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Complex life cycles of multicellular eukaryotes: new approaches based on the use of model organisms

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Abbreviations: *AGP18, ARABINOGALACTAN PROTEIN 18;* Ds, Dissociator; *LEC1, LEAFY COTYLEDON1; LEC2, LEAFY COTYLEDON2;* MADS, MCM1/AGAMOUS/DEFICIENS/SRF; *SERK, SOMATIC EMBRYOGENESIS RECEPTOR KINASE1*; *SPL, SPOROCYTELESS*; *ZmSERK, Zea mays SOMATIC EMBRYOGENESIS RECEPTOR KINASE1*

Abstract

A wide variety of life cycles can be found in the different groups of multicellular eukaryotes. Here we provide an overview of this variety, and review some of the theoretical arguments that have been put forward to explain the evolutionary stability of different life cycle strategies. We also describe recent progress in the analysis of the haploid-diploid life cycle of the model angiosperm *Arabidopsis thaliana* and show how new molecular data are providing a means to test some of the theoretical predictions. Finally, we describe an emerging model organism from the brown algae, *Ectocarpus siliculosus*, and highlight the potential of this system for the investigation of the mechanisms that regulate complex life cycles.

1. Introduction

A wide variety of different life cycles are found in the eukaryotes and one of the challenges of biology is to understand how this diversity has evolved and how (and to what degree) each type of life cycle is stably maintained on an evolutionary timescale. This review will present an overview of life cycle diversity in the eukaryotes along with some of the theoretical models and hypotheses that have been proposed to explain the existence of the different types of life cycle and recent experimental work aimed at testing these hypotheses. In 1998, when Mable and Otto reviewed the current state of theoretical and experimental work aimed at explaining the variety of life cycles among eukaryotes, they concluded that the major

important evolutionary question of the maintenance of alternation of generations with substantial development in both haploid and diploid phases remained unanswered. We will focus particularly on this problem and discuss how approaches based on the use of model organisms are being developed to address it. A number of other reviews that address several of the topics discussed here in more detail have been published recently (McCormick, 2004; Wilson and Yang, 2004; Yadegari and Drews, 2004; Zeyl, 2004; Boavida et al., 2005; Maraschin et al., 2005), including a recent discussion of the increasing attention that is being paid to ecological and evolutionary aspects of haploid-diploid life cycles (Thornber, 2006). Definitions of some of the terms used in the review are given in Table 1.

2. Life cycles of the eukaryotes

Sexual life cycles in the eukaryotes involve a cyclic alternation between diploid and haploid phases with meiosis mediating the transition from the diploid to the haploid state and cell fusion (syngamy) reconstituting a diploid genome. The wide variety of sexual life cycles found in nature share this common structure but exhibit differences in, essentially, two parameters: the temporal importance of each phase (i.e. the proportion of the time spent in the haploid or diploid phase) and the degree of mitotic activity in each phase (clonal multiplication of haploid and/or diploid cells). For the multicellular organisms that will be considered in this review, this mitotic activity not only serves a direct reproductive function, but is also required to construct the multicellular organism itself (somatic development).

Although reproduction is often linked with sex (in most animals for example) this is not always the case and asexual cycles (involving spore formation and/or either vegetative reproduction in plants or fission and fragmentation of the body in animals) can exist either

instead of, or in addition to, a sexual cycle. Asexual cycles produce a succession of genetically identical individuals with the same level of ploidy.

Three basic types of sexual life cycle are found in eukaryotes: diploid, haploid and haploid-diploid (Figure 1). These cycles differ with regard to the relative position of meiosis and syngamy. When syngamy directly follows meiosis, somatic development occurs only in the diploid generation and the gametes are the only haploid cells. This is called a diploid (or diplontic) life cycle. Conversely, in a haploid (or haplontic) life cycle, meiosis directly follows syngamy and somatic development occurs in the haploid generation with the minimal diploid phase corresponding solely to the zygote. Finally when meiosis and syngamy are separated temporally, somatic development can occur in both the haploid and the diploid phases (as seen, for example, in land plants and in some seaweeds) and the life cycle is termed haploid-diploid (or haplo-diplontic). The two generations of a haploid-diploid cycle can either be dependent (with one generation growing on the other) or independent. The alternation between two distinct types of individual observed in haploid-diploid cycles (corresponding to the gametophytic and the sporophytic generations) has been called a Hofmeister-Strasburger alternation of generations (Bell, 1994).

It should be noted here that the correspondence between ploidy and the sporophyte and gametophyte generations of the life cycle is not absolute. This is clear from a very simple and familiar example for flowering plant biologists in as far as it is the gametophyte stage that is diploid in tetraploid variants of flowering plant species (Leitch and Bennett, 1997). Moreover, and from a more general point of view, many organisms with haploid-diploid life cycles exhibit the phenomena of apospory (the transition from sporophyte to gametophyte without meiosis) and apogamy (the transition from gametophyte to sporophyte without gamete fusion)

in which there is a clear uncoupling of cellular mechanisms that affect ploidy and the alternation between the sporophyte and the gametophyte (Bell, 1992).

3. Life cycle complexity in multicellular eukaryotes

The following sections describe the various types of life cycle found in the different eukaryote groups that include complex multicellular organisms, with emphasis on red and brown macroalgae because of the particularly broad variety of life cycles in these groups.

3.1. Life cycle variety in the opistokonts (animals and fungi)

Most animals possess a sexual, diploid life cycle, the haploid phase being represented solely by the single-celled gametes. This is not an absolute rule, however, and asexual (diploid) life cycles are commonly found in several groups of metazoans. Moreover, some animal life cycles, such as the haplo-diploid life cycles of some insects, involve the production of haploid organisms via parthenogenesis in addition to diploids. Note that such life cycles are referred to as haplo-diploid to distinguish them from the haploid-diploid cycles described above.

Even when an animal has a strict diploid life cycle, its life cycle may still involve the sequential development of distinct phases that exhibit marked differences in morphology (such as, for example, the larval and the adult stages of a fly's life cycle). This phenomenon was first described by Steenstrup (1845), and is therefore referred to as a Steenstrup alternation of phases to distinguish it from the Hofmeister-Strasburger alternation of generations in haploid-diploid life cycles, described above (Bell, 1994). Thus, adoption of a

haploid-diploid life cycle is not the only way to evolve morphological dimorphism within a life cycle.

Fungal life cycles are very diverse. In general, multicellular development does not occur during the diploid phase (with the exception of some groups such as certain chytrids, which exhibit an alternation between simple, filamentous gametophytes and sporophytes). However, there is often a delay between syngamy and karyogamy (nuclear fusion), resulting in an additional, "dikaryon" phase during which there is a proliferation of cells with two unfused, haploid nuclei. This feature, which is found only in the fungi, adds a further complication to life cycles in this group (Raper and Flexer, 1970). Moreover, the dikaryon phase can be an important phase of the life cycle, both in terms of duration and developmental complexity (for example in the Basidiomycetes).

3.2. Life cycle variety in the viridiplantae (embryophyte plants and green algae)

Flowering plants have haploid-diploid life cycles with a highly reduced gametophyte generation corresponding to the pollen grain (consisting of only two or three cells) on the male side and the embryo sac (most commonly consisting of only seven cells) on the female side. The reduction of the gametophyte generation in flowering plants appears to be a derived feature because there is a tendency in other members of the green lineage, in particular in the bryophytes, for the gametophyte generation to be more complex.

In pteridophytes (ferns) the sporophyte generation is generally also the dominant generation but the gametophyte has a considerable level of complexity including features such as cell differentiation, photomorphogenesis and hormone and pheromone responses (Banks, 1999).

Moreover, in some members of this group that are under unusual selection pressures, the gametophyte appears to have acquired an even greater importance, with some species being driven towards complete reliance on this generation of the life cycle (Watkins and Farrar, 2005).

The tendency towards dominance of the gametophyte is more marked in the bryophytes (e.g. mosses), which diverged from flowering plants more than 400 million years ago. Members of this group possess a gametophyte-dominant life cycle in which the sporophyte grows "parasitically" on the gametophyte.

Work on the evolution of plant life cycles has a long history, the alternation of generations during the life cycles of Bryophyta and Pteridophyta was described in 1851 (Hofmeister, 1851) and the cycles were shown to be haploid-diploid in 1894 (Strasburger, 1894). Interestingly, botanists of this period noted a correlation between habitat and life cycle: most terrestrial plants having a well developed diploid generation, while in aquatic environments the haploid generation was often dominant (Bower, 1908). In the lineage leading to flowering plants, therefore, dominance of the diploid generation seems to be correlated both with the move to drier terrestrial environments and with increased developmental complexity. Bower (1908) suggested that the gametophyte generation was reduced in the terrestrial environment because the motile sperm required an aqueous environment for fertilisation to take place. Two principal theories have been proposed to explain the evolution of the dimorphic alternation of generations in land plants (Lang, 1909) and this question of the origin of land plants and the characteristics of their charophycean ancestors is still debated (Bennici, 2005; Blackwell, 2003; Qui *et al.*, 2006; Taylor *et al.*, 2005). The antithetic theory proposed by Bower (1908) suggests that the sporophyte generation evolved from a haploid green alga with the zygote

retained in the thallus that gave rise to the diploid phase (sporophyte). The homologous theory assumes a green algal ancestor but with an isomorphic haploid-diploid life cycle.

