428   the posterior distribution.

429   *Life history traits*

430   Four life-history traits were recorded based on a literature review: life cycle type (haploid >

431   diploid; haploid = diploid; haploid < diploid; diplont), sexual system (monoicous; dioicous;

432   monoecious; dioecious), gamete dimorphism (isogamous; anisogamous; oogamous), and the

433   occurrence of gamete parthenogenesis (no parthenogenesis; parthenogenesis in female

434   gametes only; parthenogenesis in both male and female gametes). The traits were coded as

435   discrete multi-state characters. A full explanation of each state is given in Table S2. We

436   separated the respective traits into seven additional characters. For example, we transferred

437   'gamete size' (iso-, aniso-, oogamous) into a continuous male gamete size trait. We

438   furthermore recoded multi-state traits into binary data for the correlation tests (see below),

439   such as the 'gamete dimorphism', which was recorded by separating the absence (0 =

440   oogamy) from presence (1 = iso- or anisogamy) of female flagellated gametes. We

441   categorized an additional sexual system trait as 'sexes occurring on the same thallus' (0 =

442   monoicous or monoecious) or 'separate thalli' (1 = dioicous or dioecious). The life cycle was

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443   simplified to the occurrence of a 'dominant haploid phase' (0 = haploid ≥ diploid) versus

444   dominance of the diploid phase (1 = haploid < diploid or diplontic), with dominance broadly

445   meaning size of the adult individual. Finally, the occurrence of parthenogenesis was separated

446   into two additional traits, absence (0) or presence (1) of male parthenogenesis, and absence

447   of parthenogenesis (0) versus parthenogenesis occurring in at least one of the sexes (1), most

448   commonly the female.

449   For simplicity, we coded as "isogamous" algae with physiological and behavioural anisogamy

450   but that have been described as having no size difference between male and female gametes.

451   Note that all brown algae exhibit an asymmetry between male and female, at least at the

452   level of their behaviour, and potentially all the algae scored as isogamous have in fact subtle

453   size differences but the literature is not detailed enough in this respect. For example, most

454   representatives of the order Ectocarpales have been reported to be 'isogamous' (based on

455   observations under the microscope, but without detailed measurements of gamete size), but

456   some members (*Ectocarpus* sp., *Colpomenia peregrina* Sauvageau) as well as the last species

457   branching off before the Ectocarpales, *Asterocladon interjectum*, have anisogamous male and

458   female gametes.

459   The sister group to the Ectocarpales, the order Laminariales, is almost completely oogamous,

460   with the exception of the genus *Saccharina*, which has been shown to have eggs with

461   rudimentary flagella (Motomura and Sakai 2008) being therefore considered strongly

462   anisogamous.

463   *Ancestral state reconstructions*

464   A likelihood-based method was used to reconstruct the ancestral state of each of the four

465   life-history traits. We fitted three different models of trait evolution using the function

466   *fitDiscrete* from the R package Geiger (Harmon et al. 2008). These models differed in the

467   number of transition rates as follows: *equal rates* (ER, a single transition rates between all

468   states), *symmetric* (SYM, forward and reverse transitions are the same), and *all-rates-different*

469   (ARD, each rate is a unique parameter). The corrected Akaike Information Criterion (AICc) was

470   used to compare the alternative models. Each model was estimated on each 100

471   phylogenetic trees sampled from the posterior distribution to account for uncertainty in tree

472   topology and divergence times. State probabilities at the root and transition rates were

473   summarized with the mean and standard deviation values of all iterations, to incorporate

474   phylogenetic uncertainty.

475   We inferred the number of transitions between states, and their minimum timing, using

476   stochastic character mapping (Huelsenbeck et al. 2003). One hundred stochastic mappings

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477   were performed on the posterior sample of trees, and on each we divided branch lengths into

478   time bins of 1 Myr and recorded the number of transitions from and to each state, in each bin

479   (as described in (Serrano-Serrano et al. 2017). We reported the mean and standard deviation,

480   and the time bin at which 60% of the stochastic mappings had at least one transition event as

481   the onset time for each type of transition.

