Genetic and cellular characterisation of parthenogenesis in the brown alga Ectocarpus sp.

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Caractérisation génétique et cellulaire de la parthénogenèse chez l'algue brune *Ectocarpus* sp.

Genetic and cellular characterisation of parthenogenesis in the brown alga Ectocarpus *sp.*

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Thèse de doctorat de Génétique et biologie du dévelopement

Dirigée par Susana M. Coelho et J. Mark Cock

Présentée et soutenue publiquement le 14 Décembre 2018

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À ma famille,

À Romain,

« Toute avancée des connaissances génère

autant d'interrogations qu'elle apporte de réponses. »

Pierre Joliot-Curry

Remerciements

La thèse de doctorat exige un investissement personnel, professionnel et intellectuel. C'est un travail de longue haleine qui peut paraitre fastidieux et frustrant mais terriblement intéressant et enrichissant sur le plan professionnel, intellectuel et personnel justement. Outre les compétences acquises, c'est le chemin parcouru et la manière dont nous l'avons parcouru qui constitue notre véritable expérience. Il parait évident que personne ne peut parcourir ce chemin à notre place. Il n'appartient qu'à nous de découvrir notre rapport face à l'adversité, aux doutes, à l'échec ou au succès. Néanmoins, sur ce chemin que nous empruntons seul, le travail réalisé n'est jamais solitaire et il me parait donc important et nécessaire de mettre en lumière, celles et ceux, qui ont contribué à l'aboutissement de ce travail.

En premier lieu, je voudrai remercier mes directeurs de thèse, Susana Coelho et Mark Cock, pour m'avoir offert l'opportunité de travailler au sein de leur équipe sur ce sujet passionnant. S'ils m'ont permis de m'épanouir dans le laboratoire, de gagner en autonomie et se sont montrés disponibles quand j'en avais besoin, je pense qu'ils m'ont, avant tout, particulièrement bien armée pour le métier de chercheur. Je tiens donc à leur exprimer toute ma gratitude.

En tant que jeune chercheuse, l'avis de scientifiques expérimentés est très important. Je remercie donc les membres de mon jury de thèse, le Dr Tanja Schwander, le Dr John Pannell, le Pr Christophe Destombe et le Dr Julie Jaquiéry, ainsi que les membres de mon comité de thèse, le Dr Ueli Grossniklaus, le Dr Denis Roze et le Dr Jean-Pierre Bouly, pour avoir pris le temps d'évaluer mon travail et lui avoir porté de l'intérêt et pour m'avoir prodigué de précieux conseils.

Je tiens à remercier particulièrement le Dr Komlan Avia pour m'avoir appris la technique de RAD-seq, la construction de cartes génétiques et l'analyse de données QTL. Merci d'avoir répondu à mes nombreuses questions dans la bonne humeur et merci pour les nombreux fous rires qu'on a partagé.

Ce travail n'aurait pas abouti sans la contribution du Dr Akira Peters que je remercie grandement. Il a fourni les nombreuses souches utilisées dans cette étude et à réaliser de

nombreux croisements. Je remercie également, Delphine Scornet, pour son aide pour entretenir les cultures de toutes ces souches et pour les réponses qu'elle m'a apportées quant à la culture d'*Ectocarpus*.

Au cours de cette thèse, j'ai été amenée à collaborer et notamment à travailler au sein de l'équipe du Professeur Taizo Motomura au Japon. Je tiens à le remercier ainsi que le Dr Chikako Nagasato pour m'avoir accueillie deux mois dans leur équipe. Ils m'ont enseignée, entre autre, les techniques de microscopie confocale et électronique à transmission appliquées aux algues brunes. Je les remercie d'avoir partagé leurs connaissances et compétences scientifiques ainsi que leur culture.

J'ai également pu collaborer avec le Dr Walid Djema, de l'Inria Sophia-Antipolis, qui a pris le temps d'élaborer un modèle mathématique pour la transmission des mitochondries chez *Ectocarpus*. Il a surtout eu la patience de m'expliquer très clairement et précisément comment fonctionnait ce modèle sachant que la modélisation mathématique n'est pas mon domaine d'expertise.

Je remercie les membres, passés et présents, de l'équipe « Génétique des algues » avec qui j'ai eu plaisir à travailler et échanger. Je remercie également les chercheurs, chercheuses, post-doc, techniciens et doctorants du laboratoire de biologie intégrative des modèles marins pour leur accueil et leur bonne humeur.

Il me faut remercier celles et ceux qui ont partagé ces trois années dans les rires et au cours de soirées inoubliables, en particulier Hervé Rabillé, Maria Matard-Man, Adèle James, Céline Conan, Florent Souchaux, Laetitia Mest, Anaïs Naretto, Jonathan Dorival, Marie-Mathilde Perrineau, Yacine Badis, Florian Ponteaux, Simon Bourdareau et Haiqin Yao. Merci à Zofia Nehr et Aga Lipinska pour avoir ravitailler le bureau en bonbons et chocolats pendant ces semaines de rédaction. Olivier Godfroy, merci pour ces dialogues de sourds dans le bureau, j'ai pu constater que je n'étais pas la seule à parler à mon ordinateur. Mention spéciale à Elodie Rolland et Josselin Guéno (Jojo pour les intimes), je n'oublierai pas ces magnifiques dessins disséminés sur ma paillasse. J'oublie probablement des gens et je m'en excuse grandement.

Enfin, je tiens à remercier mes parents et mon frère, qui m'ont soutenu malgré la distance et qui ont toujours été présents dans les moments difficiles. Je voudrai remercier Romain, mon fiancé, qui m'a épaulée et encouragée jusqu'à la fin de cette aventure.

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General introduction

I. Sexual versus asexual reproduction

"We do not even in the least know the final cause of sexuality;

why new beings should be produced by the union of the two sexual elements,

instead of by a process of parthenogenesis"

Charles Darwin (1862)

Reproduction is a fundamental and universal biological process by which new individuals are produced from their parent(s). Organisms may adopt two strategies to reproduce, either using sexual or asexual reproduction. Many organisms combine both strategies, alternating between asexual cycles and occasional sexual reproduction. The maintenance of sex is one of the most debated topics in evolutionary biology, as sex is costly in terms of investment per offspring but at the same time widespread among eukaryotes. A large number of hypotheses have been put forward to explain this evolutionary paradox. In the first section of the introduction, I will review studies that have focused on the origin and maintenance of sexual reproduction, including the costs and benefits of meiotic sex, but also the evolution of the sexes. I will then describe the different modes of asexual reproduction, their advantages and costs and finish by focusing on parthenogenesis, a specific mode of asexuality.

1. Evolution of sexual reproduction

1.1. Definition of sex

How do we define sex? There are at least two commonly used definitions of sex. Sex may be defined as the process leading to exchange of genetic material between individuals or simply as the occurrence of meiosis (Maynard Smith, 1978; Levin, 1988). Sex defined as an exchange of genetic material between individuals can be found in both eukaryotic and prokaryotic organisms (Maynard Smith, 1978; Levin, 1988). However, there are fundamental differences between eukaryotic sex and bacterial sex. In bacteria, sex involves the unidirectional transfer of genetic material from a donor cell to a recipient cell through conjugation or transformation.

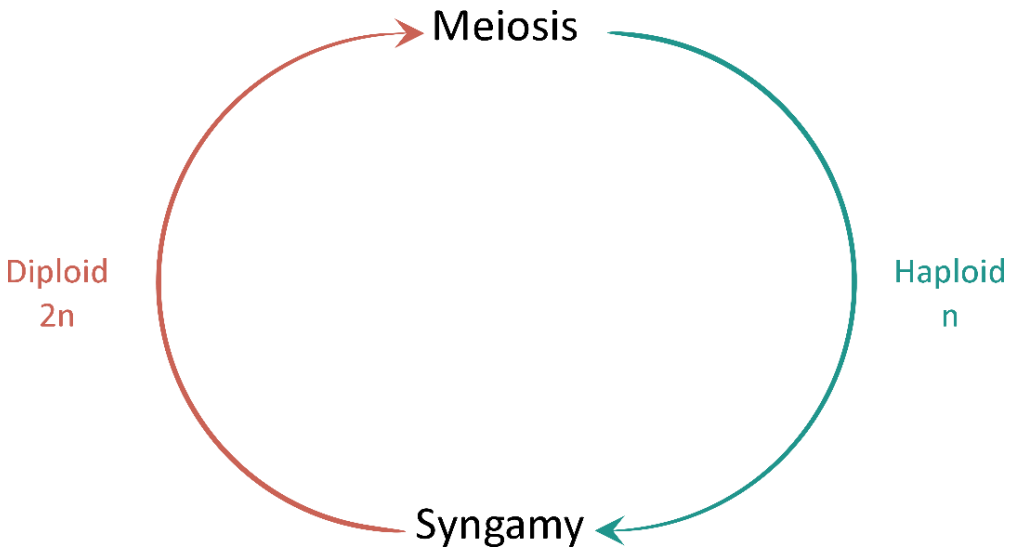

Figure 1: Simplified life cycle of organisms reproducing through meiotic sex. Organisms reproducing via meiotic sex exhibit life cycles involving two key event, meiosis and syngamy resulting in an alternation between haploid and diploid stage.

Eukaryotic sex, in contrast, involves alternation between a haploid phase resulting from meiosis and a diploid phase following the fusion of two gametes. When defined as a reproductive process associated with meiosis, sex does not include virus reproduction or bacterial conjugation (Maynard Smith *et al.*, 1991; Maynard Smith, 1990). I will refer to sex under this definition as meiotic sex. Meiotic sex is nearly universal in eukaryotes and ensures the production of new genetic combinations via two highly conserved mechanisms: meiosis (a specialized type of cell division that reduces the chromosome number by half) and syngamy (the fusion of haploid cells or gametes). Syngamy allows the admixture of two genomes while meiosis reduces ploidy and ensures recombination between the parental genomes (Figure 1).

As far as the evolution of sexual reproduction is concerned, two main questions have been raised: 1) what is the origin of sex and 2) why is sex the predominant mode of reproduction among eukaryotes? If the function of reproduction is to increase the number of individuals in a population, then *"How do we explain the origin of cell fusion, which halves cell numbers, contrary to the fundamental evolutionary drive to increase them?"* (Cavalier-smith, 2002).

1.2. The origin of sex

Dacks and Roger (Dacks and Roger, 1999; Speijer *et al.*, 2015) suggested that the Last Eukaryotic Common Ancestor (LECA) was probably a facultative sexual unicellular species and therefore that meiotic sex has a single evolutionary origin. This conclusion is supported by the observation that meiosis genes have been found in all major eukaryotic supergroups and in basal eukaryotes such as *Trichomonas*, *Giarda* (Excavata) and *Ostreoccocus* (Chlorophyta; e.g. Ramesh *et al.*, 2005; Derelle *et al.*, 2007; Schurko *et al.*, 2009; Halary *et al.*, 2011; Malik *et al.*, 2008). These observations support the idea that sex originated in basal eukaryotes more than 1.5 billion years ago (Javaux *et al.*, 2001). Along with evidence suggesting a single evolutionary origin of sex in eukaryotes came several theories to explain what mechanisms could have driven the appearance of this reproductive strategy.

Sex as a DNA repair mechanism

Meiotic sex was already present in the last common eukaryotic ancestor, and probably evolved from bacterial transformation, which also involves pairing of DNA strands, recombination and the transmission of the recombined information to progeny (Bernstein,

2013). The primary evolutionary function of transformation may be the use of homologous DNA molecules for recombinational repair of double strand DNA breaks caused by oxidative stress (Michod *et al.*, 2008; Mirzaghaderi and Hörandl, 2016). The existence of homologues of core meiosis genes in prokaryotes is a strong argument for this hypothesis (Malik *et al.*, 2008; Ramesh *et al.*, 2005). Moreover, in a broad range of facultative sexual eukaryotes, the expression of meiotic genes is triggered by oxidative stress (Bernstein and Johns, 1989; Nedelcu and Michod, 2003; Nedelcu *et al.*, 2004). Oxidative stress causes physical damage to DNA (Slupphaug *et al.*, 2003) and it has been suggested that sex could be an adaptive response and could have evolved to repair DNA damage (Bernstein *et al.*, 2011). Homologous recombination allows damaged DNA to be repaired using a sister chromatid (available in G2 phase after DNA replication) or a homologous chromosome as a template. Mutation of some of the genes involved in DNA repair has revealed that these genes are also involved in recombination during meiosis (Joyce *et al.*, 2009; Klovstad *et al.*, 2008; Staeva-Vieira *et al.*, 2003). Indeed, in a system where these genes are shut down and DNA repair is no longer active, harmful DNA damage can accumulate and be transmitted to subsequent generations. Therefore, during meiotic sex, syngamy results in diploidisation, which brings together homologous chromosomes in a single cell allowing the repair of damaged DNA using undamaged copies as a template. As discussed in Bernstein (2011), this DNA repair mechanism is particularly efficient for double strand DNA damage. However, the proposed role for recombination in the repair of damaged DNA is not sufficient to explain why sex evolved and was maintained because there are populations of asexual, diploid eukaryotes where homologous chromosomes are present allowing homologous recombination repair of DNA damage (Otto and Lenormand, 2002).

Sex and selfish DNA elements

It has been suggested that ‘selfish DNA elements’ may have driven the initial evolution of sex. On the whole, such elements, which include transposable elements, some genes and parasitic genetic elements, will tend to have a damaging effect on their host (Hurst and Werren, 2001). However, this negative effect will have limited consequences on the survival of the elements provided they are rapidly transmitted to a new host before the effects of the damage are felt by their existing host. Sex could therefore have evolved as a mechanism to promote the transmission of selfish elements to new hosts (Hartfield and Keightley, 2012; Otto and

Lenormand, 2002). Moreover, transposable elements that carry genetic information specifying sexual reproduction would spread more efficiently. Indeed, if transposable elements carrying such genetic information arise and compel their host to reproduce sexually with partners that would normally reproduce asexually, such sex drivers would spread through population as long as the driving element was represented more often among sexual offspring than among the parents (Hickey, 1982; Otto and Lenormand, 2002). This argument provides a possible explanation for why sex evolved in the first place and for why it was maintained in the short-term but it does not explain why sex persisted over the long duration of eukaryote evolution.

1.3. Maintenance of sex

The widespread occurrence of sex in eukaryotes suggests that there is a significant fitness advantage to reproducing sexually. However, the costs of sex are much easier to identify than the benefits and, consequently, the maintenance of sex is a debated topic in evolutionary biology. Because sex is costly in evolutionary terms, but at the same time widespread among eukaryotes, it presents an evolutionary paradox (Maynard Smith, 1978). Biologists struggle to explain why sex is advantageous, despite these costs. During the 1970s and 1980s, theoretical evolutionary biologists proposed more than twenty five hypotheses to explain the evolutionary advantage of sex (Schön *et al.*, 2009). The majority of these hypotheses can be divided into two major groups. The first group is based on the fact that sex is a source of novel genetic variation in offspring because it remodels genome content and structure through recombination and meiotic segregation. Such variation could produce new adaptations, for example to a changing environment, that could be acted upon by natural selection. The second group of hypotheses focus on the capacity of meiosis and recombination to remove negative and deleterious mutations. Each group of hypotheses will be developed in the following sections.

1.3.1. The benefits of sex

Sex generates variation

One of the major hypothesis to explain the maintenance of sex among eukaryotes is that meiosis and recombination efficiently generate variation on which natural selection can act (Weissmann, 1889). In accordance with Darwin, Weismann argued that genetic variability provides the basis for adaptation and therefore that sex exists because it creates variation. The generation of novel genotypes through recombination and segregation provides a means to fix beneficial mutations and eliminate deleterious mutations. This hypothesis was later elaborated by Fisher (1930) and Muller (1932) (Figure 2). The advantage of recombination is illustrated by the fact that if two different advantageous alleles arise at different loci on a chromosome in distinct individuals within a sexually reproducing population, a chromosome containing both alleles can be produced in a very limited number of generations by chromosomal segregation and recombination. However, should the same two alleles arise in individuals within an asexually reproducing population, they cannot be combined by recombination. In such a population, the only way that these two alleles could end up on the same chromosome would be if both alleles were to arise sequentially by mutation in the same genome as it is passed through subsequent generations.

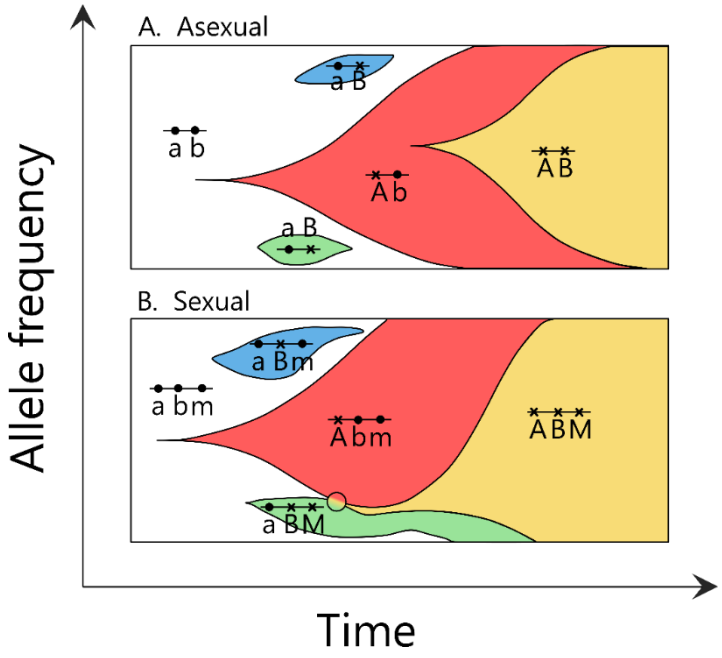

Figure 2: The Fisher-Muller argument. (A) Favourable mutations must be established sequentially in an asexual population. For example, if allele a is destined to be replaced by A in the population, then any other favourable allele that occurs at other loci (e.g. allele B) can be fixed only if it appears in the same genome as A. Different colours represent genotype frequencies in a population over time. (B) In sexual populations, beneficial mutations arising at different loci can be combined into one genome through recombination. This leads to an advantage for modifiers that control sex and recombination. As an example, favourable allele B associates with favourable allele A by recombination (circle). A modifier allele M, which is required in the process, increases its frequency by hitchhiking.

(Adapted from (Barton *et al.*, 2007); redrawn from (Barton and Charlesworth, 1998)).

The ability to generate genetic variation is expected to be particularly important is some contexts. For example, host-parasite co-evolution is thought to lead to a situation in which the host and the parasite engage in a continuous arms race, with each partner continually evolving new innovations that give them a selective advantage. This idea is known as the Red Queen hypothesis (Bell, 1982; Van Valen, 1973). Evidence for the Red Queen hypothesis has been provided by observing the long-term dynamics of parasitism in sexual and asexual populations of the snail *Potamopyrgus antipodarum* (Jokela *et al.*, 2009). Asexual female snails were abundant at the beginning of this study but became more susceptible to parasite infection over-time. The increase of the rate of parasite infections caused most of the common asexual clones to disappear entirely or to be replaced by rare clones and the entire population decreased dramatically in number within a few years of observation. In contrast, sexually reproducing snail populations persisted and remained much more stable over time.

Sex and molecular advantages of recombination

The recombination process that is associated with sexual reproduction has three main advantages. First, as mentioned above, recombination allows the repair of damaged DNA using intact, homologous chromosomes (see above). Second, recombination and outcrossing allow the masking of deleterious mutations by creating heterozygosity. The third advantage associated with recombination is the avoidance of the Muller's ratchet. Most DNA mutations are deleterious and the probability that subsequent mutations will cause reversion to the original state is low. In 1964, Muller proposed a ratchet process whereby deleterious mutations accumulate in an irreversible manner in the genomes of asexually reproducing individuals (Muller, 1964 ; Figure 3). If we consider a finite asexual population where mutations are accumulating at different rates in different individuals, the stochastic disappearance of the least mutated individuals due to drift is predicted to lead to an accumulation of deleterious mutations over time. Muller proposed that sexual reproduction may be favoured over asexual reproduction because it provides a means to prevent this ratchet process from operating. Recombination allows natural selection to act more efficiently on deleterious mutations by uncoupling their inheritance from those of other loci in the genome. Hence, sexual reproduction both allows the avoidance of Muller's ratchet by generating offspring with a reduced mutation load and provides the means to escape the effects of recessive deleterious mutations through dominance (Chasnov, 2000) or epistasis (Kimura and Maruyama, 1966; Kondrashov, 1982; Kondrashov, 1988; Charlesworth, 1990).

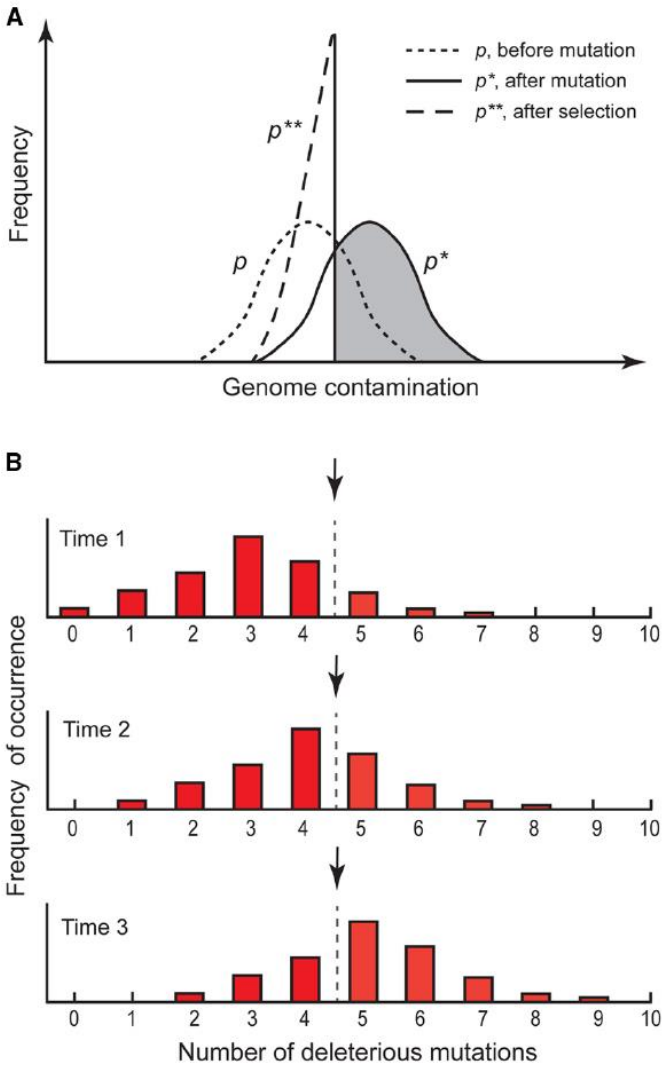

Figure 3: Principles of Muller's ratchet. (A) Scheme of distributions (p) of mutations in a sexual population. Before mutation, distribution in the population is (p), after mutation, distribution shifts upward to p*. After recombination and selection against mutants, individuals in the gray part remain sterile and die, and the distribution goes backward to p**. At equilibrium the means of p and p** are equal. (B) Scheme of mutational load distributions in an asexual population. Initially, genotypes with zero mutations exist in the population, but are lost over time by drift. Without recombination, the class with zero or few mutations cannot be restored, and consequently mutations accumulate until a threshold level of extinction (arrow) is reached.

(extracted from Hojsgaard and Hörandl, 2015: (A) (redrawn after Kondrashov, 1988) (B) redrawn after Maynard Smith, 1988)).

1.3.2. Cost of sex

As mentioned above, the evolutionary advantages of sexual reproduction have been largely debated because sex appears to be less efficient than asexual reproduction but its widespread occurrence among eukaryotes indicates that it has evolutionary benefits and advantages. The paradox of sex is amplified by the fact that several different factors make sexual reproduction costly.

Two fold cost of sex

The "two fold cost of sex" was underlined by John Maynard Smith in 1971 when he pointed out that most sexual eukaryotes have two different sexes, with only one (the female) involved in bearing offspring. In most cases, males contribute to the next generation only by providing genetic information. Consequently, females allocate much more care and resources to their offspring than males (Maynard Smith, 1978) and the number of new individuals that can be created by a population depends essentially on the number of females in that population. Therefore, because females invest half of their reproductive potential in the production of males whose only role is to transmit their genetic information to the next generation, sexual populations are expected to produce progeny half as efficiently as asexual populations (assuming a 1:1 sex ratio; Figure 4).

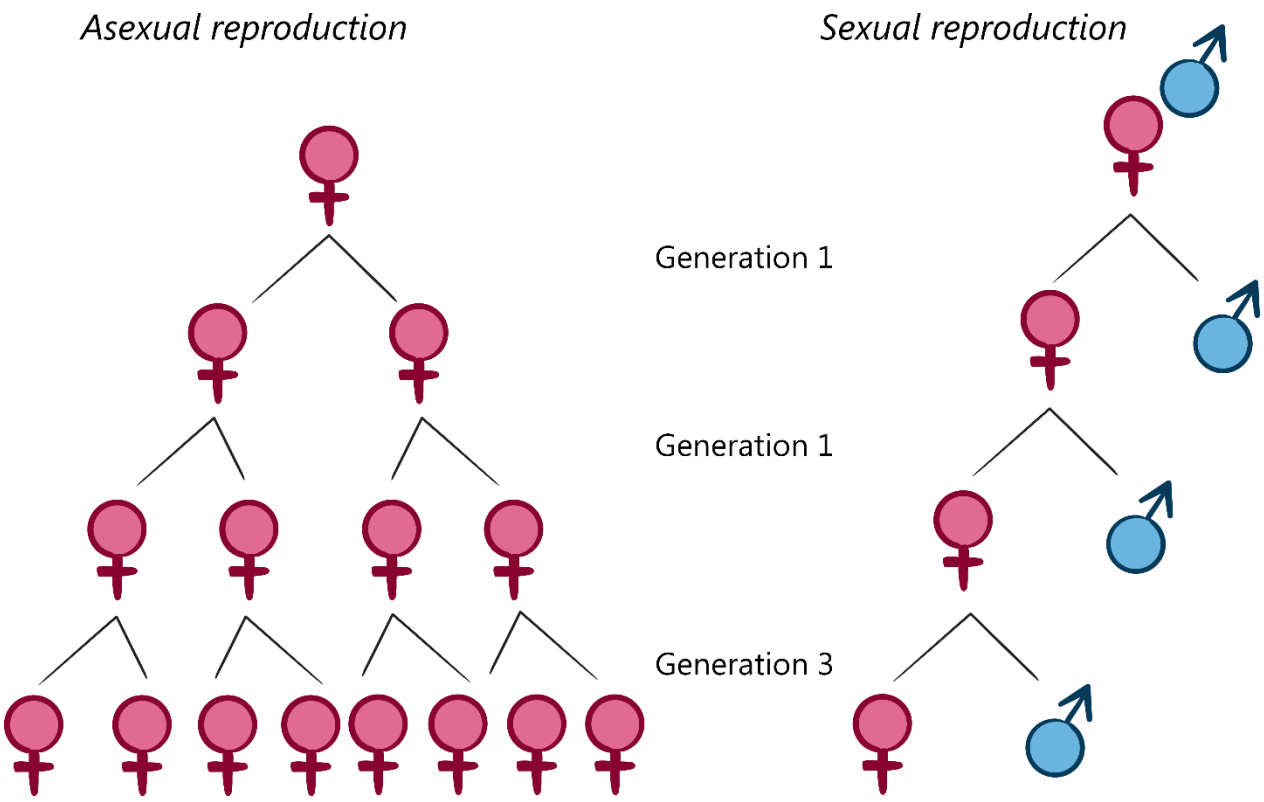

Figure 4: Spread of asexuality assuming a two-fold cost of sex. The hypothetical sexual and asexual populations are shown with offspring of three subsequent generations. The asexual population, which does not invest in males, can double in frequency over time.

Another component of the cost of sex is the decreased efficiency of transmission of genes to the next generation. Half of the genes of the female partner and half of the genes of the male partner are transferred to each offspring in a sexual population. In contrast, in an asexual population, offspring inherit the entire genome of their parent (Maynard Smith, 1978; Maynard Smith, 1971). The cost of sex can increase in situations where there is sexual conflict, particularly when female reproductive success is decreased by male competitiveness. For example, in *Drosophila melanogaster* male seminal fluids promote egg-laying, destroy sperm from other mates, reduce female receptivity to further mating and are toxic, increasing the death rate of females that mate multiple times (Chapman *et al.*, 1995). Another example is the beetle *Callosobruchus maculatus*, where the spiky genitalia of the male damage the female reproductive tract resulting in reduced female longevity (Eady *et al.*, 2007).

Hence, competition between males can heighten the cost of sex for females. On the other hand, the cost of sex can be reduced when males invest resources in their offspring, especially

in monogamous populations where males evolve traits that maximize the female's reproductive success. Isogamous organisms can also partially escape the two-fold cost of sex since the two mating-types invest the same resources in the offspring by producing gametes of equal size. Therefore, the production of isogametes results in the same output as asexual populations in the sense that the cost of producing males is avoided (Lehtonen *et al.*, 2012).

The costs of mate detection and mate choice

Sex requires the encounter of two compatible gametes and, therefore, a compatible mate needs to be found, unless the organism is capable of selfing. Mate finding can be very costly in low density populations. Eppley and Jesson showed that mate-finding efficiency is strongly correlated with breeding strategy, in particular that it is implicated in the evolution of hermaphroditism (Eppley and Jesson, 2008). The mate-finding problem probably explains the widespread occurrence of hermaphrodites, which correspond to approximately one-third of animal species excluding insects. Moreover, mate finding and attraction can also increase the risk of predation (associated with having attractive ornaments) and disease (i.e. sexually transmitted diseases), which lead to a reduction in fecundity (Daly, 1978).

The cost of meiosis

Despite the advantages of meiosis in terms of recombination and DNA repair, it also represents a cost since in many unicellular organisms, for example, the time required to effect meiosis is 5-100 times greater than for mitosis (Otto, 2009). In multicellular organisms, the cost is reduced because the relative time spent carrying out meiotic divisions is usually small compared to total life expectancy and gamete production can occur in parallel with growth and development (Lehtonen *et al.*, 2012).

The cost of recombination

Recombination has been proposed to be one of the main benefits of sex because it creates new associations of beneficial mutations more efficiently than in asexual populations (see section 'Sex and molecular advantages of recombination'). Over the course of evolution, recombination, combined with natural selection, can permit the assembly of multiple advantageous alleles in the same genome and, at the same time, the removal of harmful

mutations from that genome. However, in the short-term, recombination will also tend to break up advantageous combinations of genes that have already been selected for in the past. This process can lead to a decrease in the fitness of the progeny compare to the parents because epistasis (interaction between genes that affect the phenotype or fitness) is widespread among eukaryotes (Maynard Smith, 1978; Otto, 2009; Lehtonen *et al.*, 2012). This effect of recombination, which is probably the most general cost of sex, is also referred to as 'recombination load' (defined as "loss of fitness because recombination breaks up associations between beneficial combinations of interacting alleles" by Charlesworth and Charlesworth, 1975). Note that recombination load can affect any sexually reproducing organism, including isogamous species that do not have sexes and is independent of sexual conflict or the efficiency of mate-finding. The effects of recombination load will depend on the degree to which the direction of selection fluctuates over time and space (Lehtonen *et al.*, 2012). In a stable environment, reshuffled genotypes are more likely to exhibit reduced fitness (recombination load) compared with the parental genotypes than in a fluctuating environment.

1.4. The evolution of separate sexes

Efforts to find evolutionary benefits to sex that could balance its costs have focused on the consequences of sex in terms of genetic variations generated by meiosis (recombination) during the transition from diploid to the haploid phase of the sexual life cycle. Syngamy, the counter-part of meiosis that restores the diploid phase, is as important as meiosis and its regulation is central to the evolution of sexual eukaryotes. Syngamy is the process by which genomes are mixed by the fusion of compatible gametes (haploid cells resulting from meiosis). For many species, compatible gametes means gametes of different mating-type or sexes. This section will focus on the evolution of mating-types and sexes, and will briefly describe theories aimed at explaining the origin of two gamete classes. Some of the questions that will be explored include: why do systems with different gamete classes (i.e. systems with either anisogamy or mating types or both) exist despite the fact that they restrict the probability of finding a compatible mating partner (i.e. why is it not possible to mix genomes with all possible partners)? and why does the number of gamete classes vary from zero to thousands, with most often only two classes?

1.4.1. From mating-types to sexes: evolution of anisogamy

Species are generally considered to exhibit one of three classes of gamete dimorphism based principally on the difference in size between male and female gametes: isogamy, anisogamy and oogamy. In isogamous species, where the gametes are of equal size and morphologically identical, it is not usually possible to assign female and male functions and mating types (for example "+" or "–" mating type) are assigned to compatible strains. Anisogamous species produce female gametes that are larger than male gametes. Oogamy is a special type anisogamy where the female gametes are large and immobile whereas the male gametes are small and motile. In all classes (isogamy, anisogamy and oogamy) there is a specific recognition of one gamete type by another (a specific interaction of either male and female or plus and minus gametes) leading to cell fusion. Studies on the evolution of mating types in isogamous species are useful to understand the forces shaping the evolution of sexes, as it is widely accepted that the differentiation of two morphologically indistinguishable mating types preceded the evolution of anisogamy (Lehtonen *et al.*, 2016; Togashi and Cox, 2011).

The origin of mating types

At least seven different hypotheses have been proposed to explain the emergence of mating types (Billiard *et al.*, 2011). Among these theories we can underline the "by-product" model, which suggests that mating types have evolved from a bipolar recognition system (for example a pheromone and pheromone receptor) that played a key role in gamete syngamy (Hoekstra, 1982); the "selfish element" model, which suggests that such elements could promote cell fusion in order to move to new host cells (Bell, 1993; Hoekstra, 1990); the "inbreeding avoidance" model, which proposes that two class of gametes evolved to avoid the cost of mutation load due to mating between genetically related individuals (Charlesworth and Charlesworth, 1978; Uyenoyama, 1988a; Uyenoyama, 1988b); and the "developmental switch" model which proposes that mating types evolved because they provide complementary signals from the two mating-types that trigger the diploid program after gamete fusion (Perrin, 2012). Finally, the "organelle inheritance" model hypothesises that mating-types are required to control potential conflicts between cytoplasmic organelles (Hurst and Hamilton, 1992; Hoekstra, 1987). Heteroplasmy (mixing of cytoplasmic genetic elements from both gametes in the zygote) potentially leads to competition between

organelles which can have deleterious effects for the progeny. Under this hypothesis, mating-types would have evolved in ancestrally isogamous species because it was advantageous for the nuclear genome to limit genetic conflicts between mitochondrial or chloroplastic genomes by enforcing uniparental inheritance. Whenever mating involves cytoplasmic fusion, mating-types or sexes would be expected not only to determine who is a potential mating partner but also to determine which parent will transmit its organelles (Hurst and Hamilton, 1992; Billiard *et al.*, 2011). There is support for this hypothesis across a diversity of phyla as organelle inheritance is strongly associated with gender in plants, animals and other anisogamic groups. Organellar transmission is uniparental in the vast majority of species, and the organelles that are transmitted are usually those of the mother in sexual species (i.e. the parent with the largest gamete). There are, however, some examples of paternal inheritance, for example in gymnosperms and chytridiomycetes (Reboud and Zeyl, 1994; Xu, 2005).

There is genetic evidence that the mating type loci of some species play a role in determining the pattern of organelle inheritance. For example, in the isogamous green alga *Chlamydomonas reinhardtii* the chloroplast genome is transmitted through the *mating type +* gamete whereas the mitochondrial genome is transmitted through the *mating type* - gamete (Gillham *et al.*, 1987; Nishimura *et al.*, 2012). Similarly, in some fungi, the locus that determines mating-type (MAT) has been found to harbour genes that actively control organelle transmission (Moriyama and Kawano, 2010).

Whilst there is a considerable amount of evidence indicating that organelle inheritance could have favoured the evolution of mating types and sexes, there are a number of species where the pattern of organelle inheritance is not consistent with a role for the mating type locus and these exceptions need to be taken into account. For example, the slime mould *Dydimium irridis* (Silliker *et al.*, 2002; Scheer and Silliker, 2006) is an interesting case because, although it exhibits uniparental transmission of mitochondria, the transmission is random with respect to mating types. It seems unlikely, therefore, at least in this species that the primary role of the mating type locus is to control organelle inheritance. Biparental inheritance has been observed in some rare cases but this pattern of inheritance is usually associated with mechanisms that limit conflict. In mussels that exhibit biparental inheritance of mitochondria, the paternal haplotype is sequestered in the male germ line, preventing inter-organellar conflict (Cao *et al.*, 2004; Breton *et al.*, 2007). Heteroplasmic inheritance (i.e. transmission

from both mating-types) occurs in the yeast *Saccharomyces cerevisiae* but potential cytoplasmic conflicts are resolved by mitochondrial fusion and mitochondrial DNA recombination (Takano *et al.*, 2010). In the brown alga *Ectocarpus* mitochondria have been reported to be transmitted maternally but chloroplasts are transmitted by both the male and female parents. However, the plastids segregate into different parts of the developing sporophyte, leading to a diploid organism that is mosaic for the two parental chloroplasts but with only one parental chloroplast type being present in any given cell (Peters *et al.*, 2004). The diatom *Pseudo-niszchia* also exhibits a form of biparental plastid inheritance (Levialdi Ghiron *et al.*, 2008). In this case, laboratory crosses showed heteroplastidic inheritance in 60 out 96 zygotes. However, analyses of field-collected strains from the same species did not detect any heteroplastidic transmission, suggesting that heteroplasmy may lead to reduced fitness. In the social amoeba *Physarum polycephalum* mitochondrial inheritance is controlled by a different locus to that which controls gamete compatibility and cell fusion (Moriyama and Kawano, 2010). Finally, many lineages have maintained mating-types despite an absence of cytoplasmic fusion. In ciliates and filamentous ascomycetes, for example, the male parent provides only the nucleus, but the mating type complementarity is essential for mating. Taken together it appears that mating-types have functions other than regulating organelle inheritance and it is possible that these other functions are more fundamental and primary. Billiard (Billiard *et al.*, 2011) pointed out that a correlation is not indicative of cause and effect. It might well be that the role of mating types in organelle inheritance was superimposed on pre-existing functions and therefore that mating types evolved for another reason. A similar argument was earlier proposed by Maynard-Smith and Szathmary, based on the observation of uniparental inheritance of mitochondria from the smaller gamete in gymnosperms and chytridiomycetes (Maynard Smith and Szathmary, 1997).

The evolution of anisogamy

Syngamy involves the fusion of compatible gametes, i.e. haploid cells specialised in mating, that can be identical to each other (isomorphic) or strongly dissimilar (heteromorphic). This similarity or dissimilarity between compatible gametes is a continuous trait, often with progressive differentiation from isomorphic gametes towards highly specialised sperm and egg cells found, for example, in metazoans. Gamete size dimorphism is a fundamental trait that defines the basis for maleness and femaleness. It is found in most multicellular organisms including plants, animals, fungi, brown algae, red algae, and green algae. However, such dimorphism is not reported in many unicellular species which produce isogametes. Anisogamy is thought to have emerged independently from isogamous ancestors in various eukaryotic lineages (Bell, 1978). Theoretical studies suggested that the evolution from isogamy towards of anisogamy resulted from disruptive selection (Parker *et al.*, 1972; Bell, 1978; Charlesworth and Charlesworth, 1978; Hoekstra, 1982; Bulmer and Parker, 2002; Lehtonen and Kokko, 2011). For anisogamy to emerge, models considered opposing selective pressures to simultaneously maximize the number of gametes, their encounter rate, and the size (and subsequent survival) of resulting zygotes. Models show that under a wide set of circumstances, the fitness of both partners is maximised when one interacting gamete is small and motile while its large and immobile partner provides the resources for zygotic development. In contrast, intermediate gametes would do worse than small ones in terms of motility and numbers, and worse than large ones in terms of provisioning. Using game theory, Bulmer and Parker (2002) showed that both anisogamy and zygote size are expected to increase with organism size and complexity. Lehoten and Kokko (2011) confirmed this expectation and showed that the evolution of anisogamy also requires either some gametic competition (the system is expected to return to isogamy in the absence of local competition) or gamete limitation (e.g. due to low production rate, high gamete mortality, or low encounter rate). In addition, Charlesworth (1978) predicted that anisogamy could evolve from isogamous genetic sex-determination system with two haploid mating types if a gamete cell-size gene with dimorphic alleles is loosely linked to the mating type locus. However, recent empirical studies on volvocine green algae show that anisogamy may evolve from isogamy without the addition of a gamete-size-control gene and without increased mating type locus size and complexity (Hamaji *et al.*, 2018).

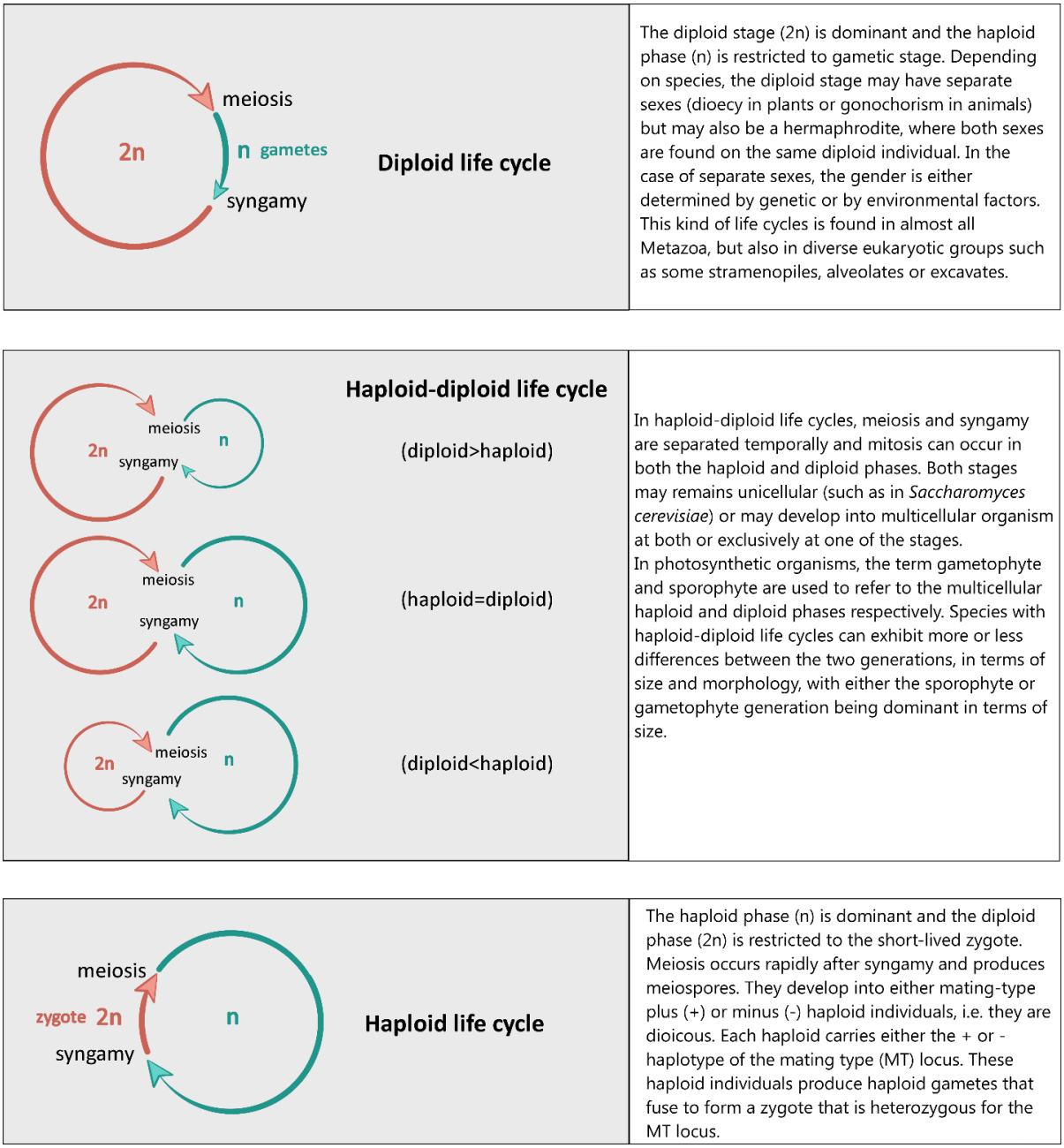

Figure 5: Main sexual life cycles. Depending on the dominant stage, haploid, diploid or both, sexual cycles can be defined as haploid, diploid, haploid-diploid life cycles respectively. (n= haploid, 2n= diploid).

(Adapted from Coelho *et al.*, 2007).

1.4.2. Evolution of sex determination systems

Meiotic sex has a single evolutionary origin whereas male and female sexes have emerged multiple times in several eukaryotic lineages via a striking diversity of mechanisms. Before describing the different mechanisms of sex determination in eukaryotes, it is necessary to present the main types of sexual life cycle. Meiotic sex is defined as the alternation of haploid and diploid phases resulting from meiosis and syngamy, respectively. Eukaryotes exhibit a broad variety of life cycles, which vary in the relative lengths of each of the two phases (haploid or diploid) and the amount of mitotic division or somatic development that occurs in each phase (Figure 5). Life cycles range from haploid-dominant to diploid-dominant, with a continuum of intermediate cases where the haploid and diploid phases are more or less dominant.

Given the diversity and complexity of eukaryotic sexual life cycles, it is not surprising that there is also a great diversity of sex determination mechanisms. Sex can be expressed in either the haploid or the diploid phase. The phase in which the sex is expressed has important consequences for the mechanisms and the evolution of sex determination. Sexes can be determined genetically, environmentally, or by a genotype-environment interaction (Pannell, 1997). For example, both abiotic or social factors have been shown to influence sex determination in several eukaryote species. The temperature of incubation of embryos influences sex determination in crocodiles, turtles and some fish (Bull and Vogt, 1979; Ospina-Álvarez and Piferrer, 2008; Woodward and Murray, 1993). In such systems, the different sexes have the same genotype and both sex determination and differentiation are therefore epigenetic. Environmental and genetic sex determination systems represent the two extremes of a continuum and between these two extremes there are systems where sex is determined by both genetic and epigenetic factors. For example, in the sea bass, both temperature and genetic factors influence the level of methylation of the gene triggering sex determination (Navarro-Martín *et al.*, 2011). The following section focuses on genetic sex determination systems.

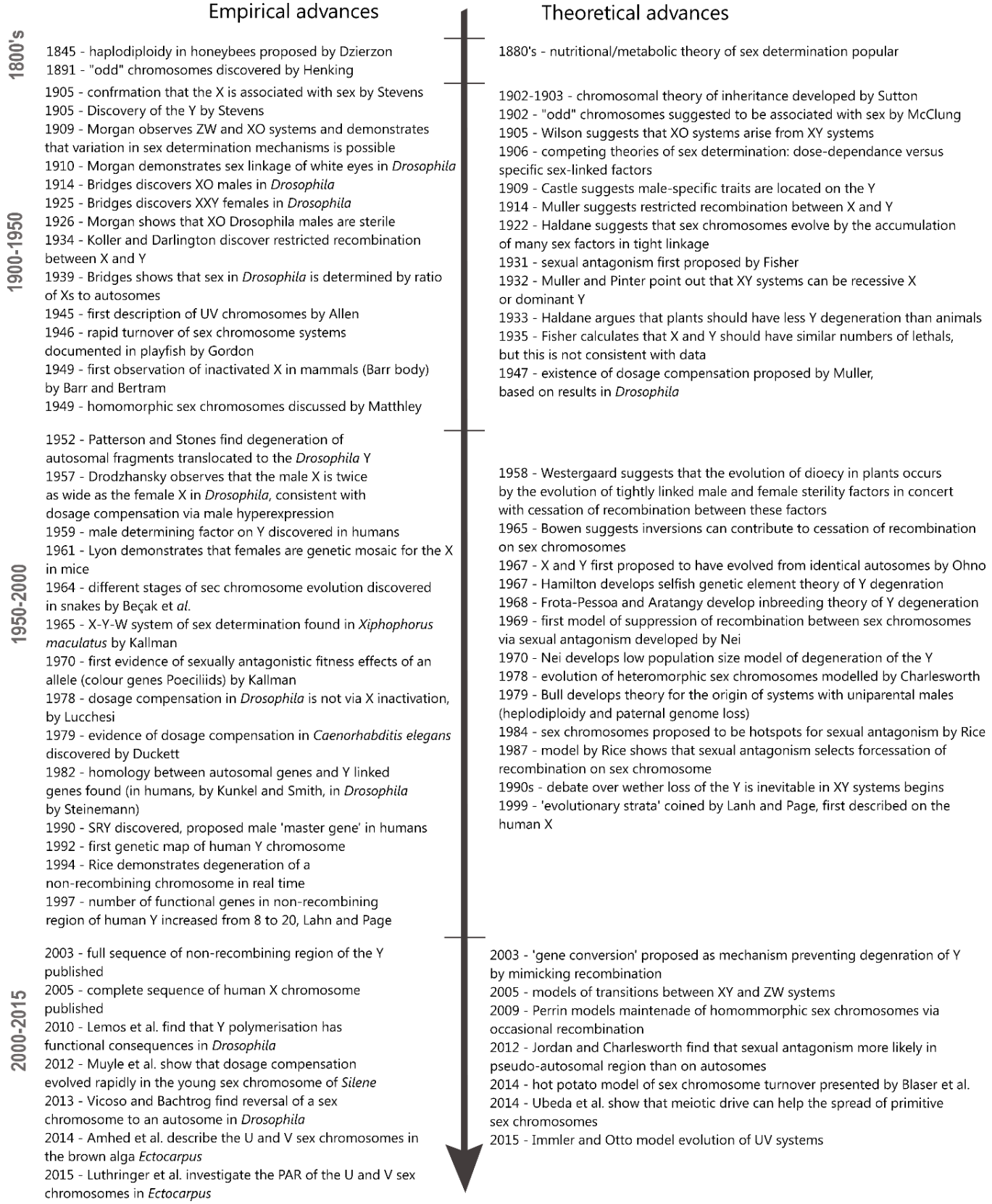

Figure 6: A historical timeline of major theoretical and empirical advances in the study of sex chromosome evolution.

(Adapted from Abbott *et al.*, 2017).

History of the discovery of genetic sex determination systems

In 1891, the German biologist Henking (Henking, 1891), was the first to notice that one element, which he named 'X', was transmitted to only half of the sperm after meiosis in the firebug (*Pyrrhocoris apterus*). Ten years later, three biologists (McClung, Stutton and Stevens, (McClung, 1902; Sutton, 1902; Stevens, 1905) postulated that this 'X' element could be a chromosome involved in sex determination. At the same time, E. B. Wilson, observed that, although males and females of several insect species had the same number of chromosomes, one pair was heteromorphic in males. The work of Bridges (Bridges, 1916; Bridges, 1925) on *Drosophila* supported the involvement of X and Y sex chromosomes in sex determination. After the discovery of XY sex chromosomes and their potential role in sex determination (by the 1920's), studies on the evolution of sex chromosome were carried out and observations of other sex determination systems were published. The first observations of ZW systems in chicken date back to 1959 (Kosin and Ishizaki, 1959) whereas the first UV system was reported by Allen (Allen, 1945) in 1945 in bryophytes (Figure 6).

Major types of genetic sex determination system

Three main types of chromosomal sex determination systems have been described in eukaryotes. Two sex determination systems (XY and ZW) are found in diploid organisms and one (the UV system) has been reported in bryophytes and algae. In organisms with XY sex determination systems, individuals carrying the heteromorphic pair of chromosomes (XY) will develop into males whereas in organisms with ZW sex determination systems individuals carrying the heteromorphic pair of chromosomes will become females. In UV systems, sex is expressed during the haploid phase of the life cycle. Females correspond to individuals carrying the U chromosome whereas individuals that inherit the V chromosome are male (Figure 7, Bachtrog *et al.*, 2011). Studies of the genetic determination of sex, mediated by sex chromosomes, have also highlighted specific features of sex chromosomes compared to autosomes. Sexual development is determined by a sex-determining factor(s) encoded by a gene within the sex-determining region (SDR) of the sex chromosome. In many species recombination is suppressed within the SDR (Beukeboom and Perrin, 2014). The SDR can be as small as a single locus or as large as a whole chromosome.

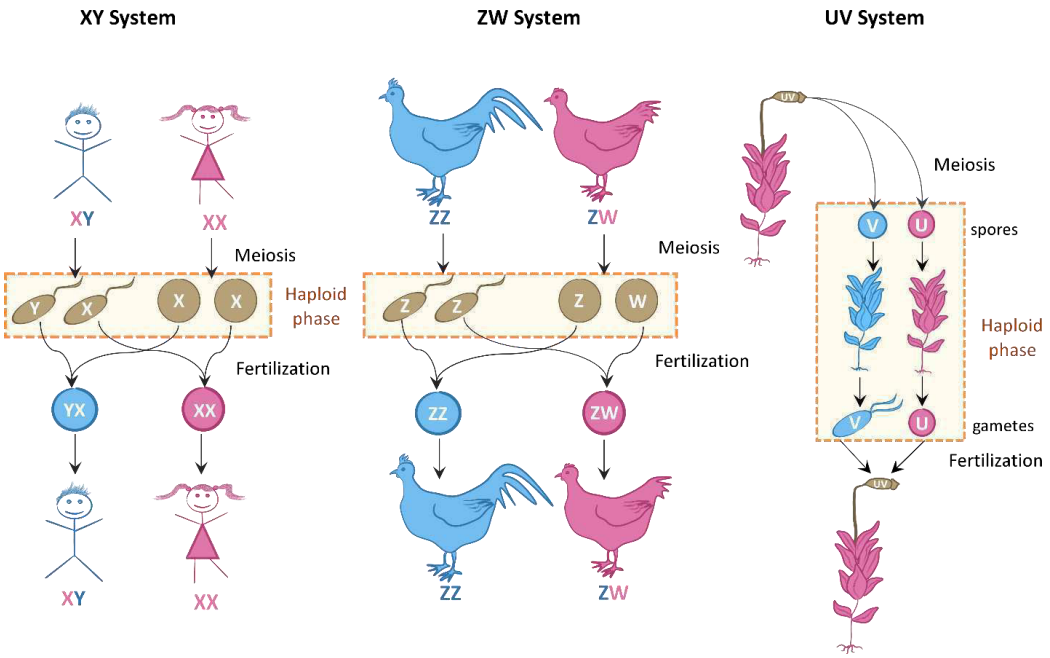

Figure 7: Diversity of types of sexual systems (XY, ZW and UV). In organisms with diploid life cycles, sex is determined in the diploid phase of the life cycle, after fertilisation. In XY systems, the sex of the embryo depends on the chromosome carried by the sperm cell, X or Y. In ZW systems, it is the female egg that determines the sex of the individual. In organisms such as some algae and mosses, that alternate between gametophyte and sporophyte generations (haploid-diploid life cycles), sex is expressed during the haploid (gametophyte) phase of the life cycle. The sexual system in this case is called UV systems. In contrast to XY and ZW systems, sex I UV systems depends on whether the spores receive a U or a V chromosome after meiosis (not at fertilisation stage).

(Extracted from (Mignerot and Coelho, 2016) (adapted from Bacthrog et al., 2011)).

Theories on sex chromosomes evolution

Over the last century, theoretical and experimental work on sex chromosomes has considerably furthered our understanding of the evolution of these unusual chromosomes. The classical model for the evolution of sex chromosomes proposes that the process starts with a pair of homologous autosomes that acquire a major sex-determining locus (Beukeboom and Perrin, 2014; Bachtrog *et al.*, 2011; Charlesworth *et al.*, 2005), for example a male sex-determining gene. In a hermaphrodite population, two mutations are needed in order to generate two separate sexes from a hermaphrodite ancestor: one mutation to suppress male fertility (recessive in X/Y systems and dominant in Z/W systems) and one mutation to suppress female fertility (dominant in X/Y systems and recessive in Z/W systems) (Figure 8 A). In UV systems, the dominance of mutations is of no consequence as sex is expressed during the haploid phase (Charlesworth and Charlesworth, 1978). At this stage, if recombination occurs between the two loci, sterile and hermaphrodite individuals will be produced because both sterility mutations will be located on one proto-sex-chromosome and both sex-determining genes on the other (e.g. *Fragaria virginiana* plant, Spigler *et al.*, 2008). Consequently, there will be a strong selective pressure to suppress recombination between the two loci (Beukeboom and Perrin, 2014). As a result of this arrest of recombination in the male-specific region of a neo-Y chromosome (in X/Y systems) or in the female-specific region of a neo-W chromosome (in Z/W systems), the region containing the sex-determining gene(s) becomes a sex-determining region (SDR) (Figure 8 B). In UV systems, both male-specific and female-specific regions do not recombine. Theoretical models predict that the non-recombining region may then expand due to the effect of sexually antagonistic selection acting on alleles of genes linked to the SDR that have different fitness effects in males and females (Charlesworth *et al.*, 2014; Otto *et al.*, 2011). If a sexually antagonistic (SA) gene arises near the non-recombining sex-determining region (SDR), extension of the non-recombining region to include this gene will allow it to be fixed in the sex for which it is advantageous and removed from the sex where it is disadvantageous ((Rice, 1996; Figure 8 C). However, the SDR does not usually expand to include the whole chromosome and a recombining region (or regions) called the pseudo-autosomal region (PAR) usually persists.

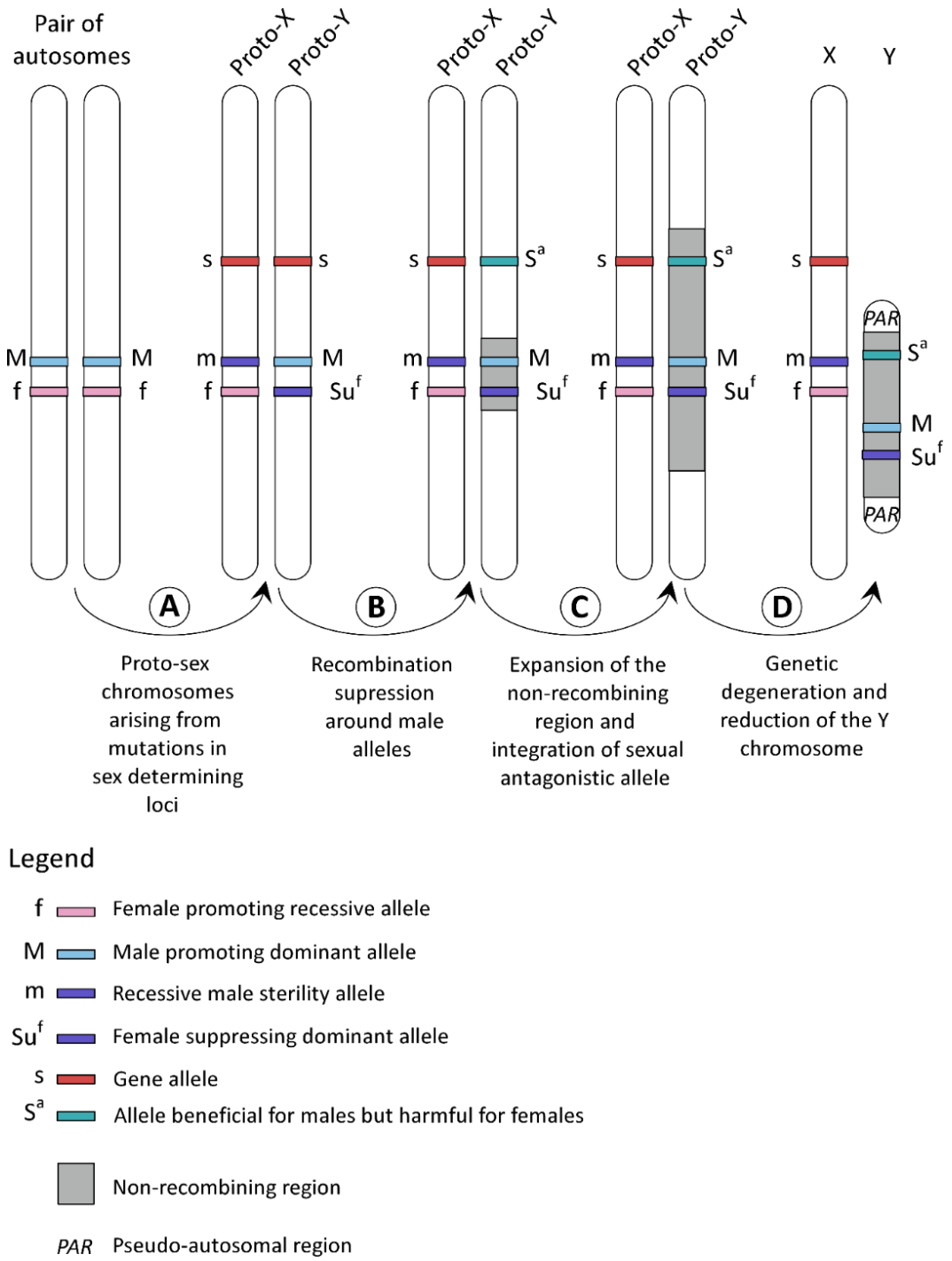

Figure 8: Sex chromosome evolution in a XY system. In a hermaphrodite population, a pair of homologous chromosomes carries the "M" and "f" alleles. (A) "M" mutates into a recessive male-sterility allele (m) which causes the emergence of females and the dichotomy of proto-x and proto-Y chromosomes. On the proto-Y, "f" mutates into a dominant "Su^f" allele, causing female-sterility and the appearance of males. (B) Between the proto-Y chromosome and its homologue the proto-X chromosome, suppression of recombination around male alleles (M and Su^f) is favoured creating a non-recombining sex determining region. The "s" gene undergoes mutation on the proto-Y chromosome to create a sexual antagonistic allele (S^a) that benefits the male but harms the female. (C) On the male proto-Y chromosome expansion of the non-recombining region to include S^a is favoured. (D) The lack of recombination on the Y chromosome induces accumulation of deleterious mutations, genetic degeneration and genes loss resulting in a smaller male Y chromosome. The non-recombining region is not spread throughout the Y chromosome, the pseudoautosomal region (PAR) can still recombine with the corresponding region on the X.

(Adapted from Charlesworth et al., 2005).

The PAR is thought to be important for sex chromosome pairing during mitosis and meiosis and a PAR is therefore present on most sex chromosomes (Otto *et al.*, 2011). Nonetheless, the expansion of the non-recombining region may result in strongly differentiated sex chromosomes, where the sex-specific, non-recombining chromosome (Y in males or W in females) experiences genetic degeneration over the long term as a consequence of the accumulation of deleterious mutations and decreased adaptation (Figure 8 D). The homogametic sex (XX females or ZZ males) possesses two copies of X- or Z-linked genes whereas there are only single copies in the heteromorphic sex (XY males or ZW females), creating the potential problem of unequal expression between sexes. Dosage compensation, which can provide a solution to this problem, can be achieved in multiple ways such as for example inactivation of one X chromosome in female mammals or hyper-expression of the X chromosome in male *Drosophila* (Graves, 2016; Lucchesi, 1978).

However, these theories are still evolving and, for example, it is currently unclear to what extent sexual antagonism is necessarily to drive expansion of the SDR. There is no clear evidence that links sexually-antagonistic alleles to the emergence of reduced recombination of Y, W, U and V chromosomes. If sex antagonistic polymorphisms do occur on the PAR, they could be maintained for long evolutionary times as a result of partial linkage to the SDR (Muyle *et al.*, 2017). Also, it is important to note that sex chromosomes do not necessarily exhibit loss of recombination leading to heteromorphic sex chromosomes. For example, the sex chromosomes of ratite birds and Boridae snakes have remained undifferentiated despite being ancient (Vicoso, Emerson, *et al.*, 2013; Vicoso, Kaiser, *et al.*, 2013). Several possible mechanisms have been proposed to explain this lack of sex chromosome degeneration, including low levels of sexual dimorphism and sexual selection limiting scope for the emergence of sexually antagonistic genes (Rice, 1984), occasional X-Y recombination, which can eliminate accumulated deleterious alleles (Stöck *et al.*, 2011), and resolution of sexual antagonism not by incorporating the sexually antagonistic allele into the SDR but through sex-biased gene expression (Vicoso, Kaiser, *et al.*, 2013). Note that the purifying selection may delay degeneration of sex chromosomes in organisms that have haploid-diploid life cycles (e.g. plants) and particularly in organisms with UV sex chromosomes. Indeed, Immler and Otto (Immler and Otto, 2015a) suggested that the SDR should degenerate more slowly in haploid compared to diploid sex determination systems because deleterious mutations are not

masked in haploid males and females. Empirical data in the brown alga *Ectocarpus* tend to support this hypothesis because degeneration of the SDR is relatively modest, presumably due to selection acting on the U and V SDR haplotypes during the gametophyte generation (Ahmed, Cock, Pessia, Luthringer, Cormier, Robuchon, Sterck, Akira F. Peters, *et al.*, 2014).

2. Asexual reproduction: reproducing without sex

Explaining the paradox of sex has been the queen of problems for evolutionary biologists. In addition to the question: why do eukaryotes reproduce through sex?, a second question has been asked: why do asexual species exist? If sex has so many advantages then, what special adaptations would allow long-term survival without it? An alternative approach to understanding the paradox of sex is to study asexual reproduction systems in an effort to understand how these systems are able to function without the proposed advantages of sexual reproduction.

2.1. A myriad of types of asexual reproduction

Definitions of asexuality

Asexual reproduction is difficult to define because this term can refer to multiple mechanisms that may or may not involve meiosis or meiosis-like processes. The definition of asexual reproduction is, *sensu stricto*, the production of offspring from a single organism where the offspring are genetically identical to that single parent at all loci (excluding sites that have been mutated between the two generations). This definition therefore includes organisms that (1) reproduce clonally through mitotic division and/or (2) no longer implement meiotic divisions. In the first category, organisms reproduce clonally through vegetative reproduction, fission or budding. Clonal reproduction is found in bacteria and archebacteria that do not reproduce through meiotic sex (although sexual reproduction exists) but it also occurs in many eukaryotes such as animals or plants. Clonal reproduction in animals is referred to as 'agametic reproduction', while the term 'vegetative reproduction' is used for plants. Both processes involve mitosis rather than meiosis, and involve the production of new individuals from somatic cells or somatic structures (i.e. structures not related to reproductive organs or gametes). Agametic asexual reproduction involving budding or fission mechanisms can be

found in unicellular organisms such as yeasts (e.g. the budding yeast *Saccharomyces cerevisiae* or the fission yeast *Schizosaccharomyces pombe* Herskowitz, 1988; Gutz *et al.*, 1974) and in multicellular organisms such as annelids (Zattara and Bely, 2016) or starfish (Achituv and Sher, 1991).