The Viridiplantae is composed of two main taxa, Streptophyta, which includes the embryophytes (land plants) and several taxa of green algae, and Chlorophyta, which includes the Ulvophyceae. With the exception of the embryophytes, all of the species within the Streptophya have haploid life cycles. This supports the Antithetic Hypothesis, which suggests that the multicellular diploid phase of the embryophyte life cycle evolved via a delay in zygotic meiosis and the insertion of mitotic cell divisions (Kenrick and Crane, 1997; Blackwell, 2003; Qui et al., 2006). The Ulvophyceae are thought to have evolved multicellularity independently of the lineage that led to the embryophytes, Life cycles in this group are variable and can be haploid or haploid-diploid, and iso- or heteromorphic (de Reviers, 2002).

Interestingly, when microspores from many flowering plant species are subjected to a stress treatment, this can induce a switch from the gametophyte to the sporophyte program of development, a type of apogamy called androgenesis (Maraschin et al., 2005). The nature of the stress treatment required to induce this phenomenon depends on the species and the variety under study. It is thought that the applied stress induces dedifferentiation of the developing microspore, allowing the subsequent initiation of a sporophyte developmental program. Apospory (sporophyte to gametophyte transition without meiosis) occurs in a number of apomictic flowering plants (Nogler et al., 1984). Apogamy and apospory have also been observed in many homosporous plants (ferns and mosses; Bell, 1992).

3.3. Life cycles of the red algae

Sexuality is not known in unicellular red algae (however, see Yoon et al., 2006 for evidence of genetic recombination in *Galdieria*) but the multicellular members of this group exhibit several different types of sexual life cycle. Members of the Bangiophyceae, such as the edible seaweed *Porphyra*, have two morphologically distinct generations with different ploidy levels. The diploid sporophyte (referred to as the conchocelis generation because it was regarded as a different species before the characterisation of the life cycle) is microscopic, creeping and filamentous. Meiosis occurs during germination of the spores, the tetrads together forming the gametophyte thallus, hence the macroscopic gametophytes may be genetically heterogeneous (Ohme and Miura, 1988; Mitman and van der Meer, 1994). They reproduce either directly by asexual mito-spores (so-called monospores), or sexually via gametes, which may be either female macrogametes (oocytes, referred to as carpogonia) or male microgametes (spermatia). Syngamy (fertilisation) occurs while the female gametes are still retained on the gametophyte (Hawkes, 1990). Zygotes divide a number of times and form diploid mito-spores (referred to as "conchospores") that are released to develop into the sporophyte and thus complete the cycle.

The second large group of multicellular red algae, the Florideophyceae, contains the majority of large red algae including economically important agarophytes (*Gracilaria, Gelidium*) and carragheenophytes (*Chondrus, Eucheuma*). Most members of this group have a "*Polysiphonia*-type" life cycle (van den Hoek et al., 1995; de Reviers, 2002). Fertilization occurs on the female individual and involves complex cytological events, resulting in the formation of a cystocarp on the female. The cystocarp releases numerous diploid mito-spores that develop into independent diploid individuals, referred to as the tetrasporophyte. Meiosis occurs during this generation, the resulting meiospores being released to re-establish the

gametophyte generation. This life cycle is referred to as "triphasic" because the cystocarp may be regarded as a sporophyte generation growing parasitically on the gametophyte. However, it is similar to a diphasic haploid-diploid life cycle, except that the cystocarp is thought to serve to amplify zygotes, a process that may be made necessary by inefficient fertilisation due to the fact that gametes (and indeed also spores) are non-flagellated in the red algae (Searles, 1978).

Deviations from this "Polysiphonia-type" life cycle are manifold and include either loss of sexual reproduction and alternation of generations, or conservation of sexuality but size reduction or suppression of the independence of generations (for reviews see Guiry, 1987; West et al., 2001)

One particularly interesting feature, found in both red and brown macroalgae is the so-called "mixed-phase" phenomenon of individuals that exhibit both sporophyte and gametophyte features in the same generation. Thalli producing tetrasporangia in addition to gametes have been observed in various florideophycean red algae (e.g. van der Meer and Todd, 1977; Destombe et al., 1989; Uwai and Masuda, 1999). In *Griffithsia*, such thalli were shown to be haploid illustrating that expression of tetrasporophytic features does not require diploidy (Lee et al., 1995).

The original life history of the red algae appears to have consisted of two isomorphic generations, with the cystocarp evolving shortly after the divergence of the Florideophyceae. The primitive lineages of red algae and the Bangiophyta do not seem to have ever possessed a cystocarp.

3.4. Life cycles of the brown algae

As a result of the recent establishment of a robust phylogeny for the brown seaweeds (Phaeophyceae; Draisma et al., 2003; Kawai et al., 2007), it has become clear that the ancestral life cycle in this group consisted of two isomorphic generations. This life history is still found in early branching lineages such as the Ishigeales, the Dictyotales, and the Sphacelariales (Cho et al., 2004) and deviations from this cycle, which involve reduction or suppression of one of the two generations, have occurred in the more recently branched lineages. In most of these variant cycles, the gametophyte is reduced and morphologically simplified but still develops independently of the sporophyte, as for example in the Laminariales which include the largest alga in the world, *Macrocystis*. However, this reduction appears to have evolved independently in different lineages. In extreme cases, such as the Fucales and *Ascoseira*, a second generation is absent due to a strong reduction of the gametophyte rendering the life history essentially diplontic and reminiscent of those of the metazoa.

The order Ectocarpales consists principally of small, ephemeral brown algae. This group exhibits a remarkably wide range of different haploid-diploid life cycles. These include cycles in which the two generations can be isomorphic (family Acinetosporaceae), slightly heteromorphic (Ectocarpaceae) or strongly heteromorphic with either the gametophyte (Chordariaceae, Adenocystaceae) or the sporophyte (Scytosiphonaceae) being microscopic (Peters and Ramírez, 2001). Moreover, as an added complication, both generations may be capable of direct, asexual replication (e.g. *Myriotrichia*, Peters et al., 2004). In addition to the meio-spores that give rise to the gametophyte, the sporophyte may produce mito-spores that appear to invariably replicate the sporophyte via an asexual cycle. Similarly, although the

gametes produced by gametophytes are capable of fusing to produce sporophytes via syngamy, they can also function as asexual propagules by developing parthenogenetically. This parthenogenetic development can either give rise to a new generation of gametophytes (e.g. *Myriotrichia*) or can produce parthenogenetic sporophytes (e.g. *Ectocarpus;* Figure 2), depending on the species. This large variability of life cycles within the Ectocarpales suggests they may be good models to study the genetic regulation of their life histories and to test theoretical predictions about life cycle evolution.

In brown algae, there seems to have been only a single observation of mixed-phase thalli. Kornmann (1956) observed an *Ectocarpus* in which gametophytes developed vegetatively on parthenogenetic sporophytes (see below for the description of the *Ectocarpus* life history).

3.5. General life cycle trends in the eukaryotes

One general conclusion that can be drawn from the distribution of the three basic types of life cycle (haploid, diploid and haploid-diploid) among eukaryote taxa is that organisms with a high level of structural complexity tend to have a diploid life cycle or at least a dominant diploid generation. The most complex organisms (seed plants and multicellular animals) show diploid dominance whilst many simple organisms (many unicellular protists for example) have haploid or haploid-diploid life cycles. This trend is found on a large scale (among phyla) and is especially clear in plants, where there is a progressive decrease in the size of the haploid generation of the life cycle, relative to the diploid generation, from Bryophyta to Pteridophyta and finally to seed plants. In other taxa such as the red and brown macroalgae the situation is less clear. In algae, the persistence of both generations suggests that haploid-diploidy is remarkably stable, rather than being a transitional state (Klinger, 1993). There are

some identifiable trends such as the emergence of “Polysiphonia-type" (triphasic) life cycles in the most highly evolved red algal species and the basal nature of haploid-diploid life cycles in the brown algae, and in the latter group there appears also to be a trend towards a reduction of the haploid generation but this is not as marked as in the green lineage (Clayton, 1988; Bell, 1997).