482   *Correlation analyses*

483   We first assessed correlation between life history traits using a reversible-jump MCMC

484   algorithm to test the correlation between two binary traits as implemented in *BayesTraits* V3

485   (Pagel *et al.* 2004). This approach compared two models, a null model assuming that the traits

486   had evolved independently, and an alternative model assuming that their evolution had been

487   correlated. Each model was run for 10 million generations using the values found in the

488   ancestral state reconstructions for the root state. The two models were compared through

489   their log marginal likelihood by estimating the log Bayes factor. This approach was used to

490   test the correlation between female parthenogenesis and the occurrence of sexes on the

491   same versus separate thalli. Tests showing a significant support for the correlated model were

492   presented as networks of evolutionary transitions using the R package qgraph (Epskamp *et al*.

493   2012). Second, we used the implementation of the threshold model, *threshBayes* in the R

494   package phytools (Revell 2014), to test for the correlation between a continuous and a

495   discrete variable. The threshold model assumes that the states of discrete phenotype are

496   governed by an unobserved continuous character called *liability.* These liabilities are assumed

497   to evolve according to a Brownian motion model (Felsenstein 2012) and translate into

498   discrete characters once they have passed certain thresholds. We used this model to test the

499   correlation between male gamete size and two discrete traits, male parthenogenesis and

500   sexes on the same or on separate thalli. For correlation analyses that were significant, we

501   fitted an Ornstein–Uhlenbeck model of evolution to further test whether the continuous trait

502   had two discrete selective regimes, determined by the discrete binary trait. We used the

503   *OUwie* from the R package Ouwie (Beaulieu *et al.* 2012), and compared the alternative

504   models using the corrected Akaike Information Criterion (AICc)-selected model.

505   FIGURES

506   **Figure 1**. Schematic illustration of sexual life cycles of representative brown algae. (A)

507   *Scytosiphon lomentaria* (Lyngbye) Link: diplohaplontic, heteromorphic life cycle, haploid

508   dominant; near-isogametes (B) *Ectocarpus* sp*.* (Dillwyn) Lyngbye: diplohaplontic, isomorphic

509   life cycle, with similar dominance in haploid and diploid phases; near-isogametes; (C)

510   *Saccorhiza polyschides* (Lightfoot) Batters: diplohaplontic, heteromorphic life cycle, with

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511 diploid dominance (D>>H); oogamous; (D) *Fucus serratus* L.: diplontic life cycle, only diploid
512 phase; oogamous. H=haploid phase; D=diploid phase.

513 **Figure 2**. Phylogenetic tree using Bayesian analyses in BEAST. Node numbers indicate the
514 posterior Bayesian support, node bars represent the 95% HPD (Highest Posterior Density) for
515 the divergence times.

516 **Figure 3.** Maximum likelihood ancestral state reconstructions for the four-brown algal life-
517 history traits. Pie charts and colours at each node represent the probabilities for each state.
518 Colours at the tips represent the species states (A, C, E, F). Estimated number of transitions
519 through time for the corresponding four life-history traits (B, D, F, H). Coloured densities
520 identify the mean number of events for each possible transition. Vertical lines and numbers
521 denote the minimum age as the point in time where at least one transition is recorded in 60%
522 of the reconstructions. A and B) Male and female gamete size; C and D) Sexual system, E and
523 F) Type of life cycle, G and H) Parthenogenetic capacity.

524 **Figure 4**. Models of the correlation between traits in brown algae. Binary states are described
525 at the bottom of each panel. Transition rates follow the nomenclature in BayesTraits software
526 (http://www.evolution.rdg.ac.uk/BayesTraitsV3.0.1/BayesTraitsV3.0.1.html). Square boxes
527 indicate the most likely ancestral state (see ancestral state estimations in Methods), sizes of
528 squares/circles represent the abundance of each state in the set of sampled species, and
529 arrow thickness is proportional to the rate of transition between states (see Table 2).

530 **Figure 5**. Phenogram for male gamete size and parameter estimation using Ornstein-
531 Uhlenbeck (OU) model. Colours denote the absence ($\theta 0$ in orange) and presence ($\theta 1$ in
532 purple) of parthenogenesis of male gametes.

533 TABLES

534 **Table 1**. Description of the traits studied, categories and discrete states. Note that some of
535 the discrete traits were also treated as continuous traits (male gamete size for instance).

536 **Table 2**. Analysis of the ancestral states for each studied trait.

537 **Table 3**. Analysis of correlations between traits.