The broad definition of asexuality given above also includes organisms that produce gametes but with an altered meiosis, and where offspring develop without fertilisation. This process is commonly referred to as parthenogenesis and occurs in both plants and animals. In these organisms, asexual reproduction involves a variant form of meiosis in the sense that chromosomes are no longer segregated, or are segregated in a very specific way that leads to the transmission of only one particular set of chromosomes, rather than random allocation into different oocytes (Dawley and Bogart, 1989). Asexuality of this type can be considered to be an alternative mode of reproduction to normal sexual reproduction. Importantly asexuality occurs both in organisms that have separate sexes and organisms that do not. Based on these definitions a wide range of eukaryotes can be consider to be asexual and the mechanisms and causes of asexual reproduction are various.

2.2. Advantages of asexual reproduction

Compared to sexually reproducing females which present a twofold cost of sex, clonally reproducing females gain a twofold advantage. Asexual females produce only daughters and do not waste effort on sons therefore they are twice as much efficient as sexually reproducing females (Maynard Smith, 1978; Butlin, 2002). Asexually reproducing organisms is also economise the costs of producing haploid cells (meiosis) and mate finding (syngamy). Meiosis generates genetic variability through recombination but it is costly in term of the time required to complete meiosis, which is 5-100 times longer than for mitosis (Otto, 2009). Moreover, asexuals economise the time spent finding a compatible gamete and the risk of predation during the search is avoided (Daly, 1978). Note that parthenogenetic organisms do not economise on meiosis but do make the economy of not having to find a mate.

2.3. Costs of asexual reproduction

Lack of recombination and genetic variation

Asexual reproduction can have severe genetic costs for offspring, the most obvious being their genotypic uniformity and the accumulation of deleterious mutations (Kondrashov, 1993; Hurst and Peck, 1996; Barton and Charlesworth, 1998). As mentioned above, sexual reproduction involves recombination and segregation during meiosis, which generate variability by randomly shuffling alleles. With selection acting on these variants, recombination speeds up adaptation to directional selection and slows down maladaptation (Otto and Lenormand, 2002; de Visser and Elena, 2007). Recombination also allows the spread of beneficial mutations in populations because they do not carry the load of deleterious mutations at linked loci (Rice and Chippindale, 2001; de Visser and Elena, 2007)Consequently, clonal organisms are expected to be slower to adapt to changing environmental conditions. In addition, the accumulation of deleterious mutations poses a threat to survival in clonally reproducing organisms. Deleterious mutations cannot be purged via recombination in asexual organisms and accumulate due to Muller's ratchet (H. J. Muller, 1932). This accumulation of deleterious mutations should lead to rapid extinction of clonal lineages and the ones found today should consequently be of recent origin (Lynch and Gabriel, 1990). For example, in the clonally reproducing freshwater fish the Amazon molly (*Poecilia Formosa*, gynogenesis), it has been estimated that Muller's ratchet should pose a threat of extinction within approximately 50,000 years (Loewe and Lamatsch, 2008) assuming a realistic mutation rate (Drake *et al.*, 1998; Baer *et al.*, 2007). Surprisingly, this freshwater fish is much older than expected from theoretical assumptions (Lampert and Schartl, 2008; Schartl, Wilde, *et al.*, 1995). Thus, other mechanisms may exist to avoid Muller's ratchet in parthenogenetic lineages (Schartl, Nanda, *et al.*, 1995; Loewe and Lamatsch, 2008). Asexual organisms are expected to have a low capacity to produce evolutionary novelty because no recombination occurs, and any advantages may therefore be short-term and thus these species are expected to be short-lived compared to sexual organisms. Asexual lineages are thought to be evolutionary dead-ends and their positions on the tree of life is consistent with this expectation. Almost all of these asexual lineages occupy terminal nodes of the tree of life (Simon *et al.*, 2003) with very rare cases of entirely asexual groups. Very few lineages have been reported to have persisted and

diversified over millions of years in the absence of sex, for example oribatid mites (Heethoff *et al.*, 2009), darwinulid ostracods (a group of freshwater crustaceans) (Schön *et al.*, 2009) and the bdelloid rotifers (Welch *et al.*, 2009). However, a recent study has shown that bdelloid rotifers have persisted and diversified in the absence of sex because they engage in an unusual form of 'parasex' that allows horizontal genetic exchange between individuals in the absence of meiosis and gamete production (Debortoli *et al.*, 2016).

II. Parthenogenesis in eukaryotes

1. Evolution of parthenogenesis

Parthenogenesis is the development of a gamete without fertilisation (i.e. without any genetic contribution of a compatible gamete). Because parthenogenesis has been reported in vertebrates, invertebrates and plants, and encompasses various mechanisms and definitions depending on which phylum is studied, I will define and describe parthenogenesis first in vertebrates and invertebrates and then in plants.

1.1. Definition and mechanisms underlying parthenogenesis in vertebrates and invertebrates

Parthenogenetic modes of reproduction in vertebrates and invertebrates

Asexual reproduction in vertebrates and invertebrates is characterised by population of female-only organisms. Various modes of asexual reproduction can lead to female-only organisms, also called unisexual organisms.

Unisexuality can be achieved through parthenogenesis or through other asexual modes of reproduction such as hybridogenesis or gynogenesis, which are not parthenogenetic development *sensus stricto* because they require fertilisation. It is important to describe these different modes of asexual reproduction to understand how parthenogenesis might have arisen in some lineages. In unisexual organisms, reproduction involve fertilisation of the egg by sperm of a close related species. Unisexuals organisms can be generated by two distinct processes either hybridogenesis or gynogenesis. In hybridogenetic species, fertilisation occurs with a male of a close species and meiosis is altered so that only the maternal genome is

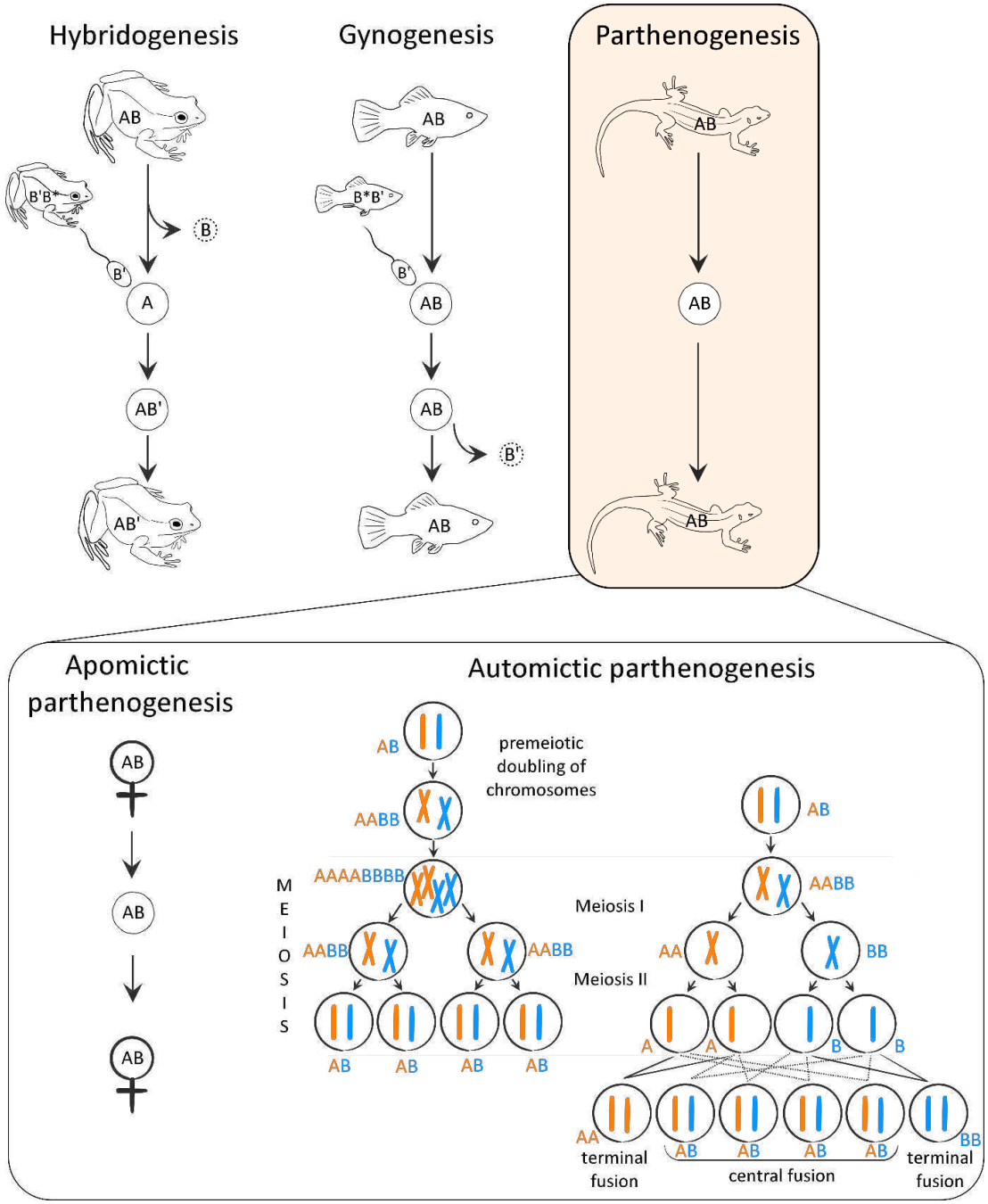

Figure 9: Modes of unisexual and parthenogenetic reproduction in vertebrates. In parthenogenesis and gynogenesis the two haploid genomes A and B inherited from the two parental species are transmitted via a diploid oocyte to the next generation. Unlike parthenogenesis, gynogenesis requires sperm (B') to stimulate development; however, male DNA does not contribute genetic information. In hybridogenesis only one ancestral genome (A) is transmitted into the oocyte and the other (B) is discarded. Diploidy is restored with a new (B') from a related sexual male. The somatic cells of the new generation contain the ancestral A genome and the new B' genome, but only the A genome will again be transmitted to the next generation. The mode of inheritance in hybridogenesis is hemiclonal in that only the maternal genome is clonally transmitted from generation to generation. In apomictic parthenogenesis, meiosis is omitted and oocytes are produced by mitosis generating offspring genetically identical to the mother. In automictic parthenogenesis with premeiotic doubling, chromosomes are doubled before meiosis and then segregated in regular meiosis. In automictic parthenogenesis without premeiotic doubling, products from the same meiosis fuse to restore diploidy. In terminal fusion, the oocyte fuses with the second polar body leading to homozygous diploid offspring. In central fusion, the oocyte fuses with the first polar body restoring heterozygisity in the diploid offspring.

(Adapted from (Neaves and Baumann, 2011; Lampert, 2008).

transferred to oocytes while the male genome is lost during meiosis. Consequently, even if the oocyte is fertilised by a closely related species, male genetic material is only present for a single generation (Figure 9).

No recombination take place during meiosis between male and female genomes and the maternal genome is transferred unaltered to the next generation (hemiclonal) (Schultz, 1969; Dawley and Bogart, 1989). Hybridogenesis is not considered to be parthenogenetic because fertilisation occurs despite the fact that only female genetic material is transmitted to the next generation. This kind of reproduction is found in various fish, salamanders and frogs (Avise, 2008; Avise, 2015; Schön *et al.*, 2009). Gynogenesis is a form of asexuality that is more similar to parthenogenesis because, although male sperm from a close related species is required to initiate the cleavage of the egg, there is no fertilisation. The male genetic material is inactivated and does not contribute to the offspring (Hubbs and Hubbs, 1932; Turner *et al.*, 1990; Schartl *et al.*, 1990). Gynogenesis occurs in many vertebrates and is especially common in fish (Schön *et al.*, 2009) and certain salamanders (Avise, 2015). While hybridogenesis and gynogenesis still depend on male sperm, there is nomore contribution of males in parthenogenetic species. Hybridogenesis, gynogenesis and parthenogenesis can be seen as a continuum where male contribution is less and less required for the next generation, up to the extreme point where it is not needed at all wich correspond to parthenogenesis.

Apomictic parthenogenesis (or apomixis) involves the suppression of meiosis so that offspring are produced from diploid unfertilised eggs by a mitotic-like cell division resulting in genetic identity with the mother (except for mutations that occur between the two generation) (Lushai and Loxdale, 2002; Schön *et al.*, 2009). Apomixis is commonly found in invertebrates such as rotifers and all major groups of arthropods and plants. In contrast with apomixis, automictic parthenogenesis (or automixis) retains meiosis with restoration of diploidy by duplication or fusion of the gametes produced by the female parent. Automixis can lead to variable offspring because recombination and segregation take place between non-identical homologous chromosomes (Mogie, 1986; Suomalainen *et al.*, 1987). Automixis occurs in many parthenogenetic stick insects and some weevils. In general, it rapidly leads to complete homozygosity. In addition to these different ways to achieve asexual reproduction, parthenogenesis can be obligate or facultative during the life history of organisms. Tychoparthenogenesis or facultative parthenogenesis occurs in organisms that are able to

switch between sexual and parthenogenetic development. The cytological mechanisms of facultative parthenogenesis are diverse and include both apomixis and automixis. Facultative parthenogenesis, which combines the advantages of asexual reproduction with the advantages of sexual reproduction, has been studied thoroughly in invertebrates. Facultative parthenogenesis is quite rare among animals. Accidental parthenogenesis, often incorrectly referred to as tychoparthenogenesis, has been observed in several vertebrates. Accidental parthenogenesis involves the parthenogenetic development of a very small portion of unfertilised eggs in a sexually reproducing species (van der Kooi and Schwander, 2015). Examples of rare parthenogenesis in vertebrates are often classified as facultative parthenogenesis when the phenomenon was observed in species kept solitary in captivity. For example, accidental parthenogenesis has been reported in sharks (Chapman *et al.*, 2007; Feldheim *et al.*, 2010), snakes (Booth *et al.*, 2011) and Komodo dragons (Watts *et al.*, 2006). *Daphnia* and aphids are the most commonly known examples of 'true' facultative parthenogenesis, in the sense that parthenogenesis has been observed in natural populations and not just in the laboratory or in captivity (Banta and Brown, 1929; Zaffagnini, 1987). Some obligate parthenogenetic species are thought to have evolved from facultative parthenogenetic species in environments in which sexual reproduction is difficult or impossible (Kramer and Templeton, 2001).

Distribution of parthenogenesis in vertebrates and invertebrates

Approximately one in every 1000 multicellular eukaryotic taxa is either unisexual or asexual (Simon *et al.*, 2003). In vertebrates, sexual reproduction predominates (Dawley and Bogart, 1989) but approximately 100 species have been reported to consist only of females who produce daughters that are genetically identical (excluding *de novo* mutations) to one another and to their mother (Avise, 2015). Obligate parthenogenesis in vertebrates is found in the order Squamata (lizards, snakes and allies). Examples of obligate parthenogenesis include several rock lizards (especially in the genus *Darevskia*) of the family Lacertidae (Murphy and Curry, 2000), various geckos of the family Gekkonidae (Moritz, 1991), whiptail lizards (especially in the genus *Aspidoscelis*) of the family Teiidae (Sites *et al.*, 1990), shinks in the family Scincidae (Adams *et al.*, 2003), and a blind snake in the family Typhlopidae (Wynn *et al.*, 1987).

In contrast to vertebrates, the frequency of obligate parthenogenesis is much higher in some invertebrate groups. Studies that focused on specific invertebrate groups found high frequencies of parthenogenesis, for example 15% of *Megastigmus* species (Boivin *et al.*, 2014) and 30% of *Aphytis* wasp species (DeBach, 1969; Rosen and DeBach, 1979). In species with haplodiploid sex determination such as hymenopterans (ants, bees and wasps) and thysanopterans (thrips), haploid males are produced from unfertilised eggs, a form of parthenogenesis called arrnhenotoky. In contrast, thelytoky is a form of parthenogenesis in which all unfertilised eggs develop into females. Facultative or obligate thelytoky occurs sporadically but is found in over 80 families of the superclass Hexapoda, and is also scattered throughout the mites. There are several families of mites (in the suborder Oribatida) that are strictly thelytokous. Although thelytoky is found in most orders of hexapods, the highest frequency of strictly thelytokous species are Thysanoptera, Psocoptera, Hemiptera (especially in the suborder Sternorrhyncha), and Phasmatodea. Thelytoky can be restricted to some families of an order, such as in weevils (Coleoptera, Curculionidae), bagworm moths (Lepidoptera, Psychidae), and chironomid midges (Diptera, Chironomidae) (Normark and Kirkendall, 2009).

1.2. Evolutionary causes and origins of parthenogenesis in vertebrate and invertebrates

Four theories have emerged to explain the possible causes and origins leading species to evolve to asexuality.

Spontaneous origin of parthenogenesis

Parthenogenesis could evolve because species lose the capacity to reproduce sexually. Spontaneous loss of sex may occur through mutations in genes involved in the production of sexual forms or required for meiosis. Emergence of parthenogenesis in such a situation would restore the capacity to reproduce but would also result in reproductive isolation of the new parthenogenetic lineage from the sexual ancestor. In an extreme case, a mutation might fix a single genotype into a strict parthenogenetic lineage. Alternatively, a less extreme mutation could generate a lineage that produces both sexual and parthenogenetic offspring, or a lineage that produces males and parthenogenetic females (Butlin, 2002; Simon *et al.*, 2002).

In many animal species, facultative parthenogenesis, which allows a small portion of unfertilised eggs to develop spontaneously into zygotes, provides a starting point for the emergence of parthenogenesis (Kramer and Templeton, 2001). Selection experiments using several sexual *Drosophila* species have shown that, in some strains, up to 6% of the eggs produced can undergo parthenogenesis (Stalker, 1954; Carson, 1967). Spontaneous origins of parthenogenesis have been demonstrated in a wide range of invertebrates including ostracods (*Cyprinotus* taxa, (Turgeon and Hebert, 1994), snails (*Campeloma* and *antipodarum*, (Johnson and Leefe, 1999; Dybdahl and Lively, 1995), aphids (*Rhopalosiphum padi*, (Delmotte, 2001) and moths (*Alsophila pometaria*, (Harshman and Futuyma, 1985). In cyclically parthenogenetic invertebrates, parthenogenesis and sexual generations alternate regularly during the life cycle. However, transitions to obligate parthenogenesis occur frequently in such organisms and these can occur via several mechanisms. In these organisms, a single loss-of-function mutation can be sufficient to suppress the sexual cycle and generate new, obligately parthenogenetic lineages (e.g. (Stelzer, 2008; Stelzer *et al.*, 2010). For example, in *Daphnia*, parthenogenesis is thought to have emerged through the action of genes that supress meiosis (Hebert, 1981; Innes and Hebert, 1988). In aphids, gene modifications that alter the responsiveness to sex-inducing environmental conditions might account for the spontaneous origin of some parthenogenetic lineages. These modifications may involve periodicity genes or genes that regulate hormonal expression (Simon *et al.*, 2002).

Hybrid origin of parthenogenesis

Asexuality has also been correlated with hybridity. Interspecific hybridisation can disrupt meiosis and create opportunities for the selection of cytological processes that rescue egg production (Vrijenhoek, 1998). In animals and plants hybridisation often favours the emergence of parthenogenetic lineages. For example, most, if not all, unisexual vertebrates have a hybrid origin (Avise *et al.*, 1992). Parthenogenesis has more diverse origins in invertebrates, but hybridisation is also a frequent factor, as has been demonstrated for snails (*Potamopyrgus*, (Johnson and Leefe, 1999), crustaceans (*Lassaea*, (Foighil and Smith, 1995) and many insects such as weevils (*Otiorhynchus saber*, (Tomiuk *et al.*, 1994), stick insects (*Bacillus lyceorum*, (Mantovani, 1998) and grasshoppers (*Warramaba virgo*, (Honeycutt and Wilkinson, 1989). The cytogenetic processes that disrupt meiosis in hybrid parthenogenetic lineages may sometimes lead to an incomplete loss of sex, for example in the fish genus

Poeciliopsis which contains six unisexual taxa that result from crosses between *P. monacha* Miller and four other bisexual species. Among these six unisexual hybrids, three are gynogenetic triploids while the remaining are hybridogenetic diploids (Vrijenhoek, 1998). As a result, unisexual *Poeciliopsis* species still require insemination from sexual relatives, which consequently constrains their habitat range (Beukeboom and Vrijenhoek, 1998). Similarly, many hybrid parthenogenetic lineages retain the potential to produce males. Hybridity and polyploidy often occur simultaneously since they both result from the fusion of non-standard gametic cells (Mogie, 1986; Dawley and Bogart, 1989) and it has been established that both plant and animal polyploids often have a hybrid ancestry (Dufresne and Hebert, 1994). Similarly, the majority of unisexual vertebrates (64% according to (Avise *et al.*, 1992) and invertebrates that have arisen via hybridization are polyploids. Polyploidy, which is common in plants, will be further described in the section "Origin of parthenogenesis in plants".

Contagious origin of parthenogenesis

A contagious origin of parthenogenesis occurs when incomplete isolation between sexual and parthenogenetic individuals generates a new parthenogenetic lineage from a pre-existing one. Parthenogenetically produced males represent one mechanism by which an asexually reproducing species can exchange genes with closely related sexually reproducing species. For example, in *Daphnia pulex*, males are commonly produced through obligate parthenogenesis, although they seem to play no known role in the life cycle. However, such parthenogenetic males can fertilise cyclically parthenogenetic females to give rise to viable hybrids (Innes and Hebert, 1988). These hybrids show that genes that supress meiosis can be transmitted to progeny because the progeny exhibit obligate parthenogenesis. Parthenogenetically produced males have also been reported in freshwater flatworms (Pongratz *et al.*, 1998), earthworms (Jaenike and Selander, 1979), ostracods (Turgeon and Hebert, 1994; Butlin, 2002; Butlin *et al.*, 1998), brine shrimp (Browne, 1992), snails (Samadi *et al.*, 1997), wasps (Plantard *et al.*, 1998; Belshaw *et al.*, 1999) and aphids (Blackman, 1972; Simon *et al.*, 1991).

The first theoretical model of contagious origin of parthenogenesis simulated the spread of a dominant gene in sexual populations that causes parthenogenetic development of eggs while allowing normal spermatogenesis (Jaenike and Selander, 1979). Such genes were predicted to spread to fixation, while there would be concomitant selection for a reduction in male

allocation. These theoretical results were validated by an experimental study of parthenogenesis in the earthworm. Studies on *Daphnia* (Hebert, 1981; Innes and Hebert, 1988) and aphids (Rispe and Pierre, 1998; Rispe *et al.*, 1998; Dedryver *et al.*, 2001) provided insights into the consequences of the spread of unisexuality genes. This contagious mechanism has a high potential for generating parthenogenetic lineages but the incidence that it has in the field is largely unknown and could be limited by several factors. First, parthenogenetically produced males must be functional. For example, many parthenogenetic populations of *Artemia* can produced a small number of males but experimental crosses with sexual females in the laboratory revealed that no offspring were obtained despite the capacity of these males to produce sperm (Browne, 1992). Some species of apomictic ascid mites occasionally produce males but these males are also non-functional (Norton and Palmer, 1991). Second, if parthenogenetic males are functional they must successfully mate with sexual females from a conspecific species. Parthenogenetically produced males might be less adept at seeking out females or may be out-competed by sexually-produced males. Third, for a contagious mechanism to be effective, parthenogenetically produced males must transmit their parthenogenesis genes to their offspring, leading to the production of parthenogenetic lineages. With *Daphnia* and aphids, when parthenogenetically produced males are mated with sexual females, these crosses generate both sexual and parthenogenetic lineages, but this phenomenon has so far only been observed in the laboratory (for *Daphnia* (Innes and Hebert, 1988); for aphids (Blackman, 1972). In conclusion, there is good evidence that contagious parthenogenesis occurs in invertebrates, but these observations need to be confirmed in the field.

Infectious origin of parthenogenesis

Another possible mode of origin of parthenogenesis is through an infectious mechanism involving vertically inherited microorganisms. In many animals, parthenogenesis is caused by parasites that are transmitted in the cytoplasm. A member of the Proteobacteria, *Wolbachia pipientis*, induces parthenogenesis in a wide range of insects, arthropods and nematodes (Stouthamer *et al.*, 1999). *Wolbachia* induces parthenogenesis in parasitoid wasps such as *Trichogramma* but also in thrips and mites (Werren, 1997; Stouthamer *et al.*, 1993; Weeks *et al.*, 2002). Females infected with this bacteria reproduce parthenogenetically and sometimes sexuality can be restored by antibiotic treatment. Though *Wolbachia* does not directly prevent

fertilisation and sexual reproduction, in populations fixed for infection, females have lost the ability to fertilize eggs and reproduce sexually (Pannebakker *et al.*, 2005). Other Proteobacteria, like *Rickettsia* sp., can also trigger parthenogenetic development, for example in eulophid wasps (*Neochrysocharis formosa* (Hagimori *et al.*, 2006); *Pnigalio soemius* (Giorgini *et al.*, 2010). Additional bacteria, unrelated to Proteobacteria, have been shown to induce parthenogenesis in *Encarsia*, a genus of parasitoid wasp (Zchori-Fein *et al.*, 2001) and in a mite species (Weeks and Breeuwer, 2001). These studies suggest that bacteria may frequently have the ability to induce parthenogenesis, although this phenomenon has only been demonstrated so far in arthropods with haplodiploid sex determination (Werren *et al.*, 2008). In these systems, unfertilised infected eggs, which would normally develop into haploid males, develop into diploid females. Parthenogenesis is caused by disruption of the cell cycle during early embryonic development, resulting in the production of diploid eggs. In both *Trichogramma* and *Leptopilina clavipes*, anaphase is abortive during the first embryonic division, resulting in one diploid nucleus rather than two haploid nuclei (Pannebakker *et al.*, 2004; Stouthamer and Kazmer, 1994). In the wasp *Muscidifurax uniraptor*, after meiosis and the first mitotic division are completed, the diploidy is restored in females by the fusion of two cell nuclei (Gottlieb *et al.*, 2002). In contrast, in the mite *Bryobia praetiosa* meiosis is altered and an apomictic parthenogenesis mechanism results in the production of diploid gametes (Weeks and Breeuwer, 2001).

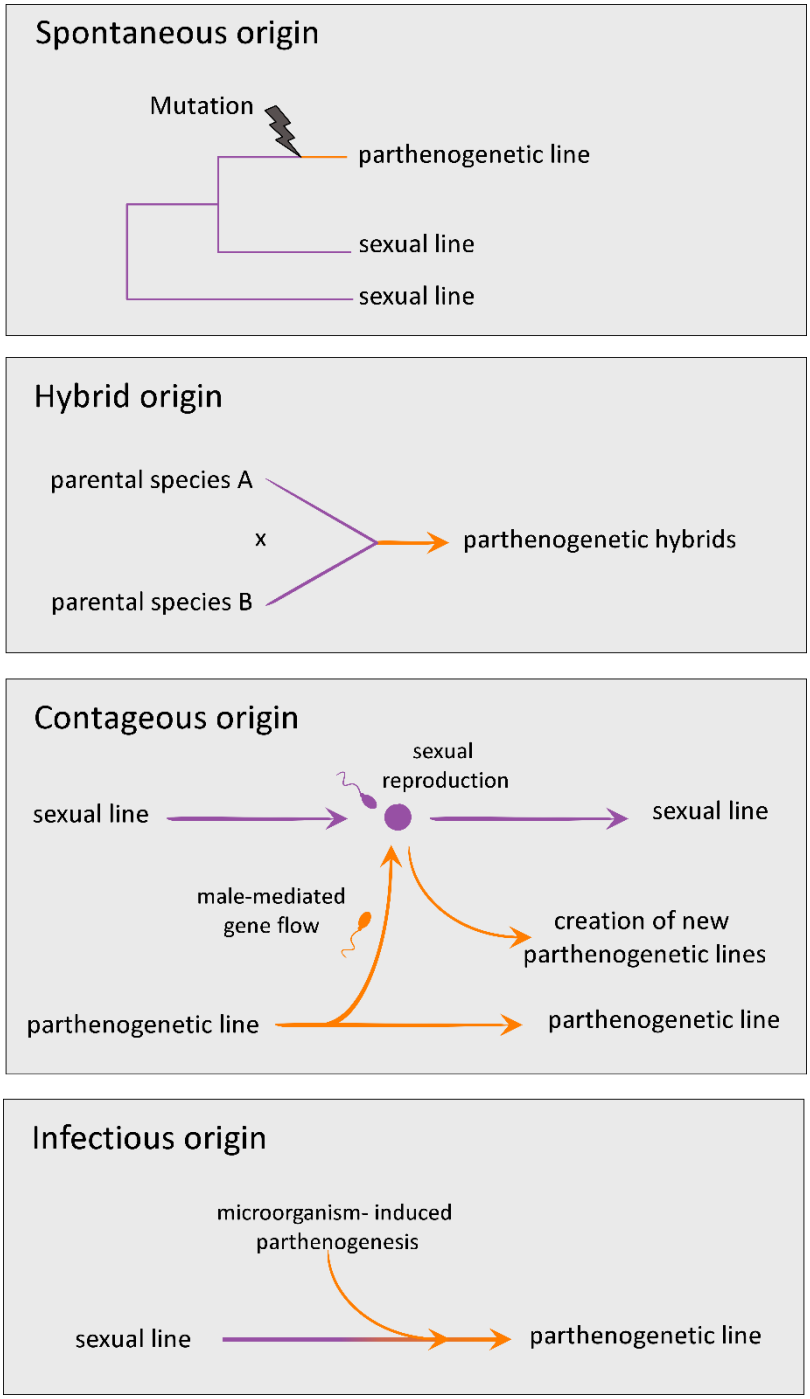

Figure 10 : Main modes of origin of parthenogenetic lineages in animals. Parthenogenetic lineages can arise from spontaneous mutation of meiotic genes in a sexual lineage. Hybridity between close species can generate parthenogenetic hybrids. Parthenogenesis can have a contagious origin where parthenogenetic line can transfer gene through sperm in sexual lineage generating new parthenogenetic lines (e.g. *Daphnia pulex*). Finally, parthenogenesis can induced in sexual line by microorganisms like the bacteria *Wolbacchia*.

(Adapted from Simon et al., 2003).

Multiple mechanisms for the origin of parthenogenesis

Parthenogenetic lineages can be generated by single mechanisms in some species such as hybrid origins in unisexual vertebrates (Figure 10), however in other species, parthenogenetic lineages can arise via multiple mechanisms. In ostracods for examples, it has been showed that diploid clones arose as a result of spontaneous loss of sex whereas polyploid clones arose following hybridisation between parthenogenetic females and males from the same or closely related species (Chaplin *et al.*, 1994). Similarly, in the freshwater snail *Campeloma*, diploid parthenogenetic lineages originated following spontaneous loss of sex while triploid parthenogenetic lineages arose from hybridisation (Johnson and Leefe, 1999). Another example, is the aphid *R. padi*, where both phenotypic (reproductive mode) and phylogenetic evidence indicate that parthenogenetic lineages arose by three different mechanisms: spontaneous, hybrid and contagious parthenogenesis (Simon *et al.*, 1999; Delmotte, 2001; Delmotte *et al.*, 2003). These are extreme examples, but they indicate that sexual reproduction is not necessarily stable, at least in the short term, and there are many situations were sexual populations can coexist with polyphyletic and diverse parthenogenetic lineages. Consequently, short-term advantages of sex are likely to be higher than its 'two-fold' cost in order to compensate for such intense competition with parthenogenesis.

1.3. Definition and mechanisms underlying parthenogenesis in plants

Parthenogenetic modes of reproduction in plants

The terminology for asexual reproduction in plants is different from that of vertebrates and invertebrates. For example, in animals, apomixis is a form of parthenogenesis, whereas parthenogenesis is an element of apomixis in plants. Apomixis in plants is defined as asexual reproduction through seeds leading to the production of clonal progeny that is genetically identical to the mother plant (Nogler, 1984). Apomixis require three developmental components to produce viable seed. First, meiosis must be modified or absent to prevent a reduction in ploidy, allowing the generation of a cell that is directly capable of forming an embryo (apomeiosis). Second, the egg cell must be activated to develop into an embryo in the absence of fertilisation (parthenogenesis).

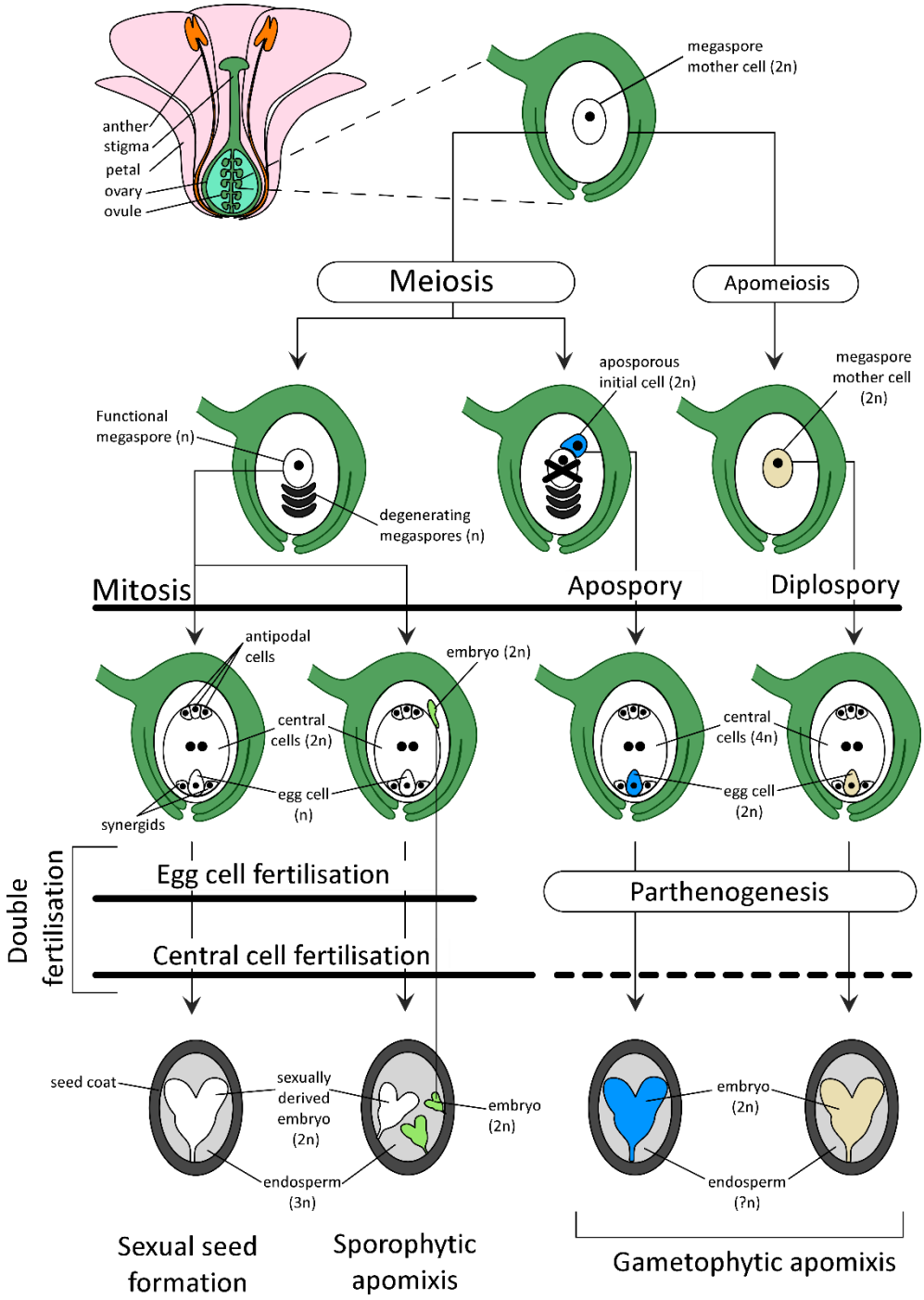

Figure 11: Mechanisms of sexual and apomictic seed development. Seed development processes occur within the ovule of the flower. This diagram compares the major differences in the seed development parthway for sexual seed formation and the apomictic mechanisms of sporophytic and gemtophytic apomixis. Meiosis, mitosis and double fertilization constitute the major components of the seed formation pathway. Arrows passing through each of these components represents the involvement of a given component whithin a particular pathway. In the process of gametophytic apomixis, embryo sac formation can accur via either apospory or diplospory, which are distinguished by different embryo sac precursor cells. In gametophytic apomixis, embryo sac formation is initiated in the absence of fertilisation (parthenogenesis); however, endosperm formation can occur either with or whithout fertilisation, which is represented by dashed line. The relative ploidy level of cells (n) is tracked for various components throughout each pathway. The ploidy level of the endosperm formed through gametophytic apomixis is variable, depends on number of factors, and is therefore represented by question mark (?). In the depicted apospory pathway, the sexual pathway is shown to terminate once the aposporous initial cell undergoes mitosis. Different colors track the precursor cells that form the embryo for each pathway: sexual (white), sporophytic apomixis (green), diplospory (yellow), and apospory (blue).

(Adapted from (Hand and Koltunow, 2014).

Finally, the development of the endosperm must be initiated either autonomously or pseudogamously (i.e. by fertilisation of the central cell) in order to support the developing embryo (Koltunow, 1993). In apomictic plants, offspring are produced from an unreduced cell that is either of sporophytic (sporophytic apomixis) or gametophytic (gametophytic apomixis) origin. Thus, the alternation of gametophytic and sporophytic generations of the plant life cycle is either bypassed, or occurs without the meiotic reduction of the somatic chromosome number. Apomixis is divided into two basic types depending on whether the unreduced cell gave rise to a megagametophyte (gametophytic apomixis) or directly to an embryo (sporophytic apomixis or adventitious embryony) (Koltunow, 1993; Savidan, 2000). Gametophytic apomixis is further subdivided on whether the megagametophyte develops from an unreduced megaspore (diplospory) or from a sporophytic cell in the nucellus (apospory) (Figure 11). These different types of apomixis can occur through various mechanisms leading to further subdivision (Crane, 2001) and can coexist within individual plants (Nogler, 1984; Savidan, 2000). For example, in *Paspalum minus*, both apospory and diplospory can occur in the same plant (Bonilla and Quarin, 1997).

Distribution of parthenogenesis in plants

Apomixis occurs in approximately 400 genera from about 40 plant families and is thought to have evolved multiple times in flowering plants given the various mechanism (Carman, 1997). Apomixis *sensu stricto* applies only to spermaphytes as it requires the production of seeds through asexual reproduction. The term applies mainly to angiosperms, because gymnosperms have rarely, if ever, been found to perform apomixis. Plants with sporophytic apomixis are generally diploid and include many tropical and sub-tropical fruit trees such as the lemon tree or the mango. In contrast, gametophytic apomicts are generally polyploid (van Dijk and Vijverberg, 2005). Apospory is found in tropical and sub-tropical grasses, including couch grass (*Cynodon*), millet (*Panicum*), Kentucky bluegrass (*Brachiaria*, *Pennisetum* and *Poa patentis*), caterpillar grass (*Hrpochloa falx*) and in some Asteracea (Compositae) such as *Hieracium*. Cases of diplospory have been described in allium (Liliacea), Asteracea (e.g. dandelion *Taraxacum*, *Antennaria*), Brassicae (*Boechera holboellii*) and also weed and forage grasses (*Eragrostis*, *Tripsacum*). Some forms of asexual reproduction in bryophytes or pteridophytes, as well as in algae, may be considered to be sporophytic or gametophytic apomixis, although these organisms do not produce any seeds.

Genetic control of apomixis in plants

The various mechanisms of apomixis and the phylogenetic positioning of apomictic species throughout many angiosperm families indicate that apomixis has evolved independently multiple times (Carman, 1997; van Dijk and Vijverberg, 2005). Evidence that apomixis can be inherited as a dominant trait was proved by genetic analyses using apomicts as pollen donors in crosses with sexual individuals as the maternal parent. Early genetic studies proposed that a single dominant locus controlled apomixis in most of the studied apomictic species. For example, in *Taraxacum* and *Erigeon* species, two independent loci have been identified that control diplospory and parthenogenesis (van Dijk *et al.*, 1999; Noyes and Rieseberg, 2000). Similarly, in *Hypericum*, *Poa*, *Hieracium* and *Cenchrus* species two independent loci control apospory and parthenogenesis (Albertini *et al.*, 2001; Catanach *et al.*, 2006; Schallau *et al.*, 2010; Conner *et al.*, 2013). In *Hieracium*, genetic studies have also demonstrated that fertilization-independent endosperm formation is a trait that segregates independently of apospory and parthenogenesis (Ogawa *et al.*, 2013). Characterisation of gamma ray deletion mutants has shown that sexual reproduction is the default pathway in apomictic *Hieracium praealtum*. Several deletion mutants were generated lacking either the apospory locus (called *LOSS OF APOMEIOSIS* or *LOA*) or a locus responsible for fertilisation-independent seed development (called *LOSS OF PARTHENOGENESIS* or *LOP*). Deletion of only one of these loci resulted in a return to the sexual pathway for that component. Plants with the *LOA* locus deleted no longer produce diploid embryo sacs via apospory as they lack the apomeiosis function. Instead, the megaspore mother cell undergoes meiosis, and as aposporous initial cells are not formed, a functional haploid embryo sac develops and in the absence of fertilisation the egg and central cell develop parthenogenetically into a haploid embryo and haploid endosperm, respectively. Deletion of both the *LOA* and the *LOP* loci results in complete reversion to sexuality (Catanach *et al.*, 2006; Koltunow *et al.*, 2011). Hence, apomixis in aposporous *Hieracium*, seems to be superimposed on the sexual developmental pathway, suggesting that apomixis may redirect the fate of cells with gametic potential, rather than being a completely independent pathway (Koltunow *et al.*, 2011). Genetic analyses of other angiosperms have demonstrated the existence of chromosomal determinants for asexuality (Dijk and Bakx-Schotman, 2004; Matzk *et al.*, 2005), some of which are thought to be "supergenes", i.e. discrete chromosomal regions carrying a number of genes under close

linkage (Grossniklaus, 2001). In the plant *Boechera holboelli* a B chromosome is suspected to be the cause of asexuality (Sharbel *et al.*, 2005). Loci genetically linked to components of apomixis have been identified in various species, and sequencing of these loci has revealed a number a candidate genes that potentially have critical roles in apomixis. Sequencing of the apospory-specific genomic region (ASGR) identified in apomictic *Pennisetum* (Akiyama *et al.*, 2004) identified 40 putative protein-coding genes, two of which had sequence similarity to the rice *BABY BOOM* (*BBM*) gene (Conner *et al.*, 2008). *BBM* was originally identified in *Brassica napus* as an AP2-domain transcription factor, and its overexpression in *Arabidopsis* results in the development of embryos from vegetative tissue (Boutilier *et al.*, 2002). The *ASPGR-BBM*-like genes are therefore strong candidates for genes with a potential role in the induction and/or maintenance of apomixis events (Conner *et al.*, 2008).

1.4. The origin and maintenance of parthenogenesis in plants

Several evolutionary features are linked with apomictic reproduction. The most prominent being that gametophytic apomixis is tightly correlated with hybridisation and polyploidisation (Asker and Jerling, 1992; Carman, 1997; Whitton *et al.*, 2008). Reduced fertility due to meiotic disfunction, especially in the F1 generation, has led traditionally to a general perception of hybridisation as maladaptive (e.g. (Arnold *et al.*, 2001). Polyploidisation of hybrids (allopolyploidy) can potentially stabilise meiosis via homologous pairing of chromosomes (Comai, 2005). Polyploids are organisms that possess more than two homologous sets of chromosomes. Hybridisation seems to be the main factor responsible for triggering apomictic developmental pathways (e.g (Delgado *et al.*, 2014; Hojsgaard *et al.*, 2014) due to conflicts in gene expression (Carman, 1997), and subsequent polyploidisation stabilises apomictic rather than sexual reproduction, for example by allowing modifications to the contribution of paternal and maternal genomes during seed formation (Hojsgaard *et al.*, 2014). Most apomictic taxa are found within genera with extensive reticulate evolution and frequent polyploidisation, especially species belonging to the Asteracea, Poaceae, and Rosaceae (Carman, 1997; Whitton *et al.*, 2008; Talent, 2009). Meiosis often fails in polyploids, particularly in those with an odd number of sets of chromosomes, due to problems during the pairing of homologous chromosomes during meiosis. Apomictic reproduction therefore provides an alternative means for polyploids to reproduce. Shifts to apomixis via asexual seed formation in plants (agamospermy) have long been claimed as a possible mechanism to

escape from hybrid sterility and to stabilise polyploidy hybrid biotypes (e.g. (Stebbins, 1950; Grant, 1981; Asker and Jerling, 1992). In apomicts, the bypassing of meiosis for embryo sac production preserves the female genetic constitution, and allows for the maintenance of highly heterozygous hybrid biotypes. Evidence for a hybrid origin of natural apomictic taxa is being provided by an increasing number of molecular studies (e.g. (Koch *et al.*, 2003; Paun *et al.*, 2006; Fehrer *et al.*, 2007; Kantama *et al.*, 2007). Nevertheless, it remains poorly understood how hybridisation and/or polyploidisation actually trigger and establish apomictic reproduction in natural populations.

2. Parthenogenesis in algae

Parthenogenesis has been reported in many species of algae but the genetic mechanisms have not been studied. Parthenogenesis occurs in both micro- and macroalgae and can be common in some alga groups. Macroalgal parthenogenesis has been reported in Chlorophyta, Rhodophyta and Phaeophyceae. For example, in the red alga *Mastocarpus papillatus* (Rhodophyta), asexual reproduction involves female gametophytes producing another generation of female gametophyte offspring without fertilisation (Fierst *et al.*, 2010). In the Chlorophyta, parthenogenetic development of gametes into either the parthenogametophytes or parthenosporophytes has been observed in several species of *Ulva* and in *Percursaria percursa* (Doust and Doust, 1990; Løvlie and Bryhni, 2009). In the red alga *Caloglossa monosticha*, a recent study indicated that hybridisation could be an underlying cause of parthenogenetic development (Kamiya and West, 2008). The spatial distribution of sexual and asexual populations of *Mastocarpus papillatus* are consistent with an effect of the topography on the dispersal dynamic and on colonisation (Fierst *et al.*, 2010). Geographic parthenogenesis, which involves the coexistence of both sexual and asexual populations, needs to be taken into account when studying the evolution of parthenogenesis. Brown algae, which are multicellular eukaryotes that have been independently evolving from animals and plants for more than a billion years, also exhibit parthenogenetic development.

2.1. Parthenogenesis in brown algae

Brown algae are particularly interesting multicellular organisms because they have an extraordinary diversity of types of life cycle, sexual systems and modes of reproduction. In oogamous brown algae species, parthenogenesis has been reported to be rare. In the Fucales for example, parthenogenesis is absent in most species, or only observed under very particular experimental conditions. However, some reports indicate that parthenogenetic development can be triggered in *Hormosira banksii* (Clayton *et al.*, 1998) and in *Fucus distichus* (Nagasato *et al.*, 2000) under laboratory conditions but the parthenotes do not develop into viable organisms. The limited potential for parthenogenesis in the Fucales suggests that the process has no ecological significance. In contrast, in the Laminariales, another oogamous brown algal species, female parthenogenesis is a relatively common phenomenon (Nakahara and Nakamura, 1973; Le Gall *et al.*, 1996; Oppliger *et al.*, 2007; Müller *et al.*, 2018). For example, parthenogenesis has been reported in two species of *Lessonia* (*Lessonia nigrescens* (Oppliger *et al.*, 2007); *Lessonia berteroana* (Müller *et al.*, 2018), two species of *Laminaria* (*Laminaria japonica* (Lewis *et al.*, 1993); *Laminaria angustata*, (Motomura, 1991) and one species of *Alaria* (*Alaria crassifolia*, (Nakahara and Nakamura, 1973). Recent work aimed at describing evolutionary traits such as parthenogenetic capacity, life cycle diversity, sexual system differences and gamete size categories in brown algae (Luthringer *et al.*, 2014), suggests an inverse correlation between gamete size and parthenogenetic capacity. Gamete size is likely to be one of the factors that determines whether a gamete is capable of developping through parthenogenesis should it fail to encounter a gamete of the opposite sex. In anisogamous and oogamous species this has led to differences between parthenogenetic capacities of male and female gametes (Figure 12). In many oogamous species, parthenogenetic capacity is absent in both male and female gamete. Notable exceptions to this trend exist (e.g. some Laminariales) but parthenogenesis is limited to the female gamete. The case of Laminariales capable of undergoing parthenogenesis was debated and evidence suggested that the gametes of parthenogenetic Laminarailes species (e.g. *Laminaria angustata*) may be considered to represent an intermediate state between anisogamy and oogamy (Motomura and Sakai, 1988; Luthringer *et al.*, 2014). In anisogamous species, parthenogenesis is widespread and occurs only in female gametes.

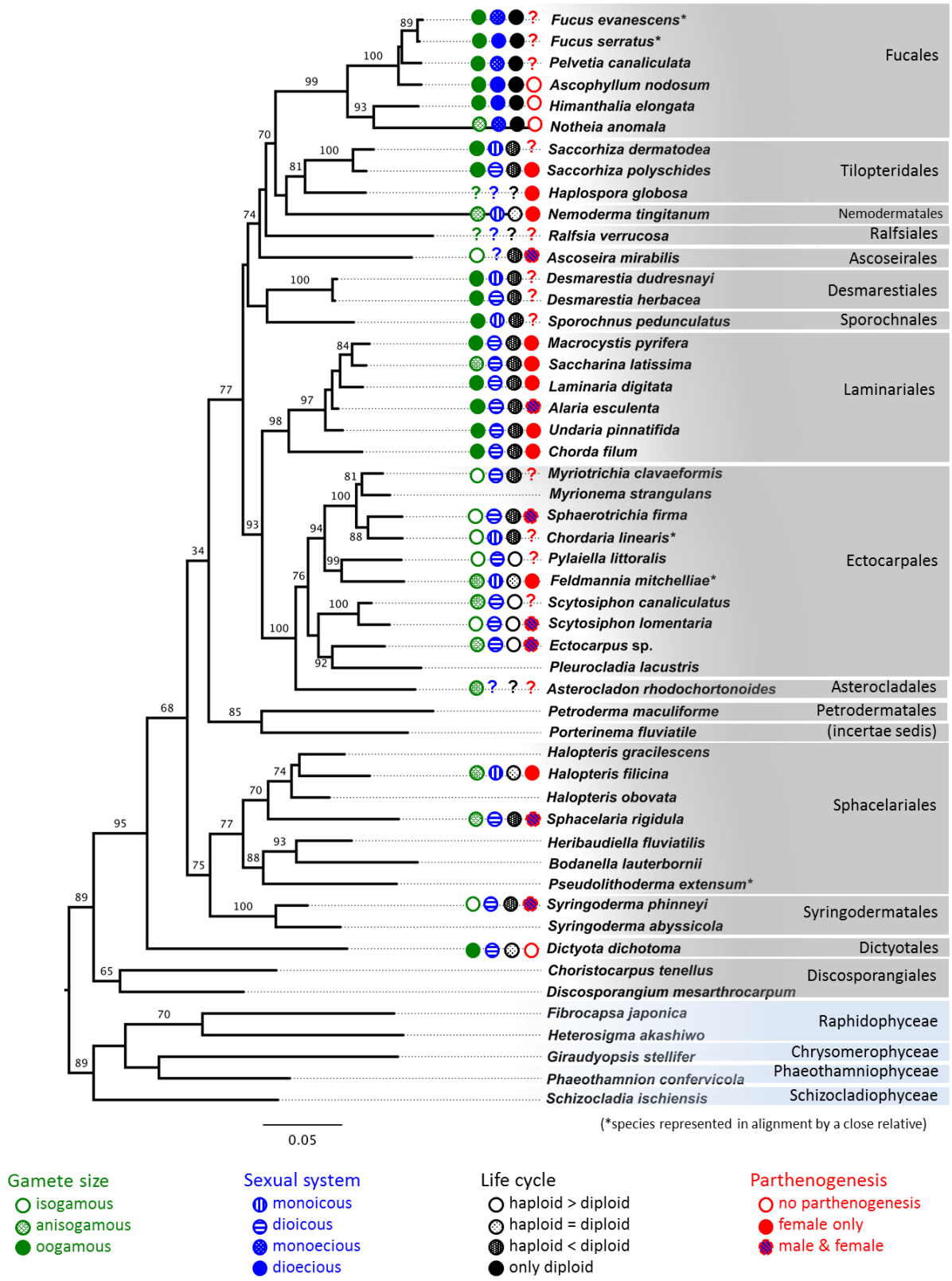

Figure 12: Distribution of gamete dimorphism, sexual system, life cycle and parthenogenetic development in brown algae. Note that gametes are considered parthenogenetic only if they develop into a functional individual (i.e. species whose gametes start to germinate but then degenerate were not scored as parthenogenetic). The question mark (?) was used when no data were available for a trait.

(Pers. Com. Svenja Heesch; adapted from Luthringer et al., 2014).

These trends suggests that in oogamous species the large female gamete is specialised for zygote production and is incapable of initiating parthenogenetic development (as in the Fucales). In anisogamous species though, parthenogenesis is perversive but tightly associated with large female gametes. However, exceptions exist like *Sphacelaria rigidula* or *Desmerestia ligulata* (Ramirez *et al.*, 1986) which are anisogamous species with both male and female gametes able to undergo parthenogenesis. In near-isogamous brown algae species, parthenogenetic capacity has been observed in both male and female gametes (Figure 12). In the near-isogamous species *Scytosiphon lomentaria*, parthenogenesis occurs in both male and female gametes (Nakamura and Tatewaki, 1975) but is prevalent in female (Han *et al.*, 2014). A recent study indicates that some populations of *S. lomentaria* from Northern Japan could be facultative asexuals, derived from sexually reproducing populations (Kogame *et al.*, 2005).

2.2. Parthenogenesis and mitochondria

Further analysis of fertilisation and parthenogenetic development in *Scytosiphon lomentaria* identified three proteins predicted to be genes involved in the mitochondria metabolic pathways suggesting that male and female gametes regulate mitochondrial metabolic pathways differentially during fertilisation and may be the reason for their physiological and behavioural differences (Han *et al.*, 2014). An interesting hypothesis was put forward in this study suggesting a possible a link between mitochondria inheritance and parthenogenetic development. In *S. lomentaria* most male gametes exhibit arrested parthenogenetic development at the 4-cell stage whereas about 95% of female gametes grow into parthenosprophyte (asexual organisms). Mitochondrial inheritance is strictly maternal in *Scytosiphon lomentaria* and Kimura et al. (Kimura *et al.*, 2010) have shown that male mitochondrial DNA gradually and selectively disappear after the 4-cell stage of germling development. Han et al (2014) therefore hypothesised that the mechanism that mediates uniparental inheritance of mitochondria could also control parthenogenetic capacity in males. When a non-parthenogenetic male gamete does not find a partner to fuse with, parthenogenetic development may be initiated but if the mechanism for selective degradation of mitochondria is also triggered, then the young partheno-sporophyte would lose its mitochondria resulting in an arrest of parthenogenetic development. Correlations between mitochondrial inheritance and parthenogenetic development have not been assessed in

plants or animals because most of the studied models exhibit parthenogenetic development from female cells and mitochondrial inheritance is maternal. Apomixis has not been described in the rare species that exhibit paternal inheritance (for example in gymnosperms).

3. *Ectocarpus* as a model to study parthenogenesis

The brown algae are key players in the intertidal ecosystems, and are very important in terms of phylogenetic position because they are one of the few eukaryotic groups that have evolved complex multicellularity. Moreover, these organisms are increasingly used in various domains such as the food-processing and pharmaceutical industry. In the last decade, a growing interest in brown algal research resulted in the development of a model for these organisms. In 2004, Peters and colleagues (Peters *et al.*, 2004) proposed *Ectocarpus* as a model organism for brown algae studies.

3.1. Ectocarpus: a genetic model for brown algae

Ectocarpus is a small filamentous brown alga, that has several advantages for genetic and genomic analyses including a short life cycle and its small size compared to the kelps, which make it easy to cultivate in the laboratory. Under laboratory conditions, *Ectocarpus* can become fertile within 1 or 2 months and usually reaches about 2 cm in length. In the wild it can reach 30 cm in length. *Ectocarpus* species are distributed throughout the temperate regions of both hemispheres but are not found in the Antarctic region. *Ectocarpus* usually grow on rocks or other substrates and can be epiphytic on other brown or red algae (Charrier *et al.*, 2008). Crosses and other genetic tools are available offering the opportunity to carry out genetic analysis. Moreover, *Ectocarpus* was the first brown alga to be sequenced (Cock *et al.*, 2010). A number of genomic tools are available for this model in addition to the well-annotated genome (Cock *et al.*, 2010; Cormier *et al.*, 2017), including transcriptomic data based on microarrays (Dittami *et al.*, 2009) and RNA-seq technologies (Ahmed, Cock, Pessia, Luthringer, Cormier, Robuchon, Sterck, Akira F. Peters, *et al.*, 2014; Luthringer *et al.*, 2015; Macaisne *et al.*, 2017), catalogues of small and long non-coding RNAs (Tarver *et al.*, 2015; Cormier *et al.*, 2017), genetic maps based on classic genetic markers (Heesch *et al.*, 2010) and on RAD sequencing (Avia *et al.*, 2017), a collection of mutants generated with ultraviolet light (Godfroy *et al.*, 2015), RNA interference (Macaisne *et al.*, 2017) and a chromatin

immunoprecipitation protocol (Bourdareau, 2018). Some genetic tools are still under development such as TILLING methodology and genetic transformation. Furthermore, *Ectocarpus* has the advantage of having a haploid-diploid life cycle, where both sporophyte and gametophyte generations are multicellular, and this feature opens the possibility to use Ectocarpus as a model to shed light into the molecular basis of developmental patterns during the life cycle of brown algae.

The alternation between gametophyte and sporophyte generations in *Ectocarpus* were therefore the focus of several studies providing new insights on the molecular mechanisms involved in the switch between the gametophyte and sporophyte programs of development (Coelho *et al.*, 2011; Peters *et al.*, 2008). *Ectocarpus* has also been more recently used as a model system to study UV sex-determination systems (Ahmed, Cock, Pessia, Luthringer, Cormier, Robuchon, Sterck, Akira F. Peters, *et al.*, 2014; Luthringer *et al.*, 2015; Avia *et al.*, 2018).

3.2. Ectocarpus exhibits parthenogenesis as part of its complex life cycle

The *Ectocarpus* life cycle consist of two independent multicellular heteromorphic generations: a diploid sporophyte generation and a haploid gametophyte generation (Figure 13.1). The sporophyte has prostrate (basal) filaments composed of round cells (Figure 13.2A and 2E) and upright (apical) filaments that grow from the basal ones, while gametophytes have highly branched upright filaments composed of cylindrical cells (Figure 13.2B and 2.F). The prostrate filaments of the sporophyte are attached to the substrate. Two types of spore-containing reproductive structure are produced on these upright filaments: plurilocular and unilocular sporangia. Plurilocular sporangia produce spores via mitosis (i.e. mito-spores), which, after germination, develop into genetically identical sporophytes. Asexual reproduction via these reproductive structures is similar to vegetative reproduction in plants. Unilocular sporangia produce haploid spore via meiosis (i.e. meio-spores) (Figure 13.2D). In each unilocular sporangium, a single meiotic event takes place producing four daughter cells that, after several mitotic divisions, produce 50 to 100 meio-spores. After release, these meio-spores germinate and develop into either male or female haploid gametophytes depending on which sex chromosome (U or V) they inherited during meiosis.

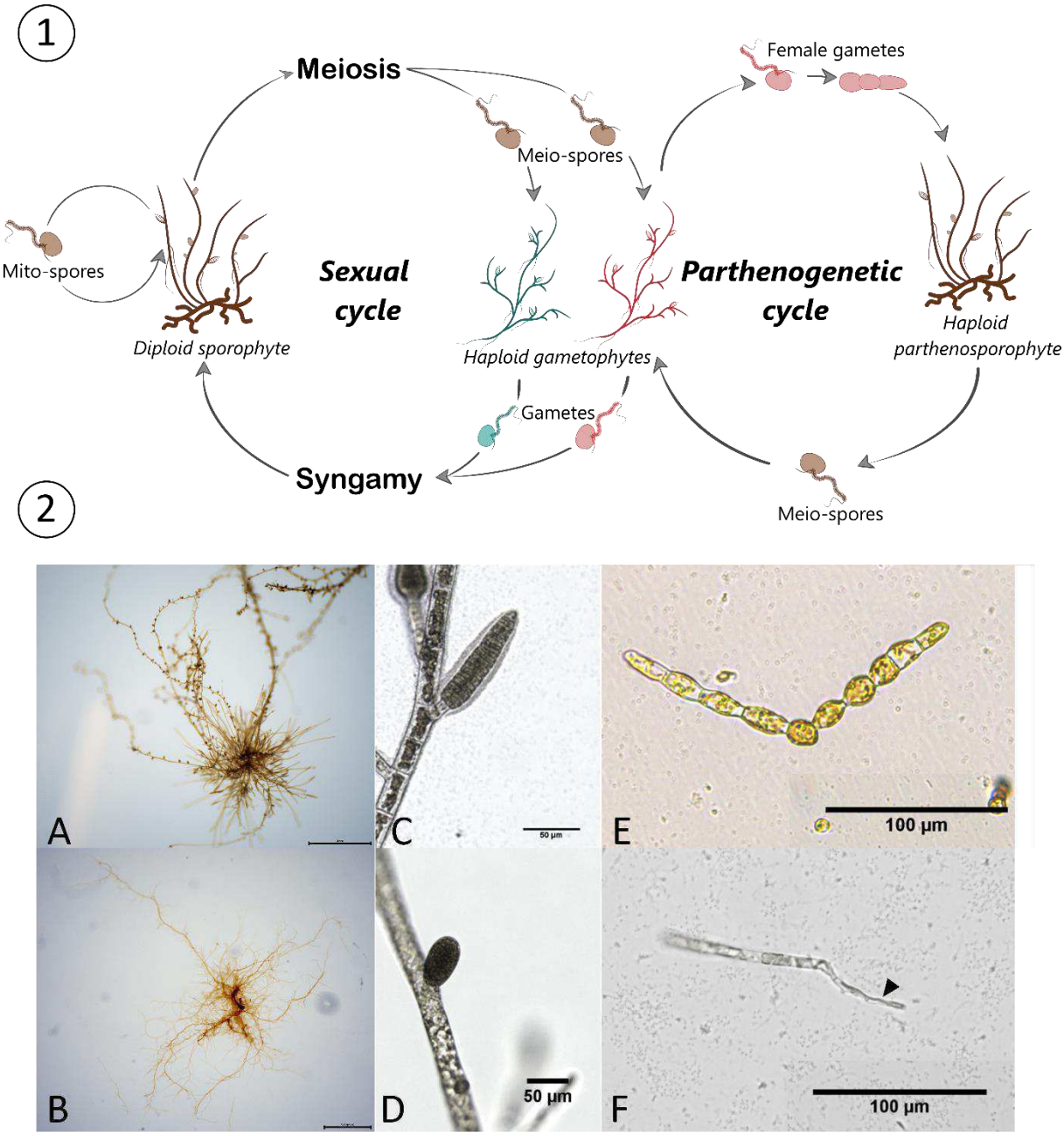

Figure 13 : *Ectocarpus* life cycle and morphological differences between sporophyte and gametophyte generations. (1) *Ectocarpus* life cycle combines a sexual life cycle and a parthenogenetic cycle. In the sexual life cycle the diploid sporophyte undergo meiosis and produce meio-spores that develop into multicellular male and female gametophytes. The gametophytes produce gamete that fuse and reform a diploid sporophyte. The sporophyte can also produce spores by mitosis (mito-spores) that develop into a sporophyte clone (asexual reproduction). When gametes fail to find a gamete of the opposite sex they can grow spontaneously through parthenogenetic devlepoment into parthenosporophyte. This parthenosporophyte can also produce mito-spores and undergo asexual reproduction (not on the figure) or produce meiospores and produce gametophytes. (2A) Mature sporophyte. (2B) Gametophyte. (2C) Plurilocular gametangia containing gametes (reproductive struture). (2D) Unilocular sporangia containing meio-spores (reproductive structure). (2E) Young sporophyte exhibiting round cells of the prostrate filament. (2F) Young gametophyte exhibiting upright filament and rhizoid (arrow).

Gametophytes carrying the U chromosome are female while those with a V are male (Ahmed, Cock, Pessia, Luthringer, Cormier, Robuchon, Sterck, Akira F. Peters, *et al.*, 2014). Male and female gametes are produced by mitosis in plurilocular gametangia (Figure 13.2C).

After release, swimming (flagellated) male and female gametes can fuse and give rise to a new diploid sporophyte, completing the sexual life cycle.

In addition to this complex life cycle involving sexual and clonal reproduction another mode of reproduction (i.e. parthenogenesis) has been observed (Figure 13.1). When gametes fail to find a compatible gamete to fuse with, they can spontaneously germinate through parthenogenesis and develop into fully functional partheno-sporophytes (Peters *et al.*, 2008). Partheno-sporophytes are genetically identical to the gametophyte from which the gamete is derived, and they are morphologically and functionally indistinguishable from diploid sporophytes. Thus, they have the capacity to produce plurilocular sporangia and undergo clonal reproduction but they can also produce unilocular sporangia. Two different processes, either endoreduplication or apomeiosis, have been suggested to be involved in the transition to the gametophyte stage (Bothwell *et al.*, 2010).