4. Theoretical hypotheses about the evolution of different types of life cycle

Because diploidy is often associated with “biological success” in the sense of attaining complex multicellularity, many arguments have been advanced to explain the evolution of a prolonged diploid phase. However, hypotheses that only predict an adaptive benefit to diploidy are powerless to satisfactorily elucidate the evolution and persistence of haploid and haploid-diploid life cycles. As a result, attention has turned to understanding the maintenance of a diversity of life cycles (Valero et al., 1992; Mable and Otto, 1998; Hughes and Otto, 1999).

4.1. Genetical models

The ploidy level of individuals affects their ability to repair DNA damage, and the distribution of genetic variation within and between individuals, which in turn affects the efficiency of natural selection. Several models have investigated how these effects may influence the evolution of life cycles.

1. DNA damage. Repair of double strand breaks requires the presence of another intact DNA molecule that can be used as a template. This is possible only in diploids, and should thus favour a longer diploid phase (Michod and Gayley, 1994).

2. Deleterious mutations. Deleterious mutations may occur frequently in multicellular organisms (Lynch et al., 1999). These mutations are often recessive, and are thus masked in diploid individuals (due to the presence of a functional copy of the gene) and this may result in selection for a diploid life cycle (Perrot et al., 1991). However, mutations tend to accumulate more in populations undergoing diploid life cycles (because they are masked). The overall effect of deleterious mutations on life cycle evolution have been studied using "modifier" models, which incorporate one or several loci at which deleterious mutations occur plus a modifier locus affecting the relative durations of the haploid and diploid phases of the life cycle (Otto and Goldstein, 1992, Jenkins and Kirkpatrick, 1995, Otto and Marks, 1996; Hall, 2000). Using such models, one can determine the conditions under which alleles that increase the proportion of the life cycle spent in the haploid (or diploid) phase are selected at the modifier locus. A general result is that diploidy has a "short-term" advantage (due to the masking of recessive deleterious alleles), while haploidy has a "longer-term" advantage, because it allows a better selective elimination of deleterious alleles. Because the advantage of diploidy is short term, it is predicted that evolution towards diploidy should be irreversible. The longer-term advantage of haploidy can overcome the short-term advantage of diploidy, but only if it is confined to individuals with a longer haploid phase, which may be the case when there is little genetic mixing in the population, *i.e.* little recombination and/or little outcrossing and/or little sexual reproduction (Otto and Goldstein, 1992; Otto and Marks, 1996). One would thus expect to observe haploid life cycles in species where inbreeding, or clonal reproduction, is common but phylogenetic studies have not detected any clear

correlation between these phenomena (Bell, 1997; Mable and Otto, 1998). Finally, it is important to note that these models cannot explain the maintenance of a haploid-diploid life cycle (they predict evolution towards haploidy or diploidy, depending on the parameter values), but see Richerd et al. (1993) and (1994). In these two last models, the maintenance of a haploid-diploid life cycle is predicted for certain values of haploid to diploid fitness ratio. The advantage of haploid-diploidy is that sex occurs half as often assuming that individual generation times are the same length as in haploid or diploid life cycles. As pointed out by Mable and Otto (1998), the model proposed by Richerd et al. (1994) predicts that haploid-diploidy would then be favoured under conditions in which the cost of sex is high.

3. Advantageous mutations. Similar arguments come into play when considering beneficial mutations. Diploid cells contain twice as many genes, increasing the probability that an advantageous mutation will occur (Paquin and Adams, 1983), or that a deleterious allele that was maintained in the population due to masking becomes advantageous after a change in environment. However, beneficial alleles are immediately expressed in haploids, whilst they are masked in diploids until they occur in the homozygous state, and thus have a greater chance of being lost from the population when rare, due to random events. As a consequence, one finds that diploidy is favoured over haploidy if beneficial mutations tend to be dominant, and if genetic mixing is frequent in the population (Orr and Otto, 1994). At present little is known about the dominance of advantageous mutations.

4. Host-parasite interactions. Nuismer and Otto (2004) recently suggested that host-parasite interactions may favour diploidy in hosts, allowing them to harbour a greater diversity of recognition molecules that may prevent infection (such as antibodies), and haploidy in parasites, in order to express as few antigens as possible.

4.2. Ecological models

Several ecological factors that may influence life cycle evolution have been discussed.

1. The nutrient-saving hypothesis. Lewis (1984) proposed that haploid cells may enjoy an advantage in nutrient-poor environments, because haploid cells are often smaller and have thus a greater surface area to volume ratio, and also because they need less energy to replicate their DNA. This effect may be more relevant for unicellular than for multicellular organisms.

2. Optimal nuclear or cell size. Cavalier-Smith (1978) presented a similar type of hypothesis, suggesting that the evolution of ploidy levels may be a by-product of selection for optimal nuclear or cell size: conditions favouring smaller individuals would select for haploid life cycles, while conditions favouring larger individuals would favour diploid cycles.

3. Difference in ecological niche between the haploid and diploid phase. Hughes and Otto (1999) constructed a model to explore the hypothesis that haploid-diploid life cycles may be favoured when organisms exploit their environment more efficiently through niche differentiation of the two ploidy phases (Stebbins and Hill, 1980; Willson, 1981). They indeed found that haploid-diploid life cycles could be selected for under a broad range of conditions when the haploid and diploid individuals presented ecological differences. One can argue that there are different ways of evolving towards an alternation of morphs that do not necessarily imply changes in ploidy (as in the alternation of larval and adult phases that occurs in many animals). However, it may be easier to evolve different morphologies starting from different ploidy phases.

4.3. Sporophytic and gametophytic alternation of "form/generation": different properties of spores and gametes

A very different argument has been advanced in the literature to explain the general evolutionary trend in vascular plants towards an increase in size of the diploid sporophyte and a concomitant reduction of the haploid gametophyte. In comparison with previous hypotheses, this argument is mainly based on the different properties of spores and gametes and not on differences between ploidy levels. This argument, first advanced by Bower (1908), was developed more recently by Keddy (1981) and Bell (1997) and relies on the different functions of gametes, which are adapted for fusion, and spores, which are adapted for dispersal. It is proposed that the growth form of the gametophyte evolved towards a reduction in size to favour close proximity, maximising fertilisation by increasing the probability of contact between male and female gametes. On the other hand, sporophytes would have been selected to be large and erect to maximise spore dispersal and hence colonisation success. Bell (1997) emphasizes that, in brown seaweeds (Phaeophyceae), the association of gametophyte and sporophyte with characteristic morphological states is not necessarily regulated by ploidy, as reflected by the very widespread occurrence of haploid sporophytes (see above). He concluded that the classic sexual cycle (when the haploid stage becomes specialised as a gametophyte and the diploid stage as a sporophyte) can be interpreted as evolving from an asexual alternation of small and large individuals through selection for the appropriate association of ploidy with vegetative stage. If this is true we can expect that the genes that mediate sporophyte and gametophyte development would be different from the genes involved in the sexual cycle (meiosis and syngamy).

4.4. Empirical support for the above theoretical hypotheses

Altogether, a considerable number of hypotheses have been generated to explain the range of life cycles found in nature but, in contrast, relatively few experimental studies have been conducted to test these hypotheses (for reviews see Perrot, 1994; Mabble and Otto, 1998; Zeyl, 2004). Most of the experiments that have been carried out have involved comparing the performances of haploids and diploids in relation to both genetic and ecological parameters. These studies have provided empirical support for genetic theories that address masking and/or repair of deleterious mutations. Diploidy has been shown to be advantageous in highly mutagenic environments by comparing haploid or diploid strains of unicellular organisms such as yeast (Waters and Moustacchi, 1975), haploid and diploid juveniles of a red seaweed (Destombe et al., 1993) and artificial haploid and diploid protoplasts of seed plants (Krumbiegel, 1979) or animals (Mezger-Freed, 1974). In addition, experimental studies of the ability to acquire advantageous mutations in haploid and diploid evolving populations have been conducted in order to test hypotheses regarding the evolution of ploidy. Most of this latter work has been done using yeast (Adams and Hansche, 1974; Hansche, 1974; Paquin and Adams, 1983; and more recently Zeyl et al. 2003, Sliva et al. 2004) apart from one study that used a simple, multicellular organism (a filamentous fungus of the genus *Aspergillus*; Schoustra et al., 2005). The results obtained in these studies were in agreement with the main predictions of the model proposed by Orr and Otto, i.e. that fixation of haploidy or diploidy depends on population size and the dominance of advantageous mutations. However, a very surprising result was published recently by Gerstein et al. (2006) that contrasts with all previous explanations of ploidy evolution (but see Perrot, 1994). Gerstein et al. followed the evolution of haploid, diploid and tetraploid lines of the budding yeast *S. cerevisiae* over 1,800 generations and showed that the haploid and tetraploid lines converged toward diploidy, the

historical state of *S. cerevisiae*. They suggest that evolutionary inertia or historical constraint might prevent shifts away from the ploidy level to which an organism has historically adapted. Nevertheless, the frequent observation of life cycle variations in natural environments for a significant number of algal species (see above) suggests that life cycle evolution may be less constrained in some groups.