538 **Table 4**. Estimated rates of correlated transitions

539 SUPPLEMENTAL FIGURES

540 **Figure S1.** Phylogenetic tree pruned to the 43 species with sexual system and life cycle
541 dominance traits recorded. Coloured squares at the tips represent the species state for sexual

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542 system (left column) and phase dominance (right column). Colour codes for each binary trait

543 are explained in the top left legend. Ancestral node states are not depicted, see Figure 3 for

544 full ancestral state reconstruction.

545 **Figure S2**. Evolutionary correlation between continuous and discrete traits using the threshold

546 model. **A,** Correlation between the size of male gametes and presence of sexes on

547 same/different individual (unisexual versus co-sexuals). **B**, Correlation between the size of

548 male gametes and male parthenogenesis. Blue histograms (left panel) denotes the posterior

549 density for the correlation between traits. Posterior liabilities, or unobserved continuous

550 traits, for each discrete trait (right panel). HPD: highest posterior density.

551 SUPPLEMENTAL TABLES

552 **Table S1**: Accession number for the sequences of the species that were not included in

553 (Silberfeld et al. 2010).

554 **Table S2**: List of detailed life cycle and reproductive traits across the brown algal species

555 SUPPLEMENTAL DATASET

556 Details of all the traits and species used in this work, including references.

557 FUNDING

560

561

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A — male gametophyte n, female gametophyte, Meiosis, Syngamy, 2n

Diplohaplontic, anisomorphic life cycle
Haploid phase dominant
e.g. *Scytosiphon lomentaria*

B — male gametophyte n, female gametophyte, Meiosis, Syngamy, 2n

Diplohaplontic, anisomorphic life cycle
Haploid phase =Diploid phase
e.g. *Ectocarpus* sp.

C — female gametophyte n, male gametophyte, Meiosis, Syngamy, 2n
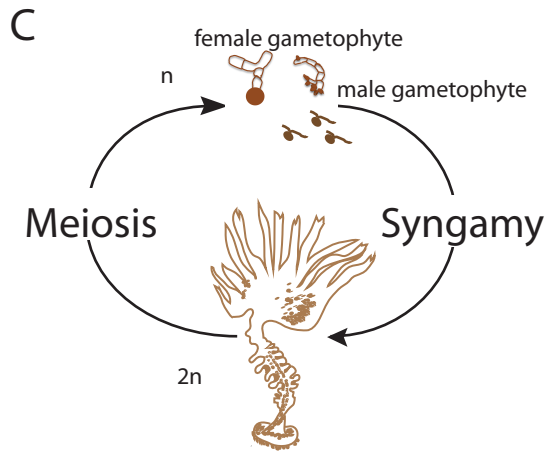
Diplohaplontic, anisomorphic life cycle
Diploid phase dominant
e.g. *Saccorhiza polyschides*