Several mutants affected in life cycle transitions have been identified and characterised in *Ectocarpus*, including for example the *ouroboros* (*oro*) mutant (Coelho *et al.*, 2011; Arun *et al.*, 2018). These mutants exhibit conversion of the sporophyte into a functional gametophyte. *oro* gametes are able to undergo parthenogenesis but instead of developing into partheno-sporophytes they produce partheno-gametophytes. Note that this mutant is not affected in its capacity to develop parthenogenetically but rather in the triggering of the diploid, sporophytic program. The molecular mechanisms underlying parthenogenesis in *Ectocarpus* remain unknown.

Objectives

The general aim of this thesis was to gain insights into the molecular, genetic and evolutionary mechanisms underlying parthenogenesis in the model brown alga *Ectocarpus*, specifically focusing on the genetic architecture of parthenogenesis and on the characterisation of the cellular mechanisms involved in this mode of asexual reproduction. The thesis also involved a study of mitochondria inheritance in *Ectocarpus*. More precisely the objectives of my thesis were:

1. To characterise the genetic architecture of parthenogenesis in the brown alga *Ectocarpus*, in particular to identify candidate genes that could be responsible for the phenotype (Chapter 2).
2. To characterise the phenotypic effect of parthenogenetic capacity on other cellular mechanisms such as zygotic growth, fusion success or mitochondria inheritance (Chapter 2 and 3).
3. To investigate the putative implication of mitochondrial inheritance on the decreased zygotic fitness delayed observed when a parthenogenetic male was used as the parental strain (Chapter 3).
4. To compare the genetic rearrangements between two close related species of *Ectocarpus* that exhibit differences in terms of parthenogenetic capacity (Chapter 4).

Chapter II

A key role for UV sex chromosomes in the regulation of parthenogenesis in the brown alga *Ectocarpus*

The brown algae have been evolving independently of animals and land plants for more than a billion years (Cock *et al.*, 2010). During that time, they independently acquired multicellularity and have become the third most complex multicellular lineage on the planet, with some species growing to more than 50 meters in length. In addition, the brown algae exhibit a remarkable diversity of growth habits, life cycles and sex determination systems. Surprisingly, whereas an enormous effort has been expended to understand the developmental and reproductive biology of animals and land plants, the brown algae have been almost completely ignored and very little is known about how these organisms function at the molecular level. Brown algae are also extremely interesting organisms because they exhibit a variety of reproductive systems, life cycles and types of sex chromosome systems. This diversity is found in a single group (brown algae), and also there appears to have been considerable switching between variants of these different features on a relatively short evolutionary timescale.

Parthenogenesis has been reported in many brown algae (Luthringer *et al.*, 2014) adding considerable interest to investigation of the origin and evolution of asexual reproduction in these organisms. *Ectocarpus* sp. is a model organism for the brown algae, for which various genetic tools have been developed (chapter 1 section II.3.1). Its complex life cycle includes a sexual and a parthenogenetic cycle with both haploid and diploid generations being multicellular (chapter 1 section II.3.2). The haploid-diploid life cycle provides experimental advantages compared to plant models (angiosperms) where the gametophyte (the pollen grain or the embryo sac) is reduced to a few cells, which, for the female, are deeply embedded in the parental sporophyte tissue. More importantly, while parthenogenesis is a component of apomixis in plants, and therefore requires other processes such as apomeiosis and spontaneous development of the endosperm, in *Ectocarpus* parthenogenesis can be studied as an isolated mechanism.

This chapter presents an investigation of the genetic architecture of parthenogenesis using a 'all-or-none' quantitative trait locus (QTL) approach. The chapter, which has been prepared in the form of a manuscript that has been submitted for publication, describes the main research project carried out in the context of this thesis. My contribution to this work consisted of cultivation of the different lines of *Ectocarpus*, generation of RAD sequencing libraries, analysis of the RAD-sequencing data, generation of the genetic map and use of JoinMap and R/qtl to detect QTLs involved in parthenogenesis in *Ectocarpus*. I also performed the GO-term enrichment analysis with the topGO package of the software R and epistasis and the statistical analyses. I provided the vcf files for the analysis of variants in the QTL intervals for the detection of polymorphisms potentially affecting gene expression or function. I carried out crosses to evaluate the growth of zygotes depending on the parthenogenetic capacity of the paternal strain and analysed the images. I participated in the writing of the manuscript and the production of figures and tables.

A key role for UV sex chromosomes in the regulation of parthenogenesis in the brown alga *Ectocarpus*

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Abstract

Although evolutionary transitions from sexual to asexual reproduction are frequent in eukaryotes, the genetic bases of these shifts remain largely elusive. Here, we used classic quantitative trait analysis, combined with genomic and transcriptomic information to dissect the genetic basis of asexual, parthenogenetic reproduction in the brown alga *Ectocarpus*. We found that parthenogenesis is controlled by the sex determining region on the sex chromosome, together with two additional autosomal loci, highlight the key role of the sex chromosome as a major regulator of asexual reproduction. Importantly, we identify several negative effects of parthenogenesis on male fitness, but also different fitness effects between parthenogenesis and life cycle generations, supporting the idea that parthenogenesis may be under both sexual selection and generation/ploidally-antagonistic selection. Overall, our data provide the first empirical illustration, to our knowledge, of a trade-off between the haploid and diploid stages of the life cycle, where distinct parthenogenesis alleles have opposing effects on sexual and asexual reproduction and may contribute to the maintenance of genetic variation. These type of fitness trade-offs have profound evolutionary implications in natural populations and may structure life history evolution in organisms with haploid-diploid life cycles.

Introduction

Although sexual reproduction, involving fusion of two gametes, is almost ubiquitous across eukaryotes, transitions to asexual reproduction have arisen remarkably frequently [1]. One type of asexual reproduction is parthenogenesis, defined as the development of an embryo from an unfertilized gamete. Parthenogenesis, which is widespread in all major eukaryotic lineages [2–7], involves the development of an embryo from an unfertilized gamete, without contribution from males [1]. In plants, parthenogenesis is a component of apomixis, which is the asexual formation of seeds, resulting in progeny that are genetically identical to the mother plant. In gametophytic apomixis, the embryo sac develops either from a megaspore mother cell without a reduction in ploidy (diplospory) or from a nearby nucellar cell (apospory) in a process termed apomeiosis. Apomeiosis is then followed by parthenogenesis, which leads to the development of the diploid egg cell into an embryo, in the absence of fertilization (reviewed in [8]).

The molecular mechanisms underlying parthenogenesis in plants and animals remain largely elusive, although the factors triggering the transition to asexual reproduction have been more intensively studied in plants than in animals, motivated by the potential use of asexual multiplication in the production of crop plants for agriculture (e.g. [9–10]). In some apomictic plants, inheritance of parthenogenesis is strictly linked to an apomeiosis locus (reviewed in [11]). In other species the parthenogenesis locus segregates independently of apomeiosis [12–14]. For example, apomixis in *Hieracium* is controlled by two loci termed *LOSS OF APOMEIOSIS* (*LOA*) and *LOSS OF PARTHENOGENESIS* (*LOP*), involved respectively in apomeiosis and parthenogenesis, respectively [15]. A third locus (*AutE*) involved in autonomous endosperm formation, was shown to be tightly linked to the LOP locus [16]. In *Pennisetum squamulatum*, apomixis segregates as a single dominant locus, the apospory-specific genomic region (ASGR), and recent work has highlighted a role for PsASGR-BABY BOOM-like, a member of the BBM-like subgroup of APETALA 2 transcription factors residing in the ASGR, in controlling parthenogenesis [17].

Parthenogenesis is also a relevant reproductive process in the brown algae, a group of multicellular eukaryotes that has been evolving independently from animals and plants for more than a billion years [18]. Once released into the surrounding seawater, gametes of brown algae may fuse with a gamete of the opposite sex, to produce a zygote which will develop into a diploid heterozygous sporophyte. Alternatively, in some brown algae, gametes that do not find a partner will develop parthenogenically, as haploid (partheno-) sporophytes (e.g. [19]). Parthenogenesis in brown algae can therefore be equated with gametophytic embryogenesis in plants, where embryos are produced from gametes [20], but in the case of brown algae the parthenogenetic gamete is haploid. The brown algae are therefore excellent models to study the molecular basis of parthenogenesis because gametes are produced directly by mitosis from the multicellular haploid gametophyte, allowing parthenogenesis to be disentangled from apomeiosis. Although parthenogenesis has been described in several species of brown algae (e.g.[21–23]), the genetic basis, the underlying mechanisms and the evolutionary drivers and consequences of this process remain obscure.

The haploid-diploid life cycles of brown algae of the genus *Ectocarpus* involve alternation between a haploid gametophyte and a diploid sporophyte, both of which consist of branched multicellular filaments (Figure 1A). Superimposed on this sexual cycle, an asexual, parthenogenetic cycle has been described for some *Ectocarpus* strains [19,21]. In this parthenogenetic cycle, gametes that fail to meet a partner of the opposite sex develop into haploid partheno-sporophytes. These partheno-sporophytes are indistinguishable morphologically from diploid sporophytes [21]. Partheno-sporophytes can produce gametophyte progeny to return to the sexual cycle through two mechanisms: 1) endoreduplication during development to produce diploid cells that can undergo meiosis or 2) individuals that remain haploid can initiate apomeiosis [21].

Here, we used a quantitative trait loci (QTL) approach to investigate the genetic basis of parthenogenesis in the brown alga *Ectocarpus siliculosus*. We show that parthenogenesis is a complex genetic trait under the control of three QTLs, one major QTL located on the sex chromosome, another on chromosome 18, with one additional minor QTL also on chromosome 18. We used genomic and transcriptomic analysis to establish a list of 89 candidate genes within the QTL intervals. Importantly, our work detected significant sex by genotype interactions for the parthenogenetic capacity, highlighting the critical role of the sex

chromosome in the control of asexual reproduction. Moreover, we identify different fitness effects between male sex and parthenogenesis and we reveal strong evidence for trade-offs between sexual and asexual reproduction during the life cycle of *Ectocarpus*. Overall, our results support the idea that parthenogenesis is a trait under sexual selection and ploidally-antagonistic selection in *Ectocarpus*.

Results

Parthenogenesis is controlled genetically

To precisely quantify the parthenogenetic capacity of two strains of *E. siliculosus*, clonal cultures of male (RB1) and female (EA1) *E. siliculosus* gametophytes were induced to release gametes under strong light (see methods) and pools of male and female gametes were allowed to settle separately, without mixing of the two sexes, on coverslips. Development of the gametes was then followed for 16 days (Figure 1B, Table S1). After 5 days, both male and female gametes had started to germinate and went through the first cell divisions. After 16 days, 94% of the female gametes had grown into >10 cell filaments, whereas 96% of the male gametes remained at the 3-4 cell stage and cell death was observed after about 20 days. Strains were therefore scored as parthenogenetic (P+) when more than 90% of the gametes have developed beyond the 10-cell stage at 16-days post release and as non-parthenogenic (P-), when less than 4% of the gametes had developed at 16d after release (Figure 1B, Table S1). In several brown algal species, unfused male and female gametes show different parthenogenetic capacity, and it is usually the female gametes that are capable of parthenogenesis whereas male gametes are non-parthenogenic (e.g. [23–24]). To investigate if there was a link between parthenogenetic capacity and sex, we crossed the female (EA1) P+ strain with the male (RB1) P- strain described above (Figure S1, Table S1). The diploid heterozygous zygote resulting from this cross (strain Ec236) was used to generate a segregating family of 272 haploid gametophytes. These 272 siblings were sexed using molecular markers [25] and their gametes phenotyped for parthenogenetic capacity (see above). The segregating population was composed of 144 females and 128 males, consistent with a 1:1 segregation pattern (chi2 test; p-value=0.33, Table S2).

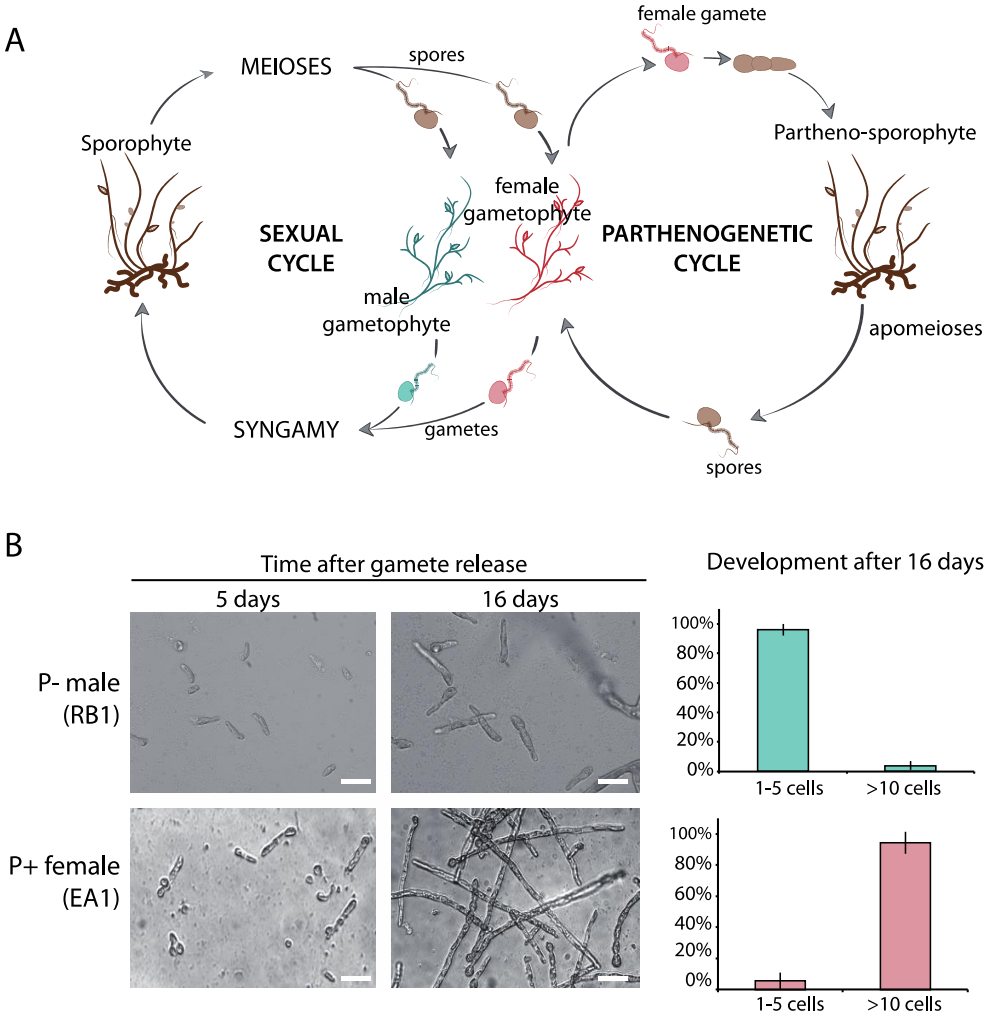

Figure 1: Life cycle of *Ectocarpus siliculosus* and phenotypes of parthenogenetic and non-parthenogenetic strains. A. Schematic representation of the life cycle of *Ectocarpus siliculosus*. *E. siliculosus* alternates between a gametophyte (haploid) and sporophyte (diploid generation). Meiosis is carried out in unilocular sporangia on the sporophyte, producing male and female meio-spores. Meio-spores develop by mitosis into male or female gametophytes, which at maturity produce male or female gametes. Syngamy reconstitutes the diploid genome. The parthenogenetic cycle involves parthenogenesis of a gamete when it fails to encounter a gamete of the opposite sex. The parthenogenetic cycle can be completed either via an apomeiosis to produce meio-spores from a haploid partheno-sporophyte (as shown) or via endoreduplication during partheno-sporophyte development, allowing meiosis to occur (not shown). **B.** Photographs of the parthenogenetic growth of gametes of non-parthenogenetic male (RB1, top) and parthenogenetic female (EA1, bottom) strains of Ectocarpus siliculosus after one day, 5 days and 16 days of development. Scale bar = 25 µm. The right panel shows the percentage of 1-5 cell and >10 cell partheno-sporophytes after 16 days of development for P- male gametes (Ec08, Ec398, Ec400, Ec409, Ec414, n=2632) and P+ female gametes (Ec399, Ec402, Ec404, Ec406, Ec410, Ec412, Ec415, n=3950).

Phenotypic assessment of the parthenogenetic capacity of the gametes released by each gametophyte revealed a significant bias in the inheritance pattern, with 84 individuals presenting a P- phenotype and 188 a P+ phenotype (Chi2 test; p-value=2.86×10^{-10}) (Table S2, S3). Strikingly, all female strains exhibited a P+ phenotype whereas 30% of the male strains were recombinants, i.e. had a P+ phenotype (Table S2). This result indicated the presence of a parthenogenesis locus or loci that was not fully linked to the sex locus, and suggested a complex relationship between gender and parthenogenetic capacity.

Stability of the parthenogenetic phenotype

A subset of the segregating family derived from the EA1 x RB1 cross was tested for phenotype stability. We cultivated two male P+ gametophytes, two male P- gametophytes and two female P+ gametophytes under different environmental conditions, varying light levels and temperature. After two weeks in culture, fertility was induced, and the parthenogenetic capacity of the gametes was scored (Table S4). The parthenogenetic phenotype of all strains was stably maintained regardless the culture conditions.

We also tested the stability of the parthenogenetic phenotype across generations: gametes of each of the three types (male P+, male P- and female P+) were allowed to develop into partheno-sporophytes. Note that this experiment is possible with P- males because a small proportion of male P- gametes (less than 4%) does not exhibit growth arrest and is able to grow to maturity. After two weeks in culture, gamete-derived partheno-sporophytes produced unilocular sporangia and released spores that developed into gametophytes. This second generation of gametophytes was again phenotyped for parthenogenetic capacity, and the results showed without exception that the parthenogenetic phenotype was stably maintained across generations (Table S4).

To further investigate the inheritance of parthenogenetic capacity, a male P+ individual was crossed with a P+ female (Figure S1). A total of 23 gametophyte lines were produced from two heterozygous sporophytes resulting from this cross. Phenotyping for sex and parthenogenesis revealed that all gametophyte lines exhibited a P+ phenotype, regardless of the sex (Table S5). We concluded that parthenogenesis is controlled by a genetic factor(s).

Generation of a genetic map for *E. siliculosus*

To produce a genetic map based on the EA1 x RB1 cross, a ddRAD-seq library was generated using 152 lines of the segregating progeny (Figure S1) and sequenced on an Illumina HiSeq 2500 platform. A total of 595 million raw reads were obtained, of which 508 million reads passed the quality filters with a Q30 of 74.1%. A catalogue of 8648 SNP loci was generated using filtered reads from the parental strains and the STACKS pipeline (version 1.44) [26]. Twenty-eight individuals were removed due to excessive missing genotypes (see Methods) and highly distorted markers were also removed. The final map constructed with 124 individuals contained 5594 markers distributed across 31 linkage groups (LGs) and spanning 2947.5 centimorgans (cM). The average spacing between two adjacent markers was 0.5 cM and the largest gap was 17.6 cM (on LG23). The lengths of the 31 LGs ranged from 174 cM with 397 markers to 13 cM with 31 markers (Figure 2A, Table S6).

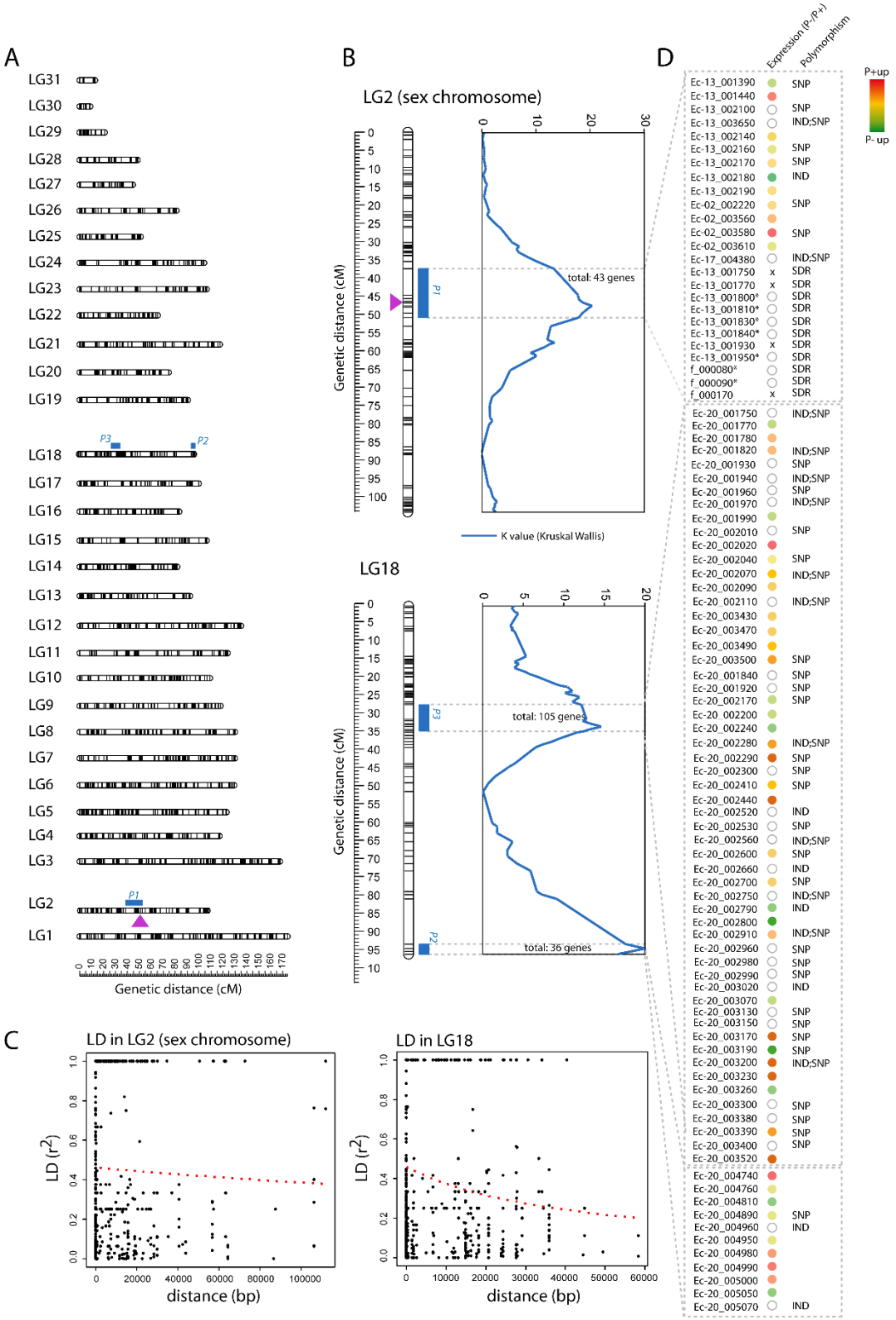

Figure 2: Quantitative trait loci identified for parthenogenetic capacity in Ectocarpus siliculosus. A. The 31 *Ectocarpus siliculosus* linkage groups showing the localization of QTLs for parthenogenesis. The position of the SDR is represented by a mauve arrow. **B.** QTLs intervals were detected using the Kruskal Wallis test (blue). **C.** Intra-chromosomal Linkage disequilibrium (LD)-decay between all pairs of markers for the sex chromosome and LG18. LD between markers (r2) is a function of marker distances (bp). D. Candidate parthenogenesis genes in each QTL interval. Genes in QTL intervals were selected based on differential expression of their orthologs in P+ versus P- in gametes, their differential expression between generation (gametophyte/partheno-sporophyte) and polymorphisms exhibited in exons and predicted to modify the protein product. *SDR gametologue; X, sex-specific gene.

Note that the Peruvian *Ectocarpus* strain that was used to generate the reference genome sequence [27] was originally taxonomically classified as *Ectocarpus siliculosus* but subsequent analysis has demonstrated that this strain actually belongs to a distinct species within the *Ectocarpus siliculosi* group [28]. The genetic map generated here using *bona fide Ectocarpus siliculosus* strains is therefore for a novel species relative to the genetic maps generated for the Peruvian strain [29,30].

QTL mapping approach to identify loci involved in parthenogenesis

To decipher the genetic architecture of parthenogenesis in *E. siliculosus*, we applied an "all-or-none" phenotyping and a quantitative trait loci (QTL) mapping approach, by considering P+ and P- as the two most 'extreme' phenotypes. We used the high-resolution genetic map to statistically associate markers with the P+ and P- phenotypes in the segregating family described above.

QTL mapping and association analysis identified three QTLs for parthenogenesis: two large-effect QTLs ($r^2 > 15\%$) and one smaller-effect QTL ($r^2=11.9\%$) (Figure 2A). Together, these three QTL explained 44.8% of the phenotypic variance. The QTLs were located on two different LGs, LG2 and LG18 (Figure 2A). LG2 was identified as the sex chromosome (Figure 2) and one of the large effect QTLs (*P1*) co-localized with the sex-determining region (SDR) of the sex chromosome. The *P1* locus was detected at the highest significance level (p-value <0.0001) with the Kruskal-Wallis statistical test (K*=20.392). The other major effect locus, which we refer to as the *P2* locus, was located on LG18, and was also detected at the highest significance level with a Kruskal-Wallis statistical test (p-value<0.0001,K*=19.993)(Table S7). A non-parametric interval mapping (IM) method also detected both *P1* and *P2* loci, and indicated a proportion of variance explained (PVE) of 16.6% for the P1 and 16.3% for the *P2* QTLs. The *P1* locus spanned 13.36 cM from 37.53 to 50.89 cM with a peak position at 47.66 cM whereas the *P2* locus spanned 2.82 cM, from 92.77 to 95.59 cM with a peak position at 93.98 cM.

The third QTL (*P3*) was detected only with the Kruskal-Wallis statistical test (K*=14.634, p-value<0.0005) and was also located on LG18. The *P3* QTL had a smaller effect than *P1* and *P2*, and explained 11.9% of the phenotypic variance (Figure 2A, 2B; Table S7).

Note that the QTL mapping described above was implemented using all 152 progeny (Figure S1), which included both male and female strains. To investigate the contribution of the sex-specific, non-recombining region of the sex chromosome, we performed the same analysis using a subset of 93 male strains. The result showed that when females were excluded, the *P1* and the *P3* QTLs were not detected, and only the QTL located on LG18 (*P2*) was significantly detected (Table S7). The absence of detection of the *P1* QTL was not due to reduced statistical power due to the small sample size, because the QTL was detected when a sub-sample of 93 male and female individuals with the same sex ratio as the full 124 samples was used (Table S7). The minor *P3* QTL was at the limit of significance when the 93 sub-sampled individuals were used, suggesting that the reduced sample size prevented the detection of this minor QTL. Taken together, our results indicate that the *P1* QTL is linked to the SDR.

To more precisely locate the three QTL intervals detected using the whole dataset, the decay of pairwise linkage disequilibrium (r^2) was estimated for each linkage group (Figure 2C). An r^2 threshold of 0.2 was used to determine approximate windows at the QTL positions to search for putative candidate genes. Based on these windows we determined the number of genes present in each QTL interval using both the reference genome of the closely related species *Ectocarpus* species 7 (strain Ec32) [18,31] and an assembly of the genome of the male parent (RB1; [32] (Table S10). The two main QTL intervals contained between 96 and 98 genes (depending on whether the female U or male V chromosome, which have slightly different gene numbers in the SDR, is considered, respectively). In total, 201/203 genes were located in the intervals corresponding to the three parthenogenesis QTLs (Figure 2D, Table S7).

Gene Ontology enrichment tools were used to test if some functional categories were over-represented in QTL regions. BLAST2GO analysis showed that the genes in the QTL intervals were significantly enriched in processes related to signalling and cell communication (p-value < 0.0001) (Figure S2, Table S8).

Epistasis analysis

An epistasis analysis was carried out to detect potential interactions between the parthenogenesis QTLs. Two analyses were performed, using either all 152 male and female progeny ('full dataset') or the subset of all the 93 male individuals.

We observed significant sex by genotype interactions for parthenogenetic capacity. The analysis of the full dataset identified an epistatic interaction between the *P2* QTL and the *P1* QTL (Figure 3). When the same analysis was carried out with only the males, this epistatic interaction was not detected (Table S9). This result indicated that the epistasis was driven by the female-specific region. In Figure 3, the B allele was inherited from the female parent, and the A allele from the male parent. All females were parthenogenetic (B allele on the *P1* locus in Figure 3) and therefore their parthenogenetic phenotype was independent of the allele carried at the *P2* locus. In contrast, the phenotype of males depended on the allele carried at the *P2* locus.

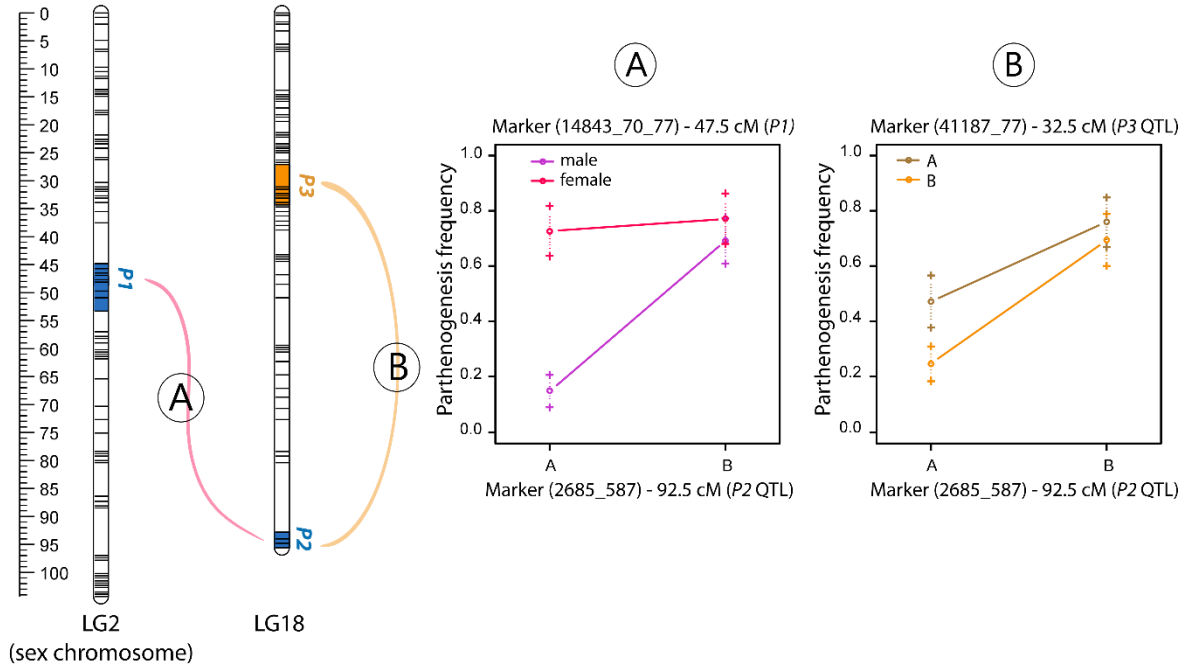

Figure 3

Figure 3: Epistatic interactions between parthenogenetic loci. A. Epistatic interactions detected between the sex-determining region (SDR) and the *P2* QTL. Females can undergo parthenogenesis independently of the allele carried at the P2 locus whereas males are only parthenogenetic if they carried the B allele at the *P2* locus. **B.** Epistatic interaction between the *P3* and *P2* loci. Individuals carrying the B allele at both loci have a higher parthenogenetic capacity than those with any other combination.

An additional interaction was detected between the *P2* QTL and the *P3* QTL. In this case, the frequency of P+ individuals was higher when the maternal B allele was present at the *P2* locus and the effect was strongest when the *P3* locus carried the maternal B allele (Figure 3B).

Several additional interactions were detected between the *P2* QTL and markers on several autosomes when the male-only dataset was analysed (Table S9).

Identification of candidate genes within the parthenogenesis QTL intervals

We used several approaches to identify candidate parthenogenesis genes within the three QTL intervals. First, we reasoned that genes involved in parthenogenesis should be expressed at least in one of the gamete types, P+ or P-, where parthenogenesis is initiated. Strains EA1 and RB1 did not produce enough gametes for RNA extraction. We therefore generated RNA-seq data from P+ female and P- male strains from another species within the *E. siliculosi* group, *Ectocarpus* species 1 [31] (see methods). We analysed the abundance of the transcripts of orthologs of the 201-203 genes within the three QTL intervals. Based on this analysis, 133/139 genes (depending on whether we consider the U or the V, respectively) were classed as being expressed in at least one of the gamete types (Table S11).

Second, we looked for genes that were significantly differentially expressed between P+ and P- gametes, again using the data for *Ectocarpus* species 1 orthologues. Overall, 4902 orthologues were differentially expressed in P+ versus P- strains across the genome, of which 64 corresponded to genes located within the QTL intervals (Figure 2D, Table S10). The QTL intervals were therefore significantly enriched in genes that we classed as being differentially expressed between P+ and P- strains (Fisher exact test; p-value=0.0165).

Third, we looked for polymorphisms with potential effects on the functions of the candidate genes. Comparison of the parental genomic sequences identified 10961 indels and 32682 SNPs within the three QTL intervals (Table S11, S12). In total, 67 genes within the QTL intervals carried SNPs or indels that corresponded to non-synonymous modifications of the coding sequence and were therefore predicted to affect protein function. The male and female SDRs do not recombine [33] and have therefore diverged considerably over evolutionary time. This has included loss and gain of genes but also strong divergence of the genes that have been

retained in both regions (gametologs). All SDR genes were therefore retained as candidates (Table S11).

We then combined the three approaches. The criteria we used were that genes involved in parthenogenesis must be expressed in gametes and they should have either differential expression in P+ versus P- gametes or carry a non-synonymous polymorphism. This reduced the number of candidates to 17/22 (U/V chromosome) genes in the *P1*, 11 genes in the *P2* and 56 genes in the *P3* QTL (Figure 2D, Table S11). Taking genes that were both differential expressed in P+ versus P- gametes and carried a non-synonymous polymorphism (Table S11, Figure 2D) further reduced the list of candidate genes to 9/14 (U/V), 1 and 16 candidates (in *P1*, *P2* and *P3* respectively).

Parthenogenetic male gametes exhibit reduced fitness in sexual crosses

It is not clear why some strains of *Ectocarpus* exhibit male gamete parthenogenesis whilst others do not. More specifically, bearing in mind that all strains tested so far exhibit parthenogenesis of female gametes, why are male gametes not parthenogenetic in some lineages? To address this question, we investigated if there were differences in fitness between P- and P+ male gametes for parameters other than parthenogenetic growth. Specifically, we examined fertilisation success (capacity to fuse with a female gamete) and growth of the resulting diploid sporophyte.

We tested several combinations of crosses between P- or P+ males and several females (Table S13). Overall, male P- gametes tended to fuse more efficiently with female gametes compared to P+ male gametes, even if the difference was not significant (Figure 4A, Student's t-test $p=0.059$). Importantly, embryos arising from a P- male gamete grew significantly faster than embryos derived from fusion with a male P+ gamete (Figure 4B, 4C, Mann-Whitney u-test $p<0.05$).

The overall size of zygotes is expected to be correlated with zygotic and diploid fitness [34–36]. We therefore hypothesised that if P- male gametes are larger, fusion with a female gamete would generate larger (and therefore fitter) zygotes. Measurements of gamete size of P+ and P- strains revealed significant differences in gamete size between different strains (Kruskal-Wallis test, Chi2=3452.395, P<2.2e-16, Table S14, Figure 4D, Figure S2). However, there was no correlation between the parthenogenetic capacity of male gametes and their size, suggesting that the increased fitness of the zygotes was unlikely to be related to the size of the male gametes.

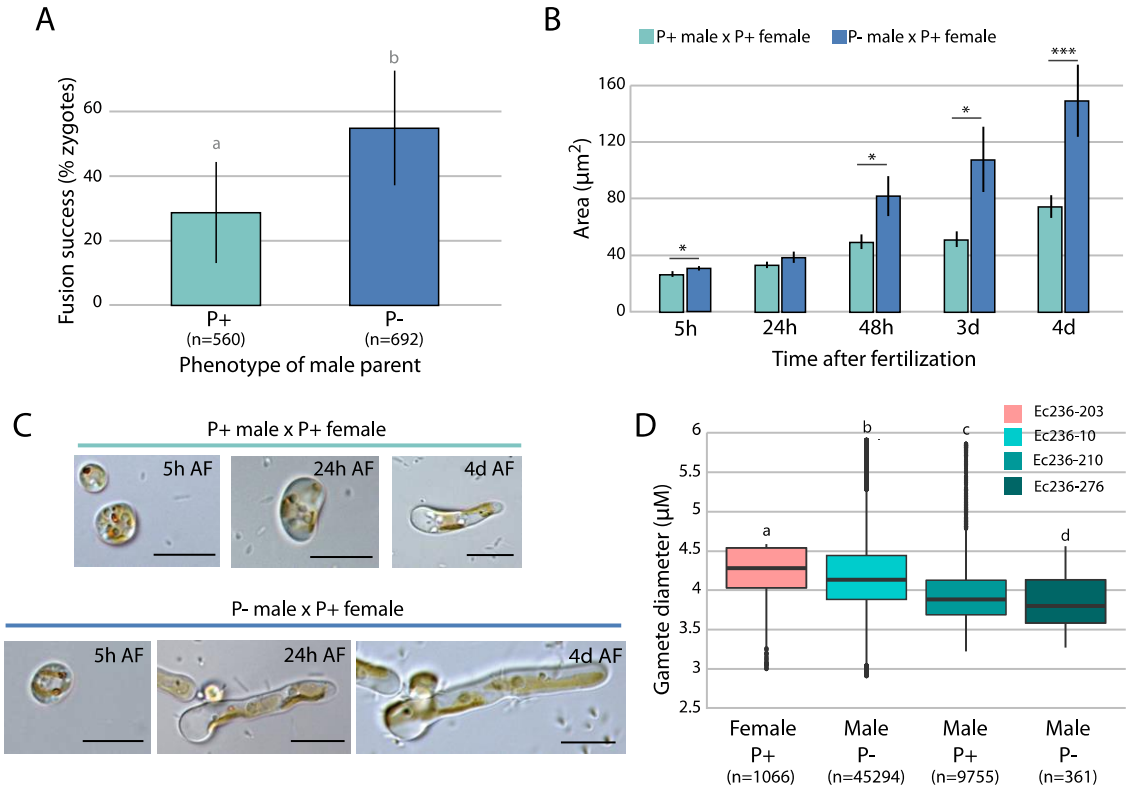

Figure 4: Fitness of parthenogenetic (P+) and non-parthenogenetic (P-) males. A. Fertilisation success was assessed by counting the proportion of zygotes obtained after crossing either parthenogenetic (Ec236-34, Ec236-245) or non-parthenogenetic (Ec236-10, Ec236-298) males with parthenogenetic females (Ec236-284, Ec236-39, Ec236-203, Ec560) (n=1252). Fusion success tended to be higher when the male parent was P- (Mann Whitney P=0.058; represented by grey letters). **B.** Growth of zygotes (from 5 hours to 4 days after fertilisation, AF) derived from crosses performed between female P+ and male P+ or male P- strains (*p-value<0.01;***p-value<0.0001). Thirteen to fourteen zygotes were scored per cross at each time point. The experiment is representative of three independent experiments performed with several parental lines (see also Figure S2). **C.** Representative images of zygotes at different developmental stages, from a male P- (RB1) x female P+ (Ec236-105) cross and from a male P+ (Ec236-154) x female P+ (Ec236-105) cross. Scale bar=10 µm. **D.** Sizes of gametes from a parthenogenetic female, a parthenogenetic male and non-parthenogenetic male. The mean diameter of female P+ (Ec236-203, n=1066), a male P+ (Ec236-210, n=9755) and two P- males (Ec236-276, n=45294 and Ec236-10 n=361) lines were measured by cytometry. The values of gamete size shown represent the mean ± s.e. for each individual.

Taken together, these analyses indicate that P+ male gametes exhibit overall reduced fitness in sexual crosses, both at the level of success of fusion with a female gamete and growth of the resulting embryo. We found no link between the size of the male gamete and the capacity to perform parthenogenesis, which excludes the possibility that the fitness decrease is due to the size of the male gamete.

Discussion

A key role for the sex chromosome in parthenogenesis

In this study, we uncover the genetic architecture of parthenogenesis in the brown alga *E. siliculosus* and demonstrate that this trait is controlled by two major and one minor QTL loci that, together, account for 44.8% of the phenotypic variation. The two main QTL loci were located in the SDR on the sex chromosome and on LG18 respectively, and the minor QTL was also located in LG18. Analysis of differential expression pattern and polymorphism for genes within the QTL intervals allowed the establishment of a list of a total of 89 candidate parthenogenesis genes: 17/22 genes within the sex chromosome QTL interval (in the U and V respectively), 11 genes within the *P2* locus and 56 within the interval of the minor *P3* locus. Interestingly, within the major *P2* QTL a strong candidate gene coded for a membrane-localized ankyrin repeat-domain palmitoyltransferase (Ec-20_004890). In *S. cerevisiae*, genes belonging to the same family are involved in the gamete pheromone response pathway, regulating the switching between vegetative and mating states [37,38].

Our results reveal a critical role for the sex chromosome in the control of parthenogenesis, with a major effect QTL being located within (or very tightly linked to) the SDR. Interactions between the SDR and the major *P2* QTL locus were detected only when the female SDR was present and parthenogenesis was triggered in females regardless of the allele carried at the *P2* or *P3* locus. The observed effects could be due to a conditional repressor of parthenogenesis in the male V-specific region or an activator of parthenogenesis in the female U-specific region. However, a recent paper on another brown alga *Undaria pinnatifida* described genetically male individuals that were capable of producing oogonia and whose

eggs were parthenogenic [39]. Similarly, several male *L. pallida* lines from a South African population had unusual reproductive structures resembling small eggs, which are also capable of parthenogenesis (Ingo Maier, pers. commun.). These results would therefore be consistent with a repressor of parthenogenesis being present on the V-specific region in these brown algae, that appears to be impaired in variant strains. Alternatively, it is also possible that parthenogenesis is switched on downstream of the female pathway, in this case these variant strains would automatically trigger parthenogenesis when becoming 'female'.

Male fitness effects of parthenogenetic capacity

Our results indicate that parthenogenetic capacity has a dramatic impact on the fitness of male gametes. Specifically, P- male gametes are fitter than P+ male gametes for sexual reproduction and this is reflected in significantly higher fertilisation success and higher growth rate of the resulting zygote. Considering that P+ males would be expected to exhibit reduced fitness in sexually reproducing populations, and the fact that females are phenotypically P+ regardless of the allele at the *P2* and *P3* QTL, how can the P+ allele be preserved in the population? In other words, how is the parthenogenesis polymorphism maintained?

Heterozygous advantage can maintain polymorphism in diploid organisms. For instance, most obligate parthenogenetic vertebrates arise from hybridization between closely related species, resulting in elevated individual heterozygosity relative to the parental genotypes [40–42]. This is considered adaptive for colonizing new areas where high genetic diversity may provide the necessary genetic tools to adjust to new conditions. In the case of *Ectocarpus*, fixing the P+ allele in the female SDR and the P- allele in the male SDR would be a way to maintain the alleles polymorphic in the sporophyte. Note however that this process would be applicable to the SDR QTL, and would not necessarily explain the polymorphism maintained at the autosomal QTLs.

One interesting possibility is that parthenogenesis is a sexually antagonistic trait (or at least differentially selected in males versus females), i.e., P+ alleles would be advantageous for females because they would be capable of reproducing even in absence of gametes of the opposite sex, so that P+ would be selected for in females, whereas P- increases male fitness because sporophytes sired by a P- male can grow more rapidly. Polymorphism could therefore be maintained by balancing selection [43–45]). Although we could not measure the effect of

parthenogenetic capacity on female gamete fitness, because all females were phenotypically P+, sexual antagonism would be consistent with the pervasiveness of the female P+ phenotype and the differences in fitness between P+ and P- males. This phenomenon would be particularly relevant in spatially heterogeneous environments, where the P+ or P- allele(s) in males would alternatively selected for, depending on female density.

Temporal or spatial changes in population density are extremely common (e.g. [56–58]), and this will probably cause strong fluctuating selection on sex-specific traits [59,60], contributing to maintaining genetic polymorphism in populations [46]. A polymorphism can be maintained by fluctuating selection when selection varies in both space and time [47] or when some genotypes are shielded from selection as in a seed bank [48–50]. This effect of sex limitation on the stability of a polymorphism is caused by a storage effect that automatically occurs when traits are expressed in only one sex. In the other sex, these alleles are sheltered from selection, because they are not expressed [50]. In the specific case of *E. siliculosus*, the P- allele would be shielded from selection because it is never expressed in females. In other words, if expression of P- allele(s) is limited to males, fluctuating selection of this sex-limited trait could therefore lead to the existence of a protected polymorphism, and contribute to explain the maintenance of genetic variance at the autosomal QTLs. The P+ allele would be maintained because it is advantageous in males when females are rare or when populations have low density.

Another potential mechanism for the maintenance of genetic variation is opposing selection during the diploid and haploid stages of biphasic life cycles, also known as ploidally-antagonistic selection [51]. Parthenogenesis could be considered an example of a trait under ploidally/generation antagonistic selection because the P- allele transmitted by the male gamete is advantageous to the diploid (sporophyte) generation (because zygotes grow faster if the father is a P-) but detrimental to the haploid (partheno-sporophyte) generation (because if they do not find a female gamete, males that carry a P- allele die). Ploidally-antagonistic selection has been proposed to have a significant impact on major evolutionary dynamics, including the maintenance of genetic variation ([51–53] and the rate of adaptation [54]. Moreover, it appears that P+ and P- are under differential selective pressures in males (when populations reproduce sexually, P- should be beneficial to males and P+ detrimental). Mathematical modelling [55] predicts that when selection differs between the sexes (and in

particular when the gametophyte-deleterious allele is neutral or slightly beneficial in one of the sexes), being close or within the SDR expands the range of parameters allowing generation-antagonistic mutations to spread. Note that conflict arising from generation-antagonism or from differences in selection in gametophytes versus sporophyte generation is best resolved by complete linkage to the SDR [55].

Is parthenogenesis adaptive?

In the brown algae, the ancestral state appears to have been sexual reproduction through fusion of strongly dimorphic gametes (oogamy) [56], that were incapable of parthenogenesis (reviewed in [24]). This suggests that gamete parthenogenesis was superimposed on a sexual cycle, having evolved secondarily possibly to ensure reproduction in conditions where populations have, for instance, low population density. In this scenario, parthenogenesis capacity could be considered a bet-hedging strategy for males, with a tradeoff between higher sexual fitness when non-parthenogenetic and lower sexual fitness (but asexual, clonal reproduction) when parthenogenetic.

A challenge for understanding the adaptive nature of gamete parthenogenesis in these organisms would be to identify the conditions under which it occurs in nature. Brown algae exhibit a remarkable degree of reproductive plasticity during their life cycle [21,57] and it is possible that this plasticity is related to capacity to adapt to new conditions, in particular low population density or very fragmented habitats where finding a partner may be problematic. It has been predicted that in marginal populations, or other situations where mates are limited, parthenogenesis could be adaptive and thus selectively favored [58]. In animals (fish, *Drosophila*) rapid transition between reproductive strategies were observed following the removal of the mate, supporting the hypothesis that parthenogenesis has a reproductive advantage under conditions of isolation from potential mates [59]. A recent study of *Ectocarpus siliculosus* populations in NW of France has shown that asexual populations are prevalent in the field, but gamete parthenogenesis does not appear to play a critical role in this population, and instead, asexual sporophytes are produced mainly from the development of diploid, asexual spores [60]. Additional population data are required, specifically for natural populations where individuals are found at different densities, for marginal versus central

populations and for different types of habitat, to further investigate whether there is an adaptive benefit to parthenogenesis.

Material and methods

E. siliculosus cultures

Gametophytes of *E. siliculosus* (Table S1) were maintained in culture as previously described [61]. *E. siliculosus* strains can be maintained in the gametophyte generation indefinitely, with weekly changes in culture media [61]. Clonal cultures of male and female gametophytes were subjected to strong light (100 μm photons/m^2/s) and low temperatures (10°C) to induce fertility resulting in the release of large numbers of gametes (>10e5). Gametes were allowed to settle on coverslips and their development was monitored under an inverted microscope (Olympus BX50).

Evaluation of parthenogenetic capacity and sex

The sex of the gametophytes was assessed using SDR-specific PCR markers [25], and parthenogenetic capacity was evaluated by scoring the capacity of released gametes to develop into adult filaments of more than 10 cells after 16 days in the absence of fusion with gametes of the opposite sex (single sex gamete cultures).

Cross design, culturing and phenotyping

A cross between a parthenogenetic female (strain EA1) and a non-parthenogenetic male (strain RB1) was carried out using a standard genetic cross protocols [62] and a diploid heterozygous sporophyte was isolated (Ec236) (Figure 1; Table S1). At maturity, the sporophyte (strain Ec236) produced unilocular sporangia, i.e, reproductive structures where meiosis takes place (Figure 1). A total of 272 unilocular sporangia were isolated, and one gametophyte was isolated from each unilocular sporangium.

The 272 strains of the EA1 x RB1 derived segregating population were cultivated in autoclaved sea water supplemented with half strength Provasoli solution [63] at 13°C, with a light dark

cycle of 12:12 (20 µmol photon m^{-2} s^{-1}) using daylight-type fluorescent tubes [61]. All manipulations were performed in a laminar flow hood under sterile conditions. We phenotyped the strains for parthenogenetic capacity (P+ or P-) and for sex (male or female). Parthenogenetic capacity was assessed by scoring the capacity of the gametes to develop into partheno-sporophytes in the absence of fertilization. In order to assess phenotype stability, gametophytes were sub-cultivated in different conditions for two weeks and then exposed to high intensity light to induce fertility. Parthenogenetic capacity was measured using the released gametes (Table S3). We monitored gamete germination every two days. In P+ strains, >96% of the gametes developed as partheno-sporophytes in the absence of fertilization whereas in P- strains, less than 4% of the gametes were capable of parthenogenesis. To test the stability of the phenotype across generations, we cultivated partheno-sporophytes and induced them to produce unilocular sporangia and release meio-spores to obtain a new generation of gametophytes. The parthenogenetic capacity of gametes derived from these second-generation gametophytes was then tested (Table S3). Note that this experiment is feasible in P- males because a very small proportion (less than 4%) of their gametes are nevertheless able to develop into mature partheno-sporophytes.

Each of the 272 gametophytes of the EA1 x RB1 segregating family was frozen in liquid nitrogen in a well of a 96 well plate. After lyophilization, tissues were disrupted by grinding. DNA of each gametophyte was extracted using the NucleoSpin® 96 Plant II kit (Macherey-Nagel) according to the manufacturer's instructions and stored at -80°C. Sexing of gametophytes was carried out using two molecular sex markers for each sex (FeScaf06_ex03 forward: CGTGGTGGACTCATTGACTG; FeScaf06_ex03 reverse: AGCAGGAACATGTCCCAAAC; 68_56_ex02 forward: GGAACACCCTGCTGGAAC; 68_56_ex02 reverse: CGCTTTGCGCTGCTCTAT) (Ahmed, Cock, Pessia, Luthringer, Cormier, Robuchon, Sterck, Akira F. Peters, *et al.*, 2014). PCR was performed with the following reaction temperatures: 94°C 2min; 30 cycles of 94°C 40s, 60°C 40s and 72°C 40s; 72°C 5min, and with the following PCR mixture 2 µL DNA, 100 nM of each primers, 200 µM of dNTP mix, 1X of Go Taq® green buffer, 2 mM of MgCl2, 0.2 µL of powdered milk at 10% and 0.5 U of Taq polymerase (Promega).

DNA extraction and library RAD sequencing

A double digest RAD sequencing (ddRAD-seq) library was generated using 152 individuals from the EA1 x RB1 segregating population. Parthenogenetic individuals were selected (37 females and 36 males) as well as non-parthenogenetic males (79 individuals). DNA extraction was performed for each individual (Macherey-Nagel, NucleoSpin® Plant II kit (GmbH & Co.KG, Germany) and DNA quantity was measured and standardized at 100 ng using a PicoGreen® (Fischer Scientific) method for quantification. The DNA quality was checked on agarose gels.

The ddRAD-seq library was constructed as in [64] using *Hha*I and *Sph*I restriction enzymes (New England Biolabs, https://www.neb.com/). Those enzymes were selected based on an *in silico* digestion simulation of the Ec32 reference genome [18] using the R package SimRAD [65]. After digestion, samples were individually barcoded using unique adapters by ligation with T4 DNA ligase (New England Biolabs, https://www.neb.com/). Then, samples were cleaned with AMPure XP beads (Beckman Coulter Genomics), and PCR was performed with the Q5® hot Start High-Fidelity DNA polymerase kit (New England Biolabs, https://www.neb.com/) to increase the amount of DNA available for each individual and to add Illumina flowcell annealing sequences, multiplexing indices and sequencing primer annealing regions. After pooling the barcoded and indexed samples, PCR products of between 550 and 800 bp were selected using a Pippin-Prep kit (Sage Science, Beverly, MA, USA), and the library was quantified using both an Agilent® 2100 Bioanalyzer (Agilent Technologies) and qPCR. The library was sequenced on two Illumina HiSeq 2500 lanes (Rapid Run Mode) by UMR 8199 LIGAN-PM Genomics platform (Lille, France), with paired-end 250 bp reads.

Quality filtering and reference mapping

The ddRAD-seq sequencing data was analysed with the Stacks pipeline (version 1.44) [26]. The raw sequence reads were filtered by removing reads lacking barcodes and restriction enzyme sites. Sequence quality was checked using a sliding window of 25% of the length of a read and reads with <90% base call accuracy were discarded. Using the program PEAR (version 0.9.10, [66]) paired-end sequencing of short fragments generating overlapping reads were identified and treated to build single consensus sequences. These single consensus sequences were added to the singleton rem1 and rem2 sequences produced by Stacks forming a unique group

of singleton sequences. For this study, paired-end reads and singleton sequences were then trimmed to 100 bp with the program TRIMMOMATIC [67]. The genome of the male parent of the population (strain RB1) was recently sequenced to generate an assembly [32] guided by the *Ectocarpus* species 7 reference genome published in 2010 [68]. We performed a *de novo* analysis running the denovo_map.pl program of Stacks. Firstly, this program assembles loci in each individual *de novo* and calls SNPs in each assembled locus. In a second step, the program builds a catalog with the parental loci and in a third step, loci from each individual are matched against the catalogue to determine the allelic state at each locus in each individual. We then used BWA (Li, H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM.arXiv:1303.3997) to align the consensus sequence of the catalog loci to the reference genome and used the Python script "integrate_alignments.py" of the Stacks pipeline to integrate alignment information back into the original de novo map output files [69]. In a final step, SNPs were re-called for all individuals at every locus and exported as a vcf file.

Genetic map construction and QTL mapping

The vcf file obtained with the Stacks pipeline was first filtered to keep only loci with maximum of 10% of missing samples and samples with a maximum of 30% of missing data. The program Lep-MAP3 (LP3) [70] was used to construct the genetic map. LP3 is suitable to analyse low-coverage datasets and its algorithm reduces data filtering and curation on the data, yielding more markers in the final maps with less manual work. In order to obtain the expected AxB segregation type for this haploid population, the pedigree file was constructed by setting the parents as haploid grand-parents and two dummy individuals were introduced for parents. The module ParentCall2 of LP3 took as input the pedigree and the vcf files to call parental genotypes. The module SeparateChromosomes2 used the genotype call file to assign markers into linkage groups (LGs). Several LOD score limits were tested to obtain an optimal LOD score of 8 giving a stable number of LGs. The module JoinSingles2All was then run to assign singular markers to existing LGs by computing LOD scores between each single marker and markers from the existing LGs. The module OrderMarkers2 then ordered the markers within each LG by maximizing the likelihood of the data given the order. Sex averaged map distances were computed and 10 runs were performed to select the best order for each LG, based on the best likelihood. This module was run with the parameters grandparentPhase=1 and

outputPhasedData=1 in order to obtain phased data for QTL mapping. This phased data was converted to fully informative genotypic data using the script map2gentypes.awk distributed with the LP3 program.

Identification and mapping of QTL were carried out using the R package R/qtl (version 1.39-5) [71] and MapQTL version 5. Because parthenogenetic capacity was phenotyped as a binary trait (either non-parthenogenetic 0 or parthenogenetic 1) non-parametrical statistics were used to identify loci involved in parthenogenesis. In R/qtl, the scanone function was used with the "binary" model to perform a non-parametrical interval mapping with the binary or Haley-Knott regression methods. In MapQTL, the Kruskal-Wallis non-parametric method was used. To determine the statistical significance of the major QTL signal, the LOD significant threshold was determined by permutation.

Analysis of linkage disequilibrium

In order to determine an approximate interval around the QTL peaks for the candidate genes search, linkage disequilibrium was calculated using vcftools [72] and the vcf file obtained from the Stacks pipeline with a minor allele frequency of 0.05.

Transcriptome data

The small number of gametes released from *Ectocarpus siliculosus* strains did not allow RNA-seq data to be obtained from this species. To analyse gene expression in P- (male) and P+ (female) gametes, we therefore used two *Ectocarpus* species 1 strains belonging to the same *Ectocarpus siliculosi* group [31], a P- male (NZKU1_3) and a P+ female (NZKU32-22-21), which produce sufficient numbers of gametes for RNA extraction.

Gametes of male and female *Ectocarpus* species 1 were concentrated after brief centrifugation, flash frozen and stored at -80°C until RNA extraction. RNA was extracted from duplicate samples using the Qiagen RNeasy plant mini kit (www.qiagen.com) with an on-column DNase I treatment. Between 69 and 80 million sequence reads were generated for each sample using Illumina HiSeq 2000 paired-end technology with a read length of 125 bp (Fasteris, Switzerland) (Table S10). Read quality was assessed with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc), and low quality bases and

adapter sequences were trimmed using Trimmomatic (leading and trailing bases with quality below 3 and the first 12 bases were removed, minimum read length 50bp) [67]. High score reads were used for transcriptome assembly generated with the Trinity *de novo* assembler (ref) with default parameters and normalized mode. RNA-seq reads were mapped to the assembled reference transcriptome using the Bowtie2 aligner [73] and the counts of mapped reads were obtained with HTSeq [74]. Expression values were represented as TPM and TPM<1 was applied as a filter to remove noise if both replicates of both samples exhibit it. Differential expression was analysed using the DESeq2 package (Bioconductor; [75]) using an adjusted p-value cut-off of 0.05 and a minimal fold-change of two. The reference transcripts were blasted to the reference genome Ec32 predicted proteins (http://bioinformatics.psb.ugent.be/orcae/overview/EctsiV2) (e-value cut-off = 10e-5) and the orthology relationship between *Ectocarpus* species 1 and Ec32 (Ectocarpus species 7) was established based on the best reciprocal blast hits.

Identification of candidate genes in the QTL intervals

We used two methods to identify putative candidate genes located in the QTL intervals. First, a marker-by-marker method, by mapping the sequences of the markers located within each QTL interval to the reference genome of the closely reference species strain Ec32 (Cock et al., 2010). When a sequence successfully mapped to the Ec32 genome, a coordinate was recorded for the marker, relative to its position on the physical map of Ec32. The linkage disequilibrium (see method above) estimated for each linkage group was used to refine the number of genes non-randomly associated with these markers, giving a first list of candidate genes within each QTL region. The second method used the same approach but was based on the reference genome of the paternal strain of the population (strain RB1). There were some differences between the two lists obtained by the two methods, which are due to the following factors: (a) because the assembly of the RB1 genome was guided by the Ec32 reference genome and its annotation was based on Ec32 transcriptomic data, the RB1 genome potentially lacks some genes that would be due to loci such as genes that are unique to the species *E. siliculosus* (RB1 strain) being omitted during the guided assembly. Hence the list obtained with the first method (using the Ec32 genome) contains genes that are absent from the RB1 genome; (b) while the two species are closely related, they are not identical, and the *E. siliculosus* genetic

map exhibited some rearrangements compared to Ec32 which placed some markers, along with associated genes, into the QTL intervals (these missing markers were located elsewhere on the Ec32 genome). In summary, the list obtained with Ec32 genome contained some genes that are missing from the RB1 genome because of its imperfect guided assembly and the list obtained with the RB1 genome contained some genes absent from the corresponding intervals on Ec32 because of rearrangements. A final, conservative list of candidate genes was obtained by merging the two lists in order not to omit any gene that were potentially located within the intervals (Table S11).

SNP and indel detection method

Draft genomes sequences are available for the parent strains RB1 and EA1 (Lipinska *et al.*, 2017). Using Bowtie2, we aligned the EA1 genome against the RB1 genome and generated an index with sorted positions. The program samtools mpileup [76] was used to extract the QTL intervals and call variants between the two genomes. The positions of variants between the two genomes were identified and filtered based on mapping and sequence quality using bcftools [72]. The annotation file generated for the RB1 genome was then used to select SNPs and indels located in exons of protein-coding genes for further study (bcftool closest command). The effect of polymorphism on modification of protein products was assessed manually using GenomeView [77], the RB1 genome annotation file (gff3) and the vcf file for each QTL region.

GO term enrichment analysis

A Gene Ontology enrichment analysis was performed using two lists of genes: a predefined list that corresponded to genes from all three QTL intervals and a reference list including all putative genes in the mapped scaffolds based on the Ec32 reference genome and that had a GO term annotation. The analysis was carried out with the package TopGO for R software (Adrian Alexa, Jörg Rahnenführer, 2016, version 2.24.0) by comparing the two lists using a Fisher's exact test based on gene counts.

Epistasis analysis

Epistasis analysis was carried out with the R package R/qtl (version 3.3.1). Two analyses were performed, one with the full data set (female and male genotypes generated with RAD-seq method) and the second with only the male individuals. For both analyses, the scantwo function from R/qtl were used with the model "binary" as the phenotypes of the individuals is either 1 (P+) or 0 (P-).

Fitness measurements

Reproductive success was assessed in the segregating population by measuring the capacity of male P+ and P- gametes to fuse with female gametes and by measuring the length of the germinating sporophytes derived from these crosses. For this, we crossed males and females as described in [62]. Briefly, we mixed the same amount of male and female gametes (app. $1x10^3$ gametes) in a suspending drop, and the proportion of gametes that succeeded in fusing was measured as in [78]. Two different P+ males (Ec236-34 and Ec236-245) and two different P- males (Ec236-10 and Ec236-298) were crossed with five different females (Ec236-39; -203; -233; -284 and Ec560) (Table S13). Between 50 and 150 cells (zygotes or unfertilised gametes) were counted for each cross. The length of zygotes derived from a cross between the female strain Ec236-105 and either the male P- strain Ec236-191 or the male P+ strain Ec236-154 was measured after 5h, 24h, 48h, 3 days and 4 days of development using Image J 1.46r [79] (13 zygotes for the P- male parent and 14 zygotes for P+ male parent). For all datasets, the assumption of normality (Shapiro test) and the homoscedasticity (Bartlett's test) were checked. The latter's assumptions were not met for zygote length, and consequently statistical significance differences at each time of development was tested with a non-parametrical test (Mann Whitney U-test, α=5%).

Measurement of gamete size

Gamete size was measured for representative strains of each parthenogenetic phenotype found in the segregating population (P+ and P-) (Table S3). Synchronous release of gametes was induced by transferring each gametophyte to a humid chamber in the dark for approximately 14 hours at 13°C followed by the addition of fresh PES-supplemented NSW

medium under strong light irradiation. Gametes were concentrated by phototaxis using unidirectional light, and collected in Eppendorf tubes. Gamete size was measured by impedance-based flow cytometry (Cell Lab QuantaTM SC MPL, Beckman Coulter®). A Kruskal-Wallis test (α=5%) followed by a posthoc Dunn's test for pairwise comparisons were performed using R software to compare female and male gamete size (Table S14).

Acknowledgements

This work was supported by the CNRS, Sorbonne Université and the ERC (grant agreement 638240). We thank Denis Roze for fruitful discussion and comments on the manuscript.

Author contributions

LM, KA, RL, SMC, AP prepared the biological material and performed experiments. LM, KA, AL performed the computational analysis. LM, KA, RL, SMC, JMC analysed data. SMC designed and coordinated the study. SMC wrote the manuscript with valuable input from LM and JMC. All authors read and approved the final manuscript.

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Supplementary tables.

Table S1 : Summary of the strains used for this study. SP: sporophyte; GA: gametophyte.

Strain name	Species	Origin	Isolation location	Generation	Sex	Parthenogenesis capacity	Parental strains	Used for
EA1	*Ectocarpus siliculosus*	field	Italy, Naples	GA	female	P+		Parental female strain, parthenogenetic, used for genetic map
RB1	*Ectocarpus siliculosus*	field	Italy, Naples	GA	male	P-		Parental male strain, non-parthenogenetic, used for genetic map
Ec236	*Ectocarpus siliculosus*	lab	Italy, Naples	SP			EA1 f x RB1 m	Progeny used for Rad seq analysis
Ec620	*Ectocarpus siliculosus*	lab	Italy, Naples	SP			Ec236-91f P+ x Ec236-202m P-	Progeny used to study stability of parthenogenesis
Ec696	*Ectocarpus siliculosus*	lab	Italy, Naples	SP			Ec236-91f P+ x Ec236-202m P-	Progeny used to study stability of parthenogenesis
EcNAP12-83	*Ectocarpus siliculosus*	field	Italy, Naples	GA	male	P-		Counting mitochondria in gametes
EcNAP12-88	*Ectocarpus siliculosus*	field	Italy, Naples	GA	female	P+		Counting mitochondria in gametes
EcNAP12-80	*Ectocarpus siliculosus*	field	Italy, Naples	GA	male	P-		Counting mitochondria in gametes
Ec560	*Ectocarpus* sp7	lab	Chili	GA	female	P+		Cross for testing fusion success
NZKU1_3	*Ectocarpus* sp1	lab	New Zealand	GA	male	P-		Transcriptome sequencing
NZKU32-22-21	*Ectocarpus* sp1	lab	New Zealand	GA	female	P+		Transcriptome sequencing

Table S2 : Contingency table for parthenogenetic capacity and sex. P+: positive parthenogenetic capacity; P- negative parthenogenetic capacity.