The major contribution of recent theoretical studies has been their focus on the maintenance of haploid-diploid life cycles (Mabble and Otto, 1998). Thornber (2006) has recently reviewed experimental studies that address the roles of the two generations of a life cycle at the physiological and the ecological level in seaweeds. Theoretically, if the sporophyte and gametophyte exploit different ecological niches this could stabilise a haploid-diploid life cycle, preventing loss of one of the two generations. There is considerable support for this hypothesis in species that have heteromorphic life cycles adapted to environments that differ in terms of temperature, light level and herbivory (Lubchenco and Cubit, 1980; Cunningham et al., 1993). Such an effect may even apply to isomorphic cycles if there are cryptic differences between the two generations (Hughes and Otto, 1999). New information is in agreement with this hypothesis since differences have been reported in isomorphic species in term of dispersal (Destombe et al., 1992), biomechanical properties (Carrington et al., 2001) and chemical defences (Cronin and Hay, 1996; Potin et al., 1999). This has stimulated efforts to understand the ecology of haploid-diploid life cycles not only in terms of functional properties but also in terms of the population dynamics of the two generations. Indeed, recent studies have provided data on parameters such as the haploid/diploid ratio, fertility and survivorship (Destombe et al., 1989; Ang and De Wreede, 1990; Engel et al., 2001; Thornber and Gaines, 2004; Fierst et al., 2005) providing parameter estimates to help test the models proposed by Richerd et al. (1994) and by Hughes and Otto (1999).

The ecological hypothesis that has received the greatest amount of experimental attention is the nutrient-saving hypothesis of Lewis (1985). The advantage of haploidy for growth in poor environments has been established, not only for unicellular yeast (Adams and Hansche, 1974; Mable, 2001) but also for multicellular organisms (growth of juveniles of the red seaweed *Gracilaria*: Destombe et al. 1993 and mycelium growth of the fungus *Aspergillus*: Perrot, 1994).

Clearly, theoretical models and empirical data have demonstrated the importance of obtaining detailed biological descriptions of the two generations of a life cycle in order to understand the mechanisms that have led to the evolution and maintenance of haploid-diploid life histories. Additional information is required not only about the ecology and the physiology of each generation but also about gene expression and function at the molecular level. However, molecular analyses of this type have only been carried out for a limited number of species so far, essentially within the viridiplantae, with the most detailed information now being available for the model angiosperm *Arabidopsis thaliana.* Genome-scale analyses of gene expression in the male and female gametophyte have recently been carried out for this model organism, complementing the wealth of data already available for the sporophyte generation. The following section summarises some of the results obtained by these studies.

5. Molecular analysis of the male and female gametophytes of flowering plants and comparison with the sporophyte generation

5.1. The male gametophyte

Functional analysis of the gametophyte generation of flowering plants is challenging because both the male and female gametophytes develop within the tissues of the sporophyte, often over a short time-span, and in both cases development is dependent on sporophyte functions (reviewed in Weterings and Russell, 2004). The haploid nature of the gametophyte also means that lethal mutations cannot be maintained in a heterozygous context. Sections 5.1 and 5.2 will describe recent advances in the characterisation of flowering plant male and female gametophytes obtained by the application of genomic and genetic approaches.

The principal function of the male gametophyte of flowering plants is to deliver the male gametes to the female gametophyte. This generation of the life cycle can be divided into two parts, the first corresponding to the development of the male gametophyte in the anther (microgametogenesis) and the second corresponding to its transfer to the female part of the flower and the delivery of the sperm cells by the pollen tube to the embryo sac. Male gametophytes are derived from diploid microsporocytes (pollen mother cells) that undergo meiosis to generate a tetrad of haploid microspores. Each microspore subsequently undergoes two mitotic divisions to generate the male gametophyte (the second of these mitotic divisions occurs either during the development of the pollen grain or in the growing pollen tube, depending on the species).

Early work on gene expression in the male gametophyte concentrated on the identification of genes with a pollen-specific pattern of expression. These studies identified both "early" genes, expressed during development, and "late" genes, expressed in mature pollen and during the pollination process (Mascarenhas 1990, McCormick, 2004). The cell-specific expression patterns of some of these genes have been studied and most are expressed specifically in the

vegetative cell (McCormick, 1993, Taylor and Hepler, 1997). This is consistent with the fact that the chromatin of the sperm cells is condensed, and suggests that the majority of gene expression occurs in the vegetative cell. However, analysis of a maize male sperm cell cDNA library has shown that the sperm cells nonetheless contain a diverse population of mRNAs, many of which are not found in the vegetative cell (Engel et al., 2003). Several of the sperm cell-specific transcripts are already present at the unicellular microspore stage, suggesting that they may be synthesised early and partitioned specifically to the sperm cell following mitosis.

The availability of the complete genome sequence for *Arabidopsis* has made genome-wide transcriptome analysis possible, providing a much more complete picture of gene expression in the male gametophyte of this species. A number of studies have now been carried out using either microarray or SAGE approaches (Becker et al., 2003; Honys and Twell, 2003; Lee and Lee, 2003; Honys and Twell, 2004; Pina et al., 2005) and although there are significant differences between the results obtained, due to differences in the experimental approaches used, some clear general conclusions can be drawn. Firstly, a large number of genes are expressed in the male gametophyte; Honys and Twell (2004) identified 13,977 male gametophyte-expressed mRNAs (61.9% of the genes tested were found to be expressed). Of these genes, only 9.7% were male gametophyte-specific indicating that a large number of genes are expressed during both the gametophyte and the sporophyte generations of the life cycle.

Analysis of different stages of development showed that there is a decline in the complexity of the transcriptome as the male gametophyte matures, particularly at the transition from bicellular to tricellular pollen (Honys and Twell, 2004). This trend was accompanied by an increase in the proportion of male gametophyte-specific transcripts. A number of transcripts

that are abundant at earlier stages become less abundant, whilst transcripts associated with the cytoskeleton, the cell wall and signalling become over-represented suggesting that these classes of gene play particularly important roles in the mature pollen grain.

Taken together, therefore, these genome-scale transcriptome studies have revealed that the male gametophyte is surprisingly complex at the genetic level, despite having a simple structure at the cellular level. A large number of genes are expressed in the male gametophyte and, although most are also expressed in the sporophyte, a significant number are expressed only in the gametophyte indicating the presence of biological processes specific to this generation of the life cycle.

In addition to these transcriptomic approaches, proteomic analyses of pollen proteins have also been carried out for a number of species, including *Arabidopsis*, providing a complementary view of gene expression at the protein level (Mayfield et al., 2001; Kerim et al., 2003; Fernando 2005; Holmes-Davis et al., 2005; Noir et al., 2005; Dai et al., 2006).

Genetic approaches, aimed at identifying genes with important functions during male gametophyte development, are producing information that is complementary to that obtained by the transcriptomic approaches described above. Table 2 lists a number of genes with key roles at specific stages of male gametophyte development that have been characterised genetically. This approach is providing essential information about the regulation of male gametophyte-specific functions both during early development and in the mature pollen grain, thereby providing important insights into male gametophyte function. The relevance of these studies to understanding the life cycle will be discussed below.

5.2. The female gametophyte

Analysis of the transcriptome of the female gametophyte is even more challenging than for the male because it is difficult to separate female gametophyte tissue from that of the surrounding sporophyte. Most of the progress in this area has come from the analysis of cDNA libraries constructed from microdissected female gametophyte cells such as the egg cell or the central cell, mainly from monocot species such as maize or wheat (Dresselhaus et al., 1994; Yang et al., 2006; Le et al., 2005; Sprunck et al., 2005). This sort of approach is not possible with *Arabidopsis* because of the small size of the embryo sac but Yu et al. (2005) were able to identify 225 female gametophyte-expressed genes by a microarray approach that compared wild type ovules with ovules of the *spl* mutant in which the embryo sac fails to develop. As expected with a differential screen of this type, expression of a large proportion of the genes identified (45%) had not been detected previously in any sporophyte tissue. Studies of this kind are providing information about gene expression in the female gametophyte and have identified many genes expressed specifically during this generation of the life cycle. However, at present they fall short of providing the genome-scale view of gene expression that is now available for the male gametophyte of *Arabidopsis*.