D — n, Meiosis, Meiosis, Syngamy, female sporophyte, male sporophyte, 2n
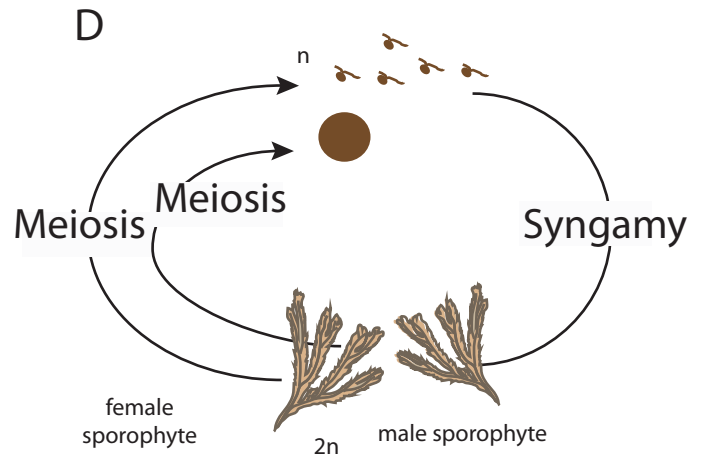
Diplontic
Diploid phase only
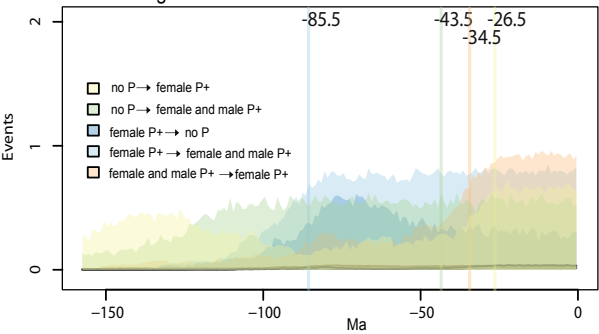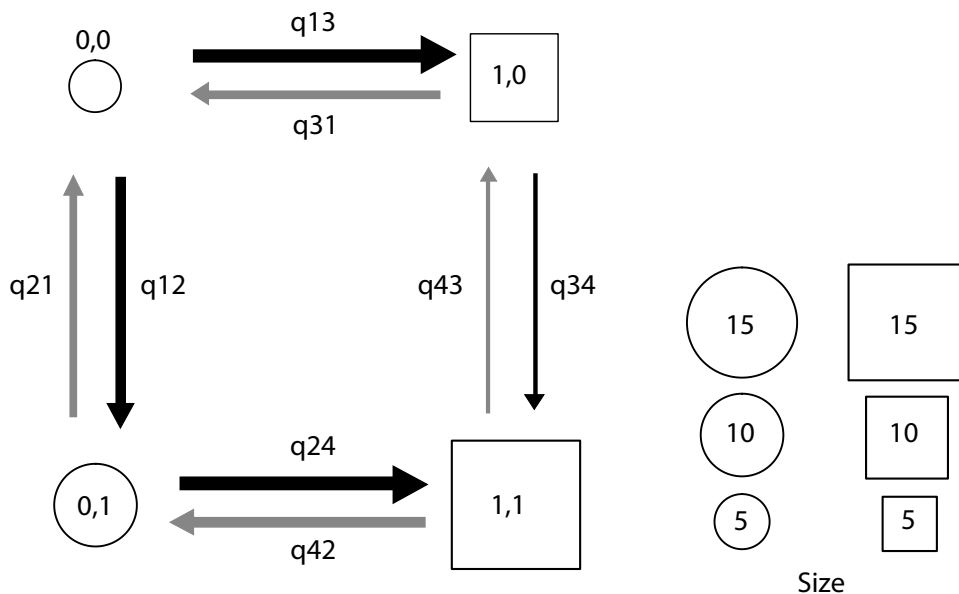e.g. *Fucus serratus*
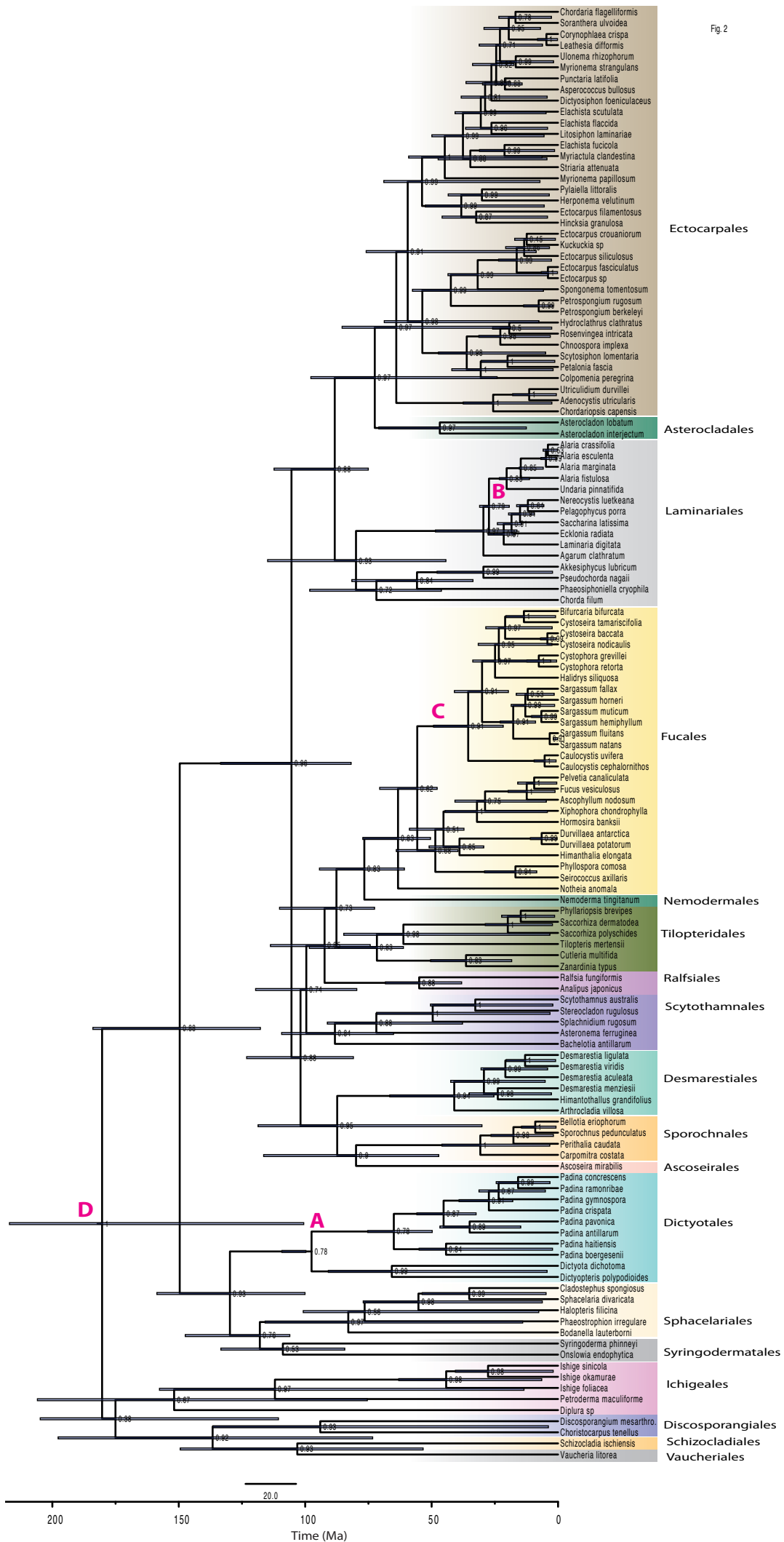
Fig. 2