	Female	Male	Total
P+	144	44	188
P-	0	84	84
Total	144	128	272

Table S3 : Parthenogenetic capacity and sex of the 272 individuals of the segregating population. Strains used for the RAD-seq, gamete size measurements, fitness measurements are marked with a cross.

E. siliculosus strain reference	Sex	Parthenogenesis capacity	RADseq	Gamete size	Fitness measurement
Ec236	female	P+			
Ec236-99	female	P+			
Ec236-97	male	P-	x		
Ec236-96	male	P-			
Ec236-95	female	P+	x		
Ec236-94	female	P+			
Ec236-92	female	P+			
Ec236-91	female	P+			
Ec236-90	male	P-	x		
Ec236-9	male	P-			
Ec236-88	female	P+			
Ec236-87	male	P-	x		
Ec236-86	female	P+			
Ec236-84	male	P-	x		
Ec236-81	female	P+			
Ec236-80	female	P+			
Ec236-8	male	P+	x		
Ec236-78	female	P+			
Ec236-77	female	P+			
Ec236-76	male	P-	x		
Ec236-75	female	P+			
Ec236-74	female	P+			
Ec236-73	male	P-	x		
Ec236-72	female	P+			
Ec236-71	female	P+			
Ec236-70	male	P-	x		
Ec236-7	male	P-	x		
Ec236-69	female	P+			
Ec236-67	female	P+			
Ec236-66	female	P+			
Ec236-65	female	P+			
Ec236-64	female	P+			
Ec236-63	male	P+	x		
Ec236-62	male	P+			
Ec236-61	male	P-	x		
Ec236-60	female	P+			
Ec236-6	female	P+			
Ec236-59	male	P-	x		
Ec236-58	female	P+			
Ec236-57	male	P+	x		
Ec236-56	male	P+	x		

E. siliculosus strain reference	Sex	Parthenogenesis capacity	RADseq	Gamete size	Fitness measurement
Ec236-55	male	P-	x		
Ec236-54	female	P+	x		
Ec236-53	male	P-	x		
Ec236-52	male	P-	x		
Ec236-50	male	P+	x		
Ec236-5	female	P+			
Ec236-48	female	P+			
Ec236-47	male	P-	x		
Ec236-46	female	P+			
Ec236-44	female	P+			
Ec236-43	female	P+	x		
Ec236-42	female	P+			
Ec236-41	female	P+			
Ec236-40	male	P+	x		
Ec236-4	female	P+			
Ec236-39	female	P+			x
Ec236-38	male	P-	x		
Ec236-37	male	P-	x		
Ec236-36	male	P-	x		
Ec236-35	female	P+			
Ec236-34	male	P+	x		x
Ec236-32	male	P-	x		
Ec236-31	male	P-	x		
Ec236-301	male	P-	x		
Ec236-300	male	P-	x		
Ec236-30	female	P+			
Ec236-3	female	P+			
Ec236-299	female	P+			
Ec236-298	male	P-	x		x
Ec236-297	male	P+			
Ec236-296	female	P+			
Ec236-295	female	P+			
Ec236-294	male	P-	x		
Ec236-292	male	P-	x		
Ec236-291	female	P+			
Ec236-290	female	P+			
Ec236-29	male	P-	x		
Ec236-289	male	P-	x		
Ec236-288	female	P+			
Ec236-287	male	P-	x		
Ec236-286	male	P+	x		
Ec236-285	female	P+			
Ec236-284	female	P+			x

E. siliculosus strain reference	Sex	Parthenogenesis capacity	RADseq	Gamete size	Fitness measurement
Ec236-282	female	P+			
Ec236-281	female	P+			
Ec236-280	female	P+			
Ec236-28	female	P+			
Ec236-279	male	P+			
Ec236-278	male	P+	x		
Ec236-277	male	P+	x		
Ec236-276	male	P-	x	x	
Ec236-274	female	P+	x		
Ec236-273	female	P+	x		
Ec236-272	male	P-	x		
Ec236-271	male	P-	x		
Ec236-270	female	P+	x		
Ec236-27	male	P+	x		
Ec236-269	female	P+			
Ec236-268	female	P+			
Ec236-267	male	P-	x		
Ec236-266	male	P-	x		
Ec236-265	female	P+	x		
Ec236-264	female	P+			
Ec236-263	male	P-	x		
Ec236-262	male	P-	x		
Ec236-261	male	P-	x		
Ec236-260	male	P+			
Ec236-26	female	P+			
Ec236-259	male	P-	x		
Ec236-257	male	P-			
Ec236-256	female	P+			
Ec236-255	male	P+			
Ec236-254	female	P+			
Ec236-253	male	P-	x		
Ec236-252	female	P+			
Ec236-25	male	P-	x		
Ec236-249	female	P+	x		
Ec236-248	female	P+	x		
Ec236-247	male	P-	x		
Ec236-246	female	P+	x		
Ec236-245	male	P+	x		x
Ec236-243	male	P-	x		
Ec236-242	male	P+	x		
Ec236-241	female	P+			
Ec236-240	male	P+	x		
Ec236-24	male	P-	x		

E. siliculosus strain reference	Sex	Parthenogenesis capacity	RADseq	Gamete size	Fitness measurement
Ec236-239	male	P-	x		
Ec236-238	female	P+	x		
Ec236-237	male	P-	x		
Ec236-235	female	P+			
Ec236-234	female	P+	x		
Ec236-233	male	P+	x		x
Ec236-232	female	P+	x		
Ec236-231	female	P+	x		
Ec236-230	male	P-	x		
Ec236-23	female	P+			
Ec236-229	male	P-	x		
Ec236-228	female	P+	x		
Ec236-227	female	P+	x		
Ec236-226	female	P+	x		
Ec236-224	female	P+	x		
Ec236-223	female	P+	x		
Ec236-222	female	P+	x		
Ec236-221	female	P+			
Ec236-220	female	P+			
Ec236-22	female	P+			
Ec236-218	female	P+			
Ec236-217	female	P+			
Ec236-216	female	P+			
Ec236-215	male	P+	x		
Ec236-214	female	P+			
Ec236-213	female	P+			
Ec236-212	male	P+	x		
Ec236-211	female	P+			
Ec236-210	male	P+		x	
Ec236-21	male	P+	x		
Ec236-209	female	P+			
Ec236-208	male	P-	x		
Ec236-207	female	P+			
Ec236-206	male	P-	x		
Ec236-205	female	P+			
Ec236-204	male	P-	x		
Ec236-203	female	P+	x	x	x
Ec236-202	male	P+	x		
Ec236-201	female	P+	x		
Ec236-200	female	P+	x		
Ec236-20	female	P+			
Ec236-2	female	P+			
Ec236-199	female	P+	x		

E. siliculosus strain reference	Sex	Parthenogenesis capacity	RADseq	Gamete size	Fitness measurement
Ec236-198	male	P-	x		
Ec236-196	male	P-	x		
Ec236-195	male	P-	x		
Ec236-194	male	P-	x		
Ec236-193	male	P-	x		
Ec236-192	male	P+	x		
Ec236-191	male	P-	x		
Ec236-190	male	P+	x		
Ec236-19	male	P-	x		
Ec236-189	female	P+	x		
Ec236-188	female	P+	x		
Ec236-187	female	P+			
Ec236-186	female	P+			
Ec236-185	female	P+			
Ec236-184	male	P-	x		
Ec236-183	female	P+			
Ec236-182	female	P+	x		
Ec236-181	male	P+	x		
Ec236-180	female	P+			
Ec236-18	male	P-			
Ec236-179	female	P+			
Ec236-178	female	P+			
Ec236-177	female	P+			
Ec236-176	male	P-	x		
Ec236-175	female	P+			
Ec236-174	female	P+			
Ec236-173	female	P+			
Ec236-172	female	P+			
Ec236-171	female	P+			
Ec236-170	female	P+			
Ec236-17	male	P-	x		
Ec236-169	male	P+	x		
Ec236-168	male	P+	x		
Ec236-167	female	P+			
Ec236-166	male	P-	x		
Ec236-165	male	P-	x		
Ec236-164	male	P+	x		
Ec236-163	male	P-	x		
Ec236-162	male	P-	x		
Ec236-161	female	P+	x		
Ec236-160	male	P-	x		
Ec236-16	male	P-	x		
Ec236-159	female	P+	x		

E. siliculosus strain reference	Sex	Parthenogenesis capacity	RADseq	Gamete size	Fitness measurement
Ec236-158	female	P+	x		
Ec236-157	female	P+	x		
Ec236-156	male	P-	x		
Ec236-155	male	P+	x		
Ec236-154	male	P+	x		
Ec236-153	male	P+			
Ec236-152	female	P+	x		
Ec236-151	male	P+	x		
Ec236-150	male	P-	x		
Ec236-149	male	P-	x		
Ec236-148	female	P+			
Ec236-147	female	P+	x		
Ec236-146	male	P+	x		
Ec236-145	female	P+	x		
Ec236-144	male	P+	x		
Ec236-143	female	P+	x		
Ec236-141	male	P+	x		
Ec236-140	female	P+			
Ec236-14	male	P+	x		
Ec236-139	male	P-	x		
Ec236-138	male	P-	x		
Ec236-136	male	P-	x		
Ec236-135	female	P+			
Ec236-134	female	P+			
Ec236-133	female	P+			
Ec236-132	male	P+	x		
Ec236-131	female	P+			
Ec236-130	female	P+			
Ec236-13	female	P+			
Ec236-129	female	P+			
Ec236-128	female	P+			
Ec236-127	female	P+			
Ec236-126	female	P+			
Ec236-125	male	P+	x		
Ec236-124	female	P+			
Ec236-123	male	P-	x		
Ec236-121	female	P+			
Ec236-120	male	P-	x		
Ec236-118	female	P+			
Ec236-117	female	P+			
Ec236-116	male	P-	x		
Ec236-115	male	P-			
Ec236-114	female	P+			

E. siliculosus strain reference	Sex	Parthenogenesis capacity	RADseq	Gamete size	Fitness measurement
Ec236-113	female	P+			
Ec236-112	male	P+			
Ec236-111	female	P+			
Ec236-110	male	P-	x		
Ec236-11	male	P+	x		
Ec236-109	male	P-	x		
Ec236-108	male	P-	x		
Ec236-107	female	P+			
Ec236-106	female	P+			
Ec236-105	female	P+	x		
Ec236-104	female	P+			
Ec236-103	male	P-	x		
Ec236-101	female	P+	x		
Ec236-100	male	P+	x		
Ec236-10	male	P-	x	x	x
Ec236-1	male	P-	x		

Table S4 : Summary of the phenotyping and sexing of strains grown under different culture conditions and after several generations.

Strain	Sex	Original phenotype	Phenotype high light	Phenotype 20°C	Phenotype 10°C
Ec236-10	male	P+	P+	P+	P+
Ec236-100	male	P+	P+	P+	P+
Ec236-101	female	P+	P+	P+	P+
Ec236-121	female	P+	P+	P+	P+
Ec236-138	male	P-	P-	P-	P-
Ec236-139	male	P-	P-	P-	P-

Strain	Sex	Phenotype1st generation	Phenotype 2nd generation
Ec236-284	female	P+	P+
Ec236-65	female	P+	P+
Ec236-34	male	P+	P+
EA1	female	P+	P+
Rb1	male	P-	P-
Ec236-154	male	P+	P+
Ec236-191	male	P-	P-
Ec236-298	male	P-	P-
Ec236-203	female	P+	P+
Ec236-10	male	P-	P-
Ec236-39	female	P+	P+
Ec236-245	male	P+	P+
Ec236-59	male	P-	P-
Ec236-276	male	P-	P-
Ec236-298	male	P-	P-
Ec236-11	male	P+	P+
Ec236-100	male	P+	P+
Ec236-168	male	P+	P+
Ec236-202	male	P+	P+
Ec236-210	male	P+	P+
Ec236-233	male	P+	P+
Ec236-245	male	P+	P+
Ec239-23	female	P+	P+
Ec236-91	female	P+	P+
Ec236-95	female	P+	P+
Ec236-179	female	P+	P+
Ec236-211	female	P+	P+
Ec236-221	female	P+	P+
Ec236-222	female	P+	P+

Table S5 : Phenotypes of the progeny derived from two different heterozygous sporophytes obtained by crossing a male P+ strain and a female P+ strain.

Parental Strains	Strain	Sex	Parthenogenetic capacity
Ec236-91 f P+ X Ec236-202 m P+ (Ec620)	Ec620-1	Female	P+
	Ec620-2	Male	P+
	Ec620-3	Female	P+
	Ec620-8	Male	P+
	Ec620-9	Female	P+
	Ec620-11	Male	P+
	Ec620-13	Female	P+
	Ec620-14	Female	P+
	Ec620-15	Male	P+
	Ec620-17	Male	P+
	Ec620-19	Male	P+
	Ec620-20	Female	P+
Ec236-91 f P+ X Ec236-202 m P+ (Ec696)	Ec696-1	Female	P+
	Ec696-2	Female	P+
	Ec696-3	Female	P+
	Ec696-4	Female	P+
	Ec696-6	Male	P+
	Ec696-7	Female	P+
	Ec696-8	Female	P+
	Ec696-9	Female	P+
	Ec696-10	Male	P+
	Ec696-11	Female	P+
	Ec696-12	Female	P+

Table S6 : Statistics for the genetic map.

LG	Number of markers	Length (cM)	Average spacing between markers (cM)	Maximum spacing between markers (cM)
1	397	174	0,4	10,2
2	362	104,3	0,3	8,6
3	316	167,7	0,5	14,1
4	289	117,2	0,4	7,7
5	295	123	0,4	12,3
6	291	129,4	0,4	9
7	262	130	0,5	17,2
8	230	130	0,6	14,1
9	237	118	0,5	9,8
10	217	109,1	0,5	6,9
11	196	123,9	0,6	14,9
12	184	135,1	0,7	9
13	173	92,4	0,5	13,2
14	176	81,7	0,5	10,6
15	180	106,4	0,6	14,5
16	169	83,4	0,5	11,5
17	177	99,7	0,6	12,3
18	176	95,6	0,5	12,3
19	157	90,6	0,6	8,1
20	157	74,6	0,5	11,5
21	145	117,4	0,8	9,4
22	124	65,4	0,5	4,9
23	119	106,4	0,9	13,6
24	117	104,2	0,9	17,6
25	105	51,1	0,5	11,9
26	81	81,1	1	11,1
27	69	44,6	0,7	10,6
28	70	48,6	0,7	7,7
29	59	21	0,4	4
30	33	8,9	0,3	2,4
31	31	13	0,4	7,7
Total	**5594**	**2947,5**	**0,5**	**17,6**

Table S7 : QTL analysis results. For each QTL, the name, the linkage disequilibrium (LD) within the chromosome, the significance obtained with the Kruskal-Wallis test and the percentage of variance explained (PVE) determined using the Interval Mapping method (IM) is given. The number of genes found in each QTL interval is also indicated. *female or male SDR.

Full dataset (males and females)

								Interval calculated with LD (bp)		
LG	QTL name	QTL interval (cM)	QTL peak (cM)	K value	Significance (KW)	% PVE	LD (bp)	Start	End	Number of genes
LG2	*P1*	37.53 - 50.89	47.66	20.392	*******	16.6	50000	2374584	3885733	60/62*
LG18	*P2*	92.77 - 95.59	93.98	19.993	*******	16.3	50000	4819726	5124637	36
LG18	*P3*	27.06 - 34.33	33.12	14.634	******	11.9	50000	1749018	3745695	105
Total						44.8				201-203

Data subset (only males)

								Interval calculated with LD (bp)		
LG	QTL name	QTL interval (cM)	QTL peak (cM)	K value	Significance (KW)	% PVE	LD (bp)	Start	End	Number of genes
LG18	*P2*	92.77 - 95.59	93.98	19.993	*******	21.4	50000	4819726	5124637	36

Table S8 : List of the top GO terms identified (TopGO) by GO enrichment analysis for genes located within the QTL intervals.

GO.ID	Term	N. of genes	N. genes with significant p-values	Expected n. genes	TopGO p-value*
GO:0008277	regulation of G-protein coupled receptor signalling pathway	57	57	1.43	<1e-30
GO:0009968	negative regulation of signal transduction	57	57	1.43	<1e-30
GO:0010648	negative regulation of cell communication	57	57	1.43	<1e-30
GO:0023021	termination of signal transduction	57	57	1.43	<1e-30
GO:0023057	negative regulation of signaling	57	57	1.43	<1e-30
GO:0038032	termination of G-protein coupled receptor signalling pathway	57	57	1.43	<1e-30
GO:0045744	negative regulation of G-protein coupled receptor signalling pathway	57	57	1.43	<1e-30
GO:0048585	negative regulation of response to stimulus	57	57	1.43	<1e-30
GO:0009966	regulation of signal transduction	67	57	1.68	<1e-30
GO:0010646	regulation of cell communication	67	57	1.68	<1e-30
GO:0023051	regulation of signaling	67	57	1.68	<1e-30
GO:0048583	regulation of response to stimulus	67	57	1.68	<1e-30
GO:0048523	negative regulation of cellular process	69	57	1.73	<1e-30
GO:0048519	negative regulation of biological process	71	57	1.78	<1e-30
GO:0007186	G-protein coupled receptor signaling pathway	84	57	2.10	<1e-30
GO:0007165	signal transduction	205	57	5.13	<1e-30
GO:0023052	signaling	205	57	5.13	<1e-30
GO:0044700	single organism signaling	205	57	5.13	<1e-30
GO:0007154	cell communication	211	57	5.28	<1e-30
GO:0051716	cellular response to stimulus	284	57	7.11	<1e-30
GO:0050896	response to stimulus	345	57	8.64	< 1e-30
GO:0050794	regulation of cellular process	481	57	12.04	4.7e-28
GO:0050789	regulation of biological process	522	57	13.07	4.0e-26
GO:0065007	biological regulation	537	57	13.45	1.8e-25
GO:0044763	single-organism cellular process	1240	57	31.05	4.4e-08
GO:0006464	cellular protein modification process	380	28	9.51	7.4e-08
GO:0036211	protein modification process	380	28	9.51	7.4e-08
GO:0043412	macromolecule modification	422	28	10.57	6.9e-07
GO:0009987	cellular process	2614	85	65.45	6.1e-06
GO:0044267	cellular protein metabolic process	671	28	16.80	0.0031
GO:0044699	single-organism process	1757	57	43.99	0.0053

*p-value obtained for the "Calssic" algorythm used with the "Fisher" statistics

Table S9 : Epistatic interactions detected for parthenogenesis loci using the full dataset (male and female individuals genotyped with the ddRAD-seq method, first table) and using a subset with only male individuals (second table). The column "interaction" indicates the the chromosomal locations of the pairs of loci that were found to interact, with "Pos1f" and "Pos2f" referring to the estimated positions of the QTL in cM. "Lod.full" indicates the improvement in the fit of the full 2-locus model over the null model. This measurement indicates evidence for at least one QTL, allowing for interaction. "Lod.fv1" measures the increase when the full model with QTLs on chromosomes j and k is compared to a single QTL on either chromosome j or k. This measurement indicates evidence for a second QTL allowing for the possibility of epistasis. "Lod int" measures the improvement in the fit of the full model over that of the additive model and so indicates evidence for interaction. "Pos1a" and "pos2a" are the estimated positions (in cM) of the QTL under the additive model. "Lod.add" measures the improvement comparing with the additive model. This measurement indicates evidence for at least one QTL assuming no interaction. "Lod.av1" measures the increase when the additive model with QTLs on chromosomes j and k is compared to the single QTL model with a single QTL on chromosome j and k. This measurement indicates evidence for a second QTL assuming no epistasis.

Interaction analysis using full dataset

Interaction	pos1f	pos2f	lod.full	lod.fv1	lod.int	pos1a	pos2a	lod.add	lod.av1
LG2 :LG18	**47.5**	**92.5**	**9.66**	**5.10**	**1.4379**	**47.5**	**92.5**	**8.224**	**3.666**
LG3 :LG9	145.0	65.0	5.82	4.11	2.7403	125.0	32.5	3.075	1.369
LG3 :LG20	102.5	60.0	5.44	4.03	3.1703	125.0	20.0	2.268	0.858
LG6 :LG6	70.0	102.5	5.27	4.03	3.0780	112.5	122.5	2.189	0.954
LG6 :LG13	0.0	27.5	5.41	4.18	3.1539	110.0	25.0	2.258	1.023
LG12:LG19	72.5	55.0	5.35	4.93	4.7218	75.0	25.0	0.624	0.209
LG18:LG18	**32.5**	**92.5**	**6.77**	**2.35**	**0.0863**	**32.5**	**90.0**	**6.688**	**2.264**

Interaction analysis using male-only dataset

Interaction	pos1f	pos2f	lod.full	lod.fv1	lod.int	pos1a	pos2a	lod.add	lod.av1
LG2 :LG10	102.5	70.0	5.07	3.50	1.0068	102.5	70.0	4.06	2.49
LG2 :LG26	102.5	80.0	4.09	2.45	0.3454	102.5	80.0	3.75	2.11
LG10 :LG18	12.5	92.5	6.60	2.66	0.4763	12.5	92.5	6.13	2.18
LG11 :LG18	57.5	92.5	6.17	2.23	0.0796	57.5	92.5	6.09	2.15

Table S10 : Summary of the sequencing methods and raw data obtained.

Sample code	Sample name	Type of expt.	Species	sex	Sequencing method	Reads	Bases	Read length	Trimming method	Reads after trimming	Accession number	Reference
GPO-44	RB1 male	WGS	*E. siliculosus (Naples)*	m	HiSeq 2500, 2x125nt paired-end1/10th lane	36 003 088	4 500 386 000	125	Trimmomatic (leading and trailing bases with quality below 3 and the first 12 bases were removed, minimum read length 50bp) (Bolger et al., 2014).	34 022 717	SRR5026351	Lipinska et al. 2017
GPO-45	EA1 female	WGS	*E. siliculosus (Naples)*	f	HiSeq 2500, 2x125nt paired-end1/10th lane	33 941 068	4 242 633 500	125	Trimmomatic (leading and trailing bases with quality below 3 and the first 12 bases were removed, minimum read length 50bp) (Bolger et al., 2014).	27 488 661	SRR5026352	Lipinska et al. 2017
GPO-47	NZKU32-22-21	RNAseq	*Ectocarpus* sp1	f	Hiseq 2000 2x125bp	77 154 180	19 288 545 000	125	Trimmomatic (leading and trailing bases with quality below 3 and the first 12 bases were removed, minimum read length 50bp) (Bolger et al., 2014).	74 403 475	SRR5242548	Arun et al. submitted
GPO-48	NZKU32-22-21	RNAseq	*Ectocarpus* sp1	f	Hiseq 2000 2x125bp	69 727 918	17 431 979 500	125	Trimmomatic (leading and trailing bases with quality below 3 and the first 12 bases were removed, minimum read length 50bp) (Bolger et al., 2014).	67 111 327	SRR5242549	Arun et al. submitted

Sample code	Sample name	Type of expt.	Species	sex	Sequencing method	Reads	Bases	Read length	Trimming method	Reads after trimming	Accession number	Reference
GPO-49	NZKU1_3	RNAseq	*Ectocarpus* sp1	m	Hiseq 2000 2x125bp	80 290 156	20 072 539 000	125	Trimmomatic (leading and trailing bases with quality below 3 and the first 12 bases were removed, minimum read length 50bp) (Bolger et al., 2014).	77 706 747	SRR5242551	Arun et al. submitted
GPO-50	NZKU1_3	RNAseq	*Ectocarpus* sp1	m	Hiseq 2000 2x125bp	74 001 348	18 500 337 000	125	Trimmomatic (leading and trailing bases with quality below 3 and the first 12 bases were removed, minimum read length 50bp) (Bolger et al., 2014).	71 714 947	SRR5242552	Arun et al. submitted

Table S11 : Predicted functions, expression patterns and polymorphisms of genes in the QTL intervals. Expression data in transcript per million (TPM) for P- (male) versus P+ (female) gametes were obtained from strains belonging to the *Ectocarpus siliculosi* group (*Ectocarpus* species 1). Information about the type of polymorphism in the parental strains of *E. siliculosus* segregating population (EA1 female and RB1 male) is also included. Genes represented in Figure 2 are highlighted in bold. "-" means that there is no best reciprocal ortholog with detectable expression in *Ectocarpus* species 1. Pseudogenes in the sex-determining region were removed except for those which have a gametologue in the opposite SDR, and these are italicised.

				TPM (Ectocarpus sp1)						
Gene in reference Ectocarpus sp.	Linkage group in E. siliculosus	QTL	Description	Female replicate 1	Female replicate 2	Male replicate 1	Male replicate 2	log2FC(P-/P+)	*padj*	Polymorphism (EA1 vs Rb1)
Ec-06_003990	LG2	*P1*	hypothetical protein	-	-	-	-	NA	*NA*	
Ec-13_001540	LG2	*P1*	hypothetical protein	113,482047	117,455524	178,837413	129,298721	0,42113	*0,216492729*	
Ec-13_001700	LG2	*P1*	PhosphatidyliNAsitol-4-phosphate 5-kinase	-	-	-	-	NA	*NA*	SNP2766985; SNP2767008
Ec-13_001390	**LG2**	***P1***	**cAMP/cGMP-dependent protein kinase**	**4,838**	**6,309**	**120,097**	**117,712**	**4,095148**	***4,78E-132***	**SNP2390005; SNP2390048**
Ec-13_001400	LG2	*P1*	Voltage-dependent anion channel	0,932	1,077	0,661	0,722	-0,866098	*0,219466252*	
Ec-13_001410	LG2	*P1*	WD40/YVTN repeat-like-containing domain	11,340	12,435	18,224	14,130	0,113356	*0,664752869*	
Ec-13_001440	**LG2**	***P1***	**hypothetical protein**	**29,421272**	**20,9326676**	**0,78783**	**1,0181002**	**-4,822141**	***0,0004387***	
Ec-13_002070	LG2	*P1*	RING-type Zinc finger domain protein	-	-	-	-	NA	*NA*	
Ec-13_002090	LG2	*P1*	Conserved hypothetical protein	0	0	0,551	0,665	4,46993807	*0,09865285*	
Ec-13_002100	**LG2**	***P1***	**P-loop containing nucleoside triphosphate hydrolase**	**152,909**	**154,422**	**230,180**	**225,410**	**0,237211**	***0,0521275***	**SNP3696417**
Ec-13_002110	LG2	*P1*	SANT: 'SWI3, ADA2, N-CoR and TFIIIB' DNA-binding domains	-	-	-	-	NA	*NA*	
Ec-13_002120	LG2	*P1*	Chromosome segregation ATPases	40,355	40,515	59,474	62,629	0,264223144	*0,09733242*	
Ec-13_002130	LG2	*P1*	hypothetical protein	-	-	-	-	NA	*NA*	
Ec-13_002140	**LG2**	***P1***	**Cation/H+ exchanger, CPA1 family**	**42,883**	**46,429**	**98,123**	**92,427**	**0,7636718**	***1,406E-06***	
Ec-13_002150	LG2	*P1*	expressed unkNAwn protein	-	-	-	-	NA	*NA*	INDEL3769388
Ec-13_002160	**LG2**	***P1***	**Threonine synthase**	**111,721**	**124,810**	**508,337**	**503,563**	**1,7695162**	***1,219E-58***	**SNP3777884**

				TPM (Ectocarpus sp1)						
Gene in reference Ectocarpus sp.	Linkage group in E. siliculosus	QTL	Description	Female replicate 1	Female replicate 2	Male replicate 1	Male replicate 2	log2FC(P-/P+)	*padj*	Polymorphism (EA1 vs Rb1)
Ec-13_002170	**LG2**	***P1***	**Signal recognition P1ticle, SRP54 subunit, GTPase domain**	**156,975**	**161,139**	**138,518**	**115,607**	**-0,6574149**	***8,027E-08***	**SNP3790159; SNP3790321; SNP3790366**
Ec-13_002180	**LG2**	***P1***	**hypothetical protein**	**0**	**0**	**20,486**	**24,752**	**9,8208236**	***2,005E-10***	**INDEL3813702; INDEL3813702**
Ec-13_002190	**LG2**	***P1***	**hypothetical protein**	**27,410**	**38,586**	**23,356**	**17,402**	**-1,0171076**	***0,0001524***	
Ec-13_002200	LG2	*P1*	Hypothetical protein	-	-	-	-	NA	*NA*	
Ec-13_002210	LG2	*P1*	Hypothetical protein	-	-	-	-	NA	*NA*	
Ec-13_002220	**LG2**	***P1***	**Nucleic acid-binding, OB-fold**	**74,672**	**71,685**	**64,171**	**62,381**	**-0,5425696**	***8,506E-05***	**SNP3829613; SNP3829641**
Ec-13_002230	LG2	*P1*	P-loop containing nucleoside triphosphate hydrolase	-	-	-	-	NA	*NA*	
Ec-13_002240	LG2	*P1*	Hypothetical protein	-	-	-	-	NA	*NA*	
Ec-13_002250	LG2	*P1*	hypothetical protein	-	-	-	-	NA	*NA*	
Ec-13_002260	LG2	*P1*	Hypothetical protein	-	-	-	-	NA	*NA*	
Ec-02_003540	LG2	*P1*	Hypothetical protein	-	-	-	-	NA	*NA*	
Ec-02_003550	LG2	*P1*	hypothetical protein	4,9743	2,3786	1,5117	1,8265	-1,49794151	*0,188976546*	
Ec-02_003560	**LG2**	***P1***	**conserved unkNAwn protein**	**88,314004**	**102,412435**	**26,07654**	**25,304223**	**-2,2196158**	***2,456E-67***	
Ec-02_003570	LG2	*P1*	hypothetical tRNA/rRNA methyltransferase	-	-	-	-	NA	*NA*	
Ec-02_003580	**LG2**	***P1***	**WD40-repeat-containing domain**	**3,2357262**	**2,57871863**	**0**	**0**	**-6,0570001**	***0,0030902***	**SNP3880590; SNP3881036; SNP3881045**
Ec-02_003590	LG2	*P1*	hypothetical protein	-	-	-	-	NA	*NA*	
Ec-02_003600	LG2	*P1*	PAS domain	-	-	-	-	NA	*NA*	SNP3900772
Ec-02_003610	**LG2**	***P1***	**Phasmid Socket Absent family member (P3a-1)**	**18,075929**	**19,4754483**	**39,76608**	**44,723495**	**0,8424149**	***4,611E-05***	
Ec-02_003620	LG2	*P1*	SWIRM domain protein	11,3194771	13,53162091	16,9825403	18,76519877	0,199577133	*0,630737868*	
Ec-02_003630	LG2	*P1*	conserved unkNAwn protein	-	-	-	-	NA	*NA*	SNP3944417
Ec-02_003640	LG2	*P1*	Uncharacterised protein family UPF0047	-	-	-	-	NA	*NA*	

				TPM (Ectocarpus sp1)						
Gene in reference Ectocarpus sp.	Linkage group in E. siliculosus	QTL	Description	Female replicate 1	Female replicate 2	Male replicate 1	Male replicate 2	log2FC(P-/P+)	*padj*	Polymorphism (EA1 vs Rb1)
Ec-02_003650	LG2	*P1*	Ribosomal L11 methyltransferase, PrmA	42,553113	44,4156335	46,08278	53,929904	-0,1264968	*0,5666982*	SNP3972288; SNP3972289; SNP3972290; SNP3972318; SNP3972336; SNP3973457; SNP3973464; SNP3973481; SNP3973640; SNP3973646; SNP3982589; INDEL3974331; INDEL3974343
Ec-00_009300	LG2	*P1*	Hypothetical protein	-	-	-	-	NA	*NA*	
Ec-17_004380	LG2	*P1*	Hypothetical protein	0	0	0,78783	0	1,0871947	*NA*	SNP3344911; SNP3344927; INDEL3344936
Ec-24_003830	LG2	*P1*	Hypothetical protein	-	-	-	-	NA	*NA*	
Ec-28_000100	LG2	*P1*	Hypothetical protein	-	-	-	-	NA	*NA*	
Ec-13_001710*	LG2	*P1*	GTPase activating protein	-	-	-	-	NA	*NA*	
Ec-13_001740	LG2	*P1*	Hypothetical protein	-	-	-	-	NA	*NA*	
Ec-13_001750	LG2	*P1*	High mobility group domain protein	0	0	2,009635	2,9137418	NA	*NA*	
Ec-13_001770	LG2	*P1*	Conserved hypothetical protein	0	0	2,813489	4,15478	NA	*NA*	
Ec-13_001800*	LG2	*P1*	Nucleotide-diphospho-sugar transferase domain protein	0	0	1,966936	2,2367312	NA	*NA*	
Ec-13_001810*	LG2	*P1*	MEMO-like domain protein	0	0	3,881624	4,1909985	NA	*NA*	
Ec-13_001830*	LG2	*P1*	chloroplast clp protease P	0	0	6,171525	5,8483707	NA	*NA*	
Ec-13_001840*	LG2	*P1*	Conserved hypothetical protein	0	0	2,696635	1,0860592	NA	*NA*	
Ec-13_001870	LG2	*P1*	Hypothetical protein	-	-	-	-	NA	*NA*	
Ec-13_001890	LG2	*P1*	Conserved hypothetical protein	-	-	-	-	NA	*NA*	

				TPM (Ectocarpus sp1)						
Gene in reference Ectocarpus sp.	Linkage group in E. siliculosus	QTL	Description	Female replicate 1	Female replicate 2	Male replicate 1	Male replicate 2	log2FC(P-/P+)	*padj*	Polymorphism (EA1 vs Rb1)
Ec-13_001910*	LG2	*P1*	STE20-like serine/threonine kinase	-	-	-	-	NA	*NA*	
Ec-13_001930/Ec13-001490**	**LG2**	***P1***	**Putative thioesterase/thiol ester dehydrase-isomerase**	**11,656584**	**8,0467454**	**9,479934**	**7,636013**	**NA**	***NA***	
Ec-13_001950*	**LG2**	***P1***	**Homoaconitate hydratase**	**0**	**0**	**24,12743**	**30,150025**	**NA**	***NA***	
Ec-13_001980	LG2	*P1*	Ubiquitin C-terminal hydrolase	-	-	-	-	NA	*NA*	
Ec-13_001990*	LG2	*P1*	Casein kinase	-	-	-	-	NA	*NA*	
Ec-13_002010*	LG2	*P1*	Hypothetical leucine rich repeat protein	-	-	-	-	NA	*NA*	
Ec-13_002030	LG2	*P1*	Conserved hypothetical protein	-	-	-	-	NA	*NA*	
*Ec-13_002040**	LG2	*P1*	*Conserved hypothetical protein*	0	0	5,04985247	3,050712581	NA	*NA*	
Ec-13_002060*	LG2	*P1*	Protein phosphatase 2C	-	-	-	-	NA	*NA*	
Ec-13_002070*	LG2	*P1*	RING-type Zinc finger domain protein	-	-	-	-	NA	*NA*	
Ec-sdr_f_000010*	LG2	*P1*	STE20 protein kinase	-	-	-	-	NA	*NA*	
Ec-sdr_f_000030*	LG2	*P1*	Homoaconitate hydratase	-	-	-	-	NA	*NA*	
*Ec-sdr_f_000040**	LG2	*P1*	*Casein kinase*	-	-	-	-	NA	*NA*	
Ec-sdr_f_000050	LG2	*P1*	Conserved hypothetical protein	-	-	-	-	NA	*NA*	
Ec-sdr_f_000060*	LG2	*P1*	Nucleotide-diphospho-sugar transferase domain protein	-	-	-	-	NA	*NA*	
*Ec-sdr_f_000070**	LG2	*P1*	*Hypothetical protein*	-	-	-	-	NA	*NA*	
Ec-sdr_f_000080*	**LG2**	***P1***	**Chloroplast clp protease P**	**3,5935899**	**3,06848451**	**0**	**0**	**NA**	***NA***	
Ec-sdr_f_000090*	**LG2**	***P1***	**GTPase activating protein**	**1,0085006**	**1,27650703**	**0**	**0**	**NA**	***NA***	
Ec-sdr_f_000100	LG2	*P1*	Hypothetical protein	-	-	-	-	NA	*NA*	
Ec-sdr_f_000110*	LG2	*P1*	LRR protein	-	-	-	-	NA	*NA*	
Ec-sdr_f_000120	LG2	*P1*	Histidine triad protein	-	-	-	-	NA	*NA*	
Ec-sdr_f_000130*	LG2	*P1*	Pseudogene : Casein kinase fragment	-	-	-	-	NA	*NA*	
Ec-sdr_f_000140	LG2	*P1*	Patched domain protein	-	-	-	-	NA	*NA*	
Ec-sdr_f_000150*	LG2	*P1*	MEMO-like domain protein	-	-	-	-	NA	*NA*	
Ec-sdr_f_000160	LG2	*P1*	Hypothetical protein	-	-	-	-	NA	*NA*	

				TPM (Ectocarpus sp1)						
Gene in reference Ectocarpus sp.	Linkage group in E. siliculosus	QTL	Description	Female replicate 1	Female replicate 2	Male replicate 1	Male replicate 2	log2FC(P-/P+)	*padj*	Polymorphism (EA1 vs Rb1)
Ec-sdr_f_000170*	**LG2**	***P1***	**Conserved hypothetical protein**	**0,4145271**	**0,9910747**	**0**	**0**	**NA**	***NA***	
*Ec-sdr_f_000200**	LG2	*P1*	*Protein phosphatase 2C*	-	-	-	-	NA	*NA*	
Ec-sdr_f_000220	LG2	*P1*	Conserved hypothetical protein	-	-	-	-	NA	*NA*	
Ec-00_002910	LG18	*P2*	Hypothetical protein	-	-	-	-	NA	*NA*	
Ec-20_004740	**LG18**	***P2***	**Serine/threonine/dual specificity protein kinase, catalytic domain**	**2,128965**	**1,15682935**	**0,392124**	**0,2368896**	**-2,7274546**	***0,020648***	
Ec-20_004750	LG18	*P2*	WD40 repeat	0,14786149	0,176757777	1,94722414	0,361955505	2,490560379	*0,241329982*	
Ec-20_004760	**LG18**	***P2***	**Thioredoxin-like fold**	**123,45798**	**134,75701**	**226,5077**	**246,01723**	**0,5454926**	***1,893E-05***	
Ec-20_004770	LG18	*P2*	Kinase phosphorylation domain	27,1088827	35,50442119	43,4138889	45,74504938	0,188121727	*0,443193863*	
Ec-20_004780	LG18	*P2*	haloacid dehalogenase (HAD) superfamily protein	-	-	-	-	NA	*NA*	
Ec-20_004790	LG18	*P2*	Hypothetical protein	1,1083465	1,722433112	1,62802503	1,356580824	-0,24049298	*0,772247609*	
Ec-20_004800	LG18	*P2*	Beta-glucosidase, family GH3	45,7780105	56,22686625	80,6183616	63,31807336	0,168456414	*0,376022972*	
Ec-20_004810	**LG18**	***P2***	**T-complex protein 10, C-terminal domain**	**3,3142382**	**0,95086403**	**8,594907**	**11,03374**	**1,8290024**	***0,0037037***	
Ec-20_004820	LG18	*P2*	Hypothetical protein	-	-	-	-	NA	*NA*	
Ec-20_004830	LG18	*P2*	Hypothetical protein	-	-	-	-	NA	*NA*	
Ec-20_004840	LG18	*P2*	conserved unkNAwn protein	-	-	-	-	NA	*NA*	
Ec-20_004850	LG18	*P2*	hypothetical protein	-	-	-	-	NA	*NA*	
Ec-20_004860	LG18	*P2*	violaxanthin de-epoxidase-related protein of unkNAwn function	0,73058544	0,476379468	0,53825222	0,243876357	-0,97090536	*0,493431909*	
Ec-20_004870	LG18	*P2*	Hypothetical protein	-	-	-	-	NA	*NA*	
Ec-20_004880	LG18	*P2*	Phosphorylated CTD interacting factor 1, WW domain	-	-	-	-	NA	*NA*	
Ec-20_004890	**LG18**	***P2***	**Ankyrin repeat-containing domain**	**60,988166**	**68,9309609**	**151,5158**	**151,53831**	**0,8949748**	***3,526E-14***	SNP4920662
Ec-20_004900	LG18	*P2*	WW domain	8,95999445	10,71102909	11,0216381	6,952134808	-0,45831666	*0,226364122*	
Ec-20_004910	LG18	*P2*	PAS domain	234,420013	256,9125183	236,935986	295,5019821	-0,20727641	*0,216355188*	
Ec-20_004920	LG18	*P2*	Elongator protein 3/MiaB/NifB	1,29345327	2,448198104	3,49411341	3,298220033	0,551619294	*0,502944726*	
Ec-20_004930	LG18	*P2*	hypothetical protein	-	-	-	-	NA	*NA*	
Ec-20_004940	LG18	*P2*	expressed unkNAwn protein	31,7684379	30,72675089	35,1076646	30,75335812	-0,25836254	*0,357286818*	

				TPM (Ectocarpus sp1)						
Gene in reference Ectocarpus sp.	Linkage group in E. siliculosus	QTL	Description	Female replicate 1	Female replicate 2	Male replicate 1	Male replicate 2	log2FC(P-/P+)	*padj*	Polymorphism (EA1 vs Rb1)
Ec-20_004950	**LG18**	***P2***	**P-loop containing nucleoside triphosphate hydrolase**	**20,507723**	**19,4723178**	**51,40257**	**50,775332**	**1,0197016**	***4,276E-06***	
Ec-20_004960	**LG18**	***P2***	**putative, spindle assembly abNArmal protein 6**	**2,9668824**	**2,48268623**	**5,409914**	**3,9945066**	**0,446212**	***0,4991968***	**INDEL4967685**
Ec-20_004970	LG18	*P2*	conserved unkNAwn protein	2,88970919	5,501514843	9,75776613	8,252794728	0,792347349	*0,118730458*	
Ec-20_004980	**LG18**	***P2***	**Cellulose synthase (UDP-forming), family GT2**	**26,824365**	**26,4249162**	**10,82625**	**11,291881**	**-1,5999059**	***4,835E-22***	
Ec-20_004990	**LG18**	***P2***	**Cellulose synthase (UDP-forming), family GT2**	**31,783687**	**25,3300751**	**8,185798**	**5,7144505**	**-2,3765454**	***1,103E-22***	
Ec-20_005000	**LG18**	***P2***	**expressed unkNAwn protein**	**12,696373**	**13,2804009**	**7,770589**	**7,3383127**	**-1,1127386**	***0,0005133***	
Ec-20_005010	LG18	*P2*	Fatty acid desaturase	-	-	-	-	NA	*NA*	
Ec-20_005020	LG18	*P2*	Glycerol:H+ symporter	1,31304419	1,046433274	1,56660756	1,857121379	0,197385448	*0,749059581*	
Ec-20_005030	LG18	*P2*	Protein phosphatase 2C (PP2C)-like domain	-	-	-	-	NA	*NA*	
Ec-20_005050	**LG18**	***P2***	**Mitochodrial transcription termination factor**	**721,87191**	**966,391489**	**2981,937**	**3168,3277**	**1,8662472**	***2,86E-36***	
Ec-20_005060	LG18	*P2*	Domain of unkNAwn function DUF4200	0,48301419	0,692890484	0,48930248	0,354716395	-0,79712833	*0,64248331*	
Ec-20_005070	**LG18**	***P2***	**expressed unkNAwn protein**	**0,0710012**	**0**	**0,287702**	**0,0869032**	**1,94527**	***0,5784457***	**INDEL5083694**
Ec-20_005080	LG18	*P2*	SGNH hydrolase-type esterase domain	0,39376157	0,094142729	0,23933273	0,385561298	-0,01925121	*0,992715095*	
Ec-20_005090	LG18	*P2*	Hypothetical protein	4,3140649	6,555704723	7,40716779	7,786172723	0,166524926	*0,72336146*	
Ec-20_001740	LG18	*P3*	CBF1-interacting co-repressor CIR, N-terminal domain	16,8121552	11,62925979	8,66613012	12,21720196	-0,45696529	*0,677664389*	
Ec-20_001750	**LG18**	***P3***	**T-complex 11**	**3,1522791**	**5,8146299**	**8,66613**	**5,0905008**	**0,6338486**	***0,6976149***	**Indel1757797; SNP1758001; SNP1760671; SNP1760672; SNP1761318**
Ec-20_001760	LG18	*P3*	Protein phosphatase 2C (PP2C)-like domain	6,30455818	5,814629895	3,93915006	1,018100164	-1,25467226	*0,490477696*	
Ec-20_001770	**LG18**	***P3***	**Hypothetical protein**	**1480,5204**	**1193,16205**	**2602,99**	**2989,1421**	**1,0634301**	***1,727E-13***	

				TPM (Ectocarpus sp1)						
Gene in reference Ectocarpus sp.	Linkage group in E. siliculosus	QTL	Description	Female replicate 1	Female replicate 2	Male replicate 1	Male replicate 2	log2FC(P-/P+)	*padj*	Polymorphism (EA1 vs Rb1)
Ec-20_001780	**LG18**	***P3***	**similar polyubiquitin**	**10792,353**	**9891,84838**	**4218,83**	**4944,9125**	**-1,1750066**	***1,841E-22***	
Ec-20_001790	LG18	*P3*	Small GTPase superfamily	0	2,325851958	2,36349003	0	0,075225604	*0,984549891*	
Ec-20_001800	LG18	*P3*	Isopenicillin N synthase-like	-	-	-	-	NA	*NA*	
Ec-20_001810	LG18	*P3*	Protein of unkNAwn function DUF1365	100,872931	89,54530038	83,5099812	77,37561244	-0,24256357	*0,560439471*	
Ec-20_001820	**LG18**	***P3***	**EF-hand domain pair**	**1372,2922**	**1264,10054**	**924,9124**	**914,25395**	**-0,519695**	***0,0002461***	Indel1822968; SNP1823095
Ec-20_001930	**LG18**	***P3***	**Peptidase M24, structural domain**	**358,30906**	**329,108052**	**269,4379**	**260,63364**	**-0,3749816**	***0,0941886***	SNP1904191
Ec-20_001940	**LG18**	***P3***	**hypothetical protein**	**358,30906**	**329,108052**	**269,4379**	**260,63364**	**-0,3749816**	***0,0941886***	Indel1907369; SNP1907390; SNP1908995; SNP1909194; SNP1909196
Ec-20_001950	LG18	*P3*	putative Glutathione S-transferase	-	-	-	-	NA	*NA*	
Ec-20_001960	**LG18**	***P3***	**Thioredoxin-like fold**	**1383,8505**	**1286,19613**	**1180,957**	**1107,693**	**-0,222063**	***0,1470995***	SNP1940537; SNP1940617
Ec-20_001970	**LG18**	***P3***	**Regulator of chromosome condensation, RCC1**	**704,009**	**689,615106**	**767,3464**	**606,7877**	**-0,0181301**	***0,9385745***	Indel1945066; SNP1945055; SNP1951841
Ec-20_001980	LG18	*P3*	Pectin lyase fold/virulence factor	-	-	-	-	NA	*NA*	
Ec-20_001990	**LG18**	***P3***	**Ribosomal protein L7/L12, C-terminal/adaptor protein ClP3-like**	**385,62881**	**379,113869**	**693,2904**	**723,86922**	**0,8893636**	***9,997E-08***	
Ec-20_002000	LG18	*P3*	expressed unkNAwn protein	-	-	-	-	NA	*NA*	
Ec-20_002010	**LG18**	***P3***	**Endoribonuclease L-P3P/chorismate mutase-like**	**0**	**0**	**2,36349**	**1,0181002**	**3,0643807**	***NA***	SNP1999545; SNP1999609
Ec-20_002020	**LG18**	***P3***	**expressed unkNAwn protein**	**26,268992**	**12,7921858**	**7,09047**	**0**	**-2,3948193**	***0,0454911***	
Ec-20_002030	LG18	*P3*	hypothetical protein	0	0	0	2,036200327	2,305098515	*NA*	

				TPM (Ectocarpus sp1)						
Gene in reference Ectocarpus sp.	Linkage group in E. siliculosus	QTL	Description	Female replicate 1	Female replicate 2	Male replicate 1	Male replicate 2	log2FC(P-/P+)	*padj*	Polymorphism (EA1 vs Rb1)
Ec-20_002040	**LG18**	***P3***	**haloacid dehalogenase-like hydrolase**	**1191,5615**	**1278,05565**	**1663,109**	**1625,906**	**0,413829**	***0,0024161***	**SNP2042772**
Ec-20_002050	LG18	*P3*	hypothetical protein	113,482047	94,1970043	103,205731	100,7919162	-0,02696659	*0,951721318*	
Ec-20_002060	LG18	*P3*	seleNAprotein O homolog, but NAt a seleNAprotein itself	373,019692	281,4280869	256,832584	214,8191345	-0,47136283	*0,05943285*	
Ec-20_002070	**LG18**	***P3***	**hypothetical protein**	**35,72583**	**52,3316691**	**14,96877**	**20,362003**	**-1,3240063**	***0,0347022***	**Indel2064455; Indel2066131; Indel2069563; SNP2063163; SNP2063245; SNP2063601; SNP2064246; SNP2064288; SNP2064301; SNP2064376; SNP2065125; SNP2066135; SNP2067335; SNP2067389**
Ec-20_002080	LG18	*P3*	Serine/threonine/dual specificity protein kinase, catalytic domain	16,8121552	17,44388969	14,1809402	21,38010344	0,045937737	*0,962085247*	
Ec-20_002090	**LG18**	***P3***	**oligopeptidase A**	**1207,3229**	**1189,67328**	**781,5274**	**649,5479**	**-0,7420845**	***1,326E-06***	
Ec-20_002100	LG18	*P3*	Kinesin motor domain	-	-	-	-	NA	*NA*	SNP2112554
Ec-20_002110	**LG18**	***P3***	**WD40 repeat**	**610,49138**	**507,035727**	**705,8957**	**645,4755**	**0,273972**	***0,1538354***	**Indel2130621; Indel2137226; Indel2142477; SNP2139737**
Ec-20_003430	**LG18**	***P3***	**Heat shock protein 40 like protein**	**1318,7034**	**1116,40894**	**845,3416**	**1023,1907**	**-0,3841518**	***0,0235881***	
Ec-20_003440	LG18	*P3*	hypothetical protein	-	-	-	-	NA	*NA*	
Ec-20_003450	LG18	*P3*	Tetratricopeptide-like helical domain	2150,9051	2274,683215	2332,76466	2451,585194	0,11233952	*0,44077801*	

				TPM (Ectocarpus sp1)						
Gene in reference Ectocarpus sp.	Linkage group in E. siliculosus	QTL	Description	Female replicate 1	Female replicate 2	Male replicate 1	Male replicate 2	log2FC(P-/P+)	*padj*	Polymorphism (EA1 vs Rb1)
Ec-20_003460	LG18	*P3*	Tetratricopeptide-like helical domain	357,258297	373,2992393	326,949455	310,5205499	-0,19550313	*0,392639398*	
Ec-20_003470	**LG18**	***P3***	**P-loop containing nucleoside triphosphate hydrolase**	**3234,2383**	**2989,88269**	**1926,244**	**1847,8518**	**-0,7216167**	***2,472E-09***	
Ec-20_003480	LG18	*P3*	conserved unkNAwn protein	-	-	-	-	NA	*NA*	
Ec-20_003490	**LG18**	***P3***	**ClpP/crotonase-like domain**	**821,69408**	**823,351593**	**562,5106**	**650,566**	**-0,4412722**	***0,0083028***	
Ec-20_003500	**LG18**	***P3***	**Glucose/ribitol dehydrogenase**	**287,90816**	**302,360755**	**174,1104**	**167,98653**	**-0,7858737**	***0,0006774***	**SNP3721507**
Ec-20_003510	LG18	*P3*	Uncharacterised domain UPF0066	0	0	0,78783001	0	1,087194685	*NA*	
Ec-15_002550	LG18	*P3*	Hypothetical protein	-	-	-	-	NA	*NA*	
Ec-20_001840	**LG18**	***P3***	**hypothetical protein**	**10,507597**	**8,14048185**	**12,60528**	**17,307703**	**0,6730844**	***0,5317049***	**SNP1849027**
Ec-20_001890	LG18	*P3*	EamA domain	0	0	0,78783001	1,018100164	2,147899781	*NA*	
Ec-20_001900	LG18	*P3*	Ribosomal L11 methyltransferase, PrmA	-	-	-	-	NA	*NA*	Indel1869372; SNP1870740
Ec-20_001920	**LG18**	***P3***	**Ankyrin repeat domain-containing protein 13**	**43,081148**	**91,8711523**	**40,96716**	**36,651606**	**-0,7911535**	***0,1424725***	**SNP1883177; SNP1883247; SNP1884580; SNP1884615**
Ec-20_002120	LG18	*P3*	chp-1 / RAR1 homologue	-	-	-	-	NA	*NA*	SNP2154003; SNP2154027
Ec-20_002140	LG18	*P3*	Soluble NSF Attachment Protein (SNAP) Receptor (SNARE)	-	-	-	-	NA	*NA*	
Ec-20_002160	LG18	*P3*	Presenilin/signal peptide peptidase	3,15227909	2,325851958	8,66613012	6,108600982	1,432389221	*0,384619688*	
Ec-20_002170	**LG18**	***P3***	**Thioredoxin-like fold**	**11031,926**	**9994,18586**	**16061,49**	**17220,146**	**0,6624113**	***3,343E-09***	**SNP2206179**
Ec-20_002180	LG18	*P3*	Nucleotide-binding alpha-beta plait domain	215,405738	220,955936	263,923054	314,5929506	0,404576953	*0,100989986*	
Ec-20_002190	LG18	*P3*	Beta-adaptin appendage, C-terminal subdomain	-	-	-	-	NA	*NA*	
Ec-20_002200	**LG18**	***P3***	**expressed unkNAwn protein**	**257,43613**	**279,102235**	**466,3954**	**591,5162**	**0,9778248**	***1,331E-06***	
Ec-20_002230	LG18	*P3*	TCP-1-like chaperonin intermediate domain	665,130888	490,7547631	578,267228	658,7108059	0,095298972	*0,687934774*	

				TPM (Ectocarpus sp1)						
Gene in reference Ectocarpus sp.	Linkage group in E. siliculosus	QTL	Description	Female replicate 1	Female replicate 2	Male replicate 1	Male replicate 2	log2FC(P-/P+)	*padj*	Polymorphism (EA1 vs Rb1)
Ec-20_002240	LG18	*P3*	expressed unkNAwn protein	72,502419	44,1911872	210,3506	173,07703	1,712162	*2,345E-07*	
Ec-20_002250	LG18	*P3*	CaroteNAid oxygenase	32,5735506	23,25851958	27,5740504	23,41630376	-0,13047733	*0,874271349*	
Ec-20_002260	LG18	*P3*	Kinesin motor domain	2,10151939	1,162925979	3,15132004	6,108600982	1,485030665	*0,488811853*	
Ec-20_002280	LG18	*P3*	Protein-tyrosine phosphatase-like	672,48621	811,722333	337,1912	359,38936	-1,0916226	*5,735E-10*	Indel2319376; Indel2319390; Indel2319750; Indel2319782; Indel2319785; Indel2319786; Indel2322146; Indel2332722; SNP2321021; SNP2326763; SNP2332682
Ec-20_002290	LG18	*P3*	P-loop containing nucleoside triphosphate hydrolase	57,791783	39,5394833	15,7566	10,181002	-1,895076	*0,0024713*	SNP2345200; SNP2345871
Ec-20_002300	LG18	*P3*	hypothetical protein	14,710636	10,4663338	7,8783	6,108601	-0,8439003	*0,4746519*	SNP2350002; SNP2351027; SNP2360212; SNP2360525
Ec-20_002360	LG18	*P3*	expressed unkNAwn protein	9,45683727	19,76974164	10,2417901	6,108600982	-0,81970819	*0,467527536*	
Ec-20_002400	LG18	*P3*	Armadillo-like helical	0	0	4,72698007	0	3,556163944	*0,366718191*	
Ec-20_002410	LG18	*P3*	mitogen-activated protein kinase	3214,2739	3382,95167	2700,681	2705,0921	-0,2872593	*0,0207227*	SNP2435265
Ec-20_002440	LG18	*P3*	RuvA domain 2-like	1205,2214	1035,00412	329,3129	295,24905	-1,8409878	*6,593E-30*	
Ec-20_002520	LG18	*P3*	acetyl-CoA carboxylase	0	6,97755587	15,7566	10,181002	1,928698	*0,1762359*	Indel2518076
Ec-20_002530	LG18	*P3*	MoNAgalactosyldiacylglycerol synthase, family GT28	70,4009	61,6350769	55,93593	68,212711	-0,0940148	*0,8605437*	SNP2565383; SNP2565602
Ec-20_002560	LG18	*P3*	mitogen-activated protein kinase, putative	112,43129	75,5901886	122,9015	120,13582	0,3671657	*0,3380931*	Indel2596504; SNP2589126
				TPM (Ectocarpus sp1)						

Gene in reference Ectocarpus sp.	Linkage group in E. siliculosus	QTL	Description	Female replicate 1	Female replicate 2	Male replicate 1	Male replicate 2	log2FC(P-/P+)	*padj*	Polymorphism (EA1 vs Rb1)
Ec-20_002600	LG18	*P3*	Transketolase-like, pyrimidine-binding domain	1356,5308	1081,52116	727,1671	686,19951	-0,7866763	*9,199E-07*	SNP2644270; SNP2644286; SNP2652504
Ec-20_002660	LG18	*P3*	expressed unkNAwn protein	5443,986	5505,29158	5524,264	6044,4607	0,0791743	*0,5680189*	Indel2673296
Ec-20_002700	LG18	*P3*	NAtch domain	7134,6583	7777,64895	5154,772	5254,4149	-0,5186158	*4,586E-06*	SNP2736999;SNP2742060; SNP2742269; SNP2742401; SNP2744073
Ec-20_002750	LG18	*P3*	hypothetical protein	0	1,16292598	1,57566	0	0,5041002	*NA*	Indel2815652; SNP2815478; SNP2818066
Ec-20_002760	LG18	*P3*	expressed unkNAwn protein	1,0507597	0	1,57566002	2,036200327	1,720042438	*0,638949339*	
Ec-20_002790	LG18	*P3*	Bromodomain	2,1015194	3,48877794	22,05924	24,434404	3,0661252	*0,0043883*	Indel2870511; Indel2871416; Indel2880029; Indel2883007; SNP2867419; SNP2875517; SNP2885716
Ec-20_002800	LG18	*P3*	Similar to 1-AmiNAcyclopropane-1-Carboxylate Deaminase	1255,6578	1133,85283	786,2544	803,28103	-0,5887516	*5,588E-05*	
Ec-20_002820	LG18	*P3*	Protein of unkNAwn function DUF1415	6,30455818	8,140481853	9,45396013	12,21720196	0,583395822	*0,64552093*	

TPM (Ectocarpus sp1)

Gene in reference Ectocarpus sp.	Linkage group in E. siliculosus	QTL	Description	Female replicate 1	Female replicate 2	Male replicate 1	Male replicate 2	log2FC(P-/P+)	*padj*	Polymorphism (EA1 vs Rb1)
Ec-20_002860	LG18	*P3*	Mannuronan C-5-epimerase C-terminal fragment	-	-	-	-	NA	*NA*	SNP2941136; SNP2941142; SNP2941954; SNP2941970; SNP2941972; SNP2941991; SNP2941992; SNP2942004
Ec-20_002870	LG18	*P3*	Protein phosphatase inhibitor 2 (IPP-2)	-	-	-	-	NA	*NA*	Indel2949630
Ec-20_002880	LG18	*P3*	Protein of unkNAwn function DUF1295	21,0151939	17,44388969	25,2105604	16,28960262	0,116453702	*0,903010702*	
Ec-20_002910	**LG18**	***P3***	**hypothetical protein**	**94,568373**	**110,477968**	**55,1481**	**33,597305**	**-1,1948752**	***0,0039488***	**Indel2982622; Indel2984006; SNP2984795; SNP2984839; SNP2984858; SNP2985688; SNP2994107; SNP2995168**
Ec-20_002950	LG18	*P3*	hypothetical protein	5,25379849	6,977555874	13,3931102	10,18100164	0,954937347	*0,44077801*	
Ec-20_002960	**LG18**	***P3***	**Protein-tyrosine phosphatase-like**	**16,812155**	**5,8146299**	**22,84707**	**30,543005**	**1,2223666**	***0,1559981***	**SNP3015415; SNP3017669**
Ec-20_002980	**LG18**	***P3***	**YihA3, YihA/EngB-like GTPase**	**80,908497**	**105,826264**	**133,9311**	**136,42542**	**0,5361998**	***0,1218493***	**SNP3048782**
Ec-20_002990	**LG18**	***P3***	**Tubby, C-terminal**	**0**	**1,16292598**	**1,57566**	**2,0362003**	**1,676159**	***0,6451815***	**SNP3067416; SNP3067747; SNP3067765**
Ec-20_003010	LG18	*P3*	conserved unkNAwn protein	3056,65996	2957,320765	3000,05668	3241,630921	0,053199396	*0,724694492*	
Ec-20_003020	**LG18**	***P3***	**Protein kinase-like domain**	**573,71479**	**640,772214**	**650,7476**	**687,21761**	**0,1397196**	***0,4726553***	**Indel3100724**
Ec-20_003030	LG18	*P3*	Janus	-	-	-	-	NA	*NA*	Indel3113979
Ec-20_003070	**LG18**	***P3***	**Similar to G-protein coupled receptors**	**644,11569**	**751,250182**	**1072,237**	**994,68386**	**0,5682868**	***0,0003305***	
				TPM (Ectocarpus sp1)						

Gene in reference Ectocarpus sp.	Linkage group in E. siliculosus	QTL	Description	Female replicate 1	Female replicate 2	Male replicate 1	Male replicate 2	log2FC(P-/P+)	*padj*	Polymorphism (EA1 vs Rb1)
Ec-20_003080	LG18	*P3*	Beta-lactamase-like	8,40607758	2,325851958	5,51481008	3,054300491	-0,32180722	*0,867544385*	
Ec-20_003100	LG18	*P3*	Pectin lyase fold	-	-	-	-	NA	*NA*	SNP3182066; SNP3182091; SNP3182166; SNP3182448; SNP3183486; SNP3184420; SNP3184427; SNP3184429; SNP3185297; SNP3185350; SNP3185366; SNP3185970; SNP3185984; SNP3188557; SNP3188564; SNP3188999; SNP3190443; SNP3190529; SNP3191097; SNP3191729; SNP3191737; SNP3192662; SNP3192684; SNP3194768; SNP3194774; SNP3194793
Ec-20_003120	LG18	*P3*	hypothetical protein	-	-	-	-	NA	*NA*	SNP3227942; SNP3227963; SNP3228493; SNP3233553; SNP3233600
TPM (Ectocarpus sp1)										

Gene in reference Ectocarpus sp.	Linkage group in E. siliculosus	QTL	Description	Female replicate 1	Female replicate 2	Male replicate 1	Male replicate 2	log2FC(P-/P+)	*padj*	Polymorphism (EA1 vs Rb1)
Ec-20_003130	**LG18**	***P3***	**RGS domain**	**178,62915**	**213,97838**	**143,3851**	**153,73312**	**-0,4020942**	***0,1683532***	**Indel3257464**
Ec-20_003140	LG18	*P3*	hypothetical protein	12,6091164	15,11803773	2,36349003	3,054300491	-2,36567748	*0,058456258*	
Ec-20_003150	**LG18**	***P3***	**ENAyl-CoA hydratase/isomerase family protein**	**1,0507597**	**0**	**1,57566**	**4,0724007**	**2,3549739**	***0,454497***	**SNP3281257**
Ec-20_003170	**LG18**	***P3***	**conserved unkNAwn protein**	**24,167473**	**23,2585196**	**5,51481**	**4,0724007**	**-2,2938947**	***0,0133792***	**Indel3290188; Indel3290294; SNP3282925**
Ec-20_003190	**LG18**	***P3***	**RGS domain**	**68,29938**	**75,5901886**	**189,867**	**199,54763**	**1,4370199**	***1,09E-06***	**SNP3330728; SNP3337249**
Ec-20_003200	**LG18**	***P3***	**caroteNAid isomerase-like protein**	**26,268992**	**27,9102235**	**4,72698**	**8,1448013**	**-2,0944214**	***0,0139286***	**Indel3350168; Indel3350376; SNP3343235**
Ec-20_003220	LG18	*P3*	Armadillo-like helical	-	-	-	-	NA	*NA*	Indel3363826; SNP3360417; SNP3361252; SNP3361375; SNP3363690; SNP3363781; SNP3363867; SNP3363904; SNP3363905; SNP3363906
Ec-20_003230	**LG18**	***P3***	**GTP binding domain**	**12517,7**	**10740,7843**	**2506,875**	**2904,6398**	**-2,104345**	***3,655E-64***	
Ec-20_003260	**LG18**	***P3***	**conserved unkNAwn protein**	**23,116713**	**16,2809637**	**87,44913**	**66,176511**	**1,9612629**	***0,0001016***	
Ec-20_003280	LG18	*P3*	Light harvesting complex protein	253,233087	301,1978286	236,349003	241,2897388	-0,21422888	*0,413091068*	
Ec-20_003290	LG18	*P3*	Hypothetical protein	-	-	-	-	NA	*NA*	SNP3440730; SNP3445332
Ec-20_003300	**LG18**	***P3***	**Kinesin motor domain**	**92,466853**	**109,315042**	**152,0512**	**150,67882**	**0,5870717**	***0,0672401***	**SNP3456918; SNP3463094**
Ec-20_003320	LG18	*P3*	Hypothetical protein	-	-	-	-	NA	*NA*	
					TPM (Ectocarpus sp1)					

Gene in reference Ectocarpus sp.	Linkage group in E. siliculosus	QTL	Description	Female replicate 1	Female replicate 2	Male replicate 1	Male replicate 2	log2FC(P-/P+)	*padj*	Polymorphism (EA1 vs Rb1)
Ec-20_003330	LG18	*P3*	Zinc finger, CCCH-type	-	-	-	-	NA	*NA*	Indel3499831; Indel3500179; SNP3500231
Ec-20_003350	LG18	*P3*	protein-coding gene	57,7917833	60,47215091	59,0872508	43,77830704	-0,19475165	*0,720636463*	
Ec-20_003380	**LG18**	***P3***	**Zinc finger, CCCH-type**	**2,1015194**	**3,48877794**	**1,57566**	**6,108601**	**0,4418961**	***0,8458208***	**SNP3567848; SNP3569123; SNP3581430; SNP3581664**
Ec-20_003390	**LG18**	***P3***	**hypothetical protein**	**98,771412**	**109,315042**	**39,3915**	**33,597305**	**-1,5057853**	***0,0001509***	**SNP3589916; SNP3593969; Indel3598467**
Ec-20_003400	**LG18**	***P3***	**Sulfotransferase domain**	**12,609116**	**22,0955936**	**10,24179**	**22,398204**	**-0,0983626**	***0,9277249***	**SNP3602859**
Ec-20_003520	**LG18**	***P3***	**mRNA decapping complex protein**	**730,27799**	**629,142955**	**163,8686**	**167,98653**	**-2,0356839**	***1,998E-27***	

**genes belonging to a gametolog pair*

***Ec13-001490 and Ec13-001490 are paralogs*

Table S12 : List of polymorphisms in coding sequence of genes located within the three parthenogenesis QTL intervals.