As was the case for the male gametophyte, genetic approaches have provided data that are complementary to those obtained by transcriptome analysis and large number of genes that are required for female gametophyte development have been identified in *Arabidopsis* (Table 2). Most of the mutants that do not show lesions on the surrounding sporophytic tissue, fall into phenotypic categories corresponding to key developmental events such as mitosis, nuclear fusion, cellularisation and cell death (Drews and Yadegari, 2002). A recent, high-throughput screen of Ds transposon insertion lines has made a significant contribution to the

list of female gametophyte mutants, identifying 130 genes with this phenotype in *Arabidopsis* (Pagnussat et al., 2005). Nearly half of these mutants were primarily defective in post fertilization processes depending on the maternal allele, suggesting that genes expressed in the female gametophyte or the maternal genome play a major role in the early development of plant embryos.

5.3. How do these molecular analyses help us understand haploid-diploid life cycles?

The principal aim of the studies described above is to obtain a deeper understanding of the biological function of the gametophyte during the plant life cycle. Differential expression studies and tissue- and cell-specific cDNA libraries are identifying genes involved in functions that are specific to each generation. In addition, mutant analyses are identifying genes that regulate specific aspects of gametophyte or sporophyte biology or that are essential for the implementation of the gametophyte or the sporophyte developmental programs. This is important because understanding the biology of the two generations of the life cycle is an essential requisite for understanding the life cycle itself. Such studies can provide important clues as to why the two different generations have been maintained. Clearly, for angiosperms, both the male and the female gametophyte play important roles in reproduction. The habitats of terrestrial plants pose specific problems because the sporophyte stage is immobile and, in many cases (particularly within the angiosperms), non-flagellated gametes must be transferred between plants (or at least between male and female organs) via a hostile, non-aqueous environment. In such species, the pollen grain has important functions in the dispersal and protection of the male sperm cells and also provides the structure (the pollen tube) that delivers the sperm cells to the female gametophyte (McCormick, 2004). The female gametophyte, on the other hand, plays an important role in attracting the pollen tube and by

providing the cellular environment in which the double fertilisation that leads to the production of two "organisms", the embryo and the endosperm, takes place (Drews and Yadegari, 2002). The female gametophyte also has a significant influence on the early development of the sporophyte generation. The grouping of pairs of gametes together within the gametophytes provides a means to coordinate double fertilisation and this may have been a factor that contributed the maintenance of the gametophyte generation in flowering plants. Transcriptome analyses have demonstrated that large numbers of genes are expressed during the gametophyte generation of the life cycle highlighting the importance of this generation in flowering plants, despite its reduced size and complexity. Moreover, significant numbers of genes are expressed specifically in both the male and the female gametophyte indicating specific functions, distinct from those carried out by the sporophyte generation.

It should be noted, however, that these studies have also shown that a large number of genes are nonetheless expressed during both the gametophyte and sporophyte generations. From a theoretical point of view, this phenomenon of "gene sharing" between gametophyte and sporophyte generations is likely to be important because purifying selection, occurring during the haploid gametophyte phase, can eliminate deleterious mutations from those genes, increasing the fitness of the sporophyte generation. Mulcahy (1979) suggested that the closed carpel of angiosperms, particularly when combined with insect pollination (which allows the transfer of large masses of pollen), creates an ideal environment for natural selection to eliminate sub-optimal haploid genomes that reduce the metabolic vigour of pollen grains, compromising their ability to compete with other pollen grains to pollinate an ovule. Selection can potentially act very efficiently during the gametophyte generation because of the large population sizes (of pollen grains) and because the haploid genome allows the genotype to be directly reflected in the phenotype. Several studies have shown that gametophytic selection

can influence the sporophyte generation (i.e. that applying a selection pressure during pollination results in improved growth during the subsequent sporophyte generation, e.g. Mulcahy and Mulcahy, 1975) and the extensive overlap between the sets of genes expressed in the *Arabidopsis* sporophyte and gametophyte generations (identified by the expression experiments described above) provides a genetic basis for this. Moreover, these transcriptomic approaches allow a measurement of the extent of the overlap between the two sets of genes expressed in the sporophyte and the gametophyte, allowing a more accurate quantification of the potential effect of purifying selection during the haploid phase on the fitness of the sporophyte. This sort of information could now be integrated into theoretical models.

Interestingly, a recent study by Seoighe et al. (2005) showed that pollen-specific genes in *Arabidopsis* have significantly shorter introns than genes expressed in the sporophyte and the authors suggest that this is because they are under a specific selection pressure to reduce the cost of transcription by reducing the size of the transcription unit. A similar, but weaker and less consistent, trend was observed for all the pollen expressed genes (ie. including those also expressed in the sporophyte) compared to genes expressed exclusively in the sporophyte. This study provided the first report of a molecular signature of strong gametophytic selection and provides further support for the potential importance of selection during the gametophyte generation.

The availability of detailed information about the genes that are expressed during each generation of the life cycle opens up the possibility of carrying out further analyses of this type in the future, using structural and compositional analyses of these genes to test models of life cycle evolution. For example, as appropriate information on gene polymorphism becomes

available, ratios of non-synonymous to synonymous mutations could be determined for the set of gametophyte-specific genes and compared with the corresponding ratios for the sporophyte-specific gene set to detect and quantify selection pressures during the two generations of the life cycle. In the longer term, it will be interesting to determine the extent of "gene sharing" between the sporophyte and gametophyte in a range of organisms with haploid-diploid life cycles in order to correlate this genetic factor with other life cycle parameters such as whether the life cycle is heteromorphic or isomorphic or whether the two generations are dependent or independent.

Molecular analyses are clearly providing new insights into the biology of the gametophyte and sporophyte generations of the life cycle and the selective pressures acting on these generations. Are they also providing information about the regulation of the life cycle? Mutations in several of the genes listed in Table 2 result in arrest at crucial stages of the transition from the sporophyte to the gametophyte generation and these genes could therefore be considered as candidate regulatory genes. Similarly, genes that are essential for fertilisation or for the initiation of embryo development (acting at the transition from the gametophyte to the sporophyte generation) are also candidate regulators of the life cycle (Berleth and Chatfield, 2002). Care must be taken in assigning a regulatory role to these genes, however, because the observed phenotypes may have other explanations. For example, the transition from the diploid to the haploid state during meiosis can result in the unmasking of deleterious mutations with the result that mutations affecting essential, general ("housekeeping") cell functions can cause a specific arrest at this stage. Similarly, embryo lethal mutations may cause early arrest by affecting "housekeeping" functions that are necessary throughout the sporophyte stage but which do not occur, or are mediated by different genes, during the gametophyte stage (Gallois, 2001). In other cases, the effect of a mutation may be indirect, for

example many mutations that cause early arrest of male gametophyte development (Wilson et al., 2001; Canales et al., 2002; Zhao et al., 2002; Yang et al., 2003; Albrecht et al., 2005; Colcombet et al. 2005; Vizcay-Barrena et al., 2006) also affect development of the tapetum (the cell layer that surrounds the developing microspores in the anther) and their effect on gametophyte development may, therefore, be indirect, as male gametophyte development is known to be highly dependent on the presence of a functional tapetum. Even when a mutation affects a gene that has a specific function during a key stage of the life cycle, the gene may be a downstream effector rather than a regulatory gene (although, the distinction between these two classes may sometimes be difficult, for example when an effector is also a key integrator of several regulatory signals). This is probably the case for many of the genes with specific roles during meiosis (Caryl et al., 2003).

One strong candidate for a life cycle regulatory gene is the *Arabidopsis SPOROCYTELESS* (*SPL*) gene. *SPL* is essential for the differentiation of sporocytes (which normally subsequently undergo meiotic divisions to form microspores and megaspores; Yang et al., 1999). On the female side, ovule primordia of *spl* mutants possess an enlarged hypodermal cell but this does not differentiate into a megasporocyte and meiosis does not occur. Similarly, on the male side, primary sporogenous cells fail to differentiate into microsporocytes. The tapetum also fails to develop but this is probably an indirect effect of the loss of sporocyte differentiation. SPL is a novel nuclear protein related to MADS box transcription factors. This is consistent with the proposition that it acts as a transcriptional regulator of sporocyte development (Yang et al., 1999).

The *Arabidopsis AGP18* gene is expressed very specifically in the megaspore mother cell, in the four meiotically-derived megaspores and in the adjacent nucellar cells. RNA interference-

induced posttrancriptional silencing of this gene resulted in the arrest of female gametophyte development at the one-cell, megaspore stage suggesting that this gene may have a very specific function during the initiation of the developmental program of the female gametophyte (Acosta-Garcia and Vielle-Calzada, 2004).