Fig. 3
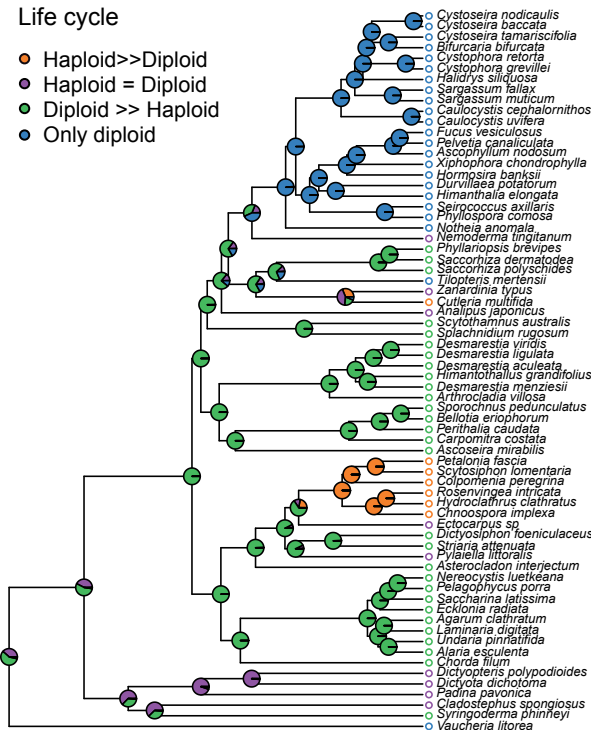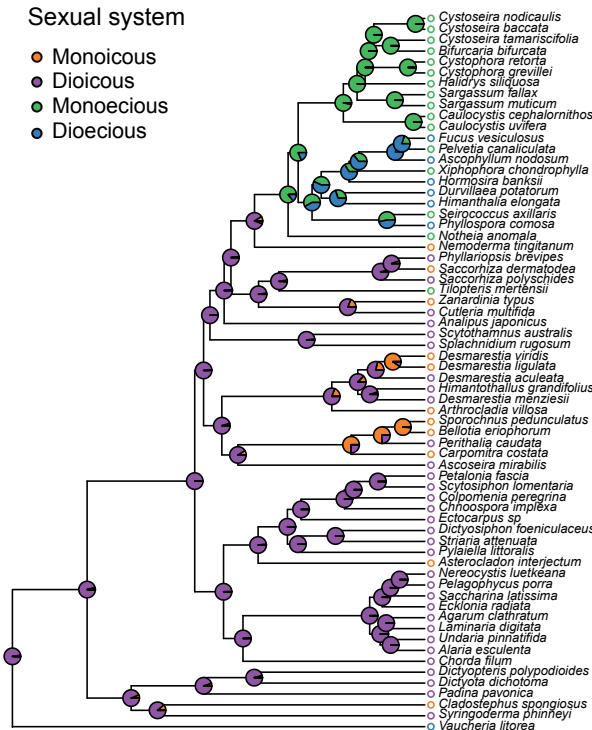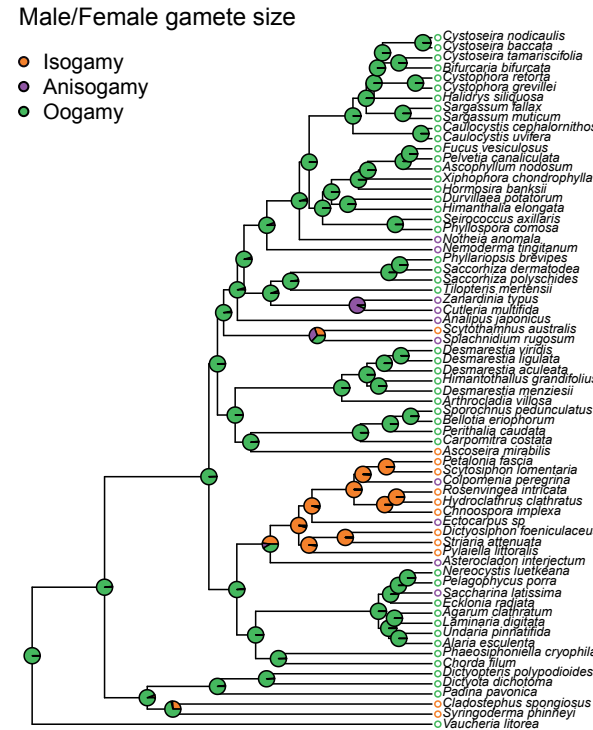
Fig. 4