LG	QTL	Name	Position in LG (bp)	Polymorphism	Localisation of polymorphism
LG18	*P2*	INDEL4967685	4967685	frame shift (LDAT>LD)	Ec-20_004960.1 exon6
LG18	*P2*	INDEL5083694	5083694	adds codon (AGC.TCC>AGC.TGC.TCC, GA>GAA on RC)	Ec-20_005070.1 exon4
LG18	*P2*	SNP4920662	4920662	AA modification (CCC>TCC, F>L)	Ec-20_004890.1 exon1 (before the ankyrin repeats)
LG2	P1	SNP2390005	2390005	AA modification (C>T, GGC>GAC=G>D on RC)	Ec-13_001390 exon2
LG2	*P1*	SNP2390048	2390048	AA modification (G>A, CCA>TCA=P>S on RC)	Ec-13_001390 exon2
LG2	*P1*	SNP2766985	2766985	AA modification (C>T, GTC>ATC=V>I on RC)	Ec-13_001700 exon1
LG2	*P1*	SNP2767008	2767008	AA modification (A>G, GTG>GCG=V>A on RC)	Ec-13_001700 exon1
LG2	*P1*	SNP3696417	3696417	AA modification (C>A, GGG>TGG=G>W on RC)	Ec-13_002100 exon8
LG2	*P1*	INDEL3769388	3769388	1 codon deleted in run of Gs (SGGGGGGGGS>SGGGGGGGS)	Ec-13_002150 exon3
LG2	*P1*	SNP3777884	3777884	AA modification (T>A, ACC>TCC=T>S on RC)	Ec-13_002160 exon8
LG2	*P1*	SNP3790159	3790159	AA modification (TCG>TTG=S>L)	Ec-13_002170 exon1
LG2	*P1*	SNP3790321	3790321	AA modification (GTA>GCA=V>A)	Ec-13_002170 exon1
LG2	*P1*	SNP3790366	3790366	AA modification (CAA>CGA=Q>R)	Ec-13_002170 exon1
LG2	*P1*	INDEL3813702	3813702	2 base deletion frame shift (SGGGGGGGGS>SGGGGGGGS)	Ec-13_002180 exon1
LG2	*P1*	INDEL3813702	3813702	5 codons inserted (QQR>QQQQQQQR)	Ec-13_002180 exon1
LG2	*P1*	SNP3829613	3829613	AA modification (AAG>CAG=K>Q)	Ec-13_002220 exon1
LG2	*P1*	SNP3829641	3829641	AA modification (GGG>GTG=G>V)	Ec-13_002220 exon1
LG2	*P1*	SNP3344911	3344911	AA modification (A>G, GTG>GCG=V>A on RC)	Ec-17_004380 exon1
LG2	*P1*	SNP3344927	3344927	AA modification (A>C, TGA>GGA=*>G on RC)	Ec-17_004380 exon1
LG2	*P1*	INDEL3344936	3344936	1 base insertion frame shift (C>CA, GGG.GGC.CAC>GGT.GGG.CCA.C=GGH>GGP on RC)	Ec-17_004380 exon1
LG2	*P1*	SNP3880590	3880590	AA modification (C>T, GAC>AAC=D>N on RC)	Ec-02_003580 exon3

LG	QTL	Name	Position in LG (bp)	Polymorphism	Localisation of polymorphism
LG2	*P1*	SNP3881036	3881036	AA modification (G>T, CGG>AGG=R>N on RC)	Ec-02_003580 exon2
LG2	*P1*	SNP3881045	3881045	AA modification (A>C, TGG>GGG=W>R on RC)	Ec-02_003580 exon2
LG2	*P1*	SNP3900772	3900772	AA modification (T>G, AAC>CAC=N>H on RC)	Ec-02_003600 exon2
LG2	*P1*	SNP3944417	3944417	AA modification (T>G, TCT>GCT=S>A)	Ec-02_003630 exon19
LG2	*P1*	SNP3972288	3972288	unclear (C>T)	Ec-02_003650 exon1
LG2	*P1*	SNP3972289	3972289	unclear (C>G)	Ec-02_003650 exon1
LG2	*P1*	SNP3972290	3972290	unclear (A>T)	Ec-02_003650 exon1
LG2	*P1*	SNP3972318	3972318	AA modification (G>A, AGG>AAG=R>K)	Ec-02_003650 exon1
LG2	*P1*	SNP3972336	3972336	AA modification (G>A, AGC>AAC=S>N)	Ec-02_003650 exon1
LG2	*P1*	SNP3973457	3973457	AA modification (A>C, ATC>CTC=I>L)	Ec-02_003650 exon2
LG2	*P1*	SNP3973464	3973464	AA modification (C>T, TCA>TTA=S>L)	Ec-02_003650 exon2
LG2	*P1*	SNP3973481	3973481	AA modification (G>A, GCC>ACC=A>T)	Ec-02_003650 exon2
LG2	*P1*	SNP3973640	3973640	AA modification (A>G, AAC>GAC=N>D)	Ec-02_003650 exon2
LG2	*P1*	SNP3973646	3973646	AA modification (G>A, GAT>AAT=D>N)	Ec-02_003650 exon2
LG2	*P1*	SNP3982589	3982589	AA modification (A>G, CAG>CGG=Q>R)	Ec-02_003650 exon7
LG2	*P1*	INDEL3974331	3974331	4 codons deleted (GGA.GAA.GAA.GAA.GAA.GAA.GAA.GAA.GA>GGA.GAA.GAA.GAA.GA, 8E>4E)	Ec-02_003650 exon3
LG2	*P1*	INDEL3974343	3974343	2 codons inserted (GAA>GAG.GGG.CTA, E>EGL)	Ec-02_003650 exon3
LG18	*P3*	INDEL1757797	1757797	insertion of 2 codons (ASSSSS>ASSSSSSS)	Ec-20_001750 exon8
LG18	*P3*	SNP1758001	1758001	AA modification (G>A, GCT>ACT=A<T)	Ec-20_001750 exon8
LG18	*P3*	SNP1760671	1760671	AA modification(A>C, AAA>AAC=K>N)	Ec-20_001750 exon12
LG18	*P3*	SNP1760672	1760672	AA modification (T>G, TGC>GGC=C>G)	Ec-20_001750 exon12
LG18	*P3*	SNP1761318	1761318	AA modification(C>A, CAG>AAG=Q>L)	Ec-20_001750 exon13
LG18	*P3*	INDEL1822968	1822968	deletion of 2 codons (GAGAGAGAA>GAGAGAA on RC)	Ec-20_001820 exon2

LG	QTL	Name	Position in LG (bp)	Polymorphism	Localisation of polymorphism
LG18	*P3*	SNP1823095	1823095	AA modification(T>C, AAC>GAC=N>D on RC)	Ec-20_001820 exon10
LG18	*P3*	SNP1849027	1849027	non-syn (C>G, ATG>ATC=M>I on RC)	Ec-20_001840 exon2
LG18	*P3*	Indel1869372	1869372	deletion of 6 codons (TGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG>TGCAGCAGCAGCAGCAGCAG, CT.GCT.GCT.GCT.GCT.GCT.GCT.GCT.GCT.GCT.GCT.GCT.GCA>CT.GCT.GCT.GCT.GCT.GCT.GCT.GCA=AAAAAAAAAAAAAA>AAAAAAA on RC)	Ec-20_001900 exon4
LG18	*P3*	SNP1870740	1870740	non-syn (C>T, GCA>ACA=A>T on RC)	Ec-20_001900 exon1
LG18	*P3*	SNP1883177	1883177	non-syn (C>G, TGG>TCG=W>S on RC)	Ec-20_001920 exon2
LG18	*P3*	SNP1883247	1883247	non-syn (T>C, AGG>GGG=R>G on RC)	Ec-20_001920 exon2
LG18	*P3*	SNP1884580	1884580	non-syn (A>C, TTG>GTG=L>V on RC)	Ec-20_001920 exon1
LG18	*P3*	SNP1884615	1884615	non-syn (C>G, GGG>GCG=G>A on RC)	Ec-20_001920 exon1
LG18	*P3*	SNP1904191	1904191	AA modification (G>C, GTC>CTC=V>L)	Ec-20_001930 exon4
LG18	*P3*	INDEL1907369	1907369	deletion of 4 codons (ACA.GAG.CGC.AAA.GCG.GAG.CGC.AAA.GCG.GAG.CGC.AAT.GCG.GAG.CGC.AAA>ACA.GAG.CGC.AAA.GCG.GAG.CGC.AAT.GCG.GAG.CGC.AAA)	Ec-20_001940 exon2
LG18	*P3*	SNP1907390	1907390	AA modification (A>T, AAA>AAT=K>N)	Ec-20_001940 exon2
LG18	*P3*	SNP1908995	1908995	AA modification (A>G, AGC>GGC=S>G but may not be coding)	Ec-20_001940 exon3
LG18	*P3*	SNP1909194	1909194	AA modification (A>G, AAC>AGC=N>S but may not be coding)	Ec-20_001940 exon3
LG18	*P3*	SNP1909196	1909196	AA modification (A>G, AGC>GGC=S>G but may not be coding)	Ec-20_001940 exon3

LG	QTL	Name	Position in LG (bp)	Polymorphism	Localisation of polymorphism
LG18	*P3*	SNP1940537	1940537	AA modification (T>C, AGC>GGC=S>G on RC)	Ec-20_001960 exon2
LG18	*P3*	SNP1940617	1940617	AA modification (G>T, ACG>AAG=T>K on RC)	Ec-20_001960 exon2
LG18	*P3*	INDEL1945066	1945066	deletion of 1 codon (AAA.AAG.AAG>AAA.AAG=KKK>KK)	Ec-20_001970 exon3
LG18	*P3*	SNP1945055	1945055	AA modification (C>T, CCT>TCT=P>S)	Ec-20_001970 exon3
LG18	*P3*	SNP1951841	1951841	AA modification (G>T, GCG>TCG=A>S	Ec-20_001970 exon12 (3 aa from end of protein)
LG18	*P3*	SNP1999545	1999545	AA modification (T>C, ATG>GTG=M>V on RC)	Ec-20_002010 exon2
LG18	*P3*	SNP1999609	1999609	AA modification (T>C, ATG>GTG=M>V on RC)	Ec-20_002010 exon2
LG18	*P3*	SNP2042772	2042772	AA modification (C>T GGG>AGG=G>R on RC)	Ec-20_002040 exon7
LG18	*P3*	INDEL2064455	2064455	insertion of 1 codon (QQQQH>QQQQQH on RC)	Ec-20_002070 exon10
LG18	*P3*	INDEL2066131	2066131	insertion of 4 codons (GGCC>GGCCGCCGCTTCCGCC, G.GCC>G.GCG.GAA.GCG.GCG.GCC on RC)	Ec-20_002070 exon10
LG18	*P3*	INDEL2069563	2069563	deletion of 5 codons (TCACCACCACCGCCACCACCACCACCGCCACCACCAC>TCACC ACCACCGCCACCACCAC, G.TGG.TGG.TGG.CGG.TGG.TGG.TGG.TGG.CGG.TGG.TGG.T GA>G.TGG.TGG.TGG.CGG.TGG.TGG.TGA on RC)	Ec-20_002070 exon3
LG18	*P3*	SNP2063163	2063163	AA modification (G>A, ACC>ATC=T>I on RC)	Ec-20_002070 exon10
LG18	*P3*	SNP2063245	2063245	AA modification (T>G AAG>CAG=K>Q on RC)	Ec-20_002070 exon10
LG18	*P3*	SNP2063601	2063601	AA modification (G>A GCG>GTG=A>V on RC)	Ec-20_002070 exon10
LG18	*P3*	SNP2064246	2064246	AA modification (C>A GGG>GTG=G>V on RC)	Ec-20_002070 exon10
LG18	*P3*	SNP2064288	2064288	AA modification (C>T CGG>CAG=R>Q on RC)	Ec-20_002070 exon10
LG18	*P3*	SNP2064301	2064301	AA modification (C>T GCC>ACC=A>T on RC)	Ec-20_002070 exon10
LG18	*P3*	SNP2064376	2064376	AA modification (C>T GCA>ACA=A>T on RC)	Ec-20_002070 exon10

LG	QTL	Name	Position in LG (bp)	Polymorphism	Localisation of polymorphism
LG18	*P3*	SNP2065125	2065125	AA modification (G>A, TCG>TTG=S>L on RC but may not be coding)	Ec-20_002070 exon10
LG18	*P3*	SNP2066135	2066135	AA modification (T>G, GAG>GCG=E>A on RC)	Ec-20_002070 exon9
LG18	*P3*	SNP2067335	2067335	AA modification (C>T, GGG>GAG=G>A on RC)	Ec-20_002070 exon7
LG18	*P3*	SNP2067389	2067389	AA modification (G>C, GCG>GGG=A>G on RC)	Ec-20_002070 exon7
LG18	*P3*	SNP2112554	2112554	AA modification (G>C, CGG>GGG=R>G on RC)	Ec-20_002100 exon18
LG18	*P3*	INDEL2130621	2130621	insertion of 4 codons (QQQQ>QQQQQQQQ)	Ec-20_002110 exon2
LG18	*P3*	INDEL2137226	2137226	deletion of 2 codons (AGC.GGC.GGC.GGC.GGC.GGC.GGC.GG>AGC.GGC.GGC.GGC.GGC.GG)	Ec-20_002110 exon7
LG18	*P3*	INDEL2142477	2142477	insertion of 1 or 2 codons? (AGC.GGC.GGC.GGC.G>AGC.GGC.GGC.GGC.GGC.GGC.G,AGC.GGC.GGC.GGC.GGC.G)	Ec-20_002110 exon13
LG18	*P3*	SNP2139737	2139737	AA modification (T>C, TCC>GCC=S>A)	Ec-20_002110 exon4
LG18	*P3*	SNP2154003	2154003	non-syn (G>A, GCC>GTC=A>V on RC)	Ec-20_002120 exon3
LG18	*P3*	SNP2154027	2154027	non-syn (G>C, GCA>GGA=A>G on RC)	Ec-20_002120 exon3
LG18	*P3*	SNP2206179	2206179	non-syn (A>T, GAA>GAT=E>D)	Ec-20_002170 exon11
LG18	*P3*	Indel2319376	2319376	deletion of 4 codons (CCCGCCGCCGCCGCCGCCGCCGCCG>CCCGCCGCCGCCG, C.GGC.GGC.GGC.GGC.GGC.GGC.GGC.GGG>C.GGC.GGC.GGC.GGG on RC)	Ec-20_002280 exon24
LG18	*P3*	Indel2319390	2319390	insertion of 2 codons (C>CTTGTGG, GGC>CCA.CAA.GGC on RC)	Ec-20_002280 exon24
LG18	*P3*	Indel2319750	2319750	insertion of 4 codons (G>GTCGCCGTTGGAA, CGG>TTC.CAA.CGG.CGA.CGG on RC)	Ec-20_002280 exon24

LG	QTL	Name	Position in LG (bp)	Polymorphism	Localisation of polymorphism
LG18	*P3*	Indel2319782	2319782	insertion of 3 codons (CGCCG>CGCCGTCCTTGCCG, C.GGC.GCG>C.GGC.AAG.GAC.GGC.GCG on RC)	Ec-20_002280 exon24
LG18	*P3*	Indel2319785	2319785	insertion of 2 codons (CG>CGTCCTTG, C.GGC>C.AAG.GAC.GGC on RC)	Ec-20_002280 exon24
LG18	*P3*	Indel2319786	2319786	insertion of 2 codons (G>GTCCTTC, GGC>GGG.AAG.GAC on RC)	Ec-20_002280 exon24
LG18	*P3*	Indel2322146	2322146	deletion of 1 codon (ACCGCCGCCGCCGCCG>ACCGCCGCCGCCG, C.GGC.GGC.GGC.GGC.GGT>C.GGC.GGC.GGC.GGT on RC)	Ec-20_002280 exon19
LG18	*P3*	Indel2332722	2332722	insertion of 1 codon (CGAGGAGGAGGAGGAGGAGGAGGA>CGAGGAGGAGGAG GAGGAGGAGGAGGA, TCC.TCC.TCC.TCC.TCC.TCC.TCC.TCG>TCC.TCC.TCC.TCC.TCC .TCC.TCC.TCC.TCG on RC)	Ec-20_002280 exon9
LG18	*P3*	SNP2321021	2321021	non-syn (C>T, GCG>ACG=A>T on RC)	Ec-20_002280 exon22
LG18	*P3*	SNP2326763	2326763	non-syn (C>T, CGG>CAG=R>Q on RC)	Ec-20_002280 exon18
LG18	*P3*	SNP2332682	2332682	non-syn (C>T, GCA>ACA=A>T on RC)	Ec-20_002280 exon10
LG18	*P3*	SNP2345200	2345200	non-syn (G>A, GGG>GAG=G>E)	Ec-20_002290 exon1
LG18	*P3*	SNP2345871	2345871	non-syn (A>C, GAC>GCC=D>A)	Ec-20_002290 exon4
LG18	*P3*	SNP2350002	2350002	non-syn (A>T, ACG>TCG=T>S)	Ec-20_002300 exon2
LG18	*P3*	SNP2351027	2351027	non-syn (G>A, GAG>AAG=E>K)	Ec-20_002300 exon4
LG18	*P3*	SNP2360212	2360212	non-syn (C>T, CGT>TGT=R>C)	Ec-20_002300 exon17
LG18	*P3*	SNP2360525	2360525	non-syn (T>G, GTC>GGC=V>G)	Ec-20_002300 exon17
LG18	*P3*	SNP2420404	2420404	non-syn (A>G, TTT>CTT=F>L on RC)	Ec-20_002390 exon1
LG18	*P3*	SNP2420579	2420579	non-syn (G>C, CAC>CAG=H>Q on RC)	Ec-20_002390 exon1

LG	QTL	Name	Position in LG (bp)	Polymorphism	Localisation of polymorphism
LG18	*P3*	SNP2420634	2420634	non-syn (G>A, CCA>CTA=P>L on RC)	Ec-20_002390 exon1
LG18	*P3*	SNP2420800	2420800	non-syn (C>T, GCG>ACG=A>T on RC)	Ec-20_002390 exon1
LG18	*P3*	SNP2421015	2421015	non-syn (A>G, GTG>GCG=V>A on RC)	Ec-20_002390 exon1
LG18	*P3*	SNP2435265	2435265	non-syn (C>G, GGA>GCA=G>A on RC)	Ec-20_002410 exon4
LG18	*P3*	Indel2518076	2518076	deletion of 1 codon (TCCCCCCCC>TCCCCC, G.GGG.GGG.GAC>G.GGG.GAC on RC)	Ec-20_002520 exon4
LG18	*P3*	SNP2565383	2565383	non-syn (C>T, GCG>ACG=A>T on RC)	Ec-20_002530 exon1
LG18	*P3*	SNP2565602	2565602	non-syn (A>G, TTC>CTC=F>L on RC)	Ec-20_002530 exon1
LG18	*P3*	Indel2596504	2596504	frame shift in reference, deletion of 4 bp (TCG.GCC.GGC.C>TCG.GCC)	Ec-20_002560 exon13
LG18	*P3*	SNP2589126	2589126	non-syn (G>C, GGG>GCG=G>A)	Ec-20_002560 exon1
LG18	*P3*	SNP2644270	2644270	non-syn (G>T, GCC>GAC=A>D on RC)	Ec-20_002600 exon17
LG18	*P3*	SNP2644286	2644286	non-syn (C>A, GCG>TCG=A>S on RC)	Ec-20_002600 exon17
LG18	*P3*	SNP2652504	2652504	non-syn (G>C, CCG>GCG=P>A on RC)	Ec-20_002600 exon9
LG18	*P3*	Indel2673296	2673296	insertion of 2 codons (GCCGGCCCCG>GCCG, C.GGG.GCC.GGC>C.GGC on RC)	Ec-20_002660 exon6
LG18	*P3*	SNP2736999	2736999	non-syn (C>T, GTT>ATT=V>I on RC)	Ec-20_002700 exon6
LG18	*P3*	SNP2742060	2742060	non-syn (T>C, AAT>GAT=N>D on RC)	Ec-20_002700 exon3
LG18	*P3*	SNP2742269	2742269	non-syn (A>G, GTA>GCA=V>A on RC)	Ec-20_002700 exon3
LG18	*P3*	SNP2742401	2742401	non-syn (C>T, AGT>AAT=S>N on RC)	Ec-20_002700 exon3
LG18	*P3*	SNP2744073	2744073	non-syn (A>T, TTT>TAT=F>Y on RC)	Ec-20_002700 exon1

LG	QTL	Name	Position in LG (bp)	Polymorphism	Localisation of polymorphism
LG18	*P3*	Indel2815652	2815652	insertion of 1 codon (TCGGCGGCGGCGGCGGCGG>TCGGCGGCGGCGGCGGCGG CGG, CCG.CCG.CCG.CCG.CCG.CCG.ATG>CCG.CCG.CCG.CCG.CCG. CCG.CCG.ATG on RC)	Ec-20_002750 exon3
LG18	*P3*	SNP2815478	2815478	non-syn (G>A CGC>TGC=R>C on RC)	Ec-20_002750 exon3
LG18	*P3*	SNP2818066	2818066	non-syn (T>C, AAG>AGG=K>R on RC)	Ec-20_002750 exon2
LG18	*P3*	Indel2870511	2870511	deletion of 6 codons (GCACTACCACCACTACTACCACTACCACCACTACTAC>GCACT ACCACCACTACTAC, GT.AGT.AGT.GGT.GGT.AGT.GGT.AGT.AGT.GGT.GGT.AGT.G CG>GT.AGT.AGT.GGT.GGT.AGT.GCG on RC)	Ec-20_002790 exon4
LG18	*P3*	Indel2871416	2871416	insertion of 2 codons (ATCCTCCTCCTCCTCCTC>ATCCTCCTCCTCCTCCTCCTCCTC, GAG.GAG.GAG.GAG.GAG.GAT>GAG.GAG.GAG.GAG.GAG.G AG.GAG.GAT on RC)	Ec-20_002790 exon5
LG18	*P3*	Indel2880029	2880029	insertion of 1 codon (ACTGCTGCTGCTGC>ACTGCTGCTGCTGCTGC, G.CAG.CAG.CAG.CAG.TCG>G.CAG.CAG.CAG.CAG.CAG.TCG on RC)	Ec-20_002790 exon5
LG18	*P3*	Indel2883007	2883007	insertion of 4 codons (GCC>GCCGCCGCCGCCCCC, G.GCG>G.GGG.GCG.GCG.GCG.GCG on RC)	Ec-20_002790 exon2
LG18	*P3*	SNP2867419	2867419	non-syn (T>C, GAG>GGG=E>G on RC)	Ec-20_002790 exon19
LG18	*P3*	SNP2875517	2875517	non-syn (T>C, AAG>AGG=K>R on RC)	Ec-20_002790 exon10
LG18	*P3*	SNP2885716	2885716	non-syn (T>C, ACC>GCC=T>A on RC)	Ec-20_002790 exon1

LG	QTL	Name	Position in LG (bp)	Polymorphism	Localisation of polymorphism
LG18	*P3*	SNP2941136	2941136	non-syn (A>G, ATT>GTT=I>V)	Ec-20_002860 exon1
LG18	*P3*	SNP2941142	2941142	non-syn (A>G, AAT>GAT=N>D)	Ec-20_002860 exon1
LG18	*P3*	SNP2941954	2941954	non-syn (G>C, TTG>TTC=L>F)	Ec-20_002860 exon2
LG18	*P3*	SNP2941970	2941970	non-syn (T>A, TAC>AAC=Y>N)	Ec-20_002860 exon2
LG18	*P3*	SNP2941972	2941972	non-syn (C>G, TAC>TAG=Y>*)	Ec-20_002860 exon2
LG18	*P3*	SNP2941991	2941991	non-syn (A>G, ATC>GTC=I>V)	Ec-20_002860 exon2
LG18	*P3*	SNP2941992	2941992	non-syn (T>A, ATC>AAC=I>N)	Ec-20_002860 exon2
LG18	*P3*	SNP2942004	2942004	non-syn (T>A, ATG>AAG=M>K)	Ec-20_002860 exon2
LG18	*P3*	Indel2949630	2949630	deletion of 1 codon (CCTCCAGCTCCAGCTCCAGCTCCAGC>CCTCCAGCTCCAGC, G.CTG.GAG.CTG.GAG.CTG.GAG.CTG.GAG.GTG>G.CTG.GAG .CTG.GAG.GTG on RC)	Ec-20_002870 exon6
LG18	*P3*	Indel2982622	2982622	deletion of 1 codon (CGCTGCTGCTGCTGC>CGCTGCTGCTGC, GC.AGC.AGC.AGC.AGC.GGC>GC.AGC.AGC.AGC.GGC on RC)	Ec-20_002910 exon12
LG18	*P3*	Indel2984006	2984006	insertion of 2 codons (TCGGCGGCGGCGG>TCGGCGGCGGCGGCGGCGG, CC.GCC.GCC.GCC.GAA>CC.GCC.GCC.GCC.GCC.GCC.GAA on RC)	Ec-20_002910 exon15
LG18	*P3*	SNP2984795	2984795	non-syn (C>T, GTC>ATC=V>I on RC)	Ec-20_002910 exon10
LG18	*P3*	SNP2984839	2984839	non-syn (C>T, GGC>GAC=G>D on RC)	Ec-20_002910 exon10
LG18	*P3*	SNP2984858	2984858	non-syn (C>T, GAC>AAC=D>N on RC)	Ec-20_002910 exon10
LG18	*P3*	SNP2985688	2985688	non-syn (T>G, CAG>CCG=Q>P on RC)	Ec-20_002910 exon10
LG18	*P3*	SNP2994107	2994107	non-syn (A>G, ATT>ACT=I>T on RC)	Ec-20_002910 exon3
LG18	*P3*	SNP2995168	2995168	non-syn (T>C, GAA>GGA=E>G on RC)	Ec-20_002910 exon1

LG	QTL	Name	Position in LG (bp)	Polymorphism	Localisation of polymorphism
LG18	*P3*	SNP3015415	3015415	non-syn (A>G, ATT>GTT=P>V)	Ec-20_002960 exon4
LG18	*P3*	SNP3017669	3017669	non-syn (T>C, TTC>CTC=F>L)	Ec-20_002960 exon8
LG18	*P3*	SNP3048782	3048782	non-syn (T>C, TAT>CAT=Y>H)	Ec-20_002980 exon2
LG18	*P3*	SNP3067416	3067416	non-syn (C>A, AAG>AAT=K>N on RC)	Ec-20_002990 exon10
LG18	*P3*	SNP3067747	3067747	non-syn (A>G, ATG>ACG=M>T on RC)	Ec-20_002990 exon10
LG18	*P3*	SNP3067765	3067765	non-syn (C>G, CGC>CCC=R>P on RC)	Ec-20_002990 exon10
LG18	*P3*	Indel3100724	3100724	insertion of 3 codons (AGCTGCTGCTGCTGCTGCTGC>AGCTGCTGCTGCTGCTG CTGCTGCTGCTGCTGC, G.CAG.CAG.CAG.CAG.CAG.CAG.CAG.CTG>G.CAG.CAG.CAG. CAG.CAG.CAG.CAG.CAG.CAG.CAG.CTG on RC)	Ec-20_003020 exon15
LG18	*P3*	Indel3113979	3113979	insertion of 2 codons (GGC>GGC.GAG.GCC)	Ec-20_003030 exon1
LG18	*P3*	Indel3257464	3257464	insertion of 5 codons (TCCTCCTCCTCCTCCCCCTCCTCCTCCTCC>TCCTCCTCCTCCTC C, G.GAG.GAG.GAG.GAG.GGG.GAG.GAG.GAG.GAG.GAG>G.G AG.GAG.GAG.GAG.GAG on RC)	Ec-20_003130 exon7
LG18	*P3*	SNP3182066	3182066	non-syn (A>C TTT>TGT=F>C on RC)	Ec-20_003100 exon18
LG18	*P3*	SNP3182091	3182091	non-syn (C>A, GTG>TTG=G>L on RC)	Ec-20_003100 exon18
LG18	*P3*	SNP3182166	3182166	non-syn (C>T, GGC>AGC=G>S on RC)	Ec-20_003100 exon18
LG18	*P3*	SNP3182448	3182448	non-syn (T>C GAT>GGT=D>G on RC)	Ec-20_003100 exon17
LG18	*P3*	SNP3183486	3183486	non-syn (C>T, GTC>ATC=V>I on RC)	Ec-20_003100 exon15
LG18	*P3*	SNP3184420	3184420	non-syn (G>A , CCA>CTA=P>L on RC)	Ec-20_003100 exon14
LG18	*P3*	SNP3184427	3184427	non-syn (T>G, ACC>CCC=T>P on RC)	Ec-20_003100 exon14
LG18	*P3*	SNP3184429	3184429	non-syn (G>A , ACG>ATG=T>M on RC)	Ec-20_003100 exon14

LG	QTL	Name	Position in LG (bp)	Polymorphism	Localisation of polymorphism
LG18	*P3*	SNP3185297	3185297	non-syn (C>T, GGG>GAG=G>E on RC)	Ec-20_003100 exon13
LG18	*P3*	SNP3185350	3185350	non-syn (G>C, GAC>GAG=D>E on RC)	Ec-20_003100 exon13
LG18	*P3*	SNP3185366	3185366	non-syn (C>T, GGG>GAG=G>E on RC)	Ec-20_003100 exon13
LG18	*P3*	SNP3185970	3185970	non-syn (G>T, GAC>GAA=D>E on RC)	Ec-20_003100 exon12
LG18	*P3*	SNP3185984	3185984	non-syn (T>C, AAC>GAC=N>D on RC)	Ec-20_003100 exon12
LG18	*P3*	SNP3188557	3188557	non-syn (G>C, AAC>AAG=N>K on RC)	Ec-20_003100 exon 9
LG18	*P3*	SNP3188564	3188564	non-syn (T>C, AAT>AGT=N>S on RC)	Ec-20_003100 exon 9
LG18	*P3*	SNP3188999	3188999	non-syn (T>C, GAA>GGA=E>G on RC)	Ec-20_003100 exon7
LG18	*P3*	SNP3190443	3190443	non-syn (C>T, GGC>GAC=G>D on RC)	Ec-20_003100 exon5
LG18	*P3*	SNP3190529	3190529	non-syn (G>C, AAC>AAG=N>K on RC)	Ec-20_003100 exon5
LG18	*P3*	SNP3191097	3191097	non-syn (T>C, GAT>GGT=D>G on RC)	Ec-20_003100 exon4
LG18	*P3*	SNP3191729	3191729	non-syn (G>T, GAC>GAA=D>E on RC)	Ec-20_003100 exon3
LG18	*P3*	SNP3191737	3191737	non-syn (T>C, ACG>GCG=T>A on RC)	Ec-20_003100 exon3
LG18	*P3*	SNP3192662	3192662	non-syn (C>A, GGG>TGG=G>W on RC)	Ec-20_003100 exon2
LG18	*P3*	SNP3192684	3192684	non-syn (G>C, AAC>AAG=N>K on RC)	Ec-20_003100 exon2
LG18	*P3*	SNP3194768	3194768	non-syn (T>C, CAG>CGG=Q>R on RC)	Ec-20_003100 exon1
LG18	*P3*	SNP3194774	3194774	non-syn (A>T, TTC>TAC=F>Y on RC)	Ec-20_003100 exon1
LG18	*P3*	SNP3194793	3194793	non-syn (A>C, TTA>GTA=L>V on RC)	Ec-20_003100 exon1
LG18	*P3*	SNP3227942	3227942	non-syn (T>C, AAT>GAT=N>D on RC)	Ec-20_003120 exon4
LG18	*P3*	SNP3227963	3227963	non-syn (C>A, GCG>TCG=A>S on RC)	Ec-20_003120 exon4
LG18	*P3*	SNP3228493	3228493	non-syn (C>T, GTC>ATC=V>I on RC)	Ec-20_003120 exon3
LG18	*P3*	SNP3233553	3233553	non-syn (C>T, GCT>ACT=A>T on RC)	Ec-20_003120 exon1
LG18	*P3*	SNP3233600	3233600	non-syn (A>G, TTG>TCG=L>S on RC)	Ec-20_003120 exon1
LG18	*P3*	SNP3281257	3281257	non-syn (G>T, CCG>CAG=P>Q on RC)	Ec-20_003150 exon1

LG	QTL	Name	Position in LG (bp)	Polymorphism	Localisation of polymorphism
LG18	*P3*	Indel3290188	3290188	insertion of 2 codons (GCC.TCC.TCC.TCC.TCC>GCC.TCC.TCC.TCC.TCC.TCC.TCC)	Ec-20_003170 exon9
LG18	*P3*	Indel3290294	3290294	deletion of 1 codon (GGT.CAT.CAT.CAT>GGT.CAT.CAT)	Ec-20_003170 exon9
LG18	*P3*	SNP3282925	3282925	non-syn (C>T, CCA>TCA=P>S)	Ec-20_003170 exon1
LG18	*P3*	SNP3330728	3330728	non-syn (T>A, TTG>ATG=R>M)	Ec-20_003190 exon4
LG18	*P3*	SNP3337249	3337249	non-syn A>C, GAC>GCC=D>A)	Ec-20_003190 exon11
LG18	*P3*	Indel3350168	3350168	insertion of 1 codon (ACCTCCTCCTCCTCCTCCTCCTCCTCCTC>ACCTCCTCCTC CTCCTCCTCCTCCTCCTC, GAG.GAG.GAG.GAG.GAG.GAG.GAG.GAG.GAG.GAG.GTT>G AG.GAG.GAG.GAG.GAG.GAG.GAG.GAG.GAG.GTT on RC)	Ec-20_003200 exon2
LG18	*P3*	Indel3350376	3350376	insertion of 2 codons (CGCT>CGCTCCTGCT, AGC.GCT>AGC.AGG.AGC.GCT on RC)	Ec-20_003200 exon2
LG18	*P3*	SNP3343235	3343235	non-syn (T>C, TAC>TGC=Y>C on RC)	Ec-20_003200 exon12
LG18	*P3*	Indel3363826	3363826	deletion of 1 codon (CCGGCGGCGGCGGCGGCGGC>CCGGCGGCGGCGGCGGC, G.CCG.CCG.CCG.CCG.CCG.CCG.GAC>G.CCG.CCG.CCG.CCG. CCG.GAC on RC)	Ec-20_003220 exon2
LG18	*P3*	SNP3360417	3360417	non-syn (T>G, AAA>CAA=K>Q on RC)	Ec-20_003220 exon6
LG18	*P3*	SNP3361252	3361252	non-syn (C>G, GGG>CGG=G>R on RC)	Ec-20_003220 exon5
LG18	*P3*	SNP3361375	3361375	non-syn (C>T, GCG>ACG=A>T on RC)	Ec-20_003220 exon2
LG18	*P3*	SNP3363690	3363690	non-syn (G>A, GCG>GTG=A>V on RC)	Ec-20_003220 exon2
LG18	*P3*	SNP3363781	3363781	non-syn (T>C, ACC>GCC=T>A on RC)	Ec-20_003220 exon2
LG18	*P3*	SNP3363867	3363867	non-syn (G>A, CCG>CTG=P>L on RC)	Ec-20_003220 exon2
LG18	*P3*	SNP3363904	3363904	non-syn (T>C, ACG>GCG=T>A on RC)	Ec-20_003220 exon2

LG	QTL	Name	Position in LG (bp)	Polymorphism	Localisation of polymorphism
LG18	*P3*	SNP3363905	3363905	non-syn (C>T, ATG>ATA=M>I on RC)	Ec-20_003220 exon2
LG18	*P3*	SNP3363906	3363906	non-syn (A>G, ATG>ACG=M>T on RC)	Ec-20_003220 exon2
LG18	*P3*	SNP3440730	3440730	non-syn (G>A, CAT>TAT=H>Y on RC)	Ec-20_003290 exon5
LG18	*P3*	SNP3445332	3445332	non-syn (A>C, TTC>TGC=F>C on RC)	Ec-20_003290 exon1
LG18	*P3*	SNP3456918	3456918	non-syn (C>G GAA>CAA=E>Q on RC)	Ec-20_003300 exon17
LG18	*P3*	SNP3463094	3463094	non-syn (G>C, CCA>CGA=P>R on RC)	Ec-20_003300 exon9
LG18	*P3*	Indel3499831	3499831	insertion of 3 codons (GCCGCCGAACCCG>GCCGCCGAACCCGCCGAACCCG, CG.GGT.TCG.GCG.GCC>CG.GGT.TCG.GCG.GGT.TCG.GCG.GCC on RC)	Ec-20_003330 exon8
LG18	*P3*	Indel3500179	3500179	insertion of 1 or 3 codons? (TCGCCGCCGCCGCCGCCG>TCGCCGCCGCCGCCGCCGCCG,TCGCCGCCGCCGCCGCCGCCGCCG, C.GGC.GGC.GGC.GGC.GGC.GAA>C.GGC.GGC.GGC.GGC.GGC.GGC.GAA,C.GGC.GGC.GGC.GGC.GGC.GGC.GGC.GAA on RC)	Ec-20_003330 exon7
LG18	*P3*	SNP3500231	3500231	non-syn (C>T, GGC>AGC=G>S on RC)	Ec-20_003330 exon7
LG18	*P3*	SNP3567848	3567848	non-syn (C>T, AGA>AAA=R>K on RC)	Ec-20_003380 exon7
LG18	*P3*	SNP3569123	3569123	non-syn (C>T, GGT>GAT=G>D on RC)	Ec-20_003380 exon5
LG18	*P3*	SNP3581430	3581430	non-syn (G>A, GCA>GTA=A>V on RC)	Ec-20_003380 exon1
LG18	*P3*	SNP3581664	3581664	non-syn (A>G, GTA>GCA=V>A on RC)	Ec-20_003380 exon1
LG18	*P3*	SNP3589916	3589916	non-syn (C>T, GCG>ACG=A>T on RC)	Ec-20_003390 exon6
LG18	*P3*	SNP3593969	3593969	non-syn (C>T, GGC>AGC=G>S on RC)	Ec-20_003390 exon3
LG18	*P3*	SNP3602859	3602859	non-syn (T>C, AAT>GAT=N>D on RC)	Ec-20_003400 exon7
LG18	*P3*	SNP3721507	3721507	AA modification (T>C, CAC>CGC=H>R on RC)	Ec-20_003500 exon9

LG	QTL	Name	Position in LG (bp)	Polymorphism	Localisation of polymorphism
LG18	*P3*	Indel3598467	3598467	insertion of 2 codons (GT>GTGCTACT, ACT>AGT.AGC.ACT on RC)	Ec-20_003390 exon2

Table S13 : Fusion success of male P- versus P+ gametes with gametes of the opposite sex. The total number of individuals corresponds to the total number of scored individuals (developing either by parthenogenesis or derived from fusion of gametes).

Female strain	Male strain	Parthenogenetic capacity male strain	Number of zygotes	Total number individuals
Ec236-284	Ec236-34	P+	28	89
Ec236-284	Ec236-34	P+	21	63
Ec236-39	Ec236-245	P+	13	61
Ec236-203	Ec236-245	P+	4	94
Ec560	Ec236-34	P+	3	253
Ec236-39	Ec236-10	P-	26	66
Ec236-39	Ec236-10	P-	82	144
Ec236-39	Ec236-298	P-	118	158
Ec236-284	Ec236-10	P-	10	70
Ec560	Ec236-10	P-	54	254

Table S14 : Pairwise comparison statistical tests carried out to determine significantly differences between P+ female, P+ male and P- male gametes. Two P- male strains (Ec236-10 and Ec236-276), one P+ female strain (Ec236-203) and one P+ male strain (Ec236-210) were used. The Kruskal-Wallis test indicated significant difference in gamete size. A posthoc Dunn's test revealed, by pairwise comparison of groups, that sizes gametes of each group (female P+, male P+ and males P-) were significantly different.

Kruskal-Wallis	
chi-squared	p-value
3452,395	<2.2e-16

Comparison by group (Benjamini-Hochberg)

	Ec236-10 m P-	Ec236-203 f P+	Ec236-210 m P+
Ec236-203 f P+	chi-squarred=-7.096046 p-value<0.00001		
Ec236-210 m P+	chi-squared=56.34901 p-value<0,0001	chi-squared=26.31408 p-value<0.00001	
Ec236-276 m P-	chi-squared=14.72313 p-value<0.00001	chi-squared=16.38673 p-value<0.0001	chi-squared=2.780366 p-value=0,0027

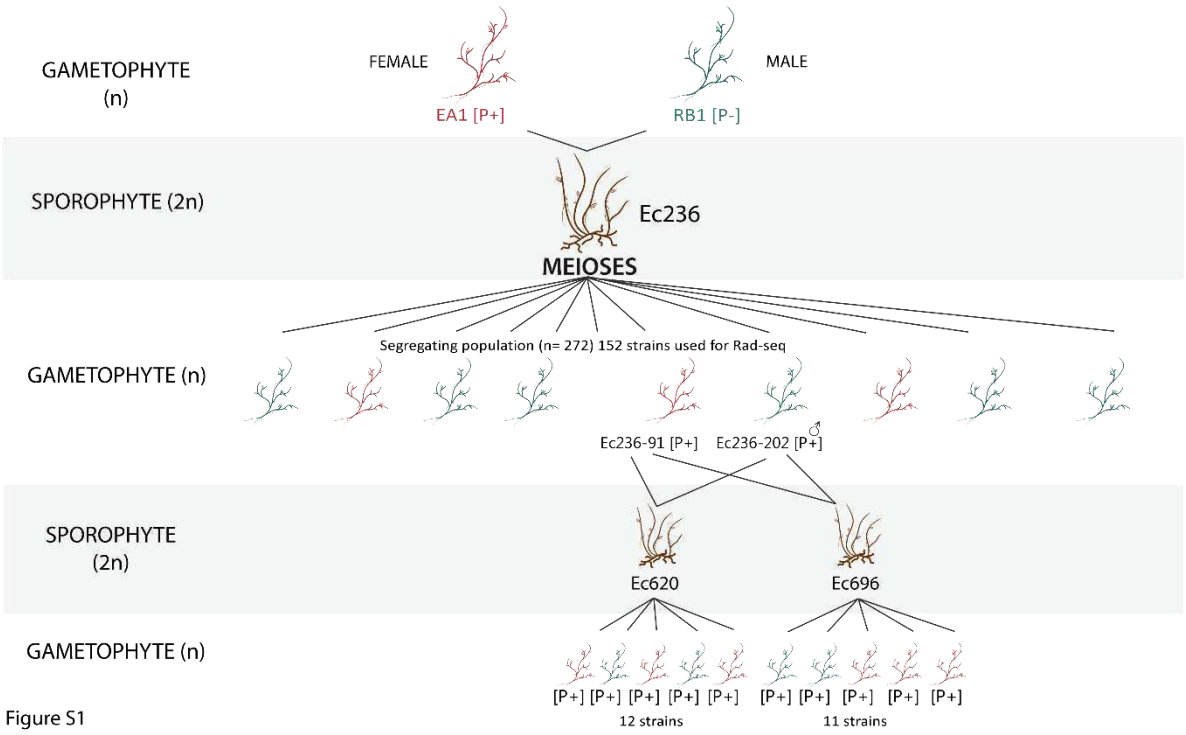

Figure S1

Figure S1. Pedigree of the strains used in this study indicating the crosses performed.

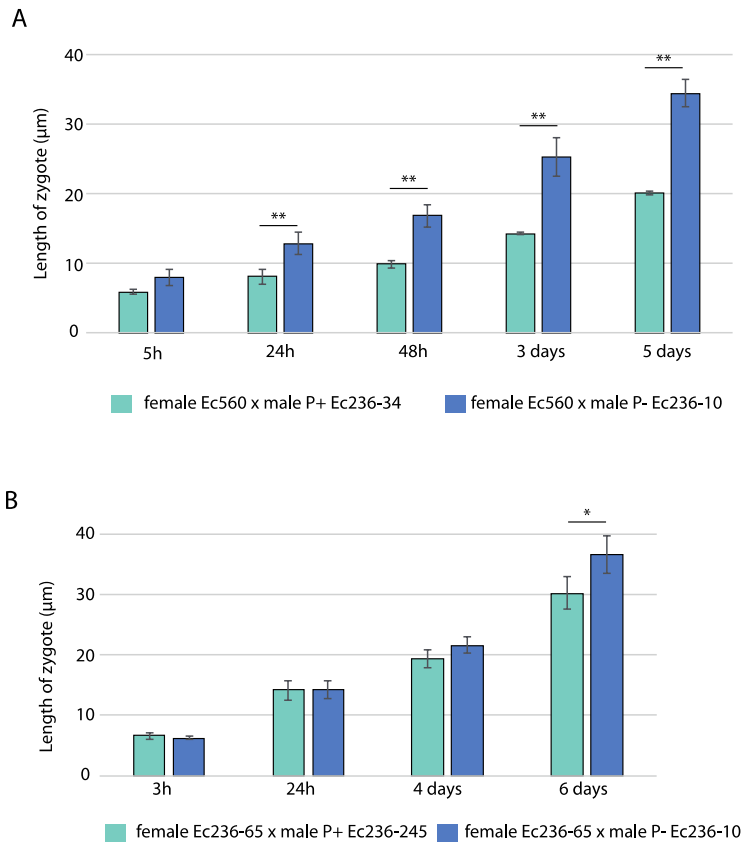

Figure S2. Fitness evaluation of several sporophytes derived from different P- and P+ male lines crossed with several female lines, at different times after fertilisation (from 5 hours to 4 days after fertilisation). A. Zygotes were derived from crosses performed between female Ec560 P+ and male Ec236-34 P+ or male Ec236-10 P- strains. **B.** Zygotes derived from the cross between female Ec236-65 P+ and male Ec236-245 P+ or male Ec236-10 P- strains. Between 4-13 zygotes were scored per cross in each of the time series. Significant differences (Wilcox rank sum test) are indicated (*p-value<0.01; **p-value<0.001).

Discussions and perspectives

Parthenogenesis was studied mainly in plants and animals, which represent only two out of the 21 major groups of eukaryotes and it is difficult to assess the diversity of mechanisms, the evolutionary causes and origins of parthenogenesis without a broader view of of this process across the tree of life. Here, *Ectocarpus* was used to determine the genetic basis of parthenogenesis because it belongs to group of multicellular organisms evolving independently from plants and animals and because its complex life cycle allows to study parthenogenesis excluding additional mechanisms (apomeiosis) necessary for viable development in plants. Since the first description of parthenogenetic development in *Ectocarpus* in 1881 (Berthold, 1881), very few information about the mechanisms controlling such developmental pathway were identified for *Ectocarpus.* In this study, we provided a first glimpse on the genetic control of parthenogenesis in *Ectocarpus*, and demonstrate that two major and one minor QTL loci control this alternative process to sexual reproduction. Analysis of differential expression pattern and polymorphism for genes within the QTLs intervals allowed to establish a list of 89 candidate genes for parthenogenesis control and further analysis provided a refined list of candidate genes, including the identification of a strong candidate gene in the *P2* QTL. Importantly, this work has highlighted the key role of the sex chromosome on the control of parthenegenesis.

While one of the major QTL that we identified is located inside the non-recombining regions, and this precludes any fine mapping, it would be interesting to further refine the QTL intervals specifically in the autosomal loci. A fine-mapping approach would allow to reduce the size of QTL interval sufficiently that the number of candidate genes is modest and functional studies (for example using RNAi or gene knockout) could be undertaken. Since there are three QTL regions that are involved in the control of parthenogenesis in *Ectocarpus* and the trait is only measurable in male (i.e. females always have a parthenogenetic phenotype, regardless of the allele present at the P2 or P3 loci), it is important to fix haplotypes in two of the QTL intervals to study the variation in only one genetic region. We have used only 124 individuals from the 1700 individuals available in the segregating population from the sporophyte Ec236. These additional individuals represent other meiotic events that would provide information on polymorphism in the QTL intervals and help for refining the interval. Therefore, genotyping

further lines based on the markers generated in our study could help to identify individuals with fixed haplotype in the two QTL intervals. For exemple, for the identification of the causal gene in the *P2* QTL, we could select individuals that inherited the paternal allele in both *P1* and *P3* QTL but the maternal allele in the *P2* QTL. Therefore, phenotyping for parthenogenesis capacity/sex more individuals and sequencing the *P2* QTL interval would provide insights on the recombination between markers in the *P2* QTL interval and the phenotypic variability of the trait would depend on this *P2* QTL only.

This study has provided marker sequences for each QTL interval, therefore these markers could be used for a targeted genotyping by sequencing experiments in the 1700-lines progeny.

Finally, the most conclusive evidence that a gene is controlling parthenogenesis would be the demonstration that replacement of the variant nucleotide results in swapping one phenotypic variant for another (i.e. an effect on parthenogenetic capacity). This test can be based on knock-in technologies (Nebert *et al.*, 2000) or knock-out followed by transgenic complementation. Currently, a protocol on genome engineering technologies based on CRISPER-associated RNA-guided endonuclease Cas9 is under development to achieve genome editing in *Ectocarpus*. This protocol would allow to directly test, for instance, the strong candidate identified in the *P2* QTL by knock-out and transgenic complementation. This candidate gene coded for a membrane localised ankyrin repeat-domain palmitoyltransferase (Ec-20_004890). In *S. cerevisiae*, genes belonging to the same family (AKR1) suppresses the mating pathway signalling in the absence of mating pheromone and promotes the maintenance of the vegetative state (Kao *et al.*, 1996; Hemsley and Grierson, 2011).

Our study has also highlighted the epistatic interactions between QTL loci. Epistasis is used by geneticist to describe three distinct processes: the functional relationship between genes, the genetic ordering of regulatory pathways and the quantitative differences of allele-specific effects (Phillips, 2008). In this study, we consider epistasis as the quantitative differences of allele-specific effects on a phenotype. Sex by genotype interactions for parthenogenetic capacity was detected only when female SDR was present suggesting that it could drive epistasis by triggering the parthenogenetic development of females regardless of the alleles carried on the *P2* or *P3* loci. Epistasis was assessed by statistical analysis, but experimental crosses could be used to pinpoint evidence of this epistatic interactions between QTL loci. In the progeny used in this study, the female genotype within each QTL interval is accessible and

could be used to select female with markers associated with the absence of parthenogenetic development (observed in males). Performing a back-cross of these genotypically 'P-' female with the male parent RB1 should allow to indirectly show that 'P-' allele can be carried by female. The expected result would be that the segregation of the male in the progeny will be biased toward non-parthenogenetic capacity. Crosses have been initiated in our laboratory using different females and males, but for two of the crosses the population size of the progeny was too small to detect any enrichment in P- males.

One important result from this work was the finding that parthenogenetic capacity has a dramatic impact on the fitness of male gametes, with P- male gametes being fitter than P+ male gametes for sexual reproduction. The effect of male parthenogenetic capacity on fitness in the diploid zygote was striking, but it would be important to determine if the 'P-' genotype in female is also affecting their sexual fitness in order to know if parthenogenesis is a sexual antagonistic trait. Experiments to study fitness (experimental crosses, measurement of fertilisation success and growth rate of the resulting zygotes) are currently on the way inour laboratory using female lines carrying the 'P-' alleles at both *P2* and *P3* QTL loci versus female lines carrying the same genotype as the parental strain (EA1).

Furthermore, females have the capacity to undergo parthenogenesis regardless of their genotype at the *P2* or *P3* QTL interval, and it would be interesting to investigate if the haploid fitness (the fitness in terms of parthenogenesis capacity, i.e., growth rate of the parthenosporophyte) of these females is similar when a female has the P- genotype or the P+ genotype at the P2 and P3 loci.

Our study of parthenogenesis in *Ectocarpus* was performed in laboratory conditions, under controlled conditions. What about parthenogenetic development in field population of *Ectocarpus*? Recent studies on the maintenance of haploid-diploid life cycles in natural populations of *Ectocarpus* indicate that *Ectocarpus siliculosus* from Roscoff population reproduce preferentially through asexual reproduction (i.e. mito-spores) whereas *Ectocarpus siliculosus* from Naples population undergo sexual reproduction (Couceiro et al., 2015). However, induction of parthenogenesis of the gametophytes derived from natural sporophytes was possible in laboratory conditions indicating that the alleles controlling parthenogenetic development were present in natural population (Table 1). Why then natural population of *Ectocarpus siliculosus* seem to not reproduce through parthenogenesis? Due to

the complex life cycle of *Ectocarpus* (i.e. a sexual reproduction cycle and two cycles through asexuality, clonally or parthenogenetically) the explanation could be that parthenogenesis occurs in marginal population and that they were not sampled. It would be therefore interesting to obtain more samples from natural populations from sites at different densities (e.g. marginal versus central populations) to undertand the significance of parthenogenesis in the field.

In addition to data provide by Couceiro et al. on reproduction in natural population, the QTL mapping from this study revealed markers closely linked with three different regions of the *Ectocarpus* genome involved in parthenogenetic development and they could be used in genome wide association study (GWAS). Several individuals from natural population could be phenotyped for parthenogenetic capacity and then used either for targeted genotyping sequencing of the QTL regions (using marker form this study) or for whole genome sequencing. The goal would then be to look for polymorphisms linked to the phenotype in the QTL regions, similarly to studies performed in birds (Slate *et al.*, 2010). This experiments would allow to (1) refine the number of of marker in the QTL region and therefore potentially find the causal autosomal loci for parthenogenesis , (2) measure the parthenogenetic capacity in individual collected from the field and (3) assess if parthenogenetic alleles are maintained (under selection) in natural populations.

Table 15 : Parthenogenetic capacity in several population/species of *Ectocarpus*. For each strain gametes germination was followed under microscope to determine their parthenogenetic capacity, either parthenogenetic (P+) or non- parthenogenetic (P-). Sex of the strain was determine PCR amplification with specific marker. The gametophyte were generated from sprophyte (SP) collected in natural populations.

Species	Localisation	Origin	Nb of male P+ gametophyte	Nb of male P- gametophyte	Nb of female P+ gametophyte	Nb of female P- gametophyte
E. siliculosus	Napples	from field SP	4	7	20	0
E. siliculosus	Perharidy	from field SP	0	5	6	0
E. crouanioru m	Perharidy	from field SP	7	0	6	0
E. fasciculatus		from field SP	0	1	1	0
Ectocarpus sp.7	Peru	laboratory culture	1	0	1	0
Ectocarpus sp.	New Zealand	laboratory culture	0	1	1	0

Chapter III

An unusual pattern of mitochondria inheritance in the brown alga *Ectocarpus*

Parthenogenesis was showed to be an antagonistic trait that seemed to not be beneficial for male in the context of sexual reproduction. The difference of zygotic growth depending on the parthenogenetic capacity of the male parent lead to hypothesise a potential correlation between mitochondria inheritance and parthenogenetic capcity. Mitochondria, i.e. organelles implicated in the production of ATP through respiration and regulation of cellular metabolism, are predominantly inherited from the maternal parent in most eukaryotes (Greiner *et al.*, 2015). A recent investigation, focused on parthenogenesis in the brown alga *Scytosiphon lomentaria,* suggested a potential correlation between the mitochondrial inheritance mechanism and the capacity of female gametes to undergo parthenogenesis (Han et al., 2014). In *Scytosiphon lomentaria*, as in *Ectocarpus siliculosus* (Chapter 2), male gametes are able to initiate parthenogenesis but rapidly stop developing after 2 or 3 mitotic divisions. Mitochondrial inheritance in *S. lomentaria* is strictly maternal and male mitochondria are selectively degraded after fertilisation (Kimura *et al.*, 2010). Therefore, it was hypothesised that male gametes were able to initiate parthenogenesis until their mitochondria disappear

probably because of the selective mechanism that ensure the maternal transmission. Mitochondrial inheritance was assessed in *Ectocarpus* by Peters et al. (Peters *et al.*, 2004), and these authors reported a strict maternal inheritance pattern. However, a recent study has demonstrated that the two parental strains used in this survey belong to two different cryptic species (Montecinos *et al.*, 2017).

We therefore decided to (1) investigate mitochondrial inheritance using intra-specific crosses to determine if the observation of Peters et al. hold for intra-specific crosses and (2) test if a correlation between parthenogenesis capacity and mitochondria inheritance existed. Mitochondria inheritance was assessed in two species of *Ectocarpus* (*Ectocarpus siliculosus* and *Ectocarpus* species 7) leading to the detection of an unusual pattern of transmission in *Ectocarpus* species 7. This transmission pattern had not been observed before in eukaryotes apart from in interspecific crosses of the slime mold *Dydimium irridis*. A mathematical model was built to determine if cell divisions could lead to this unusual inheritance pattern. This chapter presents, in the form of a manuscript, the results of the investigation of mitochondrial inheritance in the two *Ectocarpus* species along with the mathematical model, which was elaborated in cooperation with Walid Djema from INRIA (Inria Sophia-Antipolis, Côte d'Azur University, France). The characterisation of mitochondria in gametes were achieved in collaboration with Dr Chikako Nagasato and Professor Taizo Motomura (Muroran Marine Station, University of Sapporo, Japan). This chapter was prepared in the form of a manuscript that will be submitted shortly. I contributed to this work by carrying out most of the experiments and also by performing imaging and statistical analysis.

An unusual pattern of mitochondrial inheritance in the brown alga *Ectocarpus*

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Abstract

Most eukaryotes inherit their mitochondria from only one of their parents. When there are different sexes, it is almost always the maternal mitochondria that are transmitted. Indeed, maternal uniparental inheritance has been reported for the brown alga *Ectocarpus* but we show here that strains belonging to different species exhibit different patterns of inheritance: *Ectocarpus siliculosus* strains showed maternal uniparental inheritance but *Ectocarpus* species 7 strains exhibited inheritance of either maternal or paternal mitochondria, but not both. A possible correlation between the pattern of mitochondrial inheritance and male gamete parthenogenesis was investigated. Finally, in contrast to observations in the green lineage, we did not detect any change in the pattern of mitochondrial inheritance in mutant strains affected in life cycle progression.

Introduction

The sexual progeny of most eukaryotes inherit mitochondria from only one of their two parents (Birky 2001; Breton & Stewart 2015). This uniparental pattern of inheritance is thought to exist to control the spread of selfish genetic elements that may arise in the mitochondrial genome and to limit conflicts between the mitochondrial and nuclear genomes (Breton & Stewart 2015; Greiner et al. 2015; Sato & Sato 2013). In organisms with different sexes it is usually the mitochondria of the female parent (i.e. the partner with the largest gametes) that are transmitted to the progeny. One possible reason for this is that male gametes are usually more metabolically active, for example because they are motile, and this may increase the risk of oxidative damage to the paternal mitochondrial genomes (Allen 1996; Greiner et al. 2015; Roze et al. 2005; Lynch 1996). In addition, in many species the production of male gametes involves more cell divisions than the production of female gametes and this also increases the risk of mitochondrial genome mutation (Greiner et al. 2015; Crow 2000). Note that maternal mitochondrial inheritance may therefore be conducive to the production of large amounts of sperm, which will tend to improve fitness under conditions of broadcast dispersal or when sperm competition is high.

In oogamous species, where the large female gamete (the egg cell) contributes more mitochondria to the zygote than the small male gamete (sperm cell), a bottleneck phenomenon (Breton & Stewart 2015) could explain the disappearance of the paternal mitochondria. However, uniparental mitochondrial inheritance is also observed in isogamous species where the two gametes carry similar numbers of mitochondria, implying the existence of specific mechanisms that eliminate the mitochondria of one parent. For example in the unicellular green alga *Chlamydomonas reinhardtii* the mitochondrial genome contributed by the plus mating type parent is specifically eliminated during zygote maturation (Nakamura 2010) and this appears to be under genetic control (Nishimura et al. 2012). Specific mechanisms also exist to promote uniparental mitochondrial inheritance in oogamous species (Greiner et al. 2015; Mishra & Chan 2014). These mechanisms are highly diverse and can act either before or after zygote formation. Pre-zygotic mechanisms include the elimination of mitochondria from male gametes, degradation of male gamete mitochondria before fertilisation and prevention of male mitochondria from entering the egg cell during

fertilisation. Alternatively, selective degradation of the mitochondria or mitochondrial DNA of one parent can occur after formation of the zygote and again this can occur via several different mechanisms involving, for example, the ubiquitin-proteasome system or autophagy.

There is accumulating evidence that many mitochondrial inheritance systems that have been classed as uniparental actually exhibit some level of heteroplasmy (i.e. transmission of both parental mitochondrial genomes to the offspring) or parental leakage (Greiner et al. 2015; Breton & Stewart 2015). Strict uniparental inheritance of mitochondria is expected to lead to the accumulation of deleterious mutations in the mitochondrial genome due to the action of Muller's ratchet. To avoid this, it has been proposed that the mechanisms that promote uniparental inheritance are periodically relaxed over evolutionary time to allow mitochondrial genomes to recombine (Greiner et al. 2015; Takano et al. 2010). "Leakage" of paternal mitochondria through to the progeny is also expected to limit the effects of Muller's ratchet but it is not clear whether leakage alone is sufficient. The broad diversity of the mechanisms by which paternal mitochondria are eliminated (see above) is consistent with periodical relaxation of uniparental inheritance in the sense that these mechanisms would need to re-evolve after each period of relaxed inheritance.

A number of organisms exhibit patterns of mitochondrial inheritance that deviate from the usual situation of uniparental maternal inheritance. These variations are of considerable interest because they can provide insights into the evolutionary and molecular mechanisms underlying mitochondrial inheritance. Examples include strict paternal inheritance in some plants including the sequoia tree (Neale et al. 1989), banana (Fauré et al. 1994) and cucumber (Havey et al. 2004). In some organisms more than one mitochondrial genome may be transmitted to the offspring. For example, stable inheritance of maternal heteroplasmy has been described for terrestrial isopod crustaceans (Doublet et al. 2012). Biparental mitochondrial transmission has been reported for several species but there do not appear to be any cases where both maternal and paternal mitochondria are systematically transmitted to the zygote and then stably inherited throughout development (Breton & Stewart 2015). Therefore, even when inheritance is biparental, there are usually mechanisms that limit heteroplasmy, usually by ensuring that individual offspring carry either the maternal or the paternal mitochondria, but not both. In the fungus *Coprinopsis cinerea*, for example, progeny can inherit mitochondria from either one or the other parent (Wilson & Xu 2012). This pattern

of inheritance occurs because heterokaryon formation involves an exchange of parental nuclei, but not mitochondria, between mating partners. In this case, therefore, there is no stage where mitochondria from both parents are mixed in a cell fusion product and therefore no need for selective elimination of mitochondria derived from one of the parents. In the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* fusion of isogametes results in heteroplasmy but partitioning of mitochondria during budding actively promotes the formation of homoplasmic daughter cells (Birky 2001). Several bivalve species exhibit doubly uniparental inheritance, with mitochondrial being transmitted in a sex-specific manner (Zouros 2013). The female (F) genome is transmitted to females and males but the males do not transmit the F genome to their progeny, rather they transmit the male (M) genome, which is transmitted uniquely through the male line. Finally, novel patterns of mitochondrial inheritance have often been reported following inter-specific crosses in a broad range of eukaryotic taxa (Breton & Stewart 2015). Under these conditions, mitochondrial inheritance systems may exhibit breakdown due to genome incompatibilities and therefore it is possible that the patterns observed are dysfunctional.

Studies of mitochondrial inheritance in the brown algae have reported maternal inheritance (Motomura et al. 2010). In oogamous species, male mitochondria are digested by lysosomes in the zygote, whereas in the anisogamous species *Scytosiphon lomentaria* male mitochondria persist until the four-cell stage of sporophyte development.

Ectocarpus is an emerging model species for the brown algae (Peters et al. 2004; Cock et al. 2011). An earlier study indicated that mitochondrial inheritance is strictly maternal in *Ectocarpus* (Peters et al. 2004). However, a recent analysis of the species structure of the genus *Ectocarpus* (Montecinos et al. 2016) has indicated that the strains used in the 2004 study belonged to distinct cryptic species and therefore that the crosses were inter-specific. Here we analysed mitochondrial inheritance in intra-specific crosses using pairs of strains from two of the recently defined *Ectocarpus* species. For one cross we observed strict maternal inheritance, as reported previously, but, surprisingly, the progeny of the second cross inherited either only maternal mitochondria or only paternal mitochondria. We investigated a possible correlation between this unusual pattern of inheritance and the parthenogenetic capacity of male gametes. Finally, a mutation that affected the *C. reinhardtii* gene GSP1, which is required for deployment of the diploid program in this green alga, exhibited aberrant

mitochondrial DNA inheritance (Nishimura et al. 2012). In contrast, we show that equivalent life cycle mutants in *Ectocarpus* exhibit a wild type pattern of mitochondrial inheritance.

Material and methods

Ectocarpus strains, culture conditions and crosses.