Ovules of *Arabidopsis DYAD/SWITCH1* mutants contain a pair of large cells in the place of an embryosac (Siddiqi et al., 2000). At first view this phenotype could be interpreted as representing a switch from a meiotic to a mitotic division of the megaspore mother cell or, in other words, a switch from a haploid (gametophyte) to a diploid (sporophyte?) developmental program. However, detailed analysis of this mutant revealed that the two cells are actually the product of a defective meiotic division indicating that *DYAD*/*SWITCH1*'s effect on life cycle progression is mediated via an essential role during meiosis (Siddiqi et al., 2000, Mercier et al., 2003). This example clearly demonstrates the importance of careful analysis of mutant phenotypes when interpreting the effects of mutations that modify crucial steps of the life cycle.

Ectopic expression of several genes (*BABY BOOM*, *SERK*, *LEC1* and *LEC2*) has been shown to promote embryo formation suggesting that they may play important roles in the initiation of the sporophyte developmental program (Boutilier et al., 2002; Schmidt et al., 1997; Lotan et al., 1998; Stone et al., 2001). Moreover, the *Brassica napus* homologue of BABY BOOM (Boutilier et al., 2002) and the maize homologue of SERK (ZmSERK1; Baudino et al., 2001) are both expressed during microspore embryogenesis (androgenesis) where there is a transition from a gametophyte to a sporophyte pattern of development. It should be noted, however, that all four genes have a very broad pattern of expression (even if expression levels are usually higher in reproductive organs) and they may therefore act downstream of the

mechanism that controls the switch from gametophyte to sporophyte development.

Epigenetic modifications of chromatin have been shown to play important roles at many transition points during the life cycles of both plants and animals (Guitton and Berger, 2005a). In *Arabidopsis*, the FIE-MEA polycomb complex, which includes MEDEA (MEA), FERTILISATION INDEPENDENT ENDOSPERM (FIE), FERTILISATION INDEPENDENT SEED 2 (FIS2) and MULTICOPY SUPPRESSOR OF IRA 1 (MSI1), plays a key role at the transition between the gametophyte and sporophyte generations. Mutation of the genes encoding these proteins has been shown to lead to the initiation of sporophyte development resulting in the proliferation of a diploid endosperm and, in some instances, formation of an embryo-like structure from the egg cell (Chaudhury et al., 1997; Guitton and Berger, 2005b).

A number of candidate life cycle regulatory genes have therefore been identified for both the gametophyte and the sporophyte generations but these studies have not yet provided a clear picture of the regulatory pathways involved. Evolutionary models could make a useful contribution to the efforts to dissect these regulatory pathways. For example, as discussed above, it has been proposed that haploid-diploid life cycles arose in the green lineage by a two-step process in which meiosis and syngamy were synchronised with a pre-existing, asexual cycle that involved an alternation between small and large individuals (Bell, 1992). If this was the case then we might expect the regulatory pathways that control sporophyte/gametophyte alternation to be different to those that control meiosis and syngamy (with cross-regulation occurring between the two pathways). The model therefore provides a conceptual framework for the interpretation of experimental data and suggests testable hypotheses about the system.

6. New approaches for identifying genes that regulate progression through the life cycle

6.1. Limitations of current model organisms

Molecular approaches are proving to be an effective means to investigate the biological functions of the two generations of haploid-diploid life cycles, at least in model organisms such as *Arabidopsis*. They have also allowed the identification of a small number of genes that are essential for crucial steps in the life cycle such as sporocyte development and meiosis. However, for the multicellular organisms that are the subject of this review, perhaps the most interesting regulatory mechanisms are those that coordinate the sporophyte and gametophyte developmental programs with the alternation between meiosis and syngamy during the life cycle, and little progress has been made in this area.

We do not understand, for example, how organisms with haploid-diploid life cycles assure that the appropriate program of multicellular development (sporophyte or gametophyte) is deployed at the appropriate stage of the life cycle. Presumably, regulatory mechanisms exist that detect key events in the life cycle, such as meiosis and syngamy, and initiate the appropriate developmental program in response to these events. Characterisation of such regulatory mechanisms would be a crucial step towards understanding the evolution and function of haploid-diploid life cycles. Factors that influence the structure of the life cycle are expected to act via such regulatory mechanisms. Access to the corresponding genes would, therefore, allow the effects of factors such as seasonal changes or stresses to be assessed at the

molecular level providing essential clues to the function of the different generations of the life cycle.

From a practical point of view, however, identification of genes that coordinate sporophyte/gametophyte development with life cycle progression may be difficult because mutations in such genes might be expected to lead to gametophyte or embryo lethality. Mutations in this class of life cycle regulatory gene would therefore be difficult to distinguish from mutations in downstream genes that are essential for the progression of early events in the sporophyte and gametophyte developmental programs. In theory, however, a mutation in a gene that regulates the transition between two generations of a life cycle could also lead to the development of the "wrong" generation at one of the transition points in the life cycle, a gametophyte where a sporophyte would be expected for example. This would be analogous to a homeotic conversion at the tissue and organ level, although in this case occurring at the level of an individual organism. It could be argued that such a phenotype would be unlikely because of the difference in ploidy between the two generations but, as discussed above, the relationship between ploidy and the alternation of generations during the life cycle is not absolute. Redirection from a gametophyte to a sporophyte developmental program clearly occurs during androgenesis and gynogenesis in flowering plants but the regulatory mechanisms that control these processes have not yet proved to be accessible genetically. This may be because early development of both the gametophyte and the sporophyte occurs within the tissues of the parental generation, with the parent having an important influence on the development of the next generation of the life cycle, at least during the early stages. In such a situation, where multiple regulatory inputs both from within and from outside the gametophyte or sporophyte are necessary for normal developmental progression (or even if these external influences tend only to maintain the original state), mutants in which there is a

switch from one generation to the other would be very difficult to obtain.

Are there alternative model systems in which it might be easier to identify life cycle regulatory genes? Mosses are potentially interesting organisms for this type of study because both the sporophyte and the gametophyte exhibit a certain amount of developmental complexity. Mosses also possess most of the developmental patterning genes found in flowering plants (Floyd and Bowman, 2007). Many of these genes are expressed during the gametophyte generation (Nishiyama et al., 2003) and it has been proposed that gametophyte genes have been co-opted for the sporophyte developmental program during the evolution of land plants. Homologous recombination in the model moss *Physcomitrella patens* represents a powerful tool to explore gene function, particularly when combined with recently developed genomic tools including the complete genome sequence, large numbers of ESTs, and RNAinterference methodology (Quatrano et al., 2007). However, the development of the sporophyte in mosses is likely to be considerably influenced by the gametophyte generation because the former generation grows "parasitically" on the latter (although this is obviously also a problem in *Arabidopsis*, where it is the gametophyte generation that is dependant on the sporophyte generation). Moreover, classical genetic approaches involving crosses are technically difficult in *P. patens*, limiting the scope for forward genetic approaches. However, this latter limitation will be alleviated by the growing potential for high-throughput approaches based on the genome sequence.

Another potentially interesting model organism is the fern *Ceratopteris* (Hickok et al., 1995; Banks, 1999). Ferns also represent potentially interesting models to study the alternation of generations in haploid-diploid life cycles because they possess a multicellular gametophyte with differentiated cells and a specific developmental pattern together with a morphologically

complex sporophyte. Apospory can be induced in *Ceratopteris* (DeYoung et al., 1997), indicating that gametophyte/sporophyte alternation can be uncoupled from ploidy, but no mutants that affect the switch between the two generations of the life cycle have been reported so far in this system.

The diversity of the life cycles of brown and red macroalgae also makes them obvious candidates for the study of life cycle regulation but the lack of well-developed model organisms in these groups has hitherto limited the scope for investigating these systems on a molecular level. This situation is currently changing and model organisms are emerging for both the red and the brown macroalgae. For the red algae, interest is being focused on *Porphyra yezoensis* (Waaland et al., 2004; Kitade et al., 2004), whilst *Ectocarpus siliculosus* has been proposed as a model organism for the brown algae (Peters et al., 2004). *Ectocarpus* is particularly interesting because it's life cycle involves an alternation between morphologically similar sporophyte and gametophyte generations, both of which develop independently of the parent organism from a single progenitor cell that is released into the sea water environment. Moreover, both haploid sporophyte and diploid gametophyte variants have been described in *Ectocarpus* indicating a weak relationship between life cycle generation and ploidy in this organism (Müller, 1967; Figure 2). *Ectocarpus*, therefore, represents a promising system to search for mutations that affect the switch between the sporophyte and gametophyte generations.

The following section will provide an overview of the current efforts to develop *Ectocarpus* as a model organism and the subsequent section will then describe the life cycle of *Ectocarpus* in more detail and the approaches being used to investigate its regulation at the molecular level.