Correlation between sexual system and life cycle dominance

0,0 : monoicous, haploid dominant
0,1 : monoicous, diploid dominant
1,0 : dioicous, haploid dominant
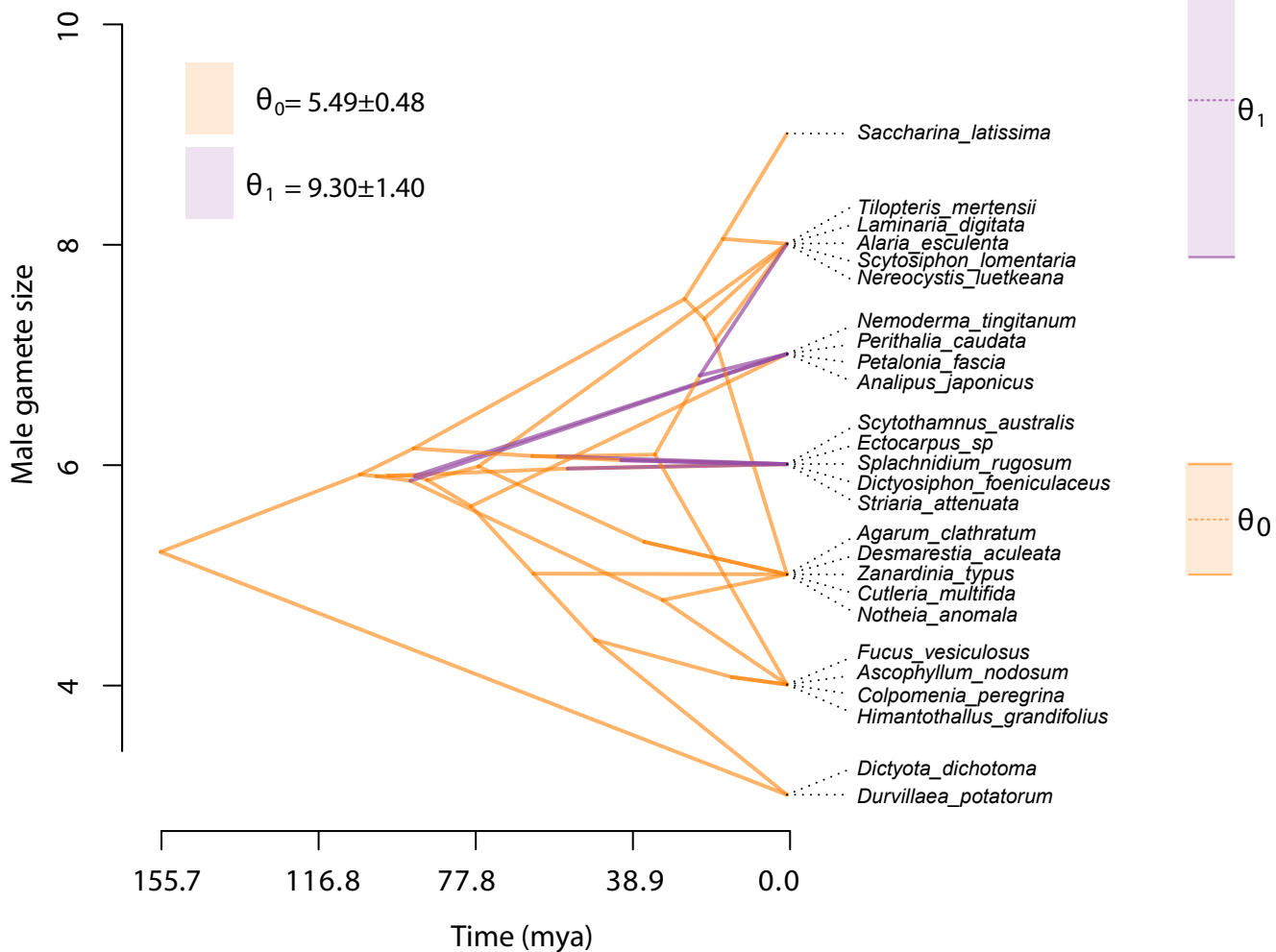1,1 : dioicous, diploid dominant