The list of species used in this study, together with their characteristics and genetic history, is shown in Table S1. Pedigrees are shown in Figure S1. The strains corresponded to two *Ectocarpus* species, Ectocarpus siliculosus sensu stricto (hereafter Ectocarpus siliculosus) and *Ectocarpus* species 7. Note that *Ectocarpus* species 7 was previously referred to as *Ectocarpus siliculosus* but actually corresponds to a distinct species (Montecinos et al. 2016). Strains Ec32, Ec568 (*Ectocarpus* species 7), EA1, RB1 and EcNAP-12-24 (*Ectocarpus siliculosus*) are all derived from independent field isolates (Figure S1). *Ectocarpus* strains were cultured in autoclaved natural sea water supplemented with half strength Provasoli solution (Starr and Zeikus, 1993) at 13°C, with a light:dark cycle of 12h:12 (20 µmol photons m −2 s −1) using daylight-type fluorescent tubes (Coelho et al. 2012). All manipulations were carried out under sterile conditions in a laminar flow hood. Crosses (listed in Table S1) were carried out using the protocol described by (Coelho et al. 2012)). Sporophytes derived from crosses were cultivated for 2 to 3 months before excision of material for DNA extraction. Genomic sequence data accession numbers for strains Ec32, Ec568, EA1, RB1 and EcNAP-12-24 are provided in Table S1.

Extraction of genomic DNA and identification of heterozygous, diploid sporophytes

Sporophyte tissue (10 to 20 mg wet weight) was frozen in liquid nitrogen and DNA was extracted using the NucleoSpin® Plant II kit (Macherey Nagel) according to the manufacturer's instructions. As some *Ectocarpus* gametes are able to undergo parthenogenesis to form haploid partheno-sporophytes, the ploidy of the sporophytes derived from each cross was assessed using sex markers (Table S2). Diploid sporophytes are expected to carry both the female (U) and the male (V) sex chromosome whereas partheno-sporophytes carry only one

sex chromosome (U or V; Fig. S2). Touchdown PCR reactions, which consisted of 2 ng of DNA, 80 nM of primer mix, 0.2 mM dNTP, 2µl of 5X Go Taq® green buffer (Promega), 2 mM $MgCl_2$, 2 mg/ml of BSA and 0.05 µl (0.25 units) of Taq polymerase (Promega), were carried out in an Applied Biosystems thermocycler under the following conditions: 3 min at 95°C, then 10 touchdown cycles of 30s 95°C; 30s at 65°C (-1°C/cycle) and 30s at 72°C followed by 25 cycles of 30s at 95°C, 30s at 55°C and 30s at 72°C and a final incubation at 72°C for 5 min before storage at 4°C. After amplification, PCR products were visualized on a 2% agarose gel after 30 minutes of migration at 100 V.

Mitochondrial genome assemblies and detection of intra-specific mitochondrial DNA polymorphisms

An earlier assembly of the *Ectocarpus* species 7 strain Ec32 mitochondrial genome was re-evaluated using high-coverage Illumina shotgun sequence data. A draft assembly of the *Ectocarpus siliculosus* strain EA1 genome, including organellar sequences, was generated using the CLC assembler (Qiagen Bioinformatics) and Illumina shotgun DNA-seq data (Table S1). Mitochondrial DNA scaffolds were identified using the *Ectocarpus* species 7 mitochondrial genome as the query in a Blastn (Altschul et al. 1997) search. These scaffolds were then assembled manually to obtain the complete circular *Ectocarpus siliculosus* strain EA1 mitochondrial genome. The genome was annotated by transferring annotation information from the *Ectocarpus* species 7 strain Ec32 mitochondrial genome (Genbank accession number FP885846). Circular maps of the Ec32 and EA1 mitochondrial genomes were generated using OGDraw (Lohse et al. 2013).

Intra-specific mitochondrial DNA polymorphisms were identified by mapping Illumina shotgun DNA-seq data (Table S1) against reference mitochondrial genome assemblies. For *Ectocarpus siliculosus*, DNA-seq data for strains RB1 and EcNAP12-024 was mapped, individually, against the EA1 reference. For Ectocarpus species 7, DNA-seq data for strain Ec568 was mapped against the Ec32 reference. Variants were detected with bcftools and verified manually by visualisation of mapping data in GenomeView (Abeel et al. 2012).

Development of dCAPS markers to study mitochondrial DNA inheritance

To identify mitochondrial genome polymorphisms, Illumina paired end whole-genome DNA reads from female strains were mapped onto the assembled mitochondrial genome sequence of male strains using Bowtie2 (Langmead et al. 2009) and SNPs identified by manual analysis of the mapping data in GenomeView (Abeel et al. 2012). Derived cleaved amplified polymorphic sequence-specific (dCAPS) markers (Neff et al. 1998) were then designed using dCAPS Finder2.0 (http://helix.wustl.edu/dcaps/) for the dCAPS primer and Primer 3 (http://primer3.ut.ee/) for the second primer of the primer pair. dCAPS primers allow the creation of a diagnostic restriction enzyme recognition site specifically in the PCR product corresponding to one allelic form of an SNP. Before use, dCAPS markers were tested on samples in which genomic DNA from the two parental strains had been mixed in different proportions (1/2; 1/5; 1/10; 1/20). Touchdown PCR reactions were carried out with dCAPS primer pairs in an Applied Biosystems thermocycler using the following conditions for *Ectocarpus* species 7: 3 min at 95°C, then 10 touchdown cycles of 30s 95°C; 30s at 65°C (-1°C/cycle) and 30s at 72°C followed by 25 cycles of 30s at 95°C, 30s at 55°C and 30s at 72°C and a final incubation at 72°C for 5 min before storage at 4°C and the following conditions for *E. siliculosus*: 3 min at 95°C, then 10 touchdown cycles of 30s 95°C; 30s at 68°C (-0.1°C/cycle) and 30s at 72°C followed by 25 cycles of 30s at 95°C, 30s at 58°C and 30s at 72°C and a final incubation at 72°C for 5 min before storage at 4°C. After amplification, 10 µl of PCR product was digested using five units of the relevant restriction enzyme (Table S2). Digestion products were analysed on a 2.5 % agarose gel after 45 minutes of migration at 100 V. All marker genotyping tests were carried out twice to ensure that the result was reproducible. The strains used for the mitochondrial counts are indicated in Table S1.

Counts of mitochondria using confocal microscopy

Mitotracker dyes (MitoTracker® Orange CMTMRos ref MT7510, Invitrogen) were used to stain mitochondria in freshly released gametes. Working solutions of mitotracker dyes were obtained by diluting 1 mM stock solutions in DMSO to 0.166 µM in freshly prepared Provasoli-enriched sea water. Gametophyte filaments carrying plurilocular gametangia were allowed to release in 20 µl of this solution on a clean coverslip and the gametes were then fixed after 20

min at room temperature under low light by addition of glutaraldehyde to a final concentration of 1%. Confocal microscopy was carried out with a Leica SP5 microscope (TCS SP5 AOBS, Merimage platform, Roscoff) and z-series of images were analysed with ImageJ/Fidji to count the number of mitochondria in each gamete.

Evaluation of parthenogenetic capacity

To evaluate parthenogenetic capacity, released gametes were allowed to settle in a Petri dish and parthenogenetic growth estimated after fifteen days in culture. Strains were scored as parthenogenetic if more than 4% of parthenotes grew beyond the ten-cell stage.

Results

Development of markers to follow mitochondrial inheritance in intraspecific crosses

A recent analysis by Montecinos et al. (2016) identified the presence of at least 15 cryptic species within the genus *Ectocarpus* and indicated that an earlier study of mitochondrial inheritance in *Ectocarpus* (Peters et al. 2004) was based on interspecific crosses. To determine whether the conclusions of the earlier study held for intraspecific crosses, we developed molecular markers to distinguish between polymorphic forms of the mitochondrial genome in two of the *Ectocarpus* species defined by Montecinos et al. (2016): *E. siliculosus sensu stricto* (hereafter *E. siliculosus*) and *Ectocarpus* species 7. Note that *Ectocarpus* species 7, which corresponds to the reference genome species (Cock et al. 2010; Cormier et al. 2017), was earlier referred to as *Ectocarpus siliculosus* under the older classification system but this nomenclature needs to be revised.

The mitochondrial genome sequence of the male *Ectocarpus* species 7 strain Ec32, which had been initially assembled using Sanger sequence data (deposited as *Ectocarpus siliculosus* with Genbank accession number FP885846.1), was re-evaluated using high-coverage Illumina shotgun sequence data and two sequencing errors were corrected. The corrected *Ectocarpus* species 7 strain Ec32 mitochondrial genome is available through the accession number FP885846.2 (Fig. 1A). The mitochondrial genome of the female *E. siliculosus* strain EA1 was

assembled using whole genome shotgun sequence data (Table S1) and the Ec32 genome as a guide (Fig. 1A). The EA1 mitochondrial genome is available through the accession number MK045263. Whole genome sequence data was then generated for independently isolated strains of both species (RB1 and EcNAP-12-24 for *E. siliculosus* and Ec568 for *Ectocarpus* species 7) and each dataset was mapped onto the corresponding, conspecific mitochondrial genome to identify intraspecific polymorphisms. This analysis identified 28 and six intra-specific SNPs for the *E. siliculosus* and *Ectocarpus* species 7 mitochondrial genomes, respectively (Fig. 1A, Table S3). Based on these SNPs, two and three dCAPS markers (Neff et al. 1998) were developed for *E. siliculosus* and *Ectocarpus* species 7, respectively (Table S2). The sensitivity of the dCAPS markers was tested by carrying out amplifications from samples in which parental DNA had been mixed in different proportions (50:50, 20:80, 10:90; 5:95). This analysis showed that the dCAPS assays distinguished between male and female alleles and were able to detect the presence of mixtures of mitochondrial DNA from the two parents (equivalent to biparental inheritance) provided they were in approximately equal proportions (Fig. 1B).

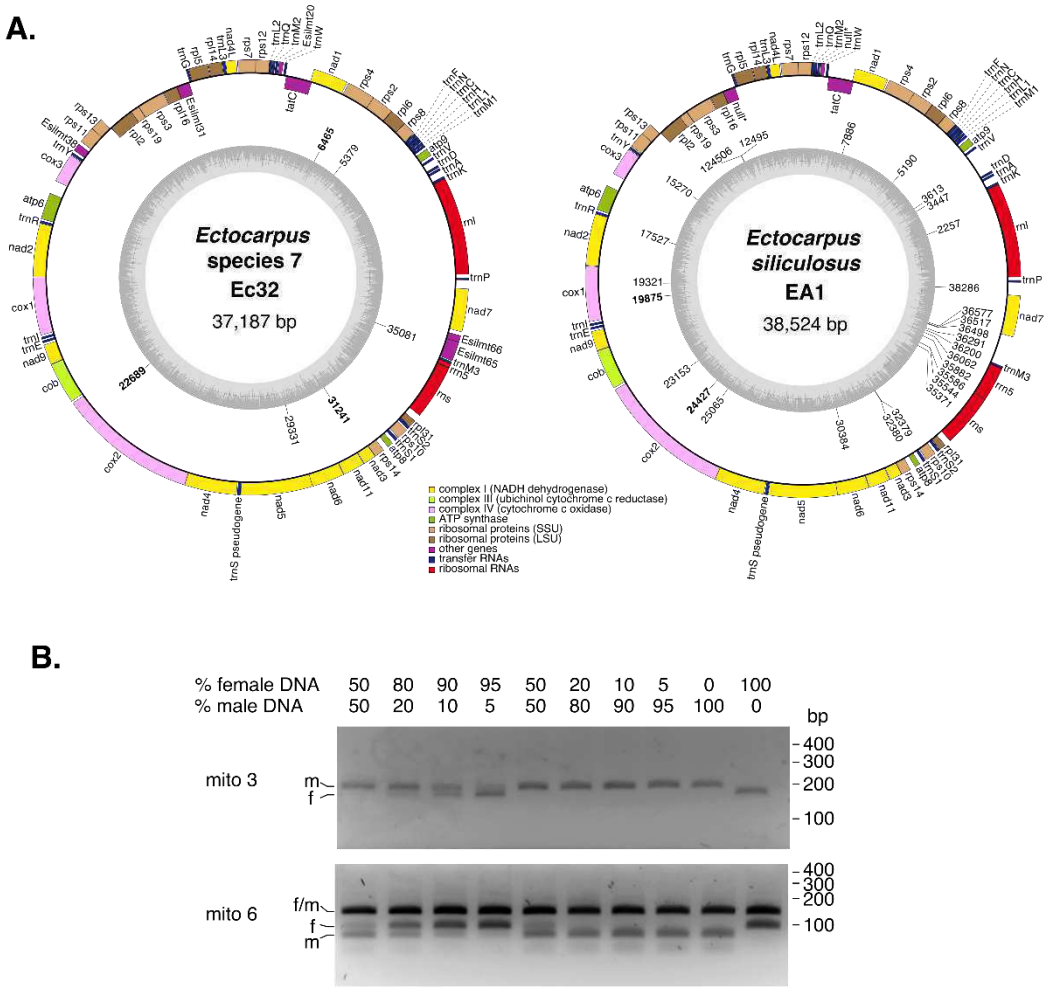

Figure 1: Mitochondrial genome analysis and development of dCAPS markers. A. Mitochondrial genomes of Ectocarpus species 7 strain Ec32 and Ectocarpus siliculosus strain EA1. The inner circles show GC content and the positions of the intra-specific polymorphisms detected by this study. Polymorphisms indicate in bold were used to develop dCAPS markers. **B.** In vitro tests of dCAPS markers. PCR amplifications were carried out using genomic DNA from the two parental strains mixed in different proportions. f, female; m, male; bp, base pairs.

Mitochondria DNA inheritance in *E. siliculosus* and *Ectocarpus* species 7

To analyse mitochondrial inheritance, crosses were carried out between male and female strains of *E. siliculosus* (EcNAP12-024 x Esil236-191) and *Ectocarpus* species 7 (Ec721-58 x Ec32). Note that Esil236-191 is a male descendent of a cross between and EA1 and RB1 and Ec721-58 is a sister of Ec568 (Fig. S2). The heterozygous sporophytes derived from the crosses were isolated and PCR amplifications were carried out to verify that they carried both the female (U) and the male (V) sex chromosome. This step allowed the elimination of any haploid individuals that had arisen via gamete parthenogenesis rather than gamete fusion and zygote formation (see Fig. S1).

dCAPS analysis of 20 sporophytes derived from the *E. siliculosus* (EcNAP12-024 x Esil236-191) cross indicated that they had all inherited their mitochondrial genomes from the mother (Figure 2 upper panel, Table S1). This result was consistent with the maternal uniparental inheritance observed by Peters et al. (2004) following interspecific crosses. Analysis of 15 sporophytes derived from the *Ectocarpus* species 7 (Ec568 x Ec32) cross also indicated uniparental inheritance but, surprisingly, approximately half of the sporophytes (7/15) had inherited their mitochondrial DNA from the father (Fig. 2 upper panel, Table S1). In *Ectocarpus* species 7, therefore, inheritance appears to be uniparental but either the maternal or paternal mitochondrial genome can be retained.

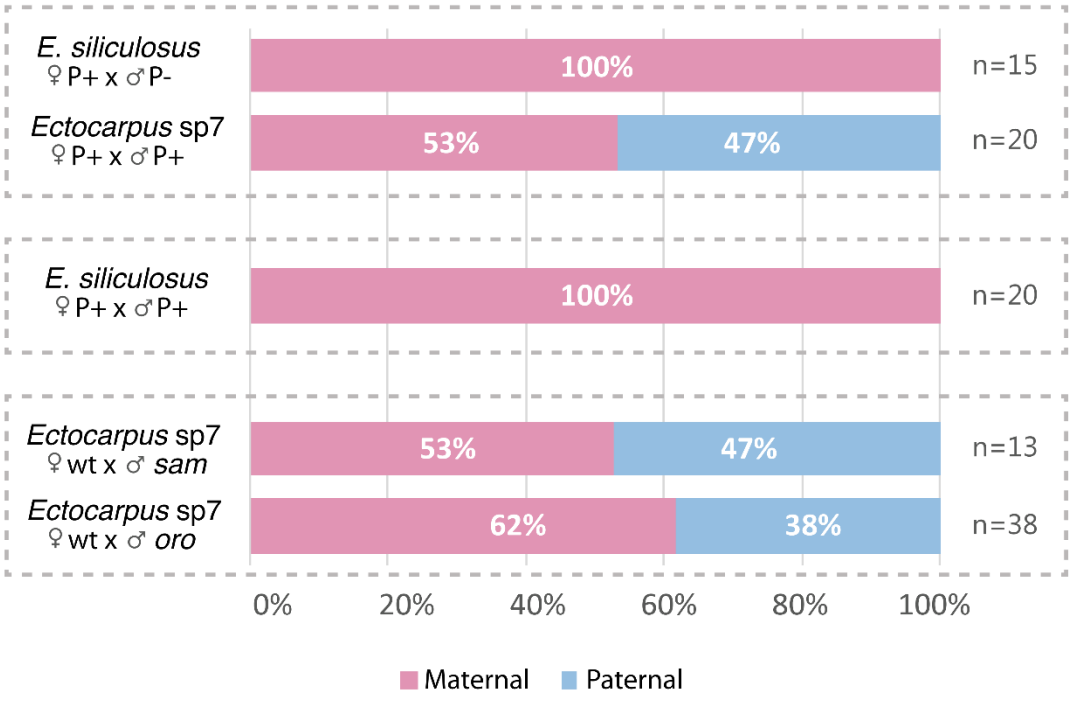

Figure 2: Inheritance of mitochondrial genomes following different intra-specific crosses. Percentage of sporophyte progeny carrying maternal (pink) or paternal (blue) mitochondrial genomes. Ectocarpus sp7, Ectocarpus species 7; P+, parthenogenetic; P-, non-parthenogenetic; wt, wild type; sam, samsara mutant; oro, ouroboros mutant.

Does male parthenogenetic capacity affect mitochondrial inheritance?

One difference between the gametes of male *Ectocarpus* species 7 strain Ec32 and those of the male *E. siliculosus* strains RB1 and EcNAP12-024 is that the latter are parthenogenetic whereas the former are not (note that the gametes of all the female strains used, EA1, EcNAP12-024 and Ec568, are parthenogenetic; Table S1). It is possible that the requirement for functional mitochondria during parthenogenesis leads to the attenuation of mechanisms that would normally prepare male mitochondria for destruction following zygote formation, resulting in a higher probability of the male mitochondria being transmitted to heterozygous sporophyte offspring (Han et al. 2014). To investigate this hypothesis, we examined whether male gametes of strain Ec32 carried more mitochondria than those of strain EcNAP12-024 by staining fixed gametes with mitotracker and counting the number of mitochondria per gamete. This analysis (Fig. 3) showed that, although in both species female gametes (which are slightly larger than male gametes; Lipinska et al. 2015) contained more mitochondria than male gametes, this difference was significant for *E. siliculosus* but not for *Ectocarpus* species 7 (Kruskal Wallis test, p-value<2.2x10-16; then Dun's post hoc-test, p-value=0.1039). This observation suggested a possible link between the number of mitochondria carried by the male gamete and transmission of the male mitochondrial genome.

Parthenogenetic male *E. siliculosus* strains exhibit maternal uniparental inheritance

Additional phenotyping of the family of gametophytes derived from the cross between *E. siliculosus* strains EA1 and RB1 (parthenogenetic female x non-parthenogenetic male) revealed that a minority of the male progeny produced parthenogenetic gametes. The number of mitochondria in the gametes of these parthenogenetic *E. siliculosus* males was intermediate between the numbers in non-parthenogenetic *E. siliculosus* and in parthenogenetic Ec32 gametes (Fig. 3) but neither difference was statistically significant. Moreover, when one of the parthenogenetic males (Esil236-154) was crossed with the female strain EcNAP12-024, mitochondrial inheritance was 100% maternal (Fig. 2 middle panel, Table S1). Taken together with the above analyses, there therefore appears to be a broad correlation between the number of mitochondria carried by a gamete and the mode of mitochondrial

DNA inheritance but there is not a simple relationship between parthenogenetic capacity and random uniparental inheritance of mitochondria.

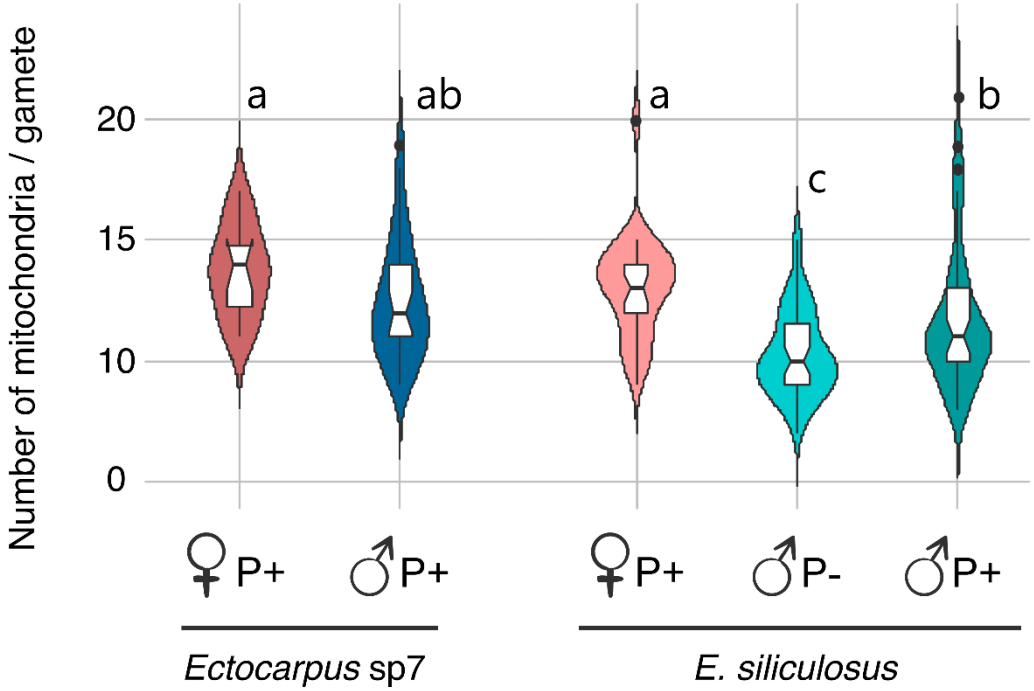

Figure 3: Number of mitochondria in gametes of different *Ectocarpus* strains. Letters indicate significant differences (Kruskal Wallis followed by Dun's post hoc-test). *Ectocarpus* sp7, *Ectocarpus* species 7; P+, parthenogenetic; P-, non-parthenogenetic.

Life cycle mutants do not exhibit altered patterns of mitochondrial inheritance

In the unicellular green algae *Chlamydomonas reinhardtii*, the deployment of the diploid program following gamete fusion is under the control of two genes *GSP1* and *GSM1* (Lee et al. 2008). Genes related to *GSP1* and *GSM1* (which both encode TALE homeodomain transcription factors) in the moss *Physcomitrella patens* have been shown to be necessary for the deployment of the diploid sporophyte generation following gamete fusion (Sakakibara et al. 2013; Horst et al. 2016), indicating that this regulatory system is conserved across the green lineage. A *C. reinhardtii* mutant in which a region of the genome including the gene *GSP1* was deleted exhibited aberrant biparental inheritance of mitochondrial DNA rather than the usual uniparental inheritance (Nishimura et al. 2012). Based on these observations, we were interested to determine whether two *Ectocarpus* life cycle mutants that also fail to deploy the

diploid sporophyte program, *ouroboros* (*oro*; Coelho et al. 2011) and *samsara* (*sam*; unpublished) exhibited defective mitochondrial inheritance. For this, male strains carrying either the *oro* or the *sam* mutations were crossed with wild type *Ectocarpus* species 7 female strains and the inheritance of mitochondrial DNA followed in the derived sporophyte generation. These experiments indicated that the presence of the oro or sam mutations did not significantly modify the pattern of mitochondrial inheritance. In both cases mitochondrial inheritance was uniparental with approximately half the sporophyte progeny inheriting the maternal mitochondrial genome and half inheriting the paternal mitochondrial genome (Fig. 2 lower panel).

Discussion

Recent work on the species structure of the genus *Ectocarpus* has provided evidence that the crosses carried out by Peters et al (2004), which indicated strict uniparental maternal inheritance of mitochondria, were between strains that belonged to different cryptic species. Inter-specific crosses can lead to aberrant patterns of organelle inheritance due to genome incompatibilities. We therefore sought to repeat these experiments using intra-specific crosses. We used complete assemblies of the mitochondrial genomes of *E. siliculosus* and *Ectocarpus* species 7, together with whole genome shotgun sequence for several *E. siliculosus* and *Ectocarpus* species 7 strains, to identify intra-specific mitochondrial DNA polymorphisms. Intra-specific crosses and genetic markers based on the intra-specific polymorphisms were then used to analyse mitochondrial inheritance in the two species. These analyses detected strict maternal inheritance of mitochondria in *E. siliculosus*, as previously reported. Mitochondrial inheritance was also uniparental in *Ectocarpus* species 7 but individuals showed either maternal or parental inheritance, depending on the gamete fusion event.

One marked difference between the *E. siliculosus* and *Ectocarpus* species 7 strains studied here was that male gametes of the latter are capable of parthenogenesis. We hypothesised that the mechanisms that allow male gametes to retain functional mitochondria for parthenogenesis may result in an increased likelihood of the male mitochondrial being transmitted through sexual crosses. However, the identification of male *E. siliculosus* strains whose gametes were capable of parthenogenesis and yet which showed a maternal pattern

of mitochondrial inheritance argued against this hypothesis. We believe nonetheless that this hypothesis would merit further investigation as it is possible that the situation regarding male gamete parthenogenesis is not the same in the two species, in the sense that parthenogenetic males appear to be rare in *E. siliculosus* but preliminary analyses indicate that they may be a more common, and perhaps universal, phenomenon in *Ectocarpus* species 7. In other words an effect on mitochondrial inheritance may only be seen when male parthenogenesis become a fixed characteristic.

We also investigated mitochondrial inheritance in two mutant strains affected in life cycle progression, *oro* and *sam*. We did not observe any effect on mitochondrial inheritance in these mutants. This observation suggests that the pleiotropic effect of the *C. reinhardtii GSP1* life cycle mutant on mitochondrial inheritance may represent a secondary function of this gene, acquired in addition to its life cycle function in the green lineage.

In conclusion, we show here that patterns of mitochondrial inheritance vary across different *Ectocarpus* isolates, with the commonly observed strict maternal inheritance pattern being observed in *E. siliculosus* strains but an unusual pattern of stochastic uniparental inheritance being observed in *Ectocarpus* species 7 strains. These observations indicate that mitochondrial inheritance patterns can vary across related species within the same genus and argue for broader analyses of inheritance using multiple strains. We would also like to underline the importance of using intra-specific mitochondrial DNA polymorphisms, which allows the analysis of intra-specific crosses and reduces the risk of observing aberrant inheritance patterns due to genome incompatibilities, as may often be the case with inter-specific crosses.

Acknowledgements

This work was supported by the CNRS, Sorbonne Université and the JSPS (Japan Society for Promotion of Science). We thank Denis Roze for fruitful discussion.

Author contributions

LM, CN, FP, TM, AP prepared the biological material and performed experiments. LM, MMP, JMC performed the computational analysis. LM, CN, FP, SMC, JMC analysed data. JMC designed and coordinated the study. JMC wrote the manuscript with valuable input from LM and SMC. All authors read and approved the final manuscript.

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Supplementary material

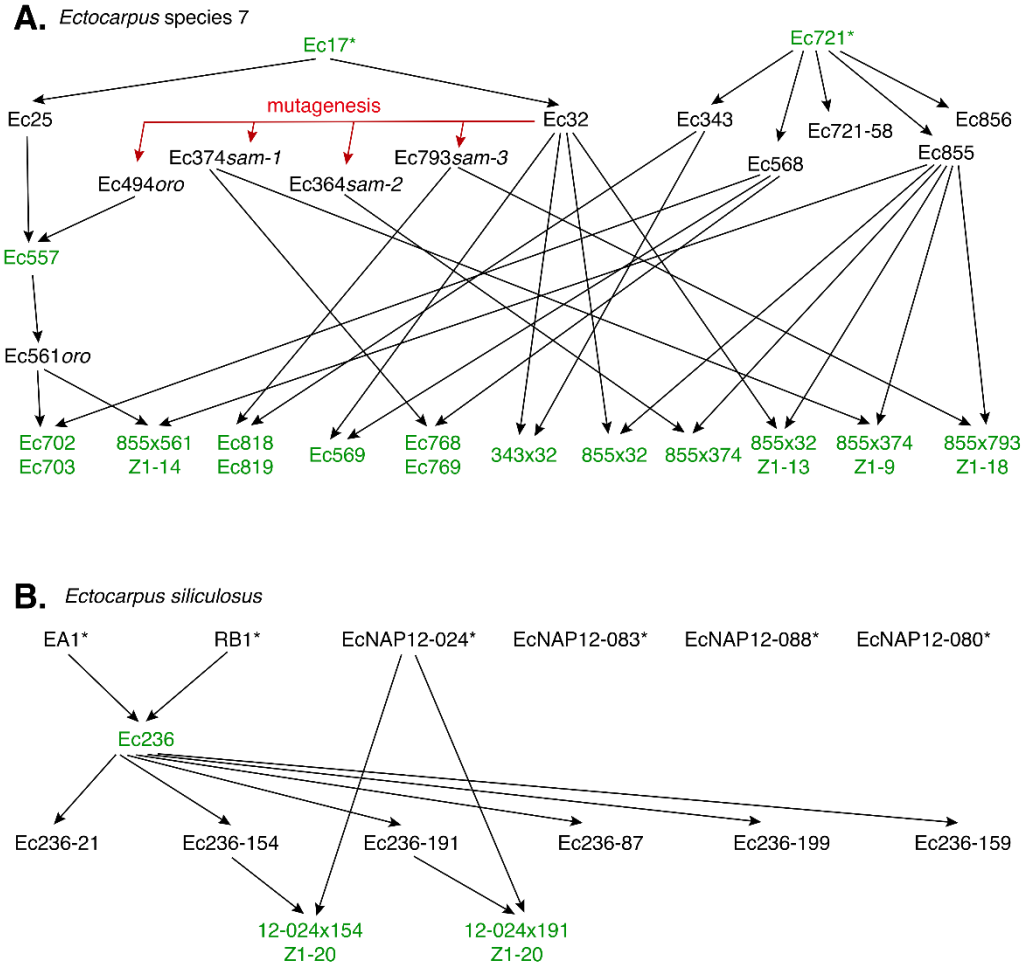

Figure S1: Pedigrees of the *Ectocarpus* strains used in this study. A. *Ectocarpus* species 7 strains. **B.** *E. siliculosus* strains. See Table S1 for further information about the strains indicated. Asterisks indicate field-isolated strains, diploid sporophytes are shown in green, gametophytes in black. oro, ouroboros mutant; sam, samsara mutants; z, zygote.

Table S1. List of Ectocarpus strains used in this study. Ec32 strain accession number for genomic sequence data: CABU01000001–CABU01013533, FN647682–FN649242, FN649726–FN649760 (Cock *et al.*, 2010). Ec568 strain accession number for genomic sequence data: SRR7692532 (Cormier *et al*., 2017). Parthenogenetic = P+; Non-parthenogenetic = P-; oro = ouroboros mutant; sam = samsara mutant; WT = Wild Type

Strain name	Species	Genetic history	Geographical origin of strain	Generation	Sex	Genotype	Mitochondrial inheritance	Parthenogenetic capacity	Strain used for gamete mitocondria counts?
Ec32	*Ectocarpus* sp. 7	From field-isolated sporophyte Ec17	San Juan de Marcona, Peru	Gametophyte	male	WT	nd	P+	Yes
Ec568	*Ectocarpus* sp. 7	From field-isolated sporophyte Ec721	Arica, Chile	Gametophyte	female	WT	nd	P+	
Ec721-58	*Ectocarpus* sp. 7	From field-isolated sporophyte Ec721	Arica, Chile	Gametophyte	female	WT	nd	P+	Yes
Ec855	*Ectocarpus* sp. 7	From field-isolated sporophyte Ec721	Arica, Chile	Gametophyte	female	WT	nd	P+	
Ec856	*Ectocarpus* sp. 7	From field-isolated sporophyte Ec721	Arica, Chile	Gametophyte	female	WT	nd	P+	
Ec343	*Ectocarpus* sp. 7	From field-isolated sporophyte Ec721	Arica, Chile	Gametophyte	female	WT	nd	P+	
Ec561	*Ectocarpus* sp. 7	Ec25 x Ec494*oro*	San Juan de Marcona, Peru	Gametophyte	male	*oro*	nd	P+ (partheno-gametophyte)	
Ec374	*Ectocarpus* sp. 7	UV mutagenised Ec32	San Juan de Marcona, Peru	Gametophyte	male	*sam-1*	nd	P+ (partheno-gametophyte)	
Ec364	*Ectocarpus* sp. 7	UV mutagenised Ec32	San Juan de Marcona, Peru	Gametophyte	male	*sam-2*	nd	P+ (partheno-gametophyte)	
Ec793	*Ectocarpus* sp. 7	UV mutagenised Ec32	San Juan de Marcona, Peru	Gametophyte	male	*sam-3*	nd	P+ (partheno-gametophyte)	
Ec569	*Ectocarpus* sp. 7	Ec568 x Ec32	Chile/Peru	Sporophyte	na	WT	Paternal	(P+ x P+)	
855x32 Z1	*Ectocarpus* sp. 7	Ec855 x Ec32	Chile/Peru	Sporophyte	na	WT	Maternal		
855x32 Z2	*Ectocarpus* sp. 7	Ec855 x Ec32	Chile/Peru	Sporophyte	na	WT	Maternal		
855x32 Z3	*Ectocarpus* sp. 7	Ec855 x Ec32	Chile/Peru	Sporophyte	na	WT	Maternal		

Strain name	Species	Genetic history	Geographical origin of strain	Generation	Sex	Genotype	Mitochondrial inheritance	Parthenogenetic capacity	Strain used for gamete mitocondria counts?
855x32 Z4	*Ectocarpus* sp. 7	Ec855 x Ec32	Chile/Peru	Sporophyte	na	WT	Maternal		
855x32 Z5	*Ectocarpus* sp. 7	Ec855 x Ec32	Chile/Peru	Sporophyte	na	WT	Paternal		
855x32 Z6	*Ectocarpus* sp. 7	Ec855 x Ec32	Chile/Peru	Sporophyte	na	WT	Maternal		
855x32 Z9	*Ectocarpus* sp. 7	Ec855 x Ec32	Chile/Peru	Sporophyte	na	WT	Paternal		
855x32 Z10	*Ectocarpus* sp. 7	Ec855 x Ec32	Chile/Peru	Sporophyte	na	WT	Paternal		
855x32 Z11	*Ectocarpus* sp. 7	Ec855 x Ec32	Chile/Peru	Sporophyte	na	WT	Paternal		
855x32 Z12	*Ectocarpus* sp. 7	Ec855 x Ec32	Chile/Peru	Sporophyte	na	WT	Maternal		
855x32 Z13	*Ectocarpus* sp. 7	Ec855 x Ec32	Chile/Peru	Sporophyte	na	WT	Paternal		
343x32 Z5	*Ectocarpus* sp. 7	Ec343 x Ec32	Chile/Peru	Sporophyte	na	WT	Paternal		
343x32 Z7	*Ectocarpus* sp. 7	Ec343 x Ec32	Chile/Peru	Sporophyte	na	WT	Maternal		
343x32 Z8	*Ectocarpus* sp. 7	Ec343 x Ec32	Chile/Peru	Sporophyte	na	WT	Maternal		
855x561 Z1	*Ectocarpus* sp. 7	Ec855 x Ec561	Chile/Peru	Sporophyte	na	*ORO/oro*	Maternal		
855x561 Z2	*Ectocarpus* sp. 7	Ec855 x Ec561	Chile/Peru	Sporophyte	na	*ORO/oro*	Maternal		
855x561 Z3	*Ectocarpus* sp. 7	Ec855 x Ec561	Chile/Peru	Sporophyte	na	*ORO/oro*	Maternal		
855x561 Z4	*Ectocarpus* sp. 7	Ec855 x Ec561	Chile/Peru	Sporophyte	na	*ORO/oro*	Paternal		
855x561 Z7	*Ectocarpus* sp. 7	Ec855 x Ec561	Chile/Peru	Sporophyte	na	*ORO/oro*	Paternal		
855x561 Z8	*Ectocarpus* sp. 7	Ec855 x Ec561	Chile/Peru	Sporophyte	na	*ORO/oro*	Paternal		
855x561 Z11	*Ectocarpus* sp. 7	Ec855 x Ec561	Chile/Peru	Sporophyte	na	*ORO/oro*	Paternal	(P+ x P+)	
856x561 Z5	*Ectocarpus* sp. 7	Ec856 x Ec561	Chile/Peru	Sporophyte	na	*ORO/oro*	Paternal		
856x561 Z12	*Ectocarpus* sp. 7	Ec856 x Ec561	Chile/Peru	Sporophyte	na	*ORO/oro*	Maternal		
856x561 Z13	*Ectocarpus* sp. 7	Ec856 x Ec561	Chile/Peru	Sporophyte	na	*ORO/oro*	Maternal		
856x561 Z14	*Ectocarpus* sp. 7	Ec856 x Ec561	Chile/Peru	Sporophyte	na	*ORO/oro*	Maternal		
Ec702	*Ectocarpus* sp. 7	Ec568 x Ec561	Chile/Peru	Sporophyte	na	*ORO/oro*	Maternal		
Ec703	*Ectocarpus* sp. 7	Ec568 x Ec561	Chile/Peru	Sporophyte	na	*ORO/oro*	Maternal		
855x793 Z1	*Ectocarpus* sp. 7	Ec855 x Ec793	Chile/Peru	Sporophyte	na	*SAM/sam*	Paternal	(P+ x P+)	

Strain name	Species	Genetic history	Geographical origin of strain	Generation	Sex	Genotype	Mitochondrial inheritance	Parthenogenetic capacity	Strain used for gamete mitocondria counts?
855x793 Z2	*Ectocarpus* sp. 7	Ec855 x Ec793	Chile/Peru	Sporophyte	na	*SAM/sam*	Paternal		
855x793 Z4	*Ectocarpus* sp. 7	Ec855 x Ec793	Chile/Peru	Sporophyte	na	*SAM/sam*	Paternal		
855x793 Z5	*Ectocarpus* sp. 7	Ec855 x Ec793	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
855x793 Z6	*Ectocarpus* sp. 7	Ec855 x Ec793	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
855x793 Z8	*Ectocarpus* sp. 7	Ec855 x Ec793	Chile/Peru	Sporophyte	na	*SAM/sam*	Paternal		
855x793 Z9	*Ectocarpus* sp. 7	Ec855 x Ec793	Chile/Peru	Sporophyte	na	*SAM/sam*	Paternal		
855x793 Z11	*Ectocarpus* sp. 7	Ec855 x Ec793	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
855x793 Z12	*Ectocarpus* sp. 7	Ec855 x Ec793	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
855x793 Z13	*Ectocarpus* sp. 7	Ec855 x Ec793	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
855x793 Z14	*Ectocarpus* sp. 7	Ec855 x Ec793	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
855x793 Z15	*Ectocarpus* sp. 7	Ec855 x Ec793	Chile/Peru	Sporophyte	na	*SAM/sam*	Paternal		
855x793 Z16	*Ectocarpus* sp. 7	Ec855 x Ec793	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
855x793 Z17	*Ectocarpus* sp. 7	Ec855 x Ec793	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
855x793 Z18	*Ectocarpus* sp. 7	Ec855 x Ec793	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
Ec818	*Ectocarpus* sp. 7	Ec343 x Ec793	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
Ec819	*Ectocarpus* sp. 7	Ec343 x Ec793	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
855x374 Z1	*Ectocarpus* sp. 7	Ec855 x Ec374	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
855x374 Z2	*Ectocarpus* sp. 7	Ec855 x Ec374	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
855x374 Z3	*Ectocarpus* sp. 7	Ec855 x Ec374	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
855x374 Z4	*Ectocarpus* sp. 7	Ec855 x Ec374	Chile/Peru	Sporophyte	na	*SAM/sam*	Paternal		
855x374 Z5	*Ectocarpus* sp. 7	Ec855 x Ec374	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
855x374 Z6	*Ectocarpus* sp. 7	Ec855 x Ec374	Chile/Peru	Sporophyte	na	*SAM/sam*	Paternal		
855x374 Z7	*Ectocarpus* sp. 7	Ec855 x Ec374	Chile/Peru	Sporophyte	na	*SAM/sam*	Paternal		
855x374 Z8	*Ectocarpus* sp. 7	Ec855 x Ec374	Chile/Peru	Sporophyte	na	*SAM/sam*	Paternal		
855x374 Z9	*Ectocarpus* sp. 7	Ec855 x Ec374	Chile/Peru	Sporophyte	na	*SAM/sam*	Paternal		

Strain name	Species	Genetic history	Geographical origin of strain	Generation	Sex	Genotype	Mitochondrial inheritance	Parthenogenetic capacity	Strain used for gamete mitocondria counts?
Ec768	*Ectocarpus* sp. 7	Ec568 x Ec374	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
Ec769	*Ectocarpus* sp. 7	Ec568 x Ec374	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
855x364 Z1	*Ectocarpus* sp. 7	Ec855 x Ec364	Chile/Peru	Sporophyte	na	*SAM/sam*	Paternal		
855x364 Z2	*Ectocarpus* sp. 7	Ec855 x Ec364	Chile/Peru	Sporophyte	na	*SAM/sam*	Paternal		
855x364 Z3	*Ectocarpus* sp. 7	Ec855 x Ec364	Chile/Peru	Sporophyte	na	*SAM/sam*	Paternal		
855x364 Z4	*Ectocarpus* sp. 7	Ec855 x Ec364	Chile/Peru	Sporophyte	na	*SAM/sam*	Paternal		
855x364 Z5	*Ectocarpus* sp. 7	Ec855 x Ec364	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
855x364 Z6	*Ectocarpus* sp. 7	Ec855 x Ec364	Chile/Peru	Sporophyte	na	*SAM/sam*	Paternal		
855x364 Z8	*Ectocarpus* sp. 7	Ec855 x Ec364	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
855x364 Z9	*Ectocarpus* sp. 7	Ec855 x Ec364	Chile/Peru	Sporophyte	na	*SAM/sam*	Maternal		
855x364 Z10	*Ectocarpus* sp. 7	Ec855 x Ec364	Chile/Peru	Sporophyte	na	*SAM/sam*	Paternal		
855x364 Z11	*Ectocarpus* sp. 7	Ec855 x Ec364	Chile/Peru	Sporophyte	na	*SAM/sam*	Paternal		
EA1	*Ectocarpus siliculosus*	Field isolate	Naples, Italy	Gametophyte	female	WT	nd	P+	
RB1	*Ectocarpus siliculosus*	Field isolate	Naples, Italy	Gametophyte	male	WT	nd	P-	
EcNAP12-24	*Ectocarpus siliculosus*	Field isolate	Naples, Italy	Gametophyte	female	WT	nd	P+	
EcNAP12-083	*Ectocarpus siliculosus*	Field isolate	Naples, Italy	Gametophyte	male	WT	nd	P-	Yes
EcNAP12-88	*Ectocarpus siliculosus*	Field isolate	Naples, Italy	Gametophyte	female	WT	nd	P+	Yes
EcNAP12-80	*Ectocarpus siliculosus*	Field isolate	Naples, Italy	Gametophyte	male	WT	nd	P-	Yes
Ec236-154	*Ectocarpus siliculosus*	EA1 x RB1	Naples, Italy	Gametophyte	male	WT	Maternal	P+	Yes
Ec236-191	*Ectocarpus siliculosus*	EA1 x RB1	Naples, Italy	Gametophyte	male	WT	Maternal	P-	Yes
Ec236-21	*Ectocarpus siliculosus*	EA1 x RB1	Naples, Italy	Gametophyte	male	WT	nd	P+	Yes
Ec236-159	*Ectocarpus siliculosus*	EA1 x RB1	Naples, Italy	Gametophyte	female	WT	nd	P+	Yes
Ec236-87	*Ectocarpus siliculosus*	EA1 x RB1	Naples, Italy	Gametophyte	male	WT	nd	P-	Yes
Ec236-199	*Ectocarpus siliculosus*	EA1 x RB1	Naples, Italy	Gametophyte	female	WT	nd	P+	Yes
12-024x154 Z1	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal	(P+ x P-)	

Strain name	Species	Genetic history	Geographical origin of strain	Generation	Sex	Genotype	Mitochondrial inheritance	Parthenogenetic capacity	Strain used for gamete mitocondria counts?
12-024x154 Z2	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x154 Z3	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x154 Z4	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x154 Z5	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x154 Z6	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x154 Z7	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x154 Z8	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x154 Z9	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x154 Z10	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x154 Z11	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x154 Z12	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x154 Z13	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x154 Z14	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x154 Z15	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x154 Z16	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x154 Z17	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x154 Z18	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x154 Z19	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x154 Z20	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-154	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x191 Z1	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal	(P+ x P+)	
12-024x191 Z2	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x191 Z3	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x191 Z4	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x191 Z5	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x191 Z6	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal		

Strain name	Species	Genetic history	Geographical origin of strain	Generation	Sex	Genotype	Mitochondrial inheritance	Parthenogenetic capacity	Strain used for gamete mitocondria counts?
12-024x191 Z7	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x191 Z8	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x191 Z9	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x191 Z10	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x191 Z11	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x191 Z12	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x191 Z13	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x191 Z14	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x191 Z15	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x191 Z16	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x191 Z17	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x191 Z18	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x191 Z19	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal		
12-024x191 Z20	*Ectocarpus siliculosus*	EcNAP12-024 x Esil236-191	Naples, Italy	Sporophyte	na	WT	Maternal		

Table S2. List of dCAPS primers used to genotype mitochondrial DNA in zygotes.

					PCR product after digestion	
Primer name	**SNP position**	**Sequence**	**Tm**	**Enzyme**	**female mtDNA**	**male mtDNA**
Mito_EcNAP 11 F	19875	TATTTTACAGCAGCAACTATGATTACTGC	58°C	*Pst*I	421 + 30 bp	451 bp
Mito_EcNAP 11 R		CGATACCATTCCAACCAGCG				
Mito_EcNAP 12 F	24427	GCAAGTATCTTTTGATAAAGCTAAAAATA	58°C	*Ssp*I	347 + 92 bp	319 + 92 + 28 bp
Mito_EcNAP 12 R		AGCAAACTCTAATTGTGCGCT				
Mito 6 F	22689	GAGTCAGCTCATTCAAATCCTGT	55°C	*Mbo*I	138 + 90 bp	138 + 60 + 30 bp
Mito 6 R		CTTACTTACTTCTGGAACCACATCAAG				
Mito 5 F	6465	GGGGGTTTGTGTTACAAAAGG	55°C	*Hpa*II	221 + 21 bp	242 bp
Mito 5 R		TAAAGCAAAAGCACACGCACC				
Mito 3 F	31241	ATTTTAGCTGTTTCAGATGCGGATAT	55°C	*Eco*RV	191 + 24 bp	215 bp
Mito 3 R		TTGAAGGTAACTGACTTAAAGAAACTG				

Table S3. Intra-specific mitochondrial DNA variants detected in this study.

Reference mitochondrial genome	Variant type	Position	EA1	RB1	EcNAP12-024	Ec32	Ec568
EA1	SNP	2257	T	C	C	n/a	n/a
EA1	Indel	3447-3448	AA	AATATA	AATATA	n/a	n/a
EA1	SNP	3613	T	C	C	n/a	n/a
EA1	SNP	5190	T	C	C	n/a	n/a
EA1	SNP	7886	T	C	C	n/a	n/a
EA1	SNP	12495	A	A	G	n/a	n/a
EA1	SNP	12506	T	G	G	n/a	n/a
EA1	SNP	15270	C	T	T	n/a	n/a
EA1	SNP	17527	G	C	C	n/a	n/a
EA1	SNP	19321	A	G	G	n/a	n/a
EA1	SNP	19875	A	G	G	n/a	n/a
EA1	SNP	23153	T	C	C	n/a	n/a
EA1	SNP	24427	C	T	T	n/a	n/a
EA1	SNP	25065	C	T	T	n/a	n/a
EA1	SNP	30384	T	G	G	n/a	n/a
EA1	SNP	32379	C	A	A	n/a	n/a
EA1	SNP	32380	T	G	G	n/a	n/a
EA1	Indel	35371-35372	CT	CTT	CTT	n/a	n/a
EA1	Indel	35544-35545	AG	AGGGG	AGGGG	n/a	n/a
EA1	SNP	35586	A	G	G	n/a	n/a
EA1	Indel	35882	T	-	-	n/a	n/a
EA1	Indel	36062-36063	GA	GTA	GTA	n/a	n/a
EA1	SNP	36200	C	A	A	n/a	n/a
EA1	SNP	36291	C	T	T	n/a	n/a

Reference mitochondrial genome	Variant type	Position	EA1	RB1	EcNAP12-024	Ec32	Ec568
EA1	Indel	36498-36499	TG	TGG	TGGG	n/a	n/a
EA1	SNP	36517	A	G	G	n/a	n/a
EA1	SNP	36577	T	C	C	n/a	n/a
EA1	Indel	38286	G	-	-	n/a	n/a
Ec32	SNP	5389	n/a	n/a	n/a	G	A
Ec32	SNP	6465	n/a	n/a	n/a	T	C
Ec32	SNP	22689	n/a	n/a	n/a	T	C
Ec32	SNP	29331	n/a	n/a	n/a	T	C
Ec32	SNP	31241	n/a	n/a	n/a	T	C
Ec32	Indel	35081	n/a	n/a	n/a	T	-

Discussions and perspectives

In the previous chapter, *Ectocarpus siliculosus* was used to determine the genetic basis of parthenogenesis and indicated that male parthenogenesis is a sexual antagonistic trait in this species because gametes of parthenogenetic males exhibited reduced fusion success and the zygotes formed grew more slowly than those derived from non-parthenogenetic males. In this chapter 3, we determine that mitochondrial inheritance was strictly maternal in *Ectocarpus siliculosus* regardless of the parthenogenetic capacity of the male parent. Mitochondrial inheritance was assessed in adult sporophytes while zygotic growth observations were performed at early stages development. Taken together these results suggest that the parthenogenetic capcity of the male parent does not have an effect on mitochondrial inheritance in offspring. However, the number of mitochondria in parthenogenetic male gametes was significantly higher than in non-parthenogenetic, which could affect the growth of zygotes at early stages, if not the selective degradation of male mitochondria over time. The reduced rate of zygotic growth observed when a parthenogenetic male was used as a parent could be due to cytoplasmic conflict following fusion of gametes because the number of male mitochondria is similar to that of the female. As a consequence, the selective degradation of male mitochondria could take longer causing a retardation of zygotic growth. The dynamics of mitochondria inheritance needs to be further investigated at early stages development to test this hypothesis. Two different Mitotracker markers could be used to independently stain male and female mitochondria in gametes before performing a cross. Then, after gamete fusion, the disappearance of male mitochondria could be assessed to determine if the rates of zygotic growth and mitochondria disappearance are correlated in *Ectocarpus siliculosus*. Previous work on *Scytosiphon lomentaria* showed that, following zygote formation, male mitochondria are eliminated by the four-cell stage leading to strictly maternal inheritance (Kimura *et al.*, 2010). These results were obtained by single cell PCR detection of a specific mitochondrial marker. Similar experiments could be conducted with *Ectocarpus siliculosus* zygotes to determine how long the mitochondrial DNA of both parents is present during zygote development. Similar experiments, i.e. assessing the disappearance of mitochondria in zygotes either by staining specically male and female mitochondria or

sequencing mtDNA from zygotes, could also be carried out to investigate the unsual transmission observed in *Ectocarpus* species 7.

Mitochondria inheritance in *Ectocarpus* species 7 was found to be uniparental but involved random transmission of either the paternal or the maternal mitochondria. To our knowledge, this surprisingly transmission pattern has not been described in any other eukaryotic lineage to date. The sensitivlity of the specific mitochondrial markers needs to be taken into account as this may have prevented the detection of some degree of biparental transmission. Nonetheless, these markers were able to detect mtDNA when it was majoritarily present in a mix of maternal and paternal mtDNA indicating that the sporophytes reported as having inherited the paternal mtDNA were presumably carrying more male than female mitochondria. The genomic DNA used in the first PCR-based mtDNA detection experiments was extracted from almost the whole individual but a second experiment was carried out in which extraction was carried out using different parts of the sporophyte to verify that the individuals were not mosaic for both maternal and paternal mitochondria (e.g as is the case for the choloroplast). For *Ectocarpus* species 7, the male parent used for crosses corresponded to strain Ec32 (reference genome) and was maintained in culture in the laboratory through the parthenogenetic cycle. Therefore, to further confirm that the transmission pattern observed in this study was not an artefact caused by laboratory culture conditions, crosses with another male strain from a natural population are needed. These crosses are in progress.

Across the eukaryotes, uniparental inheritance of maternal mitochondria DNA (mtDNA) is mediated by various mechanisms that prevent the transmission of sperm-derived paternal mitochondrial DNA to the offspring. Most of these mechanisms involve mitochondrial autophagy with paternal mitochondrial being selectively degraded following ubiquitination (Sato and Sato, 2017). Others, such as that in the Japanese rice fish (*Oryzias latipes*), trigger specific degradation of paternal mtDNA rather than the organelle (Nishimura *et al.*, 2012). However, it is difficult to evoke any of these types of mechanism to account for the random uniparental transmission of mitochondria observed in *Ectocarpus*. Rather than focus on a mechanism that selectively removes the mitochondrial DNA from one parent, we are exploring the potential of systems involving heteroplasmic transmission and subsequent uniparental inheritance mediated by differential segregation and transmissionof mitochondria through multiple cell divisions. A model of this type has been developed for

yeast (Birky *et al.*, 1978) but it is not applicable to *Ectocarpus* because yeast cell division involves a specific mechanism, called budding, which create a severe bottleneck between mother and daughter cells. We are therefore working on an alternative mathematical model based on the cell cycle to determine if the observed transmission pattern could occur solely by a processinvolving cell division and cell differentiation. At present this model proposes a possible mechanism for uniparental mitochondrial transmission but does not yet incorporate random inheritance of either maternal or paternal mitochondria.

The mathematical model, based on pre-existing models elaborated for hematopoietic stem cells (Djema *et al.*, 2018), was developed to explore possible mechanisms of mitochondrial inheritance in *Ectocarpus* species 7, in particular to propose mechanisms that could randomly lead to either maternal or parental uniparental inheritance. Development of the partheno-sporophyte was modelled as a simple process involving phases of proliferation and differentiation. The proliferation phase P corresponds to the G1, S, G2 and M phases of the cell cycle while the resting phase R corresponds to G0, which can either be temporary, allowing re-entry into the cell cycle, or permanent, i.e. differentiation (Figure 1A, 1B).

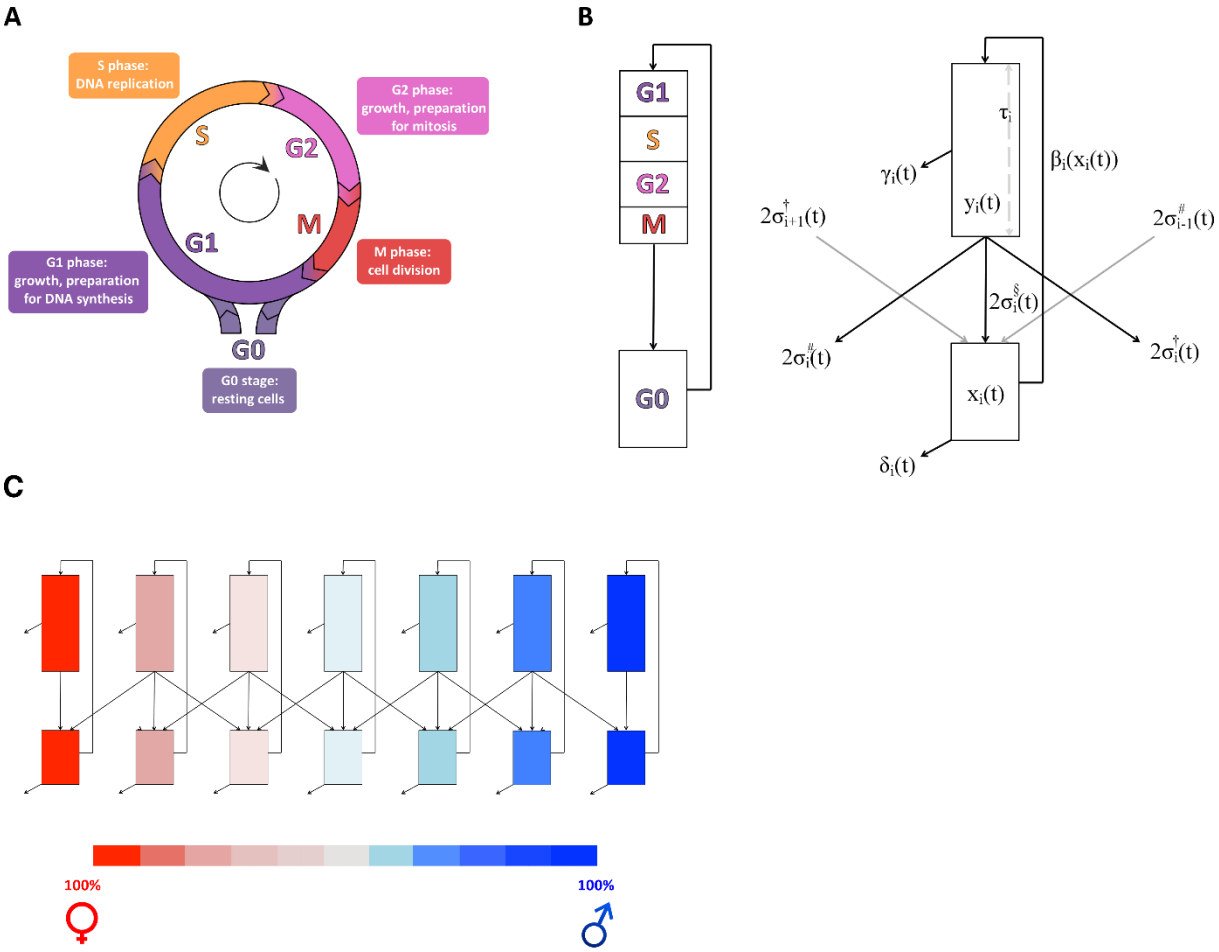

Figure 1: Representation of the biological cell cycle into mathematical modelling. A. Biological cell cycle with the proliferation phase corresponding to the G1, S, G2 and M phases. G1 correspond to cells preparing for DNA synthesis, which occurs durin the S phase. Then, cells prepare for mitosis in the G2 phase while cell division occurs in the M phase. Cells that are not inone of those phases are resting in the G0 phase. **B.** Representation of the biological cycle from the mathematical modelling with a proliferation compartment corresponding to the G1, S, G2 and M phases and the resting compartment corresponding to the G0 phase. Each compartment is defined by several mathematical equation (see text). **C.** The sporophyte organism is represented by different sub-population based on their mitochondrial content ranged from homoplasmic (100% red or 100% blue) to different degree of heteroplasmy.

The general model consists of multiple sub-populations of cells. In a cell sub-population i, the total density of proliferating cells is defined by: $y_i(t) = \int_0^{\tau_i} p_i\,(t,a)$ and the total density of resting cells is defined by: $x_i(t) = \int_0^{+\infty} r_i(t,a)da$. The dynamics of resting $r_i(t,a)$, and proliferating cells $p_i(t,a)$, of the i[th] sub-population ($i \in I_n = \{1,\dots,n\}$), of age $a > 0$, at time $t \geq 0$, are governed by a partial-differential equation (PDE) of McKendrick-type (McKendrick, 1925):

$$\begin{cases} \dfrac{\partial p_i(t,a)}{\partial t} + \dfrac{\partial p_i(t,a)}{\partial a} = -\gamma_i(t)p_i(t,a), \\ \dfrac{\partial r_i(t,a)}{\partial t} + \dfrac{\partial r_i(t,a)}{\partial a} = -\left[\delta_i(t) + \beta_i\left(\int_0^{+\infty} r_i(t,a)da\right)\right]r_i(t,a), \end{cases} \quad (1)$$

This general equation contains several parameters including $\delta_i(t)$, the natural differentiation rate of resting cells, and $\gamma_i(t)$, the death rate of proliferating cells ('apoptosis'). The latter was set at zero as no evidence of apoptosis was observed in *Ectocarpus* after fertilisation. The equation also allows a fraction of resting cells to begin a new proliferation phase with the term β_i. This term allows infinite proliferation to be excluded and normalizes the total cell density (i.e. $x_i + y_i$). In our numerical simulations we chose the Hill function (Keener and Sneyd, 2009; Mackey, 1978; Qian, 2012):

$$\beta_i(x_i) = \frac{\beta_i(0)}{1+x_i^2} \qquad (2)$$

With this model, the inheritance of mitochondria is an implicit process that depends on each cell's capacity to proliferate. We considered a discrete distribution of nine cell sub-populations with different levels of heteroplasmy, the extreme cell sub-populations being homoplasmic for only one parental type of mitochondrium, maternal or paternal (Figure 1C). We assumed that cytoplasmic conflict is not optimal for cell proliferation and therefore during proliferation cell with a mix of male and female mitochondria will tend to segregate by separating their type of mitochondria. After the proliferation process, new cell sub-populations are assigned to either the i+1 or the i-1 sub-population based on their mitochondrial content. Cells with a mitochondrial content that is predominantly paternal are assigned to the appropriate resting cell sub-population (x_i) by the term: $2\sigma_i^{\#}(t)$. In contrast, if the mitochondrial content is predominantly maternal after the proliferation process then a proportion $2\sigma_i^{\dagger}(t)$ of cells will be assigned to the x_{ith} sub-population (Figure 1C). Finally, if the proliferating cells have only maternal or only paternal mitochondria they undergo a self-renewing process defined by the term: $2\sigma_i^{\S}(t)$. These renewal conditions, which give the birth rate at the initial age $a = 0$, are introduced through the boundary conditions:

$$\begin{cases} p_i(t,0) = \beta_i\big(x_i(t)\big)x_i(t), \\ r_i(t,0) = 2\sigma_{i-1}^{\#}(t)p_{i-1}(t,\tau_{i-1}) + 2\sigma_i^{\S}(t)p_i(t,\tau_{i-1}) + 2\sigma_{i+1}^{\dagger}(t)p_{i+1}(t,\tau_{i+1}), \end{cases} \qquad (3)$$

Where, for all $i \in I_n$,

$$\sigma_i^{\dagger} + \sigma_i^{\#} + \sigma_i^{\S} = 1$$

The dynamic of the system is defined by the equation $\dot{x}(t)$:

$$\dot{x}(t) = -\Big(\delta_i(t) + \beta_i\big(x_i(t)\big)\Big)\, x_i(t) + 2\sigma_{i-1}^{\#}(t)e^{-\gamma_{i-1}\tau}\beta_{i-1}\big(x_{i-1}(t-\tau)\big)x_{i-1}(t-\tau) + 2\sigma_i^{\S}(t)e^{-\gamma_i\tau}\beta_i\big(x_i(t-\tau)\big)x_i(t-\tau) + 2\sigma_{i+1}^{\dagger}(t)e^{-\gamma_{i+1}\tau}\beta_{i+1}\big(x_{i+1}(t-\tau)\big)x_{i+1}(t-\tau). \quad (4)$$

The length of cell cycle was estimated at 4 days according to observations of mitotic division of zygotes. The Hill function for the parameter β_i was considered to be dependent on $x_i(t)$. Matlab was used to numerically integrate this system and model the equation $\dot{x}(t)$. Using this model we then formulated the optimal control problem as the minimal time to converge to homoplasmy (x_1 or x_9) using the rate of cell differentiation as a control (Figure 2). We tried to optimise the model using cell differentiation as a control. The objective was to determinate the minimal time require for the model to reach the homoplasmic state. The values of some parameters (apoptosis ($\gamma_i(t)$), and the σ terms) for each cell sub-populations are determined (Figure 2A). The cell differentiation (δ_i) was used as the control parameter allowed to vary to reach homoplasmy state. Results show a decrease from all cell sub-population except for one (x1) reaching zero in about 8 days. In contrast the homoplasmic cell sub-population (x1) persisted (Figure2 C). The model predicted that in an optimal way, in about 8 days the homoplasmic state can be reached. In the Figure 2C, homoplasmy is reach through the x1 cell sub-population but it could also occur through the x9 cell sub-population suggesting a random process.

The model qualitatively and implicitely show that homoplasmic state can be reach through cell division and cell differentiation. However, no data were collected on the cell differentiation variation and mitochondria content of cells. Despite confirming that random uniparental mitochondrial transmission can occur via a cell cycle and differentiation process, this model does not demonstrate that it this is effectively what happens in *Ectocarpus* species 7.

It is clear that we need more data on the process of mitochondrial inheritance to optimise the model. Moreover, this model is based on populations of cells and it is likely that stochastic events at early stages of development (i.e. right after fusion) influence the mitochondrial inheritance pattern.

The novel pattern of random mitochondrial inheritance raised a lot of questions concerning the mechanisms and regulation of such transmission. Despite the unusual pattern, however,

Ectocarpus species 7 nonetheless exhibits uniparental inheritance which is assumed to be the optimal mechanism of transmission to avoid cytoplasmic conflict (Greiner *et al.*, 2015).