6.2. Emergence of Ectocarpus siliculosus *as a general model organism for the brown algae*

Experimental work on *Ectocarpus* dates back to the beginning of the 19th century (Berthold, 1881) but it was principally the work of Dieter Müller's group in Konstanz, Germany over the last 4 decades that has led to the emergence of this organism as a laboratory model (Müller, 1967, 1976; Müller et al., 1971, 1990; Bräutigam et al., 1995; Delaroque et al., 2001). *Ectocarpus* has a number of features that make it well adapted as a laboratory organism. It can be grown in nutrient-enriched seawater in Petri dishes and the life cycle can be completed in the laboratory in a period of about 3 months (Müller et al., 1998; Peters et al., 2004). In culture, this alga usually becomes mature and fertile at a size of one to two centimetres (although thalli are often considerably larger in the field) making it easy to handle large numbers of organisms. *Ectocarpus* is highly fertile, producing large numbers of several different types of zoids, and controlled crosses can be carried out, allowing classical genetic methods to be used. Moreover, the *Ectocarpus* genome is relatively small (approximately 200 Mbp) compared to other model brown algae such as *Fucus serratus* or *Laminaria digitata* (1095 and 640 Mbp respectively; Peters et al., 2004).

Based on these features, we have recently proposed *Ectocarpus* as a general model organism for the brown algae with the aim of making genomic tools and gene function analysis available for this species (Peters et al., 2004). The genome of *Ectocarpus* is being sequenced as part of this project (http://www.cns.fr/externe/English/Projets/Projet_KY/organisme_KY.html) and genetic screens for a range of different types of mutant, including life cycle mutants, have been, and are being, carried out.

6.3. Ectocarpus siliculosus: *a model haploid-diploid life cycle*

The sexual part of the life cycle of *Ectocarpus* involves an alternation between a diploid sporophyte and haploid, dioecious (male and female) gametophytes (Figure 2). Sporophytes and gametophytes have a similar morphology, both consisting of branched, uniserate filaments, so that it can be difficult to distinguish between sporophytes and gametophytes in the field. In culture, however, sporophytes form fairly compact thalli that are firmly attached to the substratum whilst gametophytes have a more feathery appearance and are less strongly attached, tending to float off into the medium.

Haploid gametophytes produce plurilocular gametangia containing either male or female gametes of identical size (morphological isogamy). Zygotes formed from the fusion of a male and a female gamete develop into diploid sporophytes which, in turn, produce the meiospores that will develop to form the next gametophyte generation. Meiospores are produced in unilocular sporangia in which a single meiosis, followed by several mitotic divisions, gives rise to about 100 spores.

In addition to this sexual cycle, *Ectocarpus* can also reproduce asexually in a number of different ways. The simplest of these is via the production of mito-spores by the sporophyte (in plurilocular sporangia that are morphologically similar to the plurilocular gametangia of the gametophyte). These mito-spores represent a means of clonally multiplying the sporophyte generation. A second, particularly interesting mode of asexual reproduction is the parthenogenetic development of unfertilised gametes (i.e. gametes that have not encountered a gamete of the other sex) into sporophytes. Estimates of ploidy based on chromosome counts

indicate that in most cases these parthenosporophytes are haploid (Müller, 1967). The concept of a haploid sporophyte can be confusing but these organisms are clearly functional sporophytes; the zoids produced in their plurilocular zoidangia are mito-spores and not gametes because they are unable to fuse with gametes of the opposite sex to produce zygotes. Moreover, the parthenosporophytes produce unilocular sporangia, structures that are only found in the sporophyte generation.

The reason these parthenosporophytes are interesting is because they indicate that the "choice" to deploy the gametophyte or the sporophyte developmental program is not determined by the ploidy of the initial cell but rather is under some sort of genetic or epigenetic control. Preliminary data from mutant analyses support this interpretation. Two single-locus mutations have recently been isolated that cause partial and complete conversion, respectively, of the sporophyte generation into a gametophyte (unpublished data). Future exploitation of this type of mutant in *Ectocarpus* is expected to provide access to the regulatory mechanisms that control the switch between the two generations of the life cycle. An understanding of these mechanisms would bring us one step closer to solving the perennial mystery of the evolution and stable maintenance of haploid-diploid life cycles.

7. Conclusion

A wide range of life cycles are found in nature and a considerable amount of theoretical work has gone into trying to explain why this should be so and into modelling the potential advantages of each type of life cycle. Advantages of both diploid and haploid life cycles have been proposed based on genetic factors, such as resistance to DNA damage or the advantages

and disadvantages of masking deleterious or advantageous mutations in diploid individuals, and on indirect effects of ploidy such as cell size. Another set of models attempt to explain the evolutionary stability of haploid-diploid life cycles and invoke factors such a reduced cost of sex and the potential ability of organisms with such life cycles to exploit different ecological niches during each generation of the cycle. In contrast, only a limited number of experimental studies have been carried out to test these various hypotheses. In this review, we have underlined the potential of both established and emerging model organisms as tools to test theoretical hypotheses about the evolution and stability of different types of life cycle. By allowing genetic and genomic approaches, model organisms provide access to the molecular basis of life cycle events and the information generated by this sort of study can be used to address specific questions. For example, the identification of genes expressed specifically in either the sporophyte or the gametophyte provides vital information about the biology of each generation of the life cycle, permitting insights into potential differences between the ecological niches of the two generations. The availability of generation-specific genes also opens up the possibility of accessing the effects of selection pressure during the two generations of a life cycle. Moreover, genetic analysis of these model organisms aimed at identifying the regulatory cascades that control the progression of the life cycle are expected to provide insights into basic questions such as the relationship between ploidy and alternation of generations and how these two phenomena interact at the molecular level. Being able to answer such questions will represent an important step towards understanding the life cycles themselves. The remarkable advances in the development of technologies for the molecular analysis of model organisms in recent years has created a situation in which convergence between theoretical and experimental studies should be greatly facilitated and exciting progress can be expected in this domain in the coming years.

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Figures

Figure 1. Major types of sexual life cycle found in the eukaryotes. See text for details.

Figure 2. The complex life cycle of *Ectocarpus siliculosus*. *Ectocarpus* has a haploid-diploid cycle (grey boxes) involving an alternation between a diploid sporophyte and dioecious (male and female), haploid gametophytes. Several variations on this basic cycle have been observed in culture including the parthenogenetic development of haploid sporophytes from unfertilised gametes and the production of diploid gametophytes by tetraploid sporophytes (white boxes). Some of these variations, such as the production of tetraploid sporophytes for example, only occur rarely. Gametophytes produce gametes in plurilocular gametangia (P) whilst sporophtyes produce spores in either plurilocular (P) or unilocular (U) sporangia. Meiosis occurs in the unilocular sporangia. The presence of the male and female sex factors is indicated (in brackets for the sporophytes because the sex factor is only expressed phenotypically in the gametophyte). Adapted from Müller (1967).

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Table 1. Definitions of some of the terms used in the text.

Term used	***Definition***
phase	A stage of the any life cycle corresponding to a certain level of ploidy e.g. the diploid phase.
generation	A stage of the life cycle of a multicellular organism during which somatic development takes place e.g. the sporophyte generation (generation is used rather than phase in this case to avoid confusion between sporophyte/gametophyte alternation and changes in ploidy as these two processes are not necessarily correlated). Note, however, that other authors may have used generation differently, to refer to a complete life cycle.
gamete	A reproductive cell capable of fusing with another gamete to produce a zygote. Note that gametes of some species can also develop without sexual fusion (parthenogenesis).
spore	A reproductive cell that is not capable of fusing with another cell.
gametophyte	A gamete-producing plant
sporophyte	A spore-producing plant.

Table 2. Genes required for normal gametophyte development in flowering plants. An asterisk (*) indicates genes that act gametophytically. Those not marked with an asterisk either act sporophytically or both gametophytically and sporophytically. All genes are from *Arabidopsis* unless stated otherwise.