Fig. 5

Table 1. Description of the traits studied, categories and discrete states. Note that some of the discrete traits were also treated as continuous traits (male gamete size for instance).

	Category	States	Description
Life cycle	Haplo-diplontic	Haplo-diplontic haploid dominant[1]	Life cycle with both haploid and diploid mitosis, with dominant gametophyte haploid generation
		Haplo-diplontic haploid = diploid	Life cycle with both haploid and diploid mitosis, with equal dominance of gametophyte and sporophyte generations
		Haplo-diplontic diploid dominant[1]	Life cycle with both haploid and diploid mitosis, with dominant sporophyte (diploid) generation
	Diplontic	Diplontic	Life cycle with no haploid mitosis, the haploid phase is limited to gametes
Sexual system	Haploid sex determination	Monoicous	Haploid phase sex determination, where both gamete types are produced by the same haploid gametophyte.
		Dioicous	Haploid phase sex or mating type determination, with genetically distinct gametophytes corresponding to each sex
	Diploid sex determination	Dioecious	Diploid phase sex-determination, with genetically distinct sporophytes corresponding to each sex
		Monoecious	Diploid phase sex determination, where both male and female organs are produced by the same diploid sporophyte
Gamete size	Female gamete with flagella	Isogamous[2]	Male and female gametes with small size difference (but different behaviour/physiology)
		Anisogamous	Male and female gametes of clearly different size, both with flagella
	Female gamete without flagella	Oogamous	Female gamete much larger and lacking a flagellum
Parthenogenesis	No parthenogenesis	No parthenogenesis	No parthenogenesis capacity in either gamete
	Parthenogenesis	Female gametes only	Only female gametes capable of parthenogenesis
		Male and female gametes	Male and female gametes capable of parthenogenesis

[1]The term "dominant" is defined here as the generation that presents larger size and higher complexity in terms of morphology (number of different cell types, number of tissues and organs)

[2]For simplicity, we code as "isogamous" algae that have almost imperceptible size differences between male and female gametes, but note that in the brown algae there is allways an asymetry (at least in terms of physiology and behaviour) between male and female gametes.

Table 2. Analysis of the ancestral states for each studied trait.