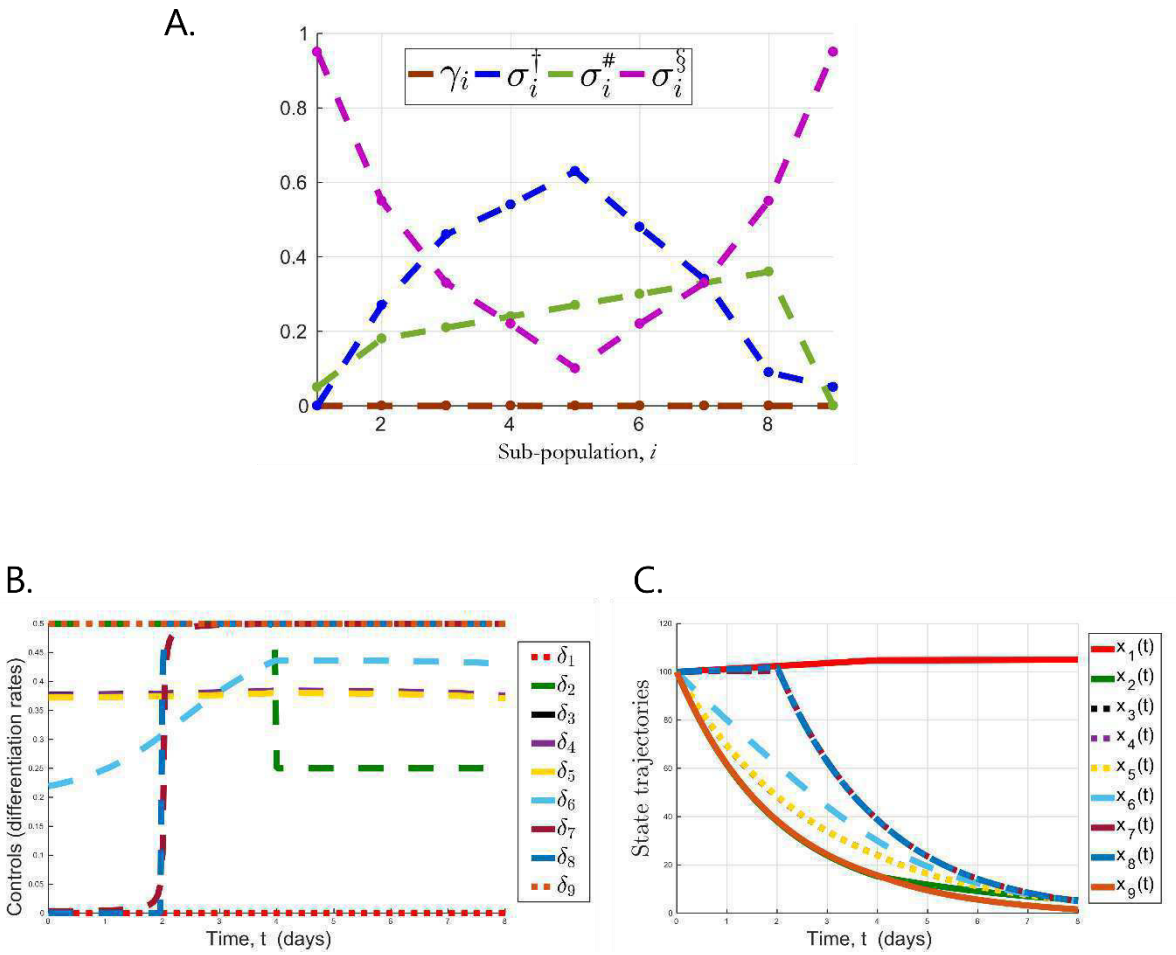

Figure 2: Simulation results of the mathemaical model for random uniparental inheritance based on cell cycle and cell differentiation. A. Evolution of the parameter depending on the cell sub-population. The apoptosis (γ_i) was set at zero as no cell death was observed in the algae. The sum of each sigma term in a cell sub-population is equal to one. **B.** The optimal control system requires a parameter that can vary to allow homoplasmy. Cell differentiation rate was choose and the variation of this parameter in each cell sub-population permit the establishment of homoplasmy in the miimal time of 8 days. **C.** Evolution of the density of resting cells in each cell sub-population over time in accordance' with the variation of cell differentiation variation given in B. Here, the variation of cell differentiation lead to homoplasmy with the survival of the x1 cell sub-population. In another simulation it could have lead to the survival of the x9 cel sub-population.

Chapter IV

Comparative analysis of the genetic maps of two closely related *Ectocarpus* species

Introduction

Over the last few decades an important investment has been made in the development of genetic and genomic approaches for the brown algae, especially since 2004 (Peters *et al*., 2004) when *Ectocarpus* sp. was proposed as a model organism for this lineage. In 2010, the *Ectocarpus* genome, the first complete genome sequence for a brown alga, was obtained (Cock *et al*., 2010) providing new possibilities to combine genetic and genomic approaches to gain insights into the biology of brown algae. The *Ectocarpus* genome has since been used to investigate genetic control of sexual reproduction (Ahmed *et al*., 2014; Luthringer *et al*., 2015), the genetic basis of environmental adaptations (Avia *et al*., 2017) and the mechanisms involved in the regulation of developmental patterning in the sporophyte and gametophyte generations (Macaisne *et al*., 2017; Godfroy *et al*., 2017).

One of the most important tools that has been developed for the reference strain (Ec32, *Ectocarpus* sp.) is a high density genetic map. A first genetic map was constructed in 2010 using 406 microsatellite markers and 60 individuals of a segregating family derived from a sporophyte parent (strain Ec569) that had been derived by crossing two *Ectocarpus* sp. strains, Ec568 and Ec32 (Heesch *et al*., 2010). This map consisted of 34 linkage groups, including 26 large linkage groups and eight smaller linkage groups. The 406 microsatellite markers allowed 325 of the longest supercontigs (70.1% of the genome sequence) of the reference genome (Cock *et al*., 2010) to be anchored onto the genetic map.

In 2017, the genetic map was significantly improved using a restriction site associated DNA sequencing (RAD-seq) approach (Avia *et al*., 2017). RAD-seq allows the complexity of a genome to be reduced in order to detect high confidence SNPs effectively (Baird *et al*., 2008; Peterson *et al*., 2012).

Three thousand five hundred and eighty SNP markers were generated from RAD-seq libraries produced for the two parents (Ec32 and Ec568) and 89 offspring of the hybrid sporophyte Ec569. The markers were distributed homogenously along 28 linkage groups (LGs). The total genetic distance covered by the 3588 markers was 2585.7 cM. The sizes of the LGs varied substantially from 152.3 cM for LG1 (with 217 markers) to 41.8 cM for the smallest linkage group LG28 (with 54 markers; Table 1). The average spacing between markers was 0.7 cM with the highest average spacing (1.5 cM) on LG21 and the lowest average spacing (0.4 cM) on LG3.

Table 1: Linkage group statistics for the *Ectocarpus* species 7 genetic map. (Extracted from Avia *et al.*, 2017).

***Ectocarpus* sp7**				
LG	Number of markers	LG length (cM)	Average spacing between markers (cM)	Maximum spacing between markers (cM)
1	217	152.3	0.7	11.2
2	194	96.5	0.5	5.3
3	202	90.4	0.4	8.5
4	180	116.7	0.7	7.6
5	168	117.9	0.7	6.7
6	171	110.4	0.6	8.8
7	170	113.4	0.7	9.3
8	152	88.7	0.6	6.3
9	155	87.8	0.6	7.6
10	138	92	0.7	8
11	120	92	0.8	10
12	127	112.4	0.9	8.9
13	124	92.6	0.8	7.6
14	124	96.7	0.8	6.3
15	119	83.1	0.7	7.8
16	106	80.7	0.8	12.8
17	111	64.9	0.6	7.6
18	116	74.8	0.7	11.5
19	107	78.5	0.7	8.7
20	112	81.8	0.7	10.1
21	87	128.9	1.5	12.8
22	103	71.4	0.7	18.3
23	91	72.4	0.8	8.8
24	90	71.6	0.8	8.8
25	96	71.9	0.8	7
26	79	112.5	1.4	16.9
27	75	91.5	1.2	19.4
28	54	41.8	0.8	20.3
overall	3588	2585.7	0.7	20.3

The maximum spacing between markers was on LG28, where 20.3 cM separated two markers. This high density genetic map was used to significantly improve the large-scale assembly of the genome *Ectocarpus* species 7 genome (strain Ec32), which together with detailed re-annotation, produced an assembly of very high quality (Cormier *et al*., 2017). The current *Ectocarpus* Ec32 pseudochromosomal assembly consists of 90.5% of the genome sequence scaffolds, assigned to 28 linkage groups. The recombination rate for *Ectocarpus* was estimated to 12.28 cM/Mb which is higher than values obtained for most terrestrial plant species.

The improved version of the *Ectocarpus* Ec32 genetic map has been instrumental a number of recent studies, including analysing sex-biased gene expression (Lipinska *et al*., 2015), investigating the evolution of candidate male reproductive genes and studying the mechanisms underlying gene movement between sex determining region and the pseudo-autosomal region of the sex chromosomes or autosomes(Lipinska *et al*., 2017), and quantitative trait loci (QTLs) involved in the responses to salinity and temperature stress (Avia *et al*., 2017).

Although the genome sequenced strain (Ec32) was initially thought to belong to the species *Ectocarpus siliculosus*, it has recently become clear that this strain corresponds to a separate species (*Ectocarpus* species 7) that had not been described previously (Montecinos *et al*., 2017). More recently, we have used *Ectocarpus siliculosus sensu stricto* strains from Naples (which correspond to *Ectocarpus* species 13 in Montecinos *et al*.) to study several aspects of the biology of Ectocarpus, including mitochondrial inheritance (chapter 3) and the genetic basis of parthenogenesis (chapter 2). As a result, it has become necessary to develop genomic tools for *E. siliculosus sensu stricto*, including a dense genetic map. Knowledge of the exact position of the borders of the SDR and PAR were particularly important in the context of a study on neutral diversity in the PAR region (Avia *et al*., 2018, Appendix 1) and the map was also required to determine the QTL intervals in the study on the genetic architecture of parthenogenesis (chapter 2). Note that in this chapter, we will distinguish *Ectocarpus siliculosus* (*E. sil*) corresponding to *Ectocarpus* species 13 and *Ectocarpus* species 7.

Although Montecinos et al established that *E. siliculosus* and *E.* species 7 were two cryptic species, the time of divergence of the two species was not determined. However, *E.* species 7 was used in a study aiming at examining the divergent times between 44 brown algal species (Kawai *et al*., 2015). Among these brown algae species and particularly among Ectocarpales

used in this study, *Kuckuckia kylinii* was the closest species to *E*. species 7, with a divergence time of 17 million years. We can therefore assume that the distance separating our two species of *Ectocarpus* is less than 17 million years.

In this Chapter, we describe in detail the construction of an *E. siliculosus sensu stricto* (*Ectocarpus* species 13) genetic map using RAD-seq markers. The availability of genetic maps for two closely related species of *Ectocarpus* provided an opportunity to look at the evolution of the genomes at a large structural scale since the divergence of the two species. This chapter also describes the comparative analysis of the *E.* species 7 (Ec32) and *Ectocarpus siliculosus sensu stricto* genetic maps.

Material and methods

Generation of an *Ectocarpus siliculosus* mapping family

An *Ectocarpus siliculosus* mapping family was generated by crossing a female strain (EA1) with a compatible male strain (RB1) to produce a diploid sporophyte, strain Ec236. This single F1 hybrid sporophyte was used to produce F2 progeny of sibling haploid gametophytes (about 1700). Each of these 1700 haploid gametophytes was derived from a single meiotic event. One hundred fifty two individuals and the parental strains were cultivated for the experiment.

Culture conditions and DNA extraction

The 152 strains of the segregating F2population derived from the cross between female EA1 and male RB1 were cultured in autoclaved sea water supplemented with half strength Provasoli solution (Starr and Zeikus, 1993) at 13°C, with a light dark cycle of 12:12 (20 µmol photon m-2 s-1) using daylight-type fluorescent tubes (Coelho *et al*., 2012). Harvested individuals were frozen and lyophilized, and DNA extraction was performed for each individual (Macherey-Nagel, NucleoSpin® Plant II kit, GmbH & Co.KG, Germany). DNA quantity was measured and standardized at 100 ng using a PicoGreen® (Fischer Scientific) method for quantification. The DNA quality was checked on agarose gels.

Generation of RAD-seq data

Restriction site associated DNA sequencing (RAD-Seq) is a fractional genome sequencing strategy that requires a choice of appropriate restriction enzymes to generate a maximum of fragments of the appropriate size according to the sequencing strategy. We choose to use an Illumina HiSeq 2500 (Rapid Run Mode) and expected to generate 500 million reads of size 250 bp (paired-end). Based on the genome size (214 Mbp) of *Ectocarpus* species 7, it was estimated that 55 555 fragments were required to obtain a 30X depth of coverage. Using the package Simrad in RStudio, an *in silico* digestion simulation was performed using the Ec32 genome (Cock *et al*., 2010) and several couples of restriction enzymes were tested to determine the best combination to generate around 55555 fragments of sizes between 550 and 800 bp. The *Hha*I and *Sph*I restriction enzymes (New England Biolabs, https://www.neb.com/) were selected as the simulation estimated the production of 49347 fragments in the required range. A double digest RAD library (ddRAD-seq) was constructed for *Ectocarpus siliculosus* as described by (Brelsford *et al*., 2016). After restriction digestion, DNA samples were individually barcoded using unique adapters by ligation with T4 DNA ligase (New England Biolabs, https://www.neb.com/). Then, samples were cleaned with AMPure XP beads (Beckman Coulter Genomics), and PCR was performed with the Q5® hot Start High-Fidelity DNA polymerase kit (New England Biolabs, https://www.neb.com/) to increase the DNA concentration for each individual and to add Illumina flowcell annealing sequences, multiplexing indices and sequencing primer annealing regions. After pooling the barcoded and indexed samples, PCR products of between 550 and 800 bp were selected using a Pippin-Prep kit (Sage Science, Beverly, MA, USA), and the library was quantified using both an Agilent® 2100 Bioanalyzer (Agilent Technologies) and qPCR. The library was sequenced on Illumina a HiSeq 2500 lanes (Rapid Run Mode) to generate 250 bp paired-end reads.

The ddRAD-seq data were analysed with the Stacks pipeline (version 1.44) (Catchen *et al*., 2013). The raw sequences reads were filtered to remove reads lacking barcodes and restriction enzyme sites. Sequence quality was checked using a sliding window of 25% of the length of a read and reads with <90% base call accuracy were discarded. Using the program PEAR (version 0.9.10, Zhang *et al*., 2014) paired-end sequences of short fragments generating overlapping reads were treated to build a single consensus sequence. PEAR identifies paired-end non-overlapping reads and generates a single consensus sequence from overlapping

reads pair. These single consensus sequences were added to the singleton rem1 and rem2 sequences produced by Stacks to form a unique group of singleton sequences. Paired-end reads and singleton sequences were then trimmed to 100 bp with the program TRIMMOMATIC (version 0.32, Bolger *et al*., 2014) because, contrary to the prediction, we obtained more fragments at 100bp than at 250 bp. The genome of the male parent of the population (strain RB1) has recently been sequenced and assembled (Lipinska *et al*., 2017) using the *Ectocarpus* species 7 reference genome as a guide (Cock *et al*., 2010). The assembled genome of *Ectocarpus siliculosus* was not used as reference genome to align the sequences obtained from the RAD-seq libraries as we wanted to look at the rearrangement between the two species. Therefore, we performed a *de novo* analysis running the denovo_map.pl program of Stacks. This program assembles loci for each individual *de novo* and calls SNPs at each assembled locus. The program then builds a catalog with the parental loci and loci from each individual are matched against this catalogue to determine the allelic state at each locus in each individual. We then used BWA (Li, 2013) to align the consensus sequence of the catalog loci to the reference genome and used the Python script "integrate_alignments.py" of the Stacks pipeline to integrate alignment information back into the original *de novo* map output files (Paris *et al*., 2017). Finally, SNPs were re-called for all individuals at each locus and exported as a vcf file.

Construction of an *Ectocarpus siliculosus* genetic map

The vcf file containing information on variant position between individuals was filtered and loci with more than 10% missing data along with individuals with more than 30% missing genotypes were removed. The program Lep-MAP3 (LP3) (Rastas, 2017) was used to construct a genetic map. LP3 is suitable for the analysis of low-coverage datasets and its algorithms reduces data filtering and curation of the data, yielding more markers in the final maps with less manual input. In order to obtain the expected AxB segregation type for this haploid population, a pedigree file was constructed by setting the parents as haploid grand-parents and two mock individuals were introduced for parents. The module ParentCall2 of LP3 used the pedigree and the vcf files as input to call parental genotypes. The module SeparateChromosomes2 used the genotype call file to assign markers to linkage groups (LGs). Several LOD score limits were tested to obtain an optimal LOD score of 8 giving a stable

number of LGs. The module JoinSingles2All was then run to assign singular markers to existing LGs by computing LOD scores between each single marker and markers from the existing LGs. The module OrderMarkers2 then ordered the markers within each LG by maximizing the likelihood of the data given the order. Sex averaged map distances were computed and 10 runs were performed to select the best order for each LG, based on the best likelihood. This module was run with the parameters grandparentPhase=1 and outputPhasedData=1 in order to obtain phased data for QTL mapping. This phased data was converted to fully informative genotypic data using the script map2gentypes.awk distributed with the LP3 program.

Comparison of the genetic maps for the two *Ectocarpus* species

Markers shared by *E. siliculosus* and *E.* species 7 were detected out by aligning the *E. siliculosus* marker sequences to the high quality Ec32 (*E.* species 7) genome. This step was carried out during the construction of the *E. siliculosus* genetic map using BWA (Li, 2013). BWA-MEM aligns sequence reads against a large reference genome. The algorithm is robust to sequencing errors and applicable to a wide range of sequence lengths from 70 to a few megabases. The algorithm works by seeding alignments with maximal exact matches (MEMs) and then extending the seed using the affine-gap Smith-Waterman algorithm. The setting options were to discard a MEM if it had more than two occurrences in the genome. Then the sequences were filtered with a minimum mapping quality cutoff of 10 and for sequences with two matches the longest match was retained. Finally, we used the Python script "integrate_alignments.py" of the Stacks pipeline to integrate alignment information back into the original *de novo* map output files. For markers common to both species, a location with coordinates on the physical map of *Ectocarpus* sp7 reference genome (2010) was noted (Table S2). This provided both information about synteny between the two maps and about rearrangements within linkage groups. The syntenic relationship between the *Ectocarpus siliculosus* genetic map and *Ectocarpus* sp7 chromosome sequences was plotted using the CIRCOS software (Krzywinski *et al*., 2009).

Results

Comparison of the *Ectocarpus siliculosus* and *Ectocarpus* species 7 genetic maps

An *Ectocarpus siliculosus* genetic map of was generated from progeny of a cross between the female strain EA1 and the male strain RB1 (chapter 2, figure 2 A). Of these 1700 individuals (Table S1), 152 offspring and the 2 parents were used to generate a ddRAD-seq library and sequenced on an Illumina HiSeq 2500 platform. A total of 595 million raw reads were obtained, with 508 million reads remaining after filtering for a Q30 of 74.1%. The filtered reads from the parental strains were used to generate a catalogue of 8648 SNP loci with the STACKS pipeline (version 1.44). Twenty-eight individuals were removed due to excessive missing genotypes (see Methods) and highly distorted markers were also removed. The final map was constructed with 124 individuals and contained 5594 markers distributed across 31 linkage groups (LGs). The genetic map spanned 2947.5 centimorgans (cM). The average spacing between two adjacent markers was 0.5 cM and the largest gap was 17.6 cM (on LG23). The lengths of the 31 LGs ranged from 174 cM with 397 markers in LG1 to 13 cM with 31 markers in LG31 (Table 2). The genetic map of *Ectocarpus* species 7 (Avia *et al*., 2017), which was generated using the same RAD seq method with 3588 markers, spanned 2585.7 cM (Table 1). The average spacing between two adjacent markers on the *Ectocarpus* species 7 genetic map was 0.7 cM. The largest spacing between two markers was 20.3 cM. The *Ectocarpus siliculosus* genetic map exhibited three additional linkage groups compared to the 28 pseudo-chromosomes defined in *Ectocarpus* species 7.

Table 16: Linkage group statistics for the *Ectocarpus siliculosus* genetic map.

Ectocarpus siliculosus				
LG	Number of markers	LG length (cM)	Average spacing between markers (cM)	Maximum spacing between markers (cM)
1	397	174	0.4	10.2
2	362	104.3	0.3	8.6
3	316	167.7	0.5	14.1
4	289	117.2	0.4	7.7
5	295	123	0.4	12.3
6	291	129.4	0.4	9
7	262	130	0.5	17.2
8	230	130	0.6	14.1
9	237	118	0.5	9.8
10	217	109.1	0.5	6.9
11	196	123.9	0.6	14.9
12	184	135.1	0.7	9
13	173	92.4	0.5	13.2
14	176	81.7	0.5	10.6
15	180	106.4	0.6	14.5
16	169	83.4	0.5	11.5
17	177	99.7	0.6	12.3
18	176	95.6	0.5	12.3
19	157	90.6	0.6	8.1
20	157	74.6	0.5	11.5
21	145	117.4	0.8	9.4
22	124	65.4	0.5	4.9
23	119	106.4	0.9	13.6
24	117	104.2	0.9	17.6
25	105	51.1	0.5	11.9
26	81	81.1	1	11.1
27	69	44.6	0.7	10.6
28	70	48.6	0.7	7.7
29	59	21	0.4	4
30	33	8.9	0.3	2.4
31	31	13	0.4	7.7
overall	5594	2947.5	0.5	17.6

Large-scale genome rearrangements during *Ectocarpus* speciation

Double digested RAD markers were not generated with the same digestion enzymes for the two *Ectocarpus* species. Therefore, the markers did not correspond to the same sequences of the genome. In order to compare the two genetic maps, sequences of *E. siliculosus* markers were aligned to the *Ectocarpus* species 7 genome to extract the orthologous regions. Of the 5594 *E. siliculosus* SNP markers, 4074 were successfully mapped on the *Ectocarpus* species 7 genome. About 1520 markers were specific to *E. siliculosus* genome.

The *Ectocarpus siliculosus* and *Ectocarpus* species 7 genetic maps were compared based on the markers that were common to the two species (Figure 1). Twenty two of the 31 *E. siliculosus* linkage groups were homologous to a single *Ectocarpus* species 7 chromosome. A high level of synteny was observed with the exception of two *Ectocarpus* species 7 chromosomes, chr_04 and chr_28, that formed a single linkage group (LG1) in *Ectocarpus siliculosus* (Figure1). Four of the *Ectocarpus* species 7 chromosomes (chr_15, chr_25, chr_12 and chr_16) in were split into two different linkage groups in the *E. siliculosus* genetic map.

Small-scale rearrangements between two *Ectocarpus* species

Small-scale rearrangements were detected by aligning *E. siliculosus* marker sequences onto the genome of *Ectocarpus* species 7 (Figure 1). Twelve markers of *Ectocarpus* species 7 chromosome 19 (1.21 cM), ended up on the linkage group 14 of *E. siliculosus* that was identified as homologous to the chromosome 23 of *Ectocarpus* species 7. Also, a segment of 13.73 cM containing 26 markers from a chromosome of unassembled sequences (chr_00) of *Ectocarpus* species 7 were linked to markers of the chr_27 of *Ectocarpus* species 7 forming the LG12 of *Ectocarpus siliculosus* genetic map. Some small-scale rearrangements corresponds to a single marker but in other cases a group of markers from a chromosome was translocated in a linkage group which is homologous to another chromosome.

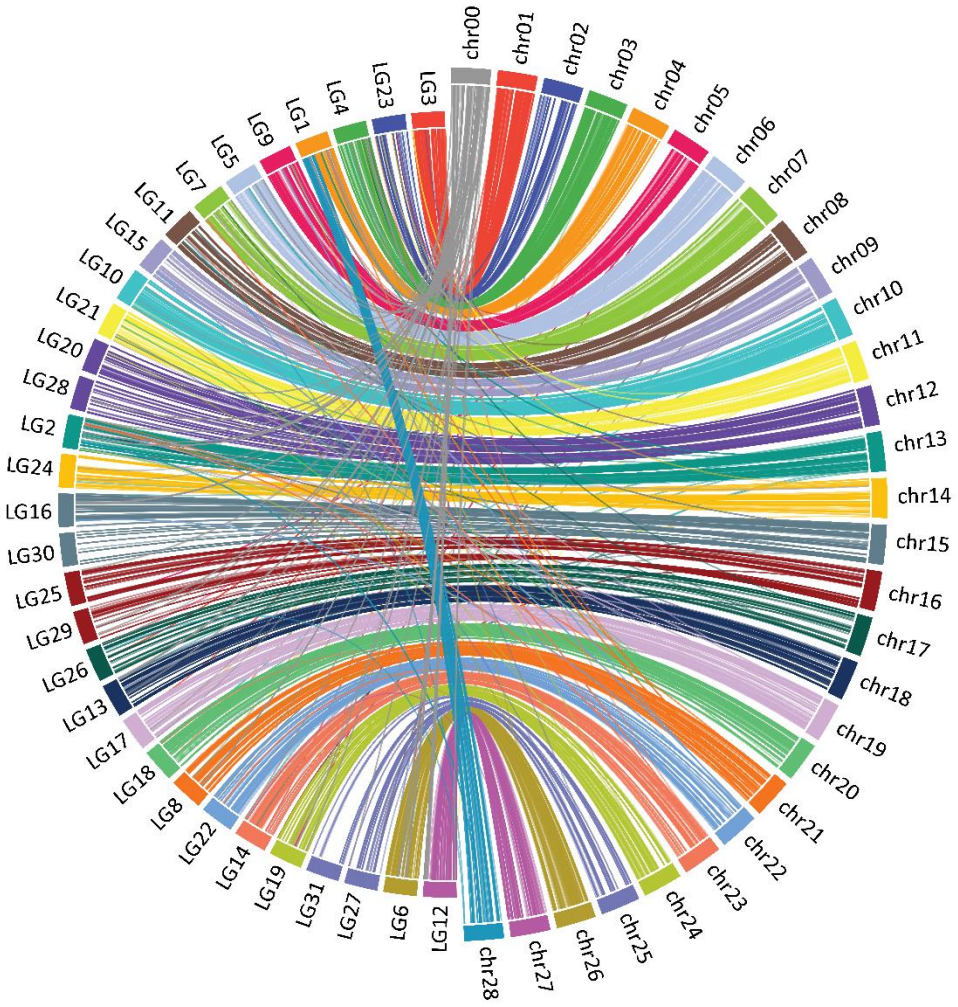

Figure 1: Synteny between the *Ectocarpus siliculosus* genetic map (left) and *Ectocarpus* sp7 chromosomes (right). Sequence surrounding markers from the *Ectocarpus siliculosus* genetic map were aligned to the *Ectocarpus* sp7 genome and the location of shared regions were reported. *Ectocarpus siliculosus* linkage groups (LG) were coloured by the most likely orthologous chromosome (chr) based on frequency of shared markers.

Discussion

ddRAD-seq libraries are an efficient method to generate dense genetic maps

The efficiency of ddRAD sequencing method to generate a great number of markers is of particular interest for the construction of dense genetic maps. The genetic map of *Ectocarpus* species 7 described in Avia et al. (2017) was constructed using 3588 SNP ddRAD-seq markers. SNP markers were distributed across 28 linkage groups, consistent with cytogenetic studies carried out using European strains of *E. siliculosus* that reported an approximate chromosome number of 25 (Muller, 1967). The quality of a genetic map depends on the density of markers used and the size of the mapping family. The quality of the genetic map is considerably impacted due to missing data so a filtering step during the mapping generation is crucial, consequently, it is very important to use a large number of offspring to counterpart the removal of missing data. For example, in *Ectocarpus siliculosus*, the RAD-seq libraries were generated using 152 individuals but the genetic map was constructed using only 124 offspring. However, 5594 SNP markers were detected for these 124 individuals whereas only 3588 SNP markers were detected for the 89 individuals of the *Ectocarpus* species 7 map. The greater the number of progeny, the more markers are detected and therefore the genetic map will be denser and have higher resolution.

Marker density can also depend on the sequencing technologies and methods used. The libraries for both the *E. siliculosus* and *Ectocarpus* species 7 genetic maps were sequenced on an Illumina HiSeq 2500 platform but the lengths of the paired-end reads were different, 250 bp reads for *E. siliculosus* and 100 bp for *Ectocarpus* species 7. However, for the construction of *E. siliculosus* genetic map the paired-end and singleton reads were trimmed to 100 bp because we had more fragments of 100 bp than 250 bp. Theoretically, if longer fragments are sequenced the probability of detection of polymorphisms between individuals should improve.

Comparative analysis of the *E. siliculosus* and *Ectocarpus* sp.7 genomes

This study provided a first insight into synteny between the genomes of two species of *Ectocarpus*. Overall, we found a relatively high degree of conservation between the two

species. Twenty six of the 31 linkage groups of *Ectocarpus siliculosus* were homologous to *Ectocarpus* sp.7 chromosomes and corresponded to a single linkage group.

Large-scale rearrangements

Based on the comparison of the genetic maps, four *Ectocarpus* species 7 chromosomes were predicted to be split into two linkage groups in *Ectocarpus siliculosus*. It is difficult to determine if these predictions represent real fragmentation or fusions of chromosomes or if the genetic map lacked resolution to link the two parts of the same chromosome in *E. siliculosus*. The *E. siliculosus* linkage groups LG30 and LG31 were quite small, corresponding to only 33 and 31 markers, respectively. Previous cytogenetic studies carried out on *E. siliculosus* reported an approximate number of 25 chromosomes (Muller, 1967) suggesting that the small linkage groups detected by cytogenetics may be artefacts. Indeed, the small size of the nuclei of *Ectocarpus* make counting metaphase chromosomes extremely challenging. Genotyping of additional offspring of the F2 progeny should allow the detection of additional markers, particularly in genomic regions where the recombination rate is lower and thereby overall improve the genetic map. In general, recombination rate is not constant along chromosomes. The most extreme example of variable recombination rates is the sex chromosome where recombination is totally supressed is the sex-determining region (SDR) (Amhed et al., 2015; Beukeboom and Perrin, 2014). Regions like centromeres where recombination rate is low could explain why chromosome are split between two linkage groups.

A putative large-scale rearrangement was detected in the LG1 of *Ectocarpus siliculosus* genetic map which seemed to be the fusion of two chromosomes (chr_04 and chr_28) of *Ectocarpus* species 7. Interestingly, comparison between *Saccharina japonica* and *Ectocarpus* species 7 is under investigation and preliminary results indicate that a part of homologous chromosome 28 and homologous chromosome 04 of *Ectocarpus* species 7 formed a linkage group in *Saccharina japonica*. A genetic map of constructed for *Saccharina japonica* using more than 7000 markers identified 31 linkage groups (Wang et al., 2015). About 90 million years separate *Ectocarpus* species 7 from *Saccharina japonica* (Kawai et al., 2015) whereas the divergence time between both *Ectocarpus* species is less than 17 million years suggesting that this large-scale rearrangement is probably either an artefact or a recent process. The putative rearrangement could be explained by a recent process that occurred in *Ectocarpus* species 7 with a split into two chromosomes (chr_04 and chr_28) while the common ancestor actually

exhibited a fusion between these two homologous chromosomes. The alternative explanation for this rearrangement could be an artefact in the genetic map of *Ectocarpus* species 7 where markers of chromosome 04 and chromosome 28 could not be assigned to the same linkage group because recombination rate was too low.

Recent molecular approaches like Hi-C could provide spatial proximity maps of genome with a high resolution (Lieberman-Aiden et al., 2009). The method is based on DNA-protein complexes that are crosslinked with formaldehyde. The DNA is then fragmented, and extracted, ligated and digested with restriction enzymes. The resulting DNA fragments are PCR-amplified and sequenced.

A recent study using genome-wide application of chromosome conformation capture (3C) (Dekker et al., 2002; Lieberman-Aiden et al., 2009) or Hi-C approach to generate an improved large-scale assembly of the *Ectocarpus* species 7 genome (unpublished) has indicated that chr_04 and chr_28 are in fact a single chromosome. This study along with the observation on rearrangement between *Saccharina lomentaria* and *Ectocarpus* species 7 provide strong evidence that chromosome 04 and chromosome 28 of *Ectocarpus* species 7 are in fact a single chromosome.

Small-scale rearrangements

Evidence for small-scale rearrangements, including translocations, was obtained by aligning *Ectocarpus siliculosus* markers to the *Ectocarpus* species 7 genome. Cases where only one marker indicated potent translocation could potentially be due to artefactual marker alignment. However, translocations were more strongly supported in cases where several markers from the same chromosome of *Ectocarpus* species 7 were assigned to a different linkage group in the *Ectocarpus siliculosus*. Twelve markers from *Ectocarpus* species 7 chromosome 19 that were found on *Ectocarpus siliculosus* LG 14 corresponding to *Ectocarpus* species 7 chromosome 23. This result suggest that small rearrangement occurred between the two species. In addition, markers from the chromosome 00 of *Ectocarpus* species 7, corresponding to unassembled sequences, were scattered on different linkage groups of the *Ectocarpus siliculosus* genetic map. For example, 26 markers corresponding to unlinked sequences of *Ectocarpus* sp.7 (chr_00) were linked to markers in *E. siliculosus* LG12 (which corresponds to *Ectocarpus* sp.7 chromosome 27). Given the high conservation between both maps, this rearrangement appears ro represent simply an improvement of the genetic map

resolution allowing the detection of linkage for these sequences to the chromosome 27. In the Hi-C map that has been recently generated for *Ectocarpus* species 7 some of the unmapped supercontigs sequences (i.e. chr_00 sequences) have been reintroduced in the contigs supporting the idea that those "rearrangements" observed between *Ectocarpus* species 7 and *Ectocarpus siliculosus* are not real rearrangements.

Overall, we found a relatively high degree of collinearity of the maps for two *Ectocarpus* species that have diverged less than 17 MY ago (Kawai *et al.*, 2015). More markers could reveal smaller-scale rearrangements, and only sequencing of long genomic contigs in *Ectocarpus siliculosus* would reveal the details of genome evolution. Interestingly, the current project Phaeoexplorer is aiming to generate transcriptome data and high quality genome assemblies for a broad range of brown algae species, including several species of the *Ectocarpus* group. Out of the 37 species that are expected to be sequenced, strains from different populations (Roscoff and Naples) of the species *Ectocarpus siliculosus* have been selected for either Illumina or both Illumina and Nanopore sequencing. These genomes and transcriptomes of the *Ectocarpus* species 13 will provide useful genetic information and undoubtfully improve the available genome assembly of *Ectocarpus siliculosus* that despite its overall high quality is not sufficiently assembled to look at small scale rearrangement at the gene level.

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Table S1: Lists of the strains used to generate the *Ectocarpus siliculosus* and *Ectocarpus* species 7 genetic maps.

Strain name	Species	Origin	Isolation location	Generation	Sex	Parental strains	Used for
EA1	*Ectocarpus siliculosus*	field	Italy, Naples	GA	female		Parental female strain, parthenogenetic, used for genetic map
RB1	*Ectocarpus siliculosus*	field	Italy, Naples	GA	male		Parental male strain, non-parthenogenetic, used for genetic map
Ec236	*Ectocarpus siliculosus*	lab	Italy, Naples	SP		EA1 f x RB1 m	Progeny used for Rad seq analysis
Ec32	*Ectocarpus* sp7	lab	Chili	GA	male		Parental male strain used for genetic map, reference genome (Cock et al., 2010)
Ec568	*Ectocarpus* sp7	lab	Peru	GA	female		Parental female strain used for genetic map
Ec569	*Ectocarpus* sp7	lab	Peru	GA		Ec568 f x Ec32 m	Progeny used for Rad seq analysis and microsatellites detection

Chapter IV

General discussions and perspectives

This PhD thesis has made several important contributions to increase our knowledge about the genetic mechanisms that control parthenogenesis in the brown algae. By investigating parthenogenesis in a multicellular organism that has independently evolved from plants and animals, the thesis has helped to assess the diversity of evolutionary mechanisms that lead to parthenogenesis.

Parthenogenesis in *Ectocarpus* is a complex genetic trait

In this thesis, the genetic architecture of *Ectocarpus siliculosus* was investigated using a QTL approach which has allowed to identify the genetic complexity of this trait. The choice of using a QTL approach for studying the genetic basis of parthenogenesis in *Ectocarpus* was well adapted in the sense that the distinction between Mendelian trait and QTL is artificial, as the techniques can be applied to both. If the trait had been a simple mendelian locus we would have detected it as such. Performing QTL approach to identify the loci controlling a developmental pathway like parthenogenesis had already been used in plants. In plants research has focused for many years on the identification of molecular factors controlling apomixis and each of its components. Three mains approaches have been used: (1) quantitative trait locus (e.g. QTL) mapping and subsequent characterisation of the locus of interest, (2) comparative transcriptomic approaches using sexual and apomictic species and (3) mutant screening for apomictic-like phenomena in sexual models (Grossniklaus *et al.*, 1998). Although these efforts have led to a multitude of candidate genes, no consensus master regulator or set of genes controlling apomixis have been identified so far. Considering the large variability in developmental routes to achieve apomixis, it is generally assumed that there exist multiple, independent molecular mechanisms that regulate the switch from sexual to apomictic development.

In *Ectocarpus*, the QTL approach has allowed to determine that parthenogenesis is controlled by three loci revealing that it is a complex trait. The idea that complex traits could be controlling asexual reproduction pathway like parthenogenesis is not new. In a review of the genetics of gametophytic apomixis, Asker (Asker, 1980) wrote: “in the author’s opinion, the constituents or elements of apomixis are – with the possible exception of apospory – to a large extent quantitative traits under polygenic control.” Example can be given in both animals and plants to illustrate that more than one locus can be involved in the genetic control of parthenogenesis. The more striking example of complex trait in the regulation of apomixis in

plants, is in *Poa patentis* which provided direct evidence that up to five separate loci interact to activate and/or suppress unreduced gamete formation and/or development (Matzk *et al.*, 2005). In animals, in the rare cases where genetic basis of parthenogenesis has been explored, *Daphnia pulex* is an example for parthenogenetic development being a complex trait controlled by four loci. In *Daphnia pulex*, association mapping on natural clones suggests that sex-limited meiosis suppression has spread in natural population conveyed by a dominant epistatic interaction among the products of four unlinked loci, with one entire chromosome being inherited through males in a nearly non-recombining fashion way (Lynch *et al.*, 2008). The implication of four loci located on different chromosomes indicate that the capacity for meiosis suppression may be conferred by the joint action of multiple loci, rather than by the single dominant mutation envisioned by Hebert (Hebert, 1981).

The originality of the complex trait in *Ectocarpus* remains in the fact that one of the three loci appears actually to be the sex determining region itself, indicating that the sex chromosomes have a key role in the switch from sexual to asexual reproductive program. This particularity has also been observed in the pea aphid where it was showed that a single genomic region linked to the X chromosome was controlling parthenogenesis (Jaquiéry *et al.*, 2014). Considering that parthenogenesis evolved independently in each lineage, knowledge of the genetic architecture of parthenogenesis from other groups of organisms would be useful to understand if there is some tendency for the parthenogenesis controlling loci to be on sex chromosomes.

Parthenogenesis in *Ectocarpus* implies a switch from gametophytic to sporophytic program

Several studies on the reproduction of *Ectocarpus* have contributed to unravel its complex life cycle (Knight, 1930; Papenfuss, 1935; Boalch, 1961) that was completed by Müller in 1972 (Müller, 1972). At that time, it has already been reported that *Ectocarpus* was not only able to undergo parthenogenesis but it was also able to switch from gametophytic to sporophytic program when reproducing by parthenogenesis. This capacity to switch from gametophytic to sporophytic program is quite interesting because parthenogenesis as an asexual mode of reproduction is by definition a way to produce offspring genetically identical to the parent. In the case of *Ectocarpus*, parthenogenetic offspring are genetically identical to the parental

gametophyte but morphologically different suggesting an additional mechanism for the switch from gametophytic to sporophytic developmental program. Interestingly this is not the case for other closely related Ectocarpus species (E. fasciculatus, (Müller, 1972) where parthenogenesis may also involve the deployment of the gametophyte program (parthenogemetophytes instead of parthenosporophytes).

In *Ectocarpus*, studies have identified mutants exhibiting a switch from gametophyte to sporophyte program (Coelho *et al.*, 2011; Arun *et al.*, 2018). Two of the *Ectocarpus* mutants (*ouroboros* and *samsara*) were identified to carry mutations into two different genes encoding TALE homedomain (HD) transcription factors. These transcription factors are particularly abundant in gametes which is interesting when considering the parthenogenetic development of gametes. In these mutants, the gametes retained their capacity to undergo parthenogenesis but they developed into partheno-gametophytes instead of partheno-sporophytes, indicating that mutations on the TALE HD genes were affecting the switch from gametophyte to sporophyte program but not the parthenogenetic development *per se*. These two transcription factors were shown to be able to interact with each other and to form a heterodimer. It remains obscure how these transcription factors work and what specific genes they might trigger, but they are probably involved in a pathway triggering the sporophyte developmental program and regulating the expression of sporophyte biased genes.

In *Chlamydomonas reinhardtii*, similar homeodomain transcription factors were reported to be expressed in either “plus” or “minus” gametes and form heterodimers after mating and trigger the switch from haploid to diploid program (Nishimura *et al.*, 2012). In *Ectocarpus*, both transcription factors are present in the male and female gametes indicating that the regulation of the switch might be more complex than in *C. reinhardtii*. Current work in *Ectocarpus* is aimed at investigating chromatin remodelling and is revealing the genes triggered by these transcription factors in parallel with genes differentially expressed between mutants and wild type strains.

In *Ectocarpus*, the switch from gametophytic to sporophytic program appears to have been superimposed on the parthenogenetic developmental pathway (Figure 1).

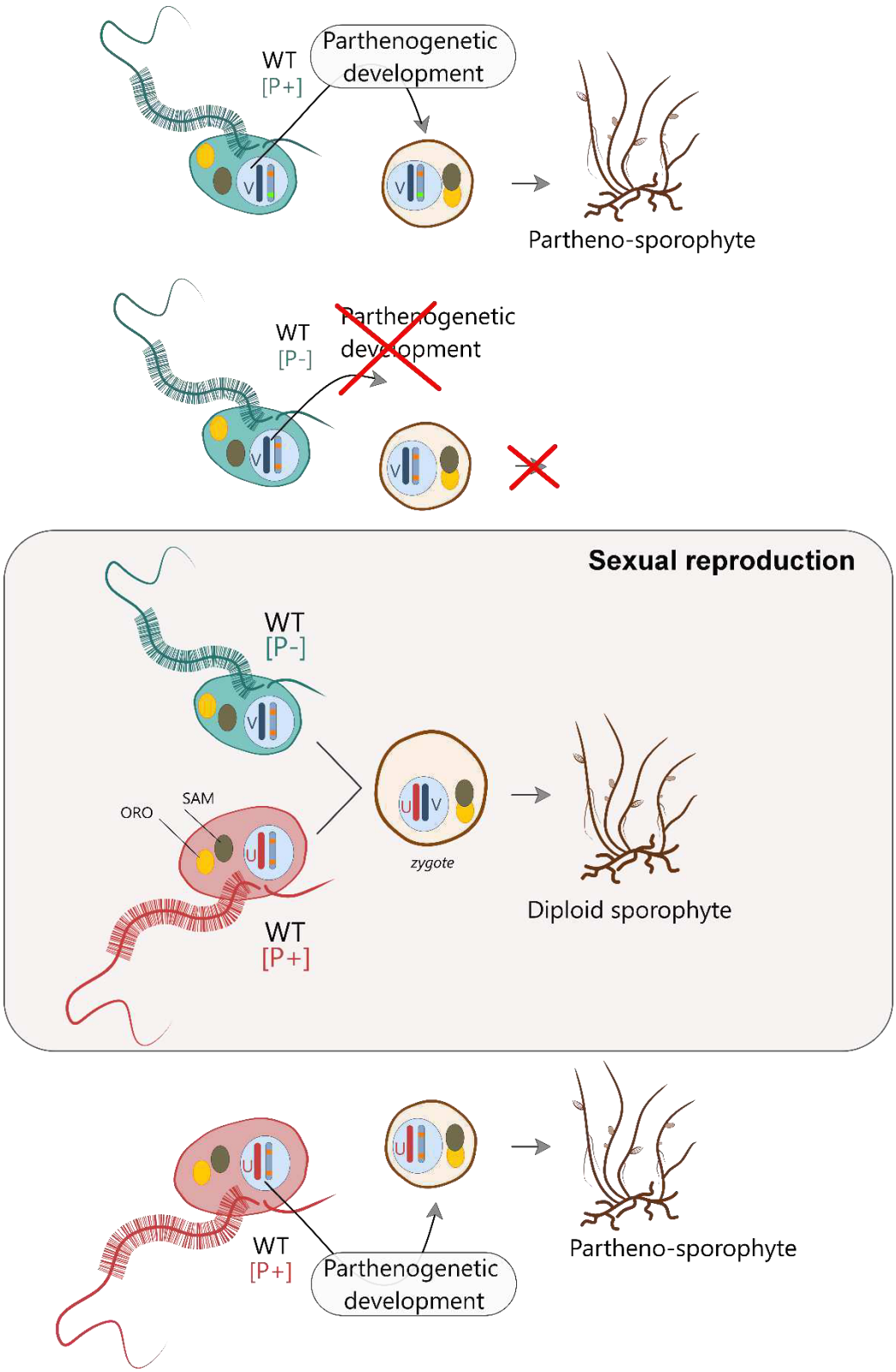

Figure 1: Potential molecular mechanism of parthenogenesis in *Ectocarpus siliculosus*. In sexual reproduction, after fusion of male and female gametes, ORO and SAM protein can form heterodimers and trigger the sporophytic program. When female gamete fail to find a male gamete, the U chromosome where a locus for parthenogenesis is located may trigger the parthenogenetic development program while after heterodimerisation the thranscription factors ORO and SAM can trigger the sporophytic program. For male gamete, failing to find a female gamete would lead to either parthenogenetic development (P+) or death (P-) depending on the P2 and P3 allele (orange or green blocks) located on the autosome chromosome (Blue chromosome = LG18).

Parthenogenesis in *Ectocarpus* affects fitness in males

We highlighted in this study that parthenogenetic capacity in males has an effect on their fitness. Parthenogenetic male gametes have less fusion success with female gametes than non-parthenogenetic male gametes. Moreover, successful crosses of parthenogenetic male gametes with female gametes revealed that the growth of the zygote was delayed compared to the growth of zygote derived from a non-parthenogenetic male gamete. The effect of parthenogenetic capacity in males on their fitness raised two questions: What is the explanation for the maintenance of the P+ allele in natural population given the effect it has on male fitness? How can we explain the difference in zygotic growth?

In *Ectocarpus*, parthenogenetic development is a facultative process that allows to avoid the asexual reproduction main cost which is the cost of recombination. In brown algae, oogamy appears to be the ancestral state (Silberfeld *et al.*, 2010) and strongly dimorphic gametes reproduce sexually through fusion and are incapable of parthenogenetic development (reviewed in (Luthringer *et al.*, 2014). This suggests that gamete capacity to undergo parthenogenesis was superimposed on a pre-existing sexual reproduction cycle. This process would have evolved secondarily possibly to ensure reproduction in conditions of low population density. Parthenogenetic development of *Ectocarpus* occurs when gametes fail to find a compatible gamete of the opposite sex and in this sense it allows the cost of finding a mate to be avoided which could be advantageous in situations of low population density or fragmented habitats. Predictions indicate that in marginal populations, or other situations where mates are limited, parthenogenesis could be adaptive and therefore selectively favoured (Lampert, 2008). In this particular conditions, parthenogenesis could be considered as a bet-hedging strategy in males, with a tradeoff between higher sexual fitness when non-parthenogenetic and lower sexual fitness (but asexual, clonal reproduction) when parthenogenetic.

Surprisingly, in natural populations of *Ectocarpus siliculosus* (Naples), a recent study indicated that sexual reproduction is prevalent (Couceiro *et al.*, 2015) and there was actually little evidence for parthenogenesis in these field populations. Additional population data are required, especially studies on natural populations where individuals are found at different densities, for example marginal versus central populations and in different types of habitat.

Can mitochondria inheritance explain the parthenogenesis effects on zygotic growth?

It is difficult to assess what mechanism could explain the significantly delay in the growth of zygotes depending on the paternal parthenogenetic capacity. Nonetheless, a hypothesis arisen with a potential implication of mitochondria inheritance. Parthenogenetic development implies that gametes are able to retain their mitochondria, which provide energy for cell division and growth of the developing zygote. The investigation on mitochondria inheritance in zygotes derived from two parthenogenetic parents revealed that male mitochondrial DNA (mtDNA) was degraded in zygotes generated from both P- or P+ crosses indicating that the inheritance was not affected by parthenogenesis. However, the zygotic growth was observed at early stages development whereas mtDNA inheritance, for technical reasons, was studied on adult sporophytes, so there is the possibility that early incompatibilities remain undetected. The characterisation of mitochondria in gametes revealed that the number of mitochondria in parthenogenetic male gamete was significantly higher than in non-parthenogenetic male gamete. One possibility is that the cytoplasmic conflict following the gamete fusion may be greater with parthenogenetic male gamete as there will be more mitochondria to degrade delaying the growth of the zygote (Figure 2). Further investigation on the mitochondria inheritance and parthenogenetic capacity needs to be addressed and the monitoring of the disappearance of male mitochondria in zygote overtime, and specifically during the early stages of development of the zygote, would provide indication on the importance of mitochondria in zygotic growth. One possibility would be to stain the male and female mitochondria using mitochondria dyes that fluoresce at different wavelenghts, and follow the fate of each of the male and female mitochondria during the early development of the diploid sporophyte.

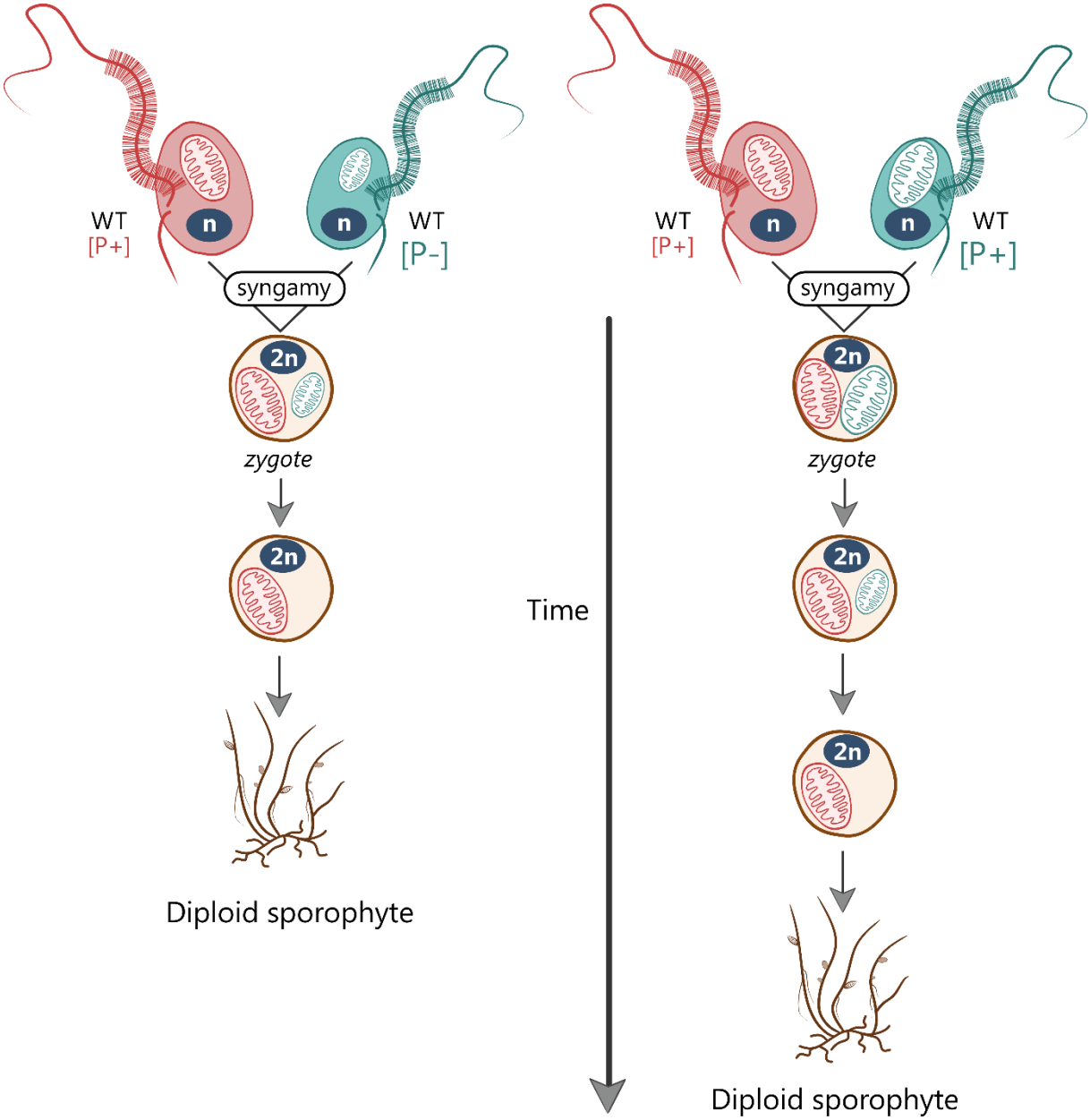

Figure 2: Cytoplasmic conflict between male and female mitochondria leading to a delayed in the gowth of zygotes (putative model). Left, a cross between a parthenogenetic female gamete and a non-parthenogenetic male gamete. The content of mitochondria in the male is significantly lower than in the female and, after fusion, the cytoplasmic conflict is resolved rapidly by the selective degradation of the male mitochondria. On the right, a cross between a parthenogenetic female gamete and a parthenogenetic male gamete. The number of mitochondria transmit by the male gamete is significantly higher than the one from a non-parthenogenetic male gamete. The male mitochondria are selectively degraded but the time needed to resolve the cytoplasmic conflict is greater and the zygotic growth is delayed.

An unusual pattern of mitochondria inheritance in *Ectocarpus* species 7

In the case of *Ectocarpus siliculosus*, mitochondria inheritance was strictly maternal as reported in Peters *et al*. (Peters *et al.*, 2004). Strikingly, mitochondria inheritance in *Ectocarpus* species 7 was uniparental but individuals showed either maternal or paternal inheritance. This is the first observation of this type of inheritance pattern in a multicellular eukaryote following intra-specific crosses and poses questions concerning the mechanisms that produce such a pattern. How, with about 15 mitochondria transmitted from each parent, do we obtain random uniparental transmission? So far attempts to model the process based on cell cycle progression rates and cell differentiation have failed to explain the random transmission pattern observed. More investigation is needed to answer this question.

Uniparental mitochondria inheritance is a major hypothesis to explain the theory on the evolution of mating types. This hypothesis suggests that mating types evolved to control potential cytoplasmic conflicts between organelles (Hurst and Hamilton, 1992) by promoting uniparental transmission. Mating types or sexes would be expected not only to determine who is a potential mating partner but also to determine which parent will transmit its organelles (Billiard *et al.*, 2011). In many taxa, uniparental maternal inheritance is predominant providing correlation with sex (Breton and Stewart, 2015; Greiner *et al.*, 2015). In contrast, our results provide evidence against the hypothesis that mating types evolved to control uniparental maternal inheritance of mitochondria because transmission in *Ectocarpus* species 7 is uniparental but random regarding the sex.

The random transmission of mitochondria observed in *Ectocarpus* species 7 raised a number of questions such as: What is the mechanism responsible for such transmission? Is there a specific mechanism to *Ectocarpus* species 7 or do other *Ectocarpus* species exhibit the same transmission pattern?

Monitoring the disappearance of mitochondria in zygotes by specific staining of male and female mitochondria (as described above) would help to understand the mechanism of transmission. Investigating the mitochondria inheritance in other *Ectocarpus* species would provide a broader view on the occurrence of such transmission pattern.

During the course of this thesis, *Ectocarpus siliculosus* and *Ectocarpus* species 7 were used as models for addressing a number of questions, and, specifically, a high quality genetic map for *Ectocarpus siliculosus* was needed in order to study both the genetic basis of parthenogenesis but also the patterns of neutral diversity across the genome (Avia *et al.*, 2018). The availability of genetic maps for these two species offered an opportunity to compare the extent of the conservation of synteny acroos two genomes. This study highlighted the high degree of collinearity between the two species and the overall superior quality of the genetic map of *Ectocarpus siliculosus*. It detected an error in the segregating linkage groups of *Ectocarpus* species 7 genetic map that was confirmed by a recent Hi-C approach. The *E. siliculosus* genetic map could also be further improved by a Hi-C approach to determinate if the small linkage group have been separated from larger one because of low recombination between markers.

In conclusion, studies on *Ectocarpus* during this PhD have allowed to increase our understanding of the genetic control of parthenogenesis, mitochondrial inheritance and the degree of collinearity between two maps of *Ectocarpus* species. Despite the fact that some technical limits remain (e.g. reverse genetic tools such as transformation), *Ectocarpus* is a particularly valuable model system to investigate mechanisms involved in the parthenogenetic life cycle, multicellular development and cellular processes such as organelle inheritance. The complex life cycle of *Ectocarpus* provides access to the different stages and generations making this model ideal for mechanistic studies on haploid-diploid multicellular organisms.

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Appendix 1

Genetic Diversity in the UV Sex Chromosomes of the Brown Alga *Ectocarpus*.

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MDPI

Article

Genetic Diversity in the UV Sex Chromosomes of the Brown Alga *Ectocarpus*

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Received: 1 May 2018; Accepted: 5 June 2018; Published: 6 June 2018

Abstract: Three types of sex chromosome system exist in nature: diploid XY and ZW systems and haploid UV systems. For many years, research has focused exclusively on XY and ZW systems, leaving UV chromosomes and haploid sex determination largely neglected. Here, we perform a detailed analysis of DNA sequence neutral diversity levels across the U and V sex chromosomes of the model brown alga *Ectocarpus* using a large population dataset. We show that the U and V non-recombining regions of the sex chromosomes (SDR) exhibit about half as much neutral diversity as the autosomes. This difference is consistent with the reduced effective population size of these regions compared with the rest of the genome, suggesting that the influence of additional factors such as background selection or selective sweeps is minimal. The pseudoautosomal region (PAR) of this UV system, in contrast, exhibited surprisingly high neutral diversity and there were several indications that genes in this region may be under balancing selection. The PAR of *Ectocarpus* is known to exhibit unusual genomic features and our results lay the foundation for further work aimed at understanding whether, and to what extent, these structural features underlie the high level of genetic diversity. Overall, this study fills a gap between available information on genetic diversity in XY/ZW systems and UV systems and significantly contributes to advancing our knowledge of the evolution of UV sex chromosomes.

Keywords: UV sex chromosomes; pseudoautosomal region; brown algae; neutral diversity

1. Introduction

Morphologically distinct sex chromosomes have evolved multiple times independently in both plants and animals [1]. Sex chromosome evolution has been mainly studied in male-heterogametic (XX/XY) and female heterogametic (ZZ/ZW) sex determination systems. A typical sex chromosome pair derives from a pair of autosomes through the acquisition of genes involved in sex determination. If more than one locus involved in sex determination is located on the chromosome, recombination between these loci is expected to be suppressed, leading to the establishment of a non-recombining

region on the nascent sex chromosome, the sex-determining region or SDR. The formation of this non-recombining region has important consequences for the evolution of this part of the genome. Repetitive DNA can accumulate, leading to an increase in SDR size (see review by Bachtrog [1]). There is also a tendency for genes within the SDR to degenerate as a consequence either of an accumulation of deleterious mutations or of a lower rate of adaptation [1,2]. At a later stage, deletion of non-functional DNA from within the SDR may lead to a decrease in the size of the SDR. Furthermore, the SDR can progressively expand into the flanking regions of the chromosome (the pseudoautosomal regions, PAR), so that it encompasses an increasingly greater proportion of the sex chromosome.

Evolutionary processes at a given site in a genome are influenced by selection acting on closely linked sites, an effect called Hill-Robertson interference [3,4]. This selection interference reduces the effective population size (N_e) experienced by the site in question [5]. As this effect is expected to be maximal in regions experiencing little or no recombination, diversity in SDRs such as Y-linked regions will be reduced compared to that of autosomes or pseudo-autosomal regions. In addition, loci on the Y chromosome are expected to experience a N_e that is one-quarter that of autosomal, and one-third that of X-linked genes. Moreover, since the level of neutral polymorphism maintained at equilibrium is proportional to the product of N_e and the neutral mutation rate, μ ($\pi = 4Ne\mu$) [6], diversity should be lower for Y-linked genes than for their X-linked counterparts and diversity in both X and Y genes should be lower than for autosomal genes [7]. Note, however, that this does not apply to genes located in the PAR, which should have the same N_e as autosomal genes. Accordingly, in *Silene latifolia* for example, diversity has been shown to be reduced in the Y-linked regions relative to X-linked regions [8]. A similar situation was observed in *Drosophila* [9] and in Saudi-Arabian hamadryas baboons [10].

In contrast to the SDR, the PARs of sex chromosomes maintain similarity between alleles of the same gene because they undergo homologous pairing and recombination. Therefore, genes in the PARs are expected to evolve in a similar manner to autosomal genes [11,12]. However, because of the proximity to the SDR, PARs are expected to display specific evolutionary dynamics [13]. One of these specificities is that linkage with the SDR widens the conditions allowing the maintenance of polymorphism at loci under sexually antagonistic selection and also increases neutral diversity due to longer coalescence times [14–16]. Diversity in PARs is therefore predicted to be high in those regions in close proximity with the SDR [17]. Increased genetic diversity, and overall footprints of balancing selection due to sexually-antagonistic selection, have been observed for several *Silene latifolia* PAR loci, although there was no evidence for an effect of proximity to the SDR [18,19].

While information (theoretical and empirical) is available for XY and ZW sex chromosome systems, we know very little about evolutionary process, and in particular about patterns of genetic diversity, in a third type of sexual system that exists in nature, UV sex chromosomes (see review by Wilson Sayres [20]). In UV systems, which are very common in non-vascular plants and red, green and brown algae, sexes are expressed during the haploid stage of the life cycle, and females carry a U chromosome whereas males carry a V chromosome [21–23]. UV sexual systems (Figure 1) have specific evolutionary and genetic properties, including the absence of homozygous or heterozygous sexes and the absence of masking of deleterious mutations during the haploid phase when sex is expressed. Another significant feature that distinguishes UV from diploid sex chromosomes is that the N_e for U- and V-specific regions is expected to be half that of autosomes [21] and both the U and the V are theoretically subject to the same mutation rate (unlike XY or ZW systems where, for example, the Y can have a higher mutation rate than the X [24,25]). The PAR region is expected to have the same N_e as autosomes.

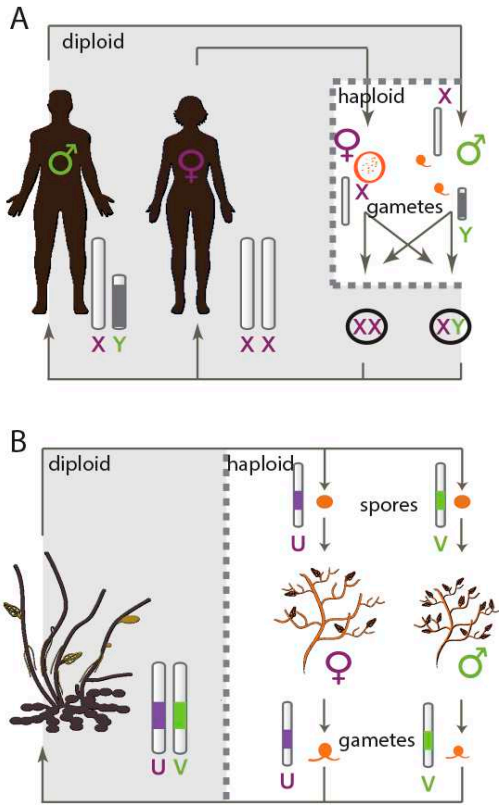

Figure 1. Comparisons of XX/XY and UV sex determination systems. (**A**) XX/XY sex determination system. The sexual individuals are diploid, and the sex of an offspring is determined after fertilization, depending on the sex chromosome contributed by the sperm. Please note that the haploid phase of the life cycle is limited to the gametic stage. Note also that ZW systems function in a similar manner to XY systems, with diploid phase sex determination, but it is the female that is the heterogametic sex; (**B**) UV sex determination system. The diploid, asexual generation (sporophyte) carries both the U and the V sex chromosomes which are passed on to the haploid spores after meiosis. Spores that receive the V sex chromosome develop into a male gametophyte whereas spores carrying U sex chromosome will produce a female gametophyte. Egg and sperm produced by the gametophyte fuse to return to the diploid generation. In UV sex determination systems, the sexual individuals are haploid and sex chromosomes function in the haploid state.

The only detailed studies focusing on the structure and evolution of both the SDR and PAR regions of UV systems have been carried out in the brown algal model organism *Ectocarpus* sp. [26–28]. In this organism, the U and V-specific regions are small, and exhibit mild degeneration despite the action of haploid purifying selection [26]. SDR genes were shown to evolve rapidly, mainly due to relaxed purifying selection [29]. Remarkably, the relatively large *Ectocarpus* PARs exhibit unique features. Although they recombine normally, these regions differ from autosomes in terms of their gene density, transposable element content and genetic structure [28]. Moreover, the PAR is significantly enriched, compared to autosomes, in genes expressed specifically or predominantly during the diploid, sporophyte phase of the life cycle (hereinafter called sporophyte-biased or SP-biased genes), and these genes have been shown to evolve faster than unbiased genes [28]. A model was proposed to explain this enrichment phenomenon, giving SP-biased genes an advantage to spread when they were partially linked to the SDR and had a positive effect on fitness in one of the sexes [28]. The model assumes that the evolution of the PAR in haploid systems is under the influence of differential selection pressures in males and females acting on alleles that are advantageous during the sporophyte generation of the life cycle.

Here we used extensive Double Digest Restriction Associated DNA sequencing (ddRAD-seq) data combined with a gene-by-gene approach to perform a comprehensive analysis of the genetic diversity

across the UV sex chromosome of the brown alga *Ectocarpus*. We show that the level of neutral diversity in the U and V SDR haplotypes is about half that of the autosomes. This observation is in line with theoretical predictions based on the reduced effective population size of the SDR (the U and V SDR each have half the N_e of the autosomes), suggesting that the influence of additional factors such as background selection or selective sweeps is minimal. Interestingly, genetic diversity in the PAR region was surprisingly elevated and there were several indications that genes in this region may be under balancing selection.

2. Materials and Methods

2.1. Quantitative Trait Loci (QTL) Mapping of the Sex Locus in Ectocarpus Siliculosus

The populations analyzed in this study belong to the species *Ectocarpus siliculosus*, a sister species to *Ectocarpus* sp. for which a reference genome sequence and genetic map with detailed coordinates of the positions of the SDR and pseudoautosomal regions are available [27,30–32]. The *Ectocarpus* sp. reference genome strain still lacks a formal species name. It was referred to as *Ectocarpus* 7 in a recent phylogenetic analysis paper [33] and we therefore also referred to it as *Ectocarpus* 7 throughout this paper. To confirm that the borders of the SDR were the same in the two species, in the absence of a complete genome sequence for *E. siliculosus*, we generated a genetic map for this species [34] focusing specifically on the sex chromosome, to investigate the location of the SDR, as described below.

A diploid sporophyte (Ec236) was generated by crossing two compatible *E. siliculosus* strains from a Naples population (EA1 and RB1) (Table S1). From this sporophyte, 152 haploid gametophytes were isolated, each arising from a unique meiotic event [35,36]. The sex of each individual was determined using sex-specific PCR markers [37]. Molecular methods, ddRAD sequencing of this population and detailed analysis of the genetic map obtained are described in the Supplementary Materials and Methods.

QTL Mapping of the Sex Locus

The genetic map obtained with the 152 haploid gametophyte progeny derived from the diploid sporophyte Ec236 was used to map the *E. siliculosus* SDR. Using the sex of the progeny as a binary trait, the SDR location was determined as a QTL in the R package R/qtl [38] with the scanone function and the "binary" model. To confirm its position, we also used MapQTL [39] with the Kruskal-Wallis non-parametric method. The R/xoi package (version 0.67–4) [40] was used to obtain a smoothed estimate of the recombination rate along the linkage groups (LGs), in 1 Mbp sliding windows.

2.2. Sequencing of the Individuals from Different Natural Populations for Population Genomics Analyses

2.2.1. Field Sample Unialgal Collections

We selected several populations from different geographical origins in order to test the repeatability of the observed pattern of neutral variation between genomic regions in face of the population history or environment conditions of this cosmopolitan species. The samples used were previously obtained from natural populations collected along the European coast of the Atlantic and Mediterranean Sea and the Pacific coast of Chile by Couceiro, et al. [41] and Montecinos, et al. [42] (Table S2). Samples were maintained in the lab as unialgal cultures as described in Couceiro, et al. [41].

2.2.2. Analyses of Neutral Diversity in Non-Recombining versus Recombining Regions

ddRAD-sequencing is a reduced-representation genome sequencing method that involves digestion of the genomic DNA with two different restriction enzymes followed by size selection, PCR amplification and sequencing of the obtained library on a sequencing platform such as Illumina MiSeq or HiSeq [43]. ddRAD-seq data were generated for 49 diploid *E. siliculosus* individuals (sporophytes), representing three European and one South American population and 6 additional haploid individuals (gametophytes) from two European and one South American population. Sample information and accession numbers are given in Table S2. Sequencing methods followed the protocols described in [30]. Sequences consisted

of paired-end reads obtained using the Illumina HiSeq 2500 system (Illumina Inc., San Diego, CA, USA). Final reads were trimmed to 70 bp. After quality (QUAL >30 and minimum genotype quality =40) and missing data filtering (not more than 40% of missing data per sample and not more than 40% of missing data per locus), 39 samples were retained for further analysis.

Quality-filtered reads were mapped to the *Ectocarpus* 7 reference genome using BWA [44] with the parameters "bwa mem -M -c 2". The genetic map of the reference *Ectocarpus* 7 genome strain provided high-quality annotation and allowed the compartmentalization of the mapped reads to autosomal, pseudoautosomal and non-recombining regions. Genotypes were called using samtools mpileup v.1.6 [45] and filtered using vcftools v.0.1.15 [46]. Only high-quality genotype calls (Phred-scaled mapping quality and genotype quality $\geq$20) were retained and sites with more than 25% missing data were excluded from the downstream analysis. Additionally, since coding sequences can experience positive or negative evolution that will affect their diversity patterns (see [47]), we excluded regions overlapping with exons to minimize the effect of selection and focused on neutrally evolving sites. We used samtools mpileup to report non-variant sites as well as polymorphic sites in order to concatenate the sequenced portion of the genome and assemble a "reduced" genomic sequence.

The method described above was validated by employing an alternative approach, which is described in the Supplementary Materials. Briefly, this method was based on using the Stacks pipeline [48] to carry out a *de novo* analysis of the ddRAD-seq data. The main difference between the first method described above and this alternative method was that, in the latter, only bi-allelic variant positions were called with the Stacks pipeline. Because both approaches gave consistently similar outputs, we focus here on the results obtained using the first method.

2.2.3. Analyses of Non-Recombining and Recombining Coding Regions

In addition to the ddRAD sequencing data, we used cDNA obtained from a total of 20 individuals (11 males and 9 females) from an *E. siliculosus* population from Naples (Italy) to provide further information about genetic diversity in the coding regions of autosomes and the sex chromosome (Table S3). Total RNA was extracted using a chloroform-isoamyl alcohol protocol (adapted from [45]). The SuperScript IV Reverse Transcription System kit (Thermo Fisher Scientific Inc., Villebon sur Yvette, France) was used to synthesize cDNA using random hexaprimers and oligo primers according to manufacturer's instructions. Primers were designed using Primer3 (Table S4). The coding regions of two autosomal, six PAR and five SDR genes (three male-specific and two female-specific) (Table 1) were amplified and sequenced on a 3130xl-3 capillary sequencer (Applied Biosystems Inc., Station Biologique de Roscoff, France). Amplicon sequences were processed with CodonCode Aligner v5.1.5 (http://www.codoncode.com).

Table 1. Selected autosomal, pseudoautosomal regions (PAR) and sex-determining region (SDR) genes analyzed in this study. In brackets after the length of the studied region, is the total length of the coding sequence.

Gene	Functional Description	No. of Sequences	Length of Region Studied (Total CDS)	Segregating Sites	No. of Haplotypes
Autosomes					
Ec-18_002220	Alpha tubulin	19	141 (1362)	1	2
Ec-20_003070	Similar to G-protein coupled receptors	20	747 (1650)	1	2
PAR					
Ec-13_003040	Expressed unknown protein	16	258 (450)	28	10
Ec-13_000140	Expressed unknown protein	19	603 (1200)	8	2
Ec-13_001070	Expressed unknown protein	12	390 (1728)	6	5
Ec-13_003030	Expressed unknown protein	9	330 (648)	5	3
Ec-13_004000	Tetratricopeptide TPR_2 repeat protein	13	423 (2997)	0	1
Ec-13_002700	Expressed unknown protein	18	606 (993)	4	4
SDR					
Ec-sdr_f_000010	STE20-like serine/threonine kinase	8	597 (1265)	0	1
Ec-sdr_f_000090	GTPase activating protein	9	543 (2547)	0	1
Ec-13_001710	GTPase activating protein	11	627 (1944)	2	3
Ec-13_001910	STE20-like serine/threonine kinase	9	1062 (1314)	1	2
Ec-13_001980	Ubiquitin C-terminal hydrolase	11	570 (1110)	0	1

2.3. Data Analysis

We used vcftools [46] and the concatenated genomic sequences described above to calculate nucleotide diversity (π) and Tajima's D for non-overlapping 1-kb windows across the different genome compartments (autosomes, PAR and SDR). Since the SDR regions of both the U and the V are rather small (ca. 1Mbp), the windowed π and Tajima's D values calculated separately for the U and V SDRs were grouped subsequently and represented as one global (concatenated) SDR region to increase the statistical power of the downstream analyses. We performed the analysis of the *E. siliculosus* species (all populations pooled) as a whole and also separately for each population where indicated.

Nucleotide diversity and its partition between synonymous (p_S) and non-synonymous (p_N) mutations and synonymous (D_S) and non-synonymous (D_N) divergence rates for the coding regions in the population from Naples were calculated in DnaSP v5.10.01 [49] using the reference genome strain (*Ectocarpus* 7) as an outgroup. Coalescent simulations were performed with recombination (where applicable) under the standard neutral model with 10,000 iterations to obtain values and 95% confidence intervals of Tajima's D and Fay and Wu's H. The direction of selection (DoS) statistic [50] was calculated using the following formula:

$$\mathrm{DoS} = D_N/[D_N + D_S] - p_N/[p_N + p_S]$$

We used the Wilcoxon test to compare the neutral diversity (π) and Tajima's D values generated per window across different genomic regions. All statistical analyses were performed in RStudio (R version 3.3.2) with graphs produced using the R package ggplot2 [51].

2.4. Data Availability

Data availability and accession numbers are described in Table S2. All sequences have been deposited in the Sequence Read Archive (SRA) under the ID SRP149054 (BioProject ID: PRJNA473288).

3. Results

3.1. Identification of the PAR and SDR in the Sex Chromosome of E. siliculosus

E. siliculosus is a closely related species to the *Ectocarpus* 7 genome-sequenced strain, whose assembly is of very high quality [27,31]. Knowledge of the exact position of the borders of the SDR and PAR is particularly important in the context of this study because theoretical models predict increased neutral diversity at the borders of the SDR and PAR regions [13,17]. To conduct the population genetic tests on the different genomic compartments, we needed to confirm that sister species *E. siliculosus* and *Ectocarpus* 7 (reference genome strain) shared the same PAR-SDR boundary. Previous studies had shown that the SDR of *E. siliculosus* and *Ectocarpus* 7 contain exactly the same genes [29]; however, the analysis focused on genes and did not determine whether the borders of the SDR were the same in the two species. We therefore used a newly generated genetic map for *E. siliculosus* [34] focusing specifically on the sex chromosome, to investigate the location of the SDR (Figure S1). A sex-specific QTL peak was detected at 50.9 cM on the linkage group 2 (362 markers over 104.3 cM). Based on the mapping of *de novo* assembled tags of *E. siliculosus* onto the reference genome, the SDR boundaries (position 2,775,867 bp to position 3,674,342 bp on the chromosome 13 of the reference genome of *Ectocarpus* 7 [52]) were found to be located overall at the same positions in the two species (Figure S1).

Taken together these analyses confirmed that the position of the SDR of the sex chromosome is similar in *E. siliculosus* and *Ectocarpus* 7 (Figure S1). Therefore, we concluded that the ddRAD-seq reads from *E. siliculosus* populations can be assigned to the different genomic compartments based on the *Ectocarpus* 7 genome annotation.

3.2. ddRAD-seq Data

We used ddRAD-seq data generated from samples collected from four populations of *E. siliculosus* (Table S2), three from Europe (Ribadeo and Gandario, in Spain and Roscoff in France) and one from South America (Pan de Azucar, Chile) to assess the extent to which genetic diversity in the male and female SDR, PAR and autosomes differed in different genomic regions.

Once demultiplexed and cleaned, the ddRAD sequencing generated between 14.6 and 28.9 million sequence reads per population (Table S2). Based on the number of uniquely mapped reads to the *Ectocarpus* 7 reference genome, we estimated that the data covered ca. 13% of the reference genome sequence. This proportion of the genome captured is in the range of typical proportions captured by Restriction Associated DNA (RAD) sequencing methods (e.g., [53]) and RAD data has been shown to provide useful information for analyses such as neutral diversity studies [54,55]. After applying stringent filtering (see material and methods), more than 2 million sites were scored across the genome for all individuals including 187,062 SNPs (Table S5).

3.3. DNA Neutral Diversity

Sequence diversity was estimated for the autosomes, the PAR and the male and female SDRs (Figure 2A). The windowed values were calculated separately for the U and V SDRs (Figure S2) and grouped subsequently to be represented as one global SDR region. Neutral diversity (π) was relatively similar across all autosomes (mean $\pi_A = 3.23 \times 10^{-3} \pm 3.47 \times 10^{-5}$ SE) (Figure S3A). Genetic diversity on the SDR was approximately half that of the autosomes (mean $\pi_{SDR} = 0.00221 \pm 3.97 \times 10^{-4}$ SE) and this difference was significant (Wilcoxon test, $p = 0.0005$) (Figure 2A), corresponding approximately to the equilibrium neutral expectations for the population size of these regions (the U and V SDR each have half of the N_e of the autosomes). Remarkably, however, the PAR exhibited significantly higher diversity (mean $\pi_{PAR} = 4.39 \times 10^{-3} \pm 3.11 \times 10^{-4}$ SE; Wilcoxon test, $p = 0.0004$) (Figure 2A and Figure S3A).

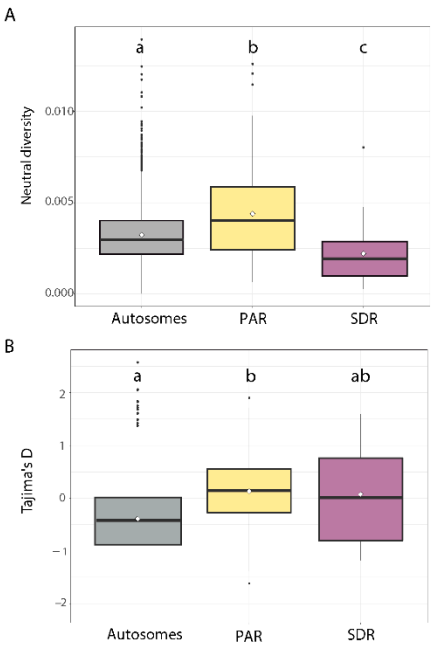

Figure 2. Population genetics statistics for the three *Ectocarpus siliculosus* genomic compartments: autosomes, pseudoautosomal regions (PAR) and sex-determining region (SDR). (**A**) Boxplots of neutral diversity (π), calculated in 1 kb windows without overlap; (**B**) Boxplots of Tajima's *D*, calculated in 1 kb windows without overlap. The mean values are represented by the diamond shape. Letters above the boxplots denote significant differences (Wilcoxon test, $p < 0.0005$).

Although π values were higher overall in the PAR compared to the autosomes (Figure 2A), sliding window analysis along the sex chromosome (Figure 3A) did not show any clear bias towards elevated π regions being located close to the SDR.

Another factor that could influence genetic diversity is gene density. Higher gene density implies more nearby sites potentially under selection and could influence the levels of diversity in the linked sites in a negative manner. Negative correlations between gene density and local levels of neutral diversity have recently been described in *Heliconius* species [47] We found a weak positive correlation between local gene density and neutral diversity along the PAR (Spearman's rho = 0.27, $p = 0.016$), but no correlation (Spearman's rho = 0.03, $p = 0.915$) for an autosomal chromosome of similar size (chr4). Positive correlation with gene density on the PAR could indicate increased polymorphism due to balancing selection. Recent work in *Silene* has shown that some of the loci on the PAR that exhibit high diversity may be under balancing selection [19], which is reflected by positive Tajima's *D* values [56]. To test the possibility that the high diversity values found in the *Ectocarpus* PAR region reflect balancing selection we therefore performed Tajima's *D* tests on the different genomic compartments (autosomes, PAR and SDRs) (Figures 2B and 3B).

Overall Tajima's *D* was negative for all autosomes (mean Tajima's $D = -0.389 \pm 0.014$ SE, Figure S3B). Strikingly, however, Tajima's *D* showed positive values for the PAR that were significantly higher than values obtained for autosomes (mean Tajima's $D = 0.130 \pm 0.090$ SE, Wilcoxon test, $p = 2.1 \times 10^{-8}$) (Figure 2B). Tajima's *D* was also elevated in the SDR (mean Tajima's $D = 0.0761 \pm 0.196$ SE) but was not significantly different from that of the autosomes.

3.4. Comparison of DNA Neutral Genetic Diversity Pattern between the Four Study Populations

The neutral diversity and Tajima's *D* pattern along the sex chromosome of *E. siliculosus* was analyzed separately for each of the four study populations (Figure S4A,B respectively). The pattern of diversity in the PAR was similar despite their different geographical origin and diverse ecological environments (Figure S4A) (Wilcoxon pairwise test between populations, $p > 0.2$). The pattern of Tajima's *D* on the other hand was more variable (Figure S4B), and this result may reflect differences among population history such as different demographic processes.

3.5. PAR Nucleotide Diversity and Generation-Biased Genes

The PAR of *Ectocarpus* exhibits unusual characteristics compared to the autosomes, including higher transposable element content, lower gene density and higher D_N/D_S rates [28,57]. Of the 455 genes on the PAR, 177 show generation-biased expression patterns (out of 6202 genome-wide [58]) and the PAR regions are significantly enriched in sporophyte-biased genes (82 compared to 2097 genome-wide) [28,57]. Interestingly, when we plotted diversity in sliding windows and compared it with the position of these generation-biased genes, the pattern of neutral diversity on the PAR was significantly correlated with the distribution of the generation-biased genes in the windows of analysis (Figure 3A) (Kendall rank correlation test: tau = 0.18, $p = 0.02$). For Tajima's *D* per window (Figure 3B), the correlation was not significant (Kendall rank correlation test: tau = -0.03, $p = 0.7$).

Based on the linkage map, the average recombination rate for the PAR was 19.2 cM/Mb. Four autosomes of similar size (chr4, chr5, chr21 and chr26) had average recombination levels in a similar range (between 17 and 20.6 cM/Mb). As shown before for *Ectocarpus* 7 [28], the PAR of *E. siliculosus* did not exhibit a significantly higher recombination rate on average than the autosomes. To investigate the possibility of a direct link between recombination rate and nucleotide diversity, we plotted recombination rate together with neutral diversity along the sex chromosome as well as for one representative autosome with a similar number of sliding windows for the π estimates (Figure S5). We also analyzed the correlation between neutral diversity and the recombination rate for those two chromosomes (Figure S6). In the sex chromosome, recombination rate was lower around the position of the SDR (as expected) and at the one of the telomeres (Figure S5). Correlation analysis indicated a weak negative correlation (r = -0.18) between recombination rate and nucleotide diversity,

which was barely significant (p = 0.049). In the autosome, we observed more variation in the recombination rate than in the pattern of nucleotide diversity along the chromosome but no significant correlation between nucleotide diversity and recombination rate was observed (Figures S5 and S6).

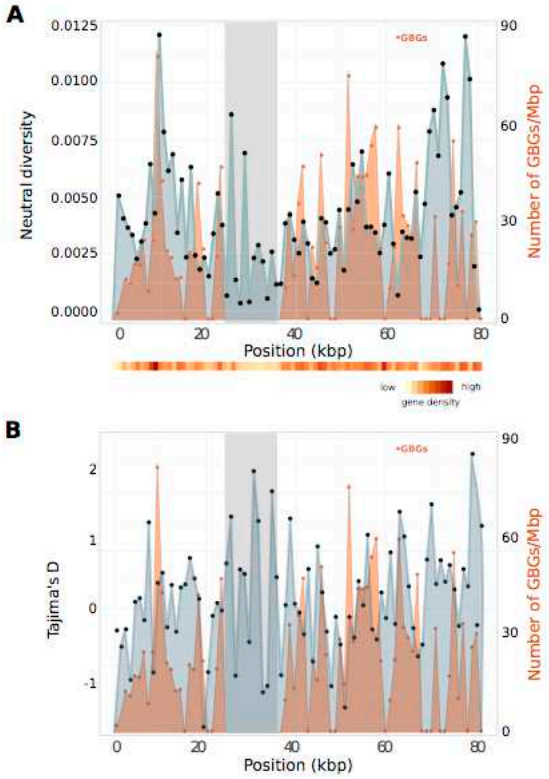

Figure 3. Population genetic statistics for the sex chromosome of *Ectocarpus siliculosus*. (**A**) Sliding window analysis of neutral diversity (π) in 1 kb non-overlapping windows along the concatenated ddRAD sequences of the sex chromosome. Values of π are indicated by black dots; (**B**) Sliding window analysis of Tajima's D in 1 kb windows (indicated by black dots) along the concatenated sex chromosome sequence. The number of genes with differential expression in the sporophyte or gametophyte generation (generation-biased genes or GBGs) normalized by the physical distance covered by the concatenated 1 kb ddRAD window are marked in orange. Global gene density along the concatenated sex chromosome sequence is represented by the heatmap. The position of the sex-determining region is shaded in gray.

3.6. Genetic Diversity in a Selected Subset of Autosomal, PAR and SDR Genes

ddRAD-seq data used in this study gave a broad overview of neutral diversity (because we removed regions corresponding to exons from the data before analysis) but did not provide information specifically for genes. To investigate diversity patterns at the gene level in the PAR and SDR, and in particular to test if the signal of high values of π in the PAR measured using the ddRAD-seq data was due to footprints of balancing selection on generation-biased genes, we determined π for a subset of six single-copy PAR genes and five single-copy SDR genes (two female-specific and three male-specific, Figure 4A) to study. For the PAR subset, we chose four generation-biased genes and two genes without significant bias in expression. We also sequenced two single-copy autosomal genes.

All 13 autosomal, PAR and SDR genes were successfully amplified from at least eight *E. siliculosus* individuals and aligned to the reference sequence from *Ectocarpus* 7 [52]. Nucleotide diversity statistics for all genes were studied based on synonymous sites (Tables 1 and 2 and Figure 4).

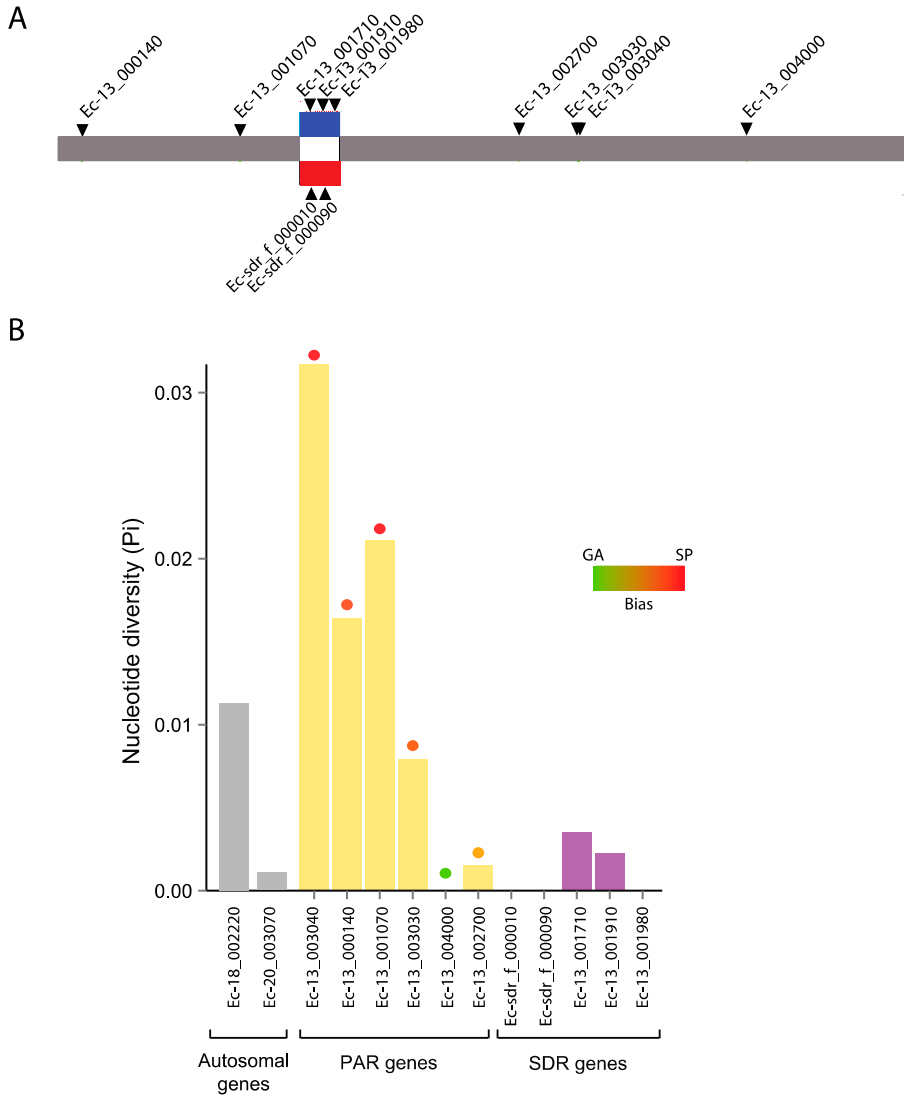

Figure 4. Neutral diversity of PAR and SDR genes in *Ectocarpus siliculosus*. (**A**) Physical position of the studied PAR genes on the sex chromosome. Gene names are indicated, the sex-determining region (SDR) is marked in red (female) and blue (male); (**B**) Diversity of the studied autosomal, PAR and SDR genes at synonymous sites. The level of differential expression (fold change) of the PAR genes in the sporophyte (SP) and gametophyte (GA) generations is represented by the colored circles (green denotes strong GA-bias and red stands for strong SP-bias).

Table 2. Measurements used to infer the evolutionary forces acting on autosomal, PAR and SDR genes. Significant *p*-values ($p < 0.05$) for the neutrality tests and for the bias in expression between generations are shown in boldface.

Gene	π_{Syn} #	D_N/D_S	DoS	Tajima's *D*	*p*-Value	Fay and Wu's *H*	*p*-Value	$\log_2$FC (SP/GA) *
Autosomes								
Ec-18_002220	0.01138	0	No replacements	0.417	0.368	0.257	0.196	−0.52
Ec-20_003070	0.00106	0.13	0.316	−0.592	0.501	**−1.516**	0.015	−0.78
PAR								
Ec-13_003040	0.032	0.512	−0.223	**−1.740**	0.0002	−3.467	0.102	**2.25**
Ec-13_000140	0.016	0.253	0.105	**2.033**	0.0007	−1.392	0.167	**6.52**
Ec-13_001070	0.02	0	0	−0.120	0.443	−0.848	0.171	**1.19**
Ec-13_003030	0.008	0.494	0.158	**−1.678**	0.013	**−4.278**	0.0073	**0.98**
Ec-13_004000	0	0.312	no polymorphism	NA	NA	NA	NA	**0.71**
Ec-13_002700	0.002	0.109	−0.265	−1.381	0.050	−0.863	0.101	−0.44

Table 2. *Cont.*

Gene	π_{Syn} #	D_N/D_S	DoS	Tajima's *D*	*p*-Value	Fay and Wu's *H*	*p*-Value	$\log_2$FC (SP/GA) *
SDR								
Ec-sdr_f_000010	0	0	no polymorphism	NA	NA	NA	NA	−0.27
Ec-sdr_f_000090	0	0.6	no polymorphism	NA	NA	NA	NA	0.41
Ec-13_001710	0.00351	0.692	0.190	0.199	0.399	0.255	0.412	−0.53
Ec-13_001910	0.00229	0.077	0.222	1.401	0.122	−0.139	0.210	0.16
Ec-13_001980	0	2.077	no polymorphism	NA	NA	NA	NA	−0.54

* SP—sporophyte, GA—gametophyte, FC—fold change in expression. # π_{Syn} values for synonymous sites.

Table 2 and Figure 4 summarize the diversity statistics for all genes, based on synonymous sites. Consistent with our ddRAD-seq data, PAR genes tended to exhibit high neutral diversity with a mean and standard error of $\pi = 0.013 \pm 0.0046$, whereas SDR genes exhibited the lowest diversity (0.0012 ± 0.0007), though it should be noted that this difference was not found to be significant by a pairwise Wilcoxon test ($p = 0.28$). The two autosomal genes showed an average $\pi = 0.0062$. Consistent with the ddRAD-seq data, no obvious correlation could be observed between the neutral diversity of PAR genes in relation to their distance from the SDR (Figure 4), i.e., the genes with the highest diversity were not necessarily located closer to the SDR border. We noted, however, that all five sporophyte-biased genes showed higher π values, whereas the two genes that were not SP-biased showed lower π (Figure 4). Note however, that statistical power was low for these analyses because of the limited number of genes studied.

3.7. Evolutionary Histories of the Selected Autosomal, PAR and SDR Genes

Most of the seven selected PAR and SDR genes that were polymorphic exhibited negative Tajima's *D* values, except for one gene in the PAR (Ec-13_000140) and two genes in the male SDR (Ec-13_001710 and Ec-13_001910) (Table 2). The positive DoS values for the latter two genes suggest that they are evolving under positive selection. However, a scenario of random differences being fixed due to the smaller effective population size (N_e) of the SDR (compared to the autosomes) cannot be ruled out [59]. Out of the six PAR genes, only Ec-13_001070 appeared to be evolving neutrally. Ec-13_004000 might also be evolving neutrally but the relatively high D_N/D_S value of 0.312 suggests positive selection.

The PAR gene Ec-13_003030 is also most likely evolving under positive selection (or has undergone a selective sweep) given the positive DoS value, and strongly negative Tajima's *D* and Fay and Wu's *H* values. Similarly, a positive DoS value suggests that Ec-13_000140 is also under adaptive selection. However, Ec-13_000140 also has a significantly positive Tajima's *D* ($p < 0.01$) which strongly suggests that the gene may be under balancing selection, a phenomenon that cannot be detected by the DoS statistic. Please note that on the other hand, Ec-13_003040 and Ec-13_002700 seem to be evolving under relaxed purifying selection, as they have much higher polymorphism than divergence (indicated by the negative DoS value). Concerning the autosomal genes, one lacked replacements sites and hence D_N/D_S could not be estimated. The second one showed a low D_N/D_S value and none of them showed a significant Tajima's *D* value.

4. Discussion

4.1. Non-Recombining Regions in U and V Chromosomes Exhibit Reduced Neutral Diversity

Reduced neutral diversity is a hallmark of Y chromosomes [60] and other non-recombining chromosomes [61]. Reduction in neutral diversity is expected to be proportional to the effective population size of the non-recombining chromosomes compared to the autosomes, leading to a prediction of $\frac{1}{4}$ as much neutral diversity on the Y chromosome compared to the autosomes in XY systems or $\frac{1}{2}$ as much neutral diversity on the V or U SDRs compared to the autosomes in UV

sex-determination systems, assuming a 1:1 sex ratio and similar levels of reproductive success in males and females. The data presented in this study was in line with this prediction, with the neutral diversity of the combined U and V sex-determining regions being 0.68 of the autosomal chromosomes.

In several species, the observed levels of π for the SDR have been lower than would have been predicted based solely on this neutral effect. In *Rumex*, for example, the low level diversity observed in the Y region is consistent with an important role of linked selection, with either effects of purifying selection alone or combined with positive selection driving loss of diversity [62]. Similarly, neutral diversity on the human Y chromosome [60] and on the W chromosome of birds [63] was drastically lower than that of autosomes (5–10 times and 8–13 times, respectively). These patterns of loss of diversity could be driven by selective sweeps due to sexual selection acting on testis-specific Y chromosome genes [64]; however, they can also occur in the absence of sexual selection as shown in the example of the flycatcher's W chromosome [63].

In contrast, the agreement between the theoretical prediction and the observed level of neutral diversity for *Ectocarpus* suggests that evolution of its SDR is not significantly driven by background selection or selective sweeps. In other words, since the observed pattern of diversity could be explained by neutral processes (e.g., stochastic processes caused by genetic drift), it is not necessary to propose additional evolutionary forces such as background selection or selective sweeps. This finding is congruent with our previous studies that failed to detect signatures of positive selection acting on a set of SDR genes that have been conserved across several brown algal species [29]. Please note that the *Ectocarpus* SDR is rather small with only 20 and 22 genes being sex-linked on the V and U chromosomes, respectively [26] and this species displays limited levels of sexual dimorphism [57]. In the absence of recombination, Hill-Robertson interference should decrease the local N_e and thereby diversity due to effects of linked selection, but the magnitude of this effect will depend on the number of linked selected sites [62,65,66]. Therefore, a small SDR may be less affected by Hill-Robertson interference than an SDR with many genes where there is a larger scope for selection.

4.2. Increased Nucleotide Diversity on the PAR Compared with Autosomes

Our data indicate that the PAR of the sex chromosome had higher median neutral diversity than any of the autosomes. Given that the PAR is also enriched in sporophyte-biased genes, one interesting possibility is that the two phenomena are connected. Such an association is supported by the observation that all of the five genes that exhibited SP-biased expression in our gene-by-gene analysis also presented high π_{syn} values and there was evidence that at least one of the genes was evolving under balancing selection.

Two of the five SP-biased genes (Ec-13_002700 and Ec-13_003040) also presented sex-biased gene expression and, overall, the PAR has been shown to be enriched in female-biased genes [28]. These observations open up the possibility that polymorphism may be maintained both by generation-antagonistic selection and by sexually-antagonistic selection, as has been proposed for *Silene* [19]. A recently proposed model to explain the spread of generation-biased alleles in the PAR of *Ectocarpus* [28] assumes that the evolution of the PAR in haploid systems is influenced by differential selection pressures in males and females acting on alleles that are advantageous during the sporophyte generation of the life cycle. One consequence of this model is that loci on the PAR that are subject to sexually-antagonistic selection would tend to be more polymorphic.

We have also considered alternative explanations for the elevated level of neutral diversity on the PAR. For example, we cannot exclude an effect of recombination rate on the variation of the nucleotide diversity [47,67,68]. Our analysis showed a weak negative correlation between recombination rate and neutral diversity, but this correlation was barely significant. No significant correlation was observed for a representative autosome. A positive correlation between DNA diversity and recombination rate has been found in many organisms including animals [69–73], plants [74–76] and fungi [77]. Although such patterns have been attributed to the action of natural selection, neutral explanations for an observed positive correlation between recombination and diversity have also

been proposed, when, in addition to intraspecific variation, recombination rate was correlated with divergence (e.g., [78]). There are also cases where such a correlation has not been found. For example, in maize, single nucleotide polymorphism and recombination rate were found to be uncorrelated [79–81]. Additional studies will be required to depict more precisely the relationship between neutral diversity and recombination rate in *Ectocarpus* genomes.

Finally, the higher diversity of the PAR could also potentially be explained by a higher mutation rate. Elevated local mutation rate (as D_S) was a strong predictor of intraspecific diversity in *Heliconius* species [47] but did not explain higher levels of π in the PAR of *Silene* [19]. In *Ectocarpus*, PAR genes have been shown to have significantly higher D_S than autosomal genes [28], so an analysis of mutation rates across the genome would be valuable to understand the role of this parameter in driving higher genetic diversity across the PAR. A thorough comparison of nucleotide variations between parents and progeny of a mapping population for instance would allow the mutation rate within the different chromosomal regions to be evaluated. Such an analysis has been carried out for the collared flycatcher [82].

4.3. Lack of a Regional Pattern of PAR Diversity in Relation to the SDR

Neutral sites very closely linked to any balanced polymorphism are expected to have higher diversity than surrounding genome regions [83]. Neutral diversity is thus expected to be elevated in the regions of the PAR that are closely linked to the SDR [17]. However, recombination during each generation will break down the association and the effect on diversity will become negligible unless both the recombination rate and the effective population size are small. This prediction is consistent with the observation that the peaks of polymorphism near sites known to be under long-term balancing selection are often confined to the gene itself. Therefore, under the neutral null hypothesis, only PAR genes very closely linked to the SDR should be affected [17]. Both ddRAD-seq and gene-by-gene analysis failed to find evidence for a pattern of elevated π close to the SDR border in *Ectocarpus*. The failure to find such a pattern could be due to the low resolution of ddRAD-seq markers or may be explained by a disruption of the PAR-SDR association due to gene movement and chromosomal rearrangements. The latter explanation was proposed to explain neutral diversity patterns in *Silene* [8]. Consistent with this, we showed recently that brown algal UV sex chromosomes are very dynamic with a lot of gene trafficking and rearrangements taking place [29]. Another possibility, again also suggested for *Silene* [8], is that the PAR used to be a non-recombining region, and recombination started recently. Under this hypothesis, the increased number of variants we found on the PAR could reflect a long evolutionary history of complete sex linkage. This hypothesis would also explain the intermediate characteristics of the PAR (between the autosome and SDR) in terms of GC content, TE content, gene density, D_S, gene size etc. [28]. Additional information about the PARs of more brown algal species is required to fully understand the evolutionary history of these interesting chromosomal regions.

5. Conclusions

We have used ddRAD-seq and gene-by-gene analyses to investigate, for the first time, patterns of neutral diversity in a UV sex determination system. Our results confirm theoretical predictions for UV SDRs, which predict that the level of genetic diversity in such regions should be about half that of the autosomes. This correlation between the theoretical prediction and experimental measurements suggests that the evolution of the SDR in *Ectocarpus* is not significantly impacted by Hill-Robertson effects.

In contrast, the PAR exhibited a higher level of diversity than the autosomes and high levels of Tajima's D, suggesting balancing selection. This observation is interesting because the PAR of *Ectocarpus* has been shown to have an unusual structure with low gene density, high transposable element content and an enrichment in both generation biased and sex-biased genes [28,57]. More work is needed to understand whether, and to what extent, these structural features underlie the high level of genetic diversity.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4425/9/6/286/s1, Table S1: Samples used for genetic mapping of the sex locus of *E. siliculosus*; Table S2: Natural populations used for ddRAD sequencing and ddRAD-seq data summary; Table S3: Samples used in the analysis of the population genetics statistics in coding regions; Table S4: PCR primers used for amplification of the PAR and SDR genes; Table S5: Summary statistics of the VCF files; Figure S1. Mapping of the sex-determining region in *Ectocarpus siliculosus*; Figure S2. Population genetics statistics for the three *Ectocarpus siliculosus* genomic compartments: autosomes, PAR and SDR; Figure S3. Population genetics statistics for the individual autosomes, PAR and SDR in *Ectocarpus siliculosus*; Figure S4. Comparison of neutral diversity patterns along the sex chromosome of *Ectocarpus siliculosus* between the four study populations; Figure S5. The landscape of neutral diversity (π) and recombination rates along the sex chromosome and autosomes in *Ectocarpus siliculosus*; Figure S6. Correlation between neutral diversity and recombination rate.

Author Contributions: A.M., A.F.P., M.J., L.M., S.A. and K.A. prepared the biological material and performed experiments. A.L., M.J. and K.A. performed the computational analysis. A.L., K.A. and S.M.C. wrote the manuscript with valuable input from M.V., D.R. and J.M.C. S.M.C. coordinated the study. All authors read and approved the final manuscript.

Funding: This work was supported by the CNRS, Sorbonne Université, the project IDEALG (France: ANR-10-BTBR-04), the Brittany Region grant SAD 2015-ECTOQTL 9119 to K.A., the Becas-Chile doctoral grant to A.E.M. (CONICYT, advanced human resources program) and the ERC (grant agreement 638240). Field collections in Chile and Italy (2012/2013) were supported by two grants from the EU FP7 "capacities" specific program ASSEMBLE to A.F.P. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Acknowledgments: We thank Thomas Broquet and Christophe Destombe for fruitful discussions, Dieter G. Muller for the isolation and maintenance of the *Ectocarpus siliculosus* strains. We thank Gildas Le Corguillé from the Roscoff Bioinformatic platform: ABiMS (http://abims.sb-roscoff.fr) for bioinformatics support. We thank Jérôme Coudret, Chloé Jollivet and Stéphane Mauger for technical support. Finally, we thank the two anonymous reviewers for their constructive comments, which helped us to improve the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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Appendix 2

The origin and evolution of the sexes: Novel insights from a distant eukaryotic linage.

C. R. Biologies 339 (2016) 252–257

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The origin and evolution of the sexes: Novel insights from a distant eukaryotic linage

L'origine et l'évolution des sexes : de nouvelles idées à partir d'une lignée eukaryote lointaine

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ARTICLE INFO

Article history:
Received 5 March 2016
Accepted after revision 14 April 2016
Available online 25 May 2016

Keywords:
Evolution
Sex determination
Brown algae
Sex chromosomes
Reproductive system

ABSTRACT

Sexual reproduction is an extraordinarily widespread phenomenon that assures the production of new genetic combinations in nearly all eukaryotic lineages. Although the core features of sexual reproduction (meiosis and syngamy) are highly conserved, the control mechanisms that determine whether an individual is male or female are remarkably labile across eukaryotes. In genetically controlled sexual systems, gender is determined by sex chromosomes, which have emerged independently and repeatedly during evolution. Sex chromosomes have been studied in only a handful of classical model organism, and empirical knowledge on the origin and evolution of the sexes is still surprisingly incomplete. With the advent of new generation sequencing, the taxonomic breadth of model systems has been rapidly expanding, bringing new ideas and fresh views on this fundamental aspect of biology. This mini-review provides a quick state of the art of how the remarkable richness of the sexual characteristics of the brown algae is helping to increase our knowledge about the evolution of sex determination.

Mots clés :
Évolution
Déterminisme du sexe
Algues brunes
Chromosomes sexuels
Systèmes de reproduction

RÉSUMÉ

La reproduction sexuée est un phénomène extrêmement répandu, qui assure la production de nouvelles combinaisons génétiques dans presque toutes les lignées eucaryotes. Bien que les caractéristiques de base de la reproduction sexuée (méiose et syngamie) soient hautement conservées, les mécanismes de contrôle qui déterminent si un individu est mâle ou femelle sont remarquablement labiles chez les eucaryotes. Dans les systèmes sexuels génétiquement contrôlés, le sexe est déterminé par les chromosomes sexuels, qui ont émergé de façon indépendante et à plusieurs reprises au cours de l'évolution. Les chromosomes sexuels ont été étudiés dans seulement une poignée d'organismes modèles classiques, et la connaissance empirique sur l'origine et l'évolution des sexes est encore étonnamment incomplète. Avec l'avènement des nouvelles méthodes de séquençage, la représentativité taxonomique des systèmes modèles s'est rapidement développée,

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http://dx.doi.org/10.1016/j.crvi.2016.04.012

apportant des idées et des points de vue nouveaux sur cet aspect fondamental de la biologie. Cette mini-revue fournit un constat rapide de la façon dont la richesse remarquable des caractéristiques sexuelles des algues brunes contribue à accroître nos connaissances quant à l'évolution des déterminismes du sexe.

1. Introduction: a myriad of sex determination mechanisms

Sexual reproduction is an extraordinarily widespread phenomenon that assures the production of new genetic combinations in nearly all eukaryotic lineages. Although the core mechanisms of sexual reproduction (meiosis and gamete fusion or syngamy) are highly conserved, the pathways that determine the male and female specificities are remarkably diverse across eukaryotes and appear to have had rapid turnover rates during evolution [1].

Genetic determination of sex is mediated by sex chromosomes, whose key feature is the sex-determining region (SDR) that carries the sex-determining factor(s) and usually does not recombine in the heterogametic sex. The SDR can be as small as a single locus or as large as an entire chromosome. Sex chromosomes are derived from autosomes and have independently evolved many times in different eukaryotic lineages. Sex chromosomes can arise in species with separate sexes in which sex is determined by environmental cues (such as in turtles, where temperature determines the sex of developing embryos) and can also arise in hermaphrodites (that is, individuals with both male and female sex organs). Sex chromosomes are subject to unique evolutionary forces including sex-specific selection, asymmetrical sheltering of deleterious mutations, hemizygosity, or dosage compensation [2]. Besides sex determination, sex chromosomes play a prominent role in several evolutionary processes such as speciation and adaptation [3].

Traditionally, most of our knowledge on the biology and evolution of sex chromosomes stems from a few well-studied model organisms, notably mammals, birds, *Drosophila* and the plant *Silene latifolia*. Animals and plants, however, represent just two out of the 21 major groups of Eukaryotes (Fig. 1A) and the universality of the mechanisms driving the evolution of the sexes is difficult to assess without a broader view that takes into account the scope of the tree of life. In this context, the brown algae, a group of complex multicellular eukaryotes, are extraordinary comparative models to look at how the sexes have originated and evolved in a lineage that has been independently evolving from animals and plants for more than a billion years.

Moreover, while studies so far have focused on diploid sex determination systems (the classical XY or ZW systems, Fig. 1B), haploid phase sex-determination systems (UV systems such as those of mosses and algae) [4] have been less studied. Contrasting the properties of XY/ZW and UV chromosomes, together with comparisons with species that lack sex chromosomes entirely, provides an outstanding chance to assess the relative importance of the forces driving the evolution of each system.

2. Evolution of sex chromosomes: new insights from UV systems

In recent years, comparative studies of sex chromosomes of different ages from both animals and plants have led to the emergence of a model for the evolution of sex chromosomes [5]. A typical sex chromosome pair derives from a pair of autosomes through the acquisition of genes involved in sex determination. If more than one locus involved in sex determination is located on the chromosome, recombination between loci is suppressed, leading to the establishment of a non-recombining region on the nascent sex chromosome, the sex-determining region or SDR. The formation of this non-recombining region has important consequences for the evolution of this part of the genome: repetitive DNA tends to accumulate, leading to an increase in SDR size and degeneration of genes within the non-recombining region. At a later stage, deletion of non-functional DNA from within the SDR may lead to a decrease in the physical size of the SDR. The evolutionary processes driving the expansion of the SDRs (and corresponding shrinkage of the pseudoautosomal region, PAR) have been the subject of research, mostly theoretical, for many years and the currently accepted view is that this process is driven by the recruitment of genes with differential selective benefits for the two sexes (sexually antagonistic genes) into the SDR [6], to resolve sexual conflicts.

The evolution of sex chromosomes is largely impacted by the genetic mechanism of sex determination. In organisms where sex is expressed in the diploid phase, such as most animals and land plants, one sex is heterogametic (XY or ZW) whilst the other is homogametic (XX or ZZ). In these systems only the Y or W contain non-recombining regions because the X and Z recombine in the homogametic sex. In contrast, in some algae and bryophytes the male and female sexes are genetically determined after meiosis, during the haploid phase of the life cycle (Fig. 1B). This type of UV sexual system exhibits specific evolutionary and genetic properties that have no exact equivalent in diploid systems. A number of verbal predictions regarding UV evolution had been formulated, but they have long lacked empirical support. The U and the V have been predicted to show similar characteristics because they function in different individuals and both are non-recombining; furthermore, degeneration should be

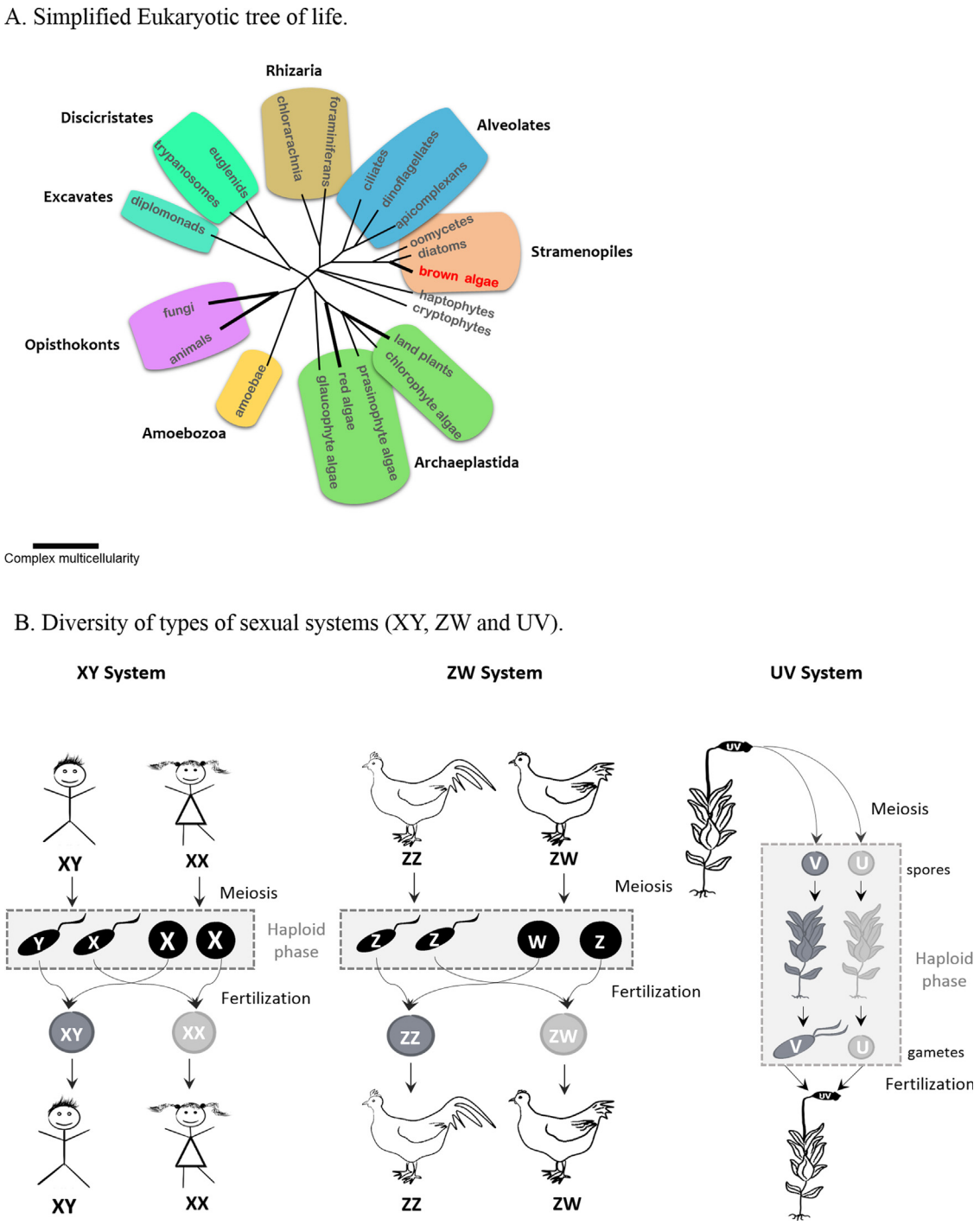

Fig. 1. A. Simplified Eukaryotic tree of life, adapted from [24]. B. Diversity of types of sexual systems (XY, ZW and UV). In organisms with diploid life cycles, sex is determined in the diploid phase of the life cycle, after fertilization. In XY systems, the sex of the embryo depends on the chromosome carried by the sperm cell, X or Y. In ZW it is the female egg that determines the sex of the individual. In organisms such as some algae and mosses, that alternate between gametophyte and sporophyte generations (haploid-diploid life cycles), sex is expressed during the haploid (gametophyte) phase of the life cycle. The sexual system in this case is called UV systems. In contrast to XY and ZW systems, sex in UV systems depends on whether the spores receive a U or a V chromosome after meiosis (not at the fertilisation stage).

modest because purifying selection is expected to act on the *SDR* genes during the haploid phase of the life cycle, and the U and V are expected to experience mainly gene gain [7].

Recent work using the model brown alga *Ectocarpus* (Box 1) has made an important contribution to the empirical validation of predictions in relation to sex chromosomes. The *Ectocarpus* UV system displays some

Box 1. *Ectocarpus*, a model organism for the brown algae

The development of *Ectocarpus* (Fig. 2), a small filamentous brown alga (**A**), as a model brown alga was closely linked to an international effort to sequence the complete male genome of this alga [19] and, more recently, two female *Ectocarpus* strains [8] that, together, have allowed the identification of the U and V sex chromosomes [8]. The advantage of such a model lies in having a haploid-diploid life cycle, which involves alternation between two independent multicellular generations: the haploid gametophyte and the diploid sporophyte. Sex of gametophytes depends on whether they receive a U or a V chromosome after meiosis. Male and female gametes are produced by mitosis in specialised cells on the gametophyte called plurilocular sporangia (**B**). Further tools currently existing for this model organism include a large EST collection, whole genome tiling arrays, deep sequencing of mRNAs during several stages of the life cycle, small and miRNAs, a genetic map, stramenopile-adapted bioinformatic tools and proteomics and metabolomics methodologies [19–22]. A number of genetic tools have also been developed, including protocols for mutagenesis, phenotypic screening methods, genetic crosses, methods for handling large populations, a large number of genetic markers and defined strains for genetic mapping and a TILLING mutant collection [20,23]. To date, more than 4400 *Ectocarpus* mutant lines have been generated for this resource. A large *Ectocarpus* strain collection (over 2500 strains, not including large segregating populations obtained in laboratory) has been established in Roscoff (Behzin Rosko), and this collection is currently being integrated in the Roscoff Culture Collection making it therefore available for the scientific community. This collection includes strains from all around the world and represents an important resource for studying genetic variation within the genus.

striking similarities with XY and ZW systems, such as low gene density and accumulation of repeated DNA in the non-recombining, sex-determining region. This highlights the universality of some of the processes shaping sex chromosome evolution across distant lineages. The *Ectocarpus* system has, however, had a distinct evolutionary trajectory. Remarkably, although this UV system is very old ($>$ 100 Myr), the SDR has remained small [8,9]. A possible explanation for this feature was suggested by the low number of sex-biased genes, implying that sexual conflict in *Ectocarpus* may be insufficient to drive extensive SDR expansion [8,10]. This result is therefore consistent with the view that sex antagonism may drive SDR expansion. Surprisingly, both the male and female SDR haplotypes showed signs of degeneration despite the action of purifying selection during the haploid phase of the life cycle, which is expected to purge mildly deleterious mutations and therefore prevent degeneration.

Taken together, we can say that studies using the SDR of ''exotic'' organisms have increased our knowledge on the evolutionary trajectory of UV systems, and have provided, and will certainly continue to provide, a broader phylogenetic dimension to sex chromosome evolutionary models.

While the genomic and evolutionary characteristics of the sex-specific regions are increasingly well understood, the recombining regions (the 'pseudoautosomal region' or PAR) of the sex chromosomes have been less studied. The PARs are expected to behave like autosomes and recombine during meiosis; therefore genes in this region are inherited in an autosomal rather than a strictly sex-linked fashion. Genomic-scale analyses have been carried out for the PARs of a less than a handful of old XY or ZW sexual systems [11]. Recent work using the well assembled PAR of the UV system of *Ectocarpus* showed that although PARs recombine at a rate that is not different from any other region of its genome, this region exhibits a number of structural and evolutionary features that are typically associated with regions of suppressed recombination [10]. The PAR of this UV system has accumulated genes that are differentially expressed during the sporophyte versus gametophyte generation of the life cycle, compared to the rest of the genome, and these generation-specific genes exhibited clear signs of accelerated evolution. Generation-antagonistic forces may therefore affect the evolution of the PARs [10]. This, and the work described above on the sex-determining region of the *Ectocarpus* UV sex chromosomes, has brought to light the critical impact of the life history of the organisms, in particular the type of life cycles, in the evolution of sex chromosomes.

3. Beyond *Ectocarpus*: brown algae as models to understand the origin and evolution of sexual systems

In this context, the brown algae are emerging as interesting models for investigating the origins and evolution of sex chromosomes because they exhibit a bewildering variety of life history traits, e.g. reproductive systems, types of life cycle and sex chromosome systems in a single group, and there appears to have been considerable switching between variants of these different features on an evolutionary timescale. The maintenance of this range of variability in a single group is actually quite unique among the eukaryotes, and clearly points to a complex evolutionary history of the underlying sex-determination systems.

The brown algae represent a unique group for studies of the evolution of the sexes, particularly with regard to the evolution of gamete size sexual dimorphism as this group exhibits a high level of variability for this trait, ranging from isogamous, through anisogamous, to oogamous systems [12]. Anisogamy has evolved several times in this group and somewhat surprisingly, oogamy seems to be the ancestral state in brown algae [13]. This opens the intriguing possibility that oogamy may evolve towards isogamy, despite the fact that transitions from oogamy towards isogamy are difficult to explain from a theoretical point of view [14]. The diversity of levels of gamete dimorphism and the multiple transitions between gamy levels within the brown algae are highly interesting in the context of recent views on the evolution of gamete size dimorphism and its consequences for the evolution of the SDRs. Evidence has been reported for linkage between

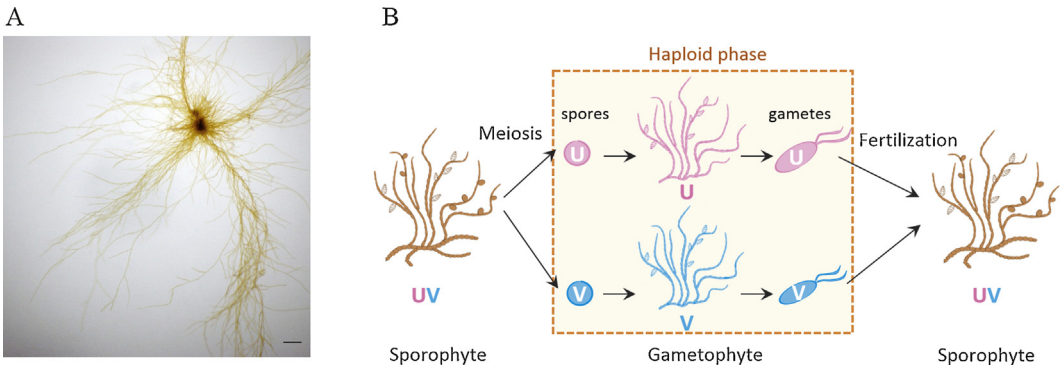

Fig. 2. A. *Ectocarpus* gametophyte. B. The haploid-diploid life cycle of *Ectocarpus*.

the mating type (MT) locus and a gene(s) controlling gamete size in the green alga *Chlamydomonas*, and it has been suggested that the MT locus in a related oogamous species underwent significant expansion correlated with the evolution of gamete size sexual dimorphism [15]. Yet, the molecular basis of gamete size control and its link to sex determination remains unclear. The brown algae, thus, may represent great comparative systems to complement work done in the green lineage.

Differences in gamete size in anisogamous and oogamous brown algal species may influence other reproductive characteristics. In particular, gamete size may be one of the factors that determine whether a gamete is capable of undergoing asexual reproduction through parthenogenesis should it fail to encounter a gamete of the opposite sex. In isogamous, anisogamous and oogamous brown algal species there are marked differences between the relative parthenogenetic capacities of male and female gametes [13,16]. A trend is apparent: usually both male and female gametes of isogamous brown algal species are capable of parthenogenesis whereas only the female gametes of anisogamous species are parthenogenetic (i.e. in the latter parthenogenesis is a sexually dimorphic trait). Neither the male nor the female gametes undergo parthenogenesis in oogamous species. Overall, these tendencies suggest that gamete size may influence parthenogenetic capacity up to a point, but that in oogamous species the large female gamete is specialised for zygote production and is no longer capable of initiating parthenogenetic development [12].

The brown algae are the Eukaryotic lineage that exhibits the broadest diversity of types of life cycle and a widest range of different sexual systems. For example, sexuality is expressed during the diploid phase of the life cycle in brown algae with diploid life cycles (dioecy) such as the fucoids, whereas it is the haploid gametophyte generation that exhibits sexuality (dioicy) in algae such as *Ectocarpus* that have haploid-diploid life cycles [12]. The selective pressures leading to the evolution of these different systems are distinct: whilst dioecy might evolve from monoecy to limit inbreeding (due, in the latter, to the fertilisation of female gametes by male gametes produced by the same organism), this is unlikely to be the case for dioicy because deleterious mutations should be efficiently purged during the extensive haploid phase of the life cycle. Similarly, genetic sex determination is expected to operate differently, with XY or ZW systems occurring in dioecious species but UV systems occurring in dioicous species. When the different types of brown algal life cycle are mapped onto a phylogenetic tree, the distribution pattern suggests that there has been considerable switching between different life cycle strategies and sex chromosome systems during the evolution of this group [13,16], and that dioicy was the ancestral state in the brown algae, and the transition to dioecy presumably required an intermediate state of co-sexuality (e.g., monoecy) with epigenetic sex differentiation (as opposed to genetic sex determination). Phylogenetic comparative methods will be valuable to fully characterise and reveal correlations between these life cycle and reproductive traits and their relationships with the evolution of sex chromosomes across the brown algal lineage.

4. Master sex-determining genes across lineages

Sex chromosomes harbour the master-switch gene that determines gender. A few master sex determination genes have been identified across the eukaryotes [17]. The transcription factor DMRT1 is involved in sex determination in deeply divergent taxa such as Drosophila, *C. elegans*, medaka fish and frogs. High-mobility group (HMG)-domain proteins have been implicated in gender determination in both vertebrates and fungi, and the mating type (MT) locus of the green alga Volvox also contains a HMG-domain gene [18]. Remarkably, a HMG-domain gene has been identified in the SDR of *Ectocarpus* and this gene represents therefore a strong candidate for the sex-determining gene in this species. If this gene acts as the master sex determination gene in *Ectocarpus*, which is very distantly related to previously identified species with HMG sex-determining genes (more than a billion years of independent evolutionary history), this will raise very important questions about the evolution of sex-determination gene networks across the Eukaryotes, suggesting shared or convergent mechanisms in brown algae, fungi and animals.

Disclosure of interest

The authors declare that they have no competing interest.

Acknowledgements

Work in the Algal Genetics group is supported by the CNRS, the ANR (project Bi-cycle ANR-10-BLAN-1727 and project Idealg ANR-10-BTBR-04-01), the Interreg program France (Channel)-England (project Marinexus), the UPMC and the European Research Council (grant agreement 638240). LM had a PhD grant (ARED) from the Région Bretagne.

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Glossary

Isogamy: production of gametes of opposite sexes morphologically similar in terms of shape and size
Anisogamy: production of gametes of different size and/or form, usually the female gamete being larger than the male gamete
Oogamy: a form of anisogamy that involves the production of a small motile male gamete and a large non-motile female gamete
Gametophyte: the gamete-producing generation of a plant life cycle
Sporophyte: the spore-producing generation of a plant life cycle
Monoicous and monoecious: refers to species that have both male and female organs in the same individual
Dioicous and dioecious: refers to species where there is distinct male and female individual organism. The term "monoicous" and "dioicous" are used to describe organisms where the sex is expressed during the gametophyte generation whereas the terms "monoecious" and "dioecious" are used for organisms where the sex is expressed during the sporophyte generation.
Heterogametic sex: the sex that carries two different sex chromosomes (male, in XY systems, and female in the case of ZW systems)

Appendix 3

Résumé en français

La reproduction est un processus fondamental essentiel à tout organisme vivant et qui représente un défi majeur pour toute espèce devant assurer sa pérennité. Après des millions d'années d'évolution, les organismes vivants ont adopté deux stratégies fondamentales, radicalement opposées pour se reproduire, soit par reproduction sexuée ou par reproduction asexuée. La reproduction sexuée est extrêmement répandue et fréquente chez les eucaryotes et implique l'échange de matériel génétique entre deux organismes contrairement à la reproduction asexuée produisant des individus génétiquement identiques à l'organisme parental. Chez les eucaryotes, contrairement aux organismes procaryotes, la reproduction sexuée implique l'alternance entre une phase haploïde, résultant d'un mécanisme de méiose, et une phase diploïde faisant intervenir la fusion de deux gamètes (syngamie) **(Beukeboom and Perrin, 2014)**. La prédominance de ce mode de reproduction chez les eucaryotes suggère un avantage évolutif à se reproduire par voie sexuée. L'un des avantages de la reproduction sexuée est qu'il s'agit d'une source de brassage génétique permettant l'apparition de nouvelles combinaisons génétiques par recombinaison et ségrégation à la méiose. Ces nouvelles combinaisons génétiques permettraient de générer de nouveaux génotypes adaptés, par exemple à un changement environnemental, et pourraient être fixée dans les populations par sélection naturelle. Les mécanismes de méiose et de recombinaison permettraient également d'éliminer les mutations délétères. La reproduction sexuée est, cependant, considérée comme un véritable paradoxe évolutif **(Maynard Smith, 1978)** car malgré les avantages qu'elle présente, de nombreux coûts lui sont également associés. Le principal désavantage évolutif du sexe par rapport à la reproduction asexuée (clonalité) est appelé « le double coût du sexe ». Ce coût est basé sur la comparaison du nombre de descendant produit par les femelles sexuées ou par les femelles asexuées. Généralement, la reproduction sexuée chez les eucaryotes fait intervenir deux sexes (mâle et femelle) avec un des sexes (femelle) allouant beaucoup plus de temps et de ressources à la production et au développement de la descendance. La contribution des mâles pour la production de la descendance se limitent au transfert de leur matériel génétique lors de la fécondation. Ainsi, le nombre de nouveaux individus produit par reproduction sexuée dépend essentiellement du nombre de femelles dans la population qui produisent pour moitié des individus de sexe mâle dans leur descendance. *A contrario*, une femelle se reproduisant par voie asexuée, ne dépend pas des mâles et produira exclusivement des femelles capable elles aussi de se cloner. Ainsi, la reproduction asexuée apparait comme plus efficace que la reproduction sexuée en termes

de production d'individus. Sans compter que le coût de production des mâles n'est pas le seul désavantage associé à la reproduction sexuée **(Otto and Lenormand, 2002)**. En effet, se reproduire par voie sexuée implique de trouver un partenaire sexuel, ce qui peut s'avérer être une tâche ardue, qui expose l'individu à des risques de prédation, de contracter des maladies, et qui demande une énergie considérable. Pourquoi alors la majorité des eucaryotes se reproduisent par voie sexuée ? Expliquer et comprendre pourquoi la plupart des organismes vivants continuent à pratiquer le sexe malgré les coûts qui lui sont associés est une véritable énigme scientifique. Aussi, comprendre pourquoi certaines espèces sont capables de se reproduire par voie asexuée et éviter les coûts associés au sexe, constitue une approche alternative pour expliquer ce paradoxe évolutif que représente la reproduction sexuée. Bien que la reproduction sexuée nous apparaisse comme un système naturel et répandu, de nombreuses espèces se reproduisent soit essentiellement par voie asexuée ou en combinant les deux stratégies **(Savidan YH, 2000)**. La reproduction asexuée comprend une multitude de processus différents selon les espèces, mais assure, pour un moindre coût énergétique, un plus grand nombre de descendants génétiquement identiques. Elle permet notamment d'économiser l'énergie et le temps passé à la production de cellules haploïdes ou encore d'éviter le coût de production d'individus mâles. Néanmoins, la reproduction asexuée présente un inconvénient majeur qui est le manque de variabilité génétique. En effet, par principe, elle assure la production d'individus génétiquement identique, aux mutations près, et cette uniformité génotypique ne permet pas de produire assez rapidement de nouvelles adaptations et peut conduire à la disparition de la population si les conditions environnementales ne sont pas maintenues constantes. De plus, le manque de recombinaison et d'évènement de ségrégation dans les populations asexuées, favorisent l'accumulation de mutations négatives voire délétères. Les organismes asexués devraient présenter une plus faible capacité à produire de nouvelles adaptations du fait du manque de recombinaisons, et ce mode de reproduction ne présenterait des avantages qu'à court termes. Ainsi, les espèces se reproduisant par voie asexuée devraient être éphémère comparé aux organismes sexués. Les lignées asexuées sont très souvent considérées comme des culs de sac évolutifs et leur distribution au sein de l'arbre du vivant appuie cette hypothèse. En effet, presque toutes les lignées asexuées occupent les branches terminales de l'arbre du vivant avec seulement de rares exceptions