Gene	***Function***	***Reference***
Female gametophyte		
*PROLIFERA (PRL)**	Required for the first cell division during megagametogenesis. Female specific.	Springer et al., 1995
*HADAD (HDD)**	Required for the first cell division during megagametogenesis. Female specific.	Moore et al., 1997
*LACHENSIS (LIS)**	Restriction of gametic cell fate.	Gross-Hardt et al., 2007
GFA2	Involved in synergid cell death.	Christensen et al., 2002
CYTOKININ-INDEPENDENT1 (CKI1)	Histidine kinase homolog required for megagametogenesis.	Pischke et al., 2002; Hejatko et al., 2003
DYAD / SWITCH1	Female-specific arrest in meiosis I.	Siddiqi et al., 2000; Agashe et al., 2002
NOMEGA	Role in cell cycle progression during gametophyte development.	Kwee and Sundaresan, 2003
RETINOBLASTOMA RELATED 1 (RBR1)	Cell cycle control during female gametogenesis.	Ebel et al., 2004
SLOW WALKER1 (SWA1)	Progression of the mitotic division cycles of the female gametophyte is disrupted in the mutant.	Shi et al., 2005,
CHROMATIN REMODELLING PROTEIN 11 (CHR11)	Essential for haploid nuclear proliferation during megagametogenesis.	Huanca-Mamani et al., 2005
ARABINOGALACTAN PROTEIN 18 (AGP18)	Expressed specifically in cells that spatially and temporally define the sporophytic to gametophytic transition and during early stages of seed development; essential for the initiation of female gametogenesis.	Acosta-Garcia and Vielle-Calzada, 2004)
MYB98	Expressed exclusively in the synergid cells, mutations in this gene affect only the female gametophyte.	Kasahara et al., 2005
NUCLEAR FUSION DEFECTIVE1 (NFD1)	Required for karyogamy during female gametophyte development.	Portereiko et al., 2006b
STERILE APETALA (SAP)	Transcription regulator involved in completion of megasporocyte meiosis and in determining floral organ number.	Byzova et al., 1999
FEM111 / AGL80	In *fem111* female gametophytes, the central cell's nucleolus and vacuole fail to mature correctly.	Portereiko et al., 2006a
AINTEGUMENTA (ANT)	Integuments missing in mutant and defects in female meiosis.	Klucher et al., 1996; Elliot et al., 1996
BELL1 (BEL1)	Integuments abnormal in the mutant.	Reiser et al., 1995
ELONGATE1	Maize gene, absence of meiosis II with one of the dyad cells directly initiating megagametogenesis in the mutant.	Barrell and Grossniklaus, 2005

INDETERMINATE GAMETOPHYTE1 (IG1)	Maize gene that encodes a LOB domain protein required for embryo sac and leaf development; restricts the proliferative phase of female gametophyte development.	Evans et al., 2007 Huang and Sheridan, 1996
CONSTITUTIVE TRIPLE RESPONSE (CTRL1)	Ethylene-response mutant defective in female gametophyte development.	Kieber et al 1993
MULTIPLE ARCHESPORIAL CELLS1 (MAC1)	Maize gene involved in the formation of multiple archeosporial cells in ovules.	Sheridan et al., 1996
GAMETOPHYTIC FACTOR1-6 (GFA1-6)	Required for megagametogenesis.	Feldmann et al 1997
GAMETOPHYTIC FACTOR (GF)	Required for megagametogenesis.	Redei 1965; Christensen et al 1997
FEMALE GAMETPHYTE 1-4 (FEM1-4)	*fem1* and *fem2* mutations affect only the female gametophyte, while the *fem3* and *fem4* mutations affect both the female and male gametophyte.	Christensen et al 1998
FEM5-38	Mutation affects the female gametophyte.	Christensen et al., 2002
LYSOPHOSPHATIDYL ACYLTRANSPHERASE 2 (LPAT2)	Essential for female gametophyte development, expressed in the male and female gametophyte.	Kim et al., 2005
Female/Male gametophyte		
SPOROCYTLESS (SPL) / NOZZLE (NZZ)	Required for the production of male and female sporocytes; MADS box-related transcription factor.	Yang et al., 1999; Schiefthaler et al., 1999
GLUCOSE 6-PHOSPHATE/PHOSPHATE TRANSLOCATOR1 (GPT1)	Mutants show inability to complete pollen and ovule development.	Niewiadomski et al., 2005
ASYNAPTIC1 (ASY1)	Mutant affected during male and female synapsis.	Caryle et al., 2000; Amstrong et al., 2002
UNGUD	Affects both male and female gametogenesis.	Lalanne et al 2004
SYN1/DIF1	Expressed specifically in male and female meiocytes, encodes a protein that is similar to cyclins.	Bai et al., 1999; Bhatt et al., 1999
SOLO DANCERS (SDS)	Required for the interaction between homologous chromosomes during meiotic prophase I (male and female meiocytes).	Azumi et al., 2002
AtDMC1	Abnormal meiosis in pollen mother cells and in megaspore mother cells	Klimyuk and Jones, 1997; Couteau et al., 1999
AtMND1	Essential for male and female meiosis.	Kerzendorfer et al., 2006
Male Gametophyte		
STUD (STD) /TETRASPORE (TES)	Required for male meiotic cytokinesis.	Hulskamp et al., 1997; Spielman et al., 1997
QUARTET (QRT)	Required for microspore separation.	Rhee and Somerville, 1998; Rhee et al., 2003
*SIDECAR (SCP)**	Required for the normal cell division pattern during pollen development.	Chen and McCormick, 1996
TWO IN ONE (TIO)	Required for cytokinesis at pollen meiosis I.	Twell et al., 1998
MOR1/GEMINI POLLEN1 (GEM1)*	Required for microspore polarity, division asymmetry and involved pollen cell fate.	Park et al., 1998; Twell et al., 2002
*LIMPET POLLEN (LIP)**	Required for generative cell migration after pollen meiosis I.	Howden et al., 1998

*MALE GERM UNIT DISPLACED (MUD)**	Required for the correct positioning of the male germ unit in the mature pollen grain.	Lalanne and Twell, 2002
*GERM UNIT MALFORMED (GUM)**		
*SETH**	Essential for male progamic development. Male specific.	Lalanne et al 2004
*HALFMAN (HAM)**	Male gametophytic; required for pollen development.	Oh et al 2003
SOMATIC EMBRYOGENESIS RECEPTOR KINASE1/2 (SERK1/2)	Required for tapetum development and microspore maturation.	Colcombet et al., 2005; Albrecht et al., 2005
MALE STERILE5(MS5)/TDM/POLLENLESS3	Mutations in *TDM* cause the formation 'polyads' (tetrads with more than four pools of chromosomes after male meiosis).	Glover et al., 1998
ARABIDOPSIS SPK1-LIKE1 (ASK1)	Essential for early nuclear reorganization events during meiosis.	Zhao et al., 2006; Yang et al., 2006
MALE STERILITY1 (MS1)	Transcriptional regulator of male gametogenesis, mutant has altered tapetal cell death and pollen wall development.	Wilson et al., 2001; Vizcay-Barrena et al., 2006
DEFECTIVE-POLLEN3/ MEIOSIS1 (MEI1)	Chromosomes are fragmented during meiosis in the mutant resulting in aberrant microspores.	Sanders et al., 1999; He and Mascarenhas, 1998
CYCLIN-DEPENDENT KINASEA-1 (CDKA-1)	*Cdc2* homologue; essential for division of the generative cell during male gametogenesis.	Iwakawa et al., 2006
EXTRA SPOROGENOUS CELLS (EXS)/EXCESS MICROSPOROCYTES1 (EMS1)	Regulates germline cell number and tapetal identity, and promotes seed development.	Canales et al., 2002; Zhao et al., 2002
DYSFUNCTIONAL TAPETUM1 (DYT1)	Controls anther development and function, *DYT1* acts downstream of SPL/NZZ and EMS1/EXS.	Zhang et al., 2006
TAPETUM DETERMINANT1 (TPD1)	Required for the tapetal cell fate.	Yang et al., 2003
ABORTED MICROSPORES (AMS1)	MYC class transcription factor with a role in tapetal cell development and the post-meiotic transcriptional regulation of microspore development.	Sorensen et al., 2003
UNDEVELOPED TAPETUM1 (Udt1) (rice)	Rice gene required for the maturation of tapetal cells.	Jung et al., 2005
GUS NEGATIVE1 (GNE1)	Required for tapetum and middle-layer cell formation.	Sorensen et al., 2002
MALE MEIOCYTE DEATH (MMD) / DUET	Required for male meiosis.	Reddy et al., 2003; Yang et al., 2003
DESYNAPTIC1 (DSY1)	Mutant affected during synapsis.	Ross et al., 1997
PARTING DANCERS (PTD)	Mutant has defective meiosis.	Wijeratne et al., 2006
SPO11	Disruption of *SPO11* causes defects in meiotic recombination, synaptonemal complex formation and bivalent formation.	Grelon et al., 2001
RAD50	Involved in meiosis and DNA repair.	Gallego et al., 2001; Gallego and White, 2001
MRE11	Mutant shows chromosome fragmentation and the absence of synapsis in the initial stages of meiosis of pollen mother cells.	Puizina et al., 2004
BRCA2	Involved in meiosis, interacts with *DCM1*, *RAD51* and *DSS1*.	Siaud et al., 2004; Dray et

		al., 2006
*RARING-TO-GO (RTG)**	Pollen grains germinate precociously within the anther	Johnson and McCormick 2001
TARDY ASYNCHRONOUS MEIOSIS (TAM)	Delayed and asynchronous cell divisions during male meiosis producing dyad pollen (two gametophytes within one exine wall)	Magnard et al., 2001
MULTIPLE SPOROCYTE1 (MSP1)	Crucial roles in restricting the number of cells entering into male and female sporogenesis and in initiating anther wall formation in rice	Nonomura et al., 2003