Trait	Description	No. species	Best Model	Mean rates (SD)[1]	Root state (prob)[2]	Mean No. transitions (SD)	Min time of transition (60% recons, and SD)[3]
Life cycle	0: Hap>>Dip, 1: Hap=Dip, 2: Hap<<Dip, 3: Dip	68	ER	0.00135 (1.1e-4)	0: 0.00117, 1: 0.39560, 2: 0.56764, 3: 0.03559	1→0: 1 (0.6), 1→2: 1 (0.9), 1→3 :1 (0.7), 2→0: 1 (0.7), 2→1: 5 (1.5), 2→3: 2 (0.8)	2→0: 57.5 My (5.05), 2→1: 81.5 My (23.55), 2→3: 74.5 My (21.41)
Sexual system	0: monoicous, 1: dioicous, 2: monoecious, 3: dioecious	66	SYM	0>1: 0.00515 (4.7e-4), 0>2: 0.00000 (4.1e-6), 0>3: 0.00057 (5.1e-4), 1>2: 0.00102 (1.3e-4), 1>3: 0.00000 (0.000), 2>3: 0.01180 (1.2e-3)	0: 0.02361, 1: 0.96346, 2: 0.01228, 3: 0.00065	1→0: 8 (0.9), 1→2: 2 (0.4), 1→3 :1 (0.2), 2→0: 1 (0.2), 2→3: 3 (1.8), 3→2: 2 (1.3)	1->0: 110.5 My (30.02), 1->2: 74.5 My (21.79), 2->3: 17.5 My (5.34), 3->2: 25.5 My (7.65)
Gamete size	0: isogamy, 1: anisogamy, 2: oogamy	69	ER	0.00236 (1.8e-04)	0:0.00118, 1:0.00029, 2:0.99854	0→1: 2 (0.8), 1→0: 3 (0.8), 2→0: 2 (0.8), 2→1: 8 (1.1)	0->1: 56.5 My (16.6), 2->0: 113.5 My (33.05), 2->1: 87.5 My (25.28)
Partenogenesis	0: Nobody, 1: only female, 2: female and male	40	ER	0.00288 (2.4e-4)	0: 0.59786, 1: 0.16131, 2: 0.24082	0->1: 1 (1.2), 0→2: 2 (1.4), 1→0: 2 (1.4), 1→2: 4 (1.9), 2→1: 1 (1.8)	0->1: 26.5 My (7.94), 0->2: 43.5 My (14.86), 1->2: 85.5 My (24.97), 2->1: 34.5 My (10.25)

[1]Rates are the mean and sd values of the optimization in a sample of 100 posterior trees.

[2]Probability of the root is the mean over 100 reconstructions

[3]Stochastic mapping analysis and time where 60% of the reconstructions that have at least 1 transition

ER: a single transition rate between all states

SYM: forward and reverse transitions are the same

ARD: each rate is a unique parameter

Table 3. Analysis of correlations between traits.

Continuous versus discrete

Trait 1	Trait 2	Hypothesis	No. of species	Assumptions on the root	r	HPD r	ESS
Male gamete size (continuous)	Separate sexes/co-sexuals	Separate sexes may tend to have smaller and more abundant gametes to ensure reproduction	38	NA	0.0908	-0.3670, 0.5566	525
Male gamete size (continuous)	Male parthenogenesis capacity	There is a minimum male gamete size where partenogenesis is not possible	26	NA	0.4242[**]	-0.0616, 0.8494	390

Two discrete binary traits

Trait 1	Trait 2	Hypothesis	No. of species	Assumptions on the root	lnL Indep	lnL Dep	Log BF[*]
Separate sexes/co-sexuals	Partenogenesis in female	Separate sexes may promote partenogenesis as a mechanism to ensure reproduction	40	1,0 = Sexes on separate thallus, no partenogenesis in female	-38.9826	-40.8219	-3.6787
Sexual system (only monoicous and dioicous taxa)	Life cycle dominance	Monoicous species are expected to have haploid dominant phase	43	1,0 and 1,1 = Dioicous, but for the life cycle dominance both states could be the root.	-50.7461	-48.9906	3.5110
Level of gamy (isogamous versus anisogamous)	Life cycle dominance	Anisogamous species are expected to have dominant diploid stages	68	1,1=anisogamy, diploid dominant	-50.7400	-50.7940	-0.1080

*BF > 2 is considered evidence of the dependent model
ESS: effective sample size
HPD: highest posterior density
**significant different from zero according to the threshold model

Table 4. Estimated rates of correlated transitions

Rate	State from	State to	Sexual system and life cycle dominance	
			Mean	sd
q12	0,0	0,1	0.0118	0.1683
q13	0,0	1,0	0.0153	0.1682
q21	0,1	0,0	0.0080	0.0090
q24	0,1	1,1	0.0160	0.1682
q31	1,0	0,0	0.0094	0.1683
q34	1,0	1,1	0.0036	0.1681
q42	1,1	0,1	0.0105	0.1682
q43	1,1	1,0	0.0034	0.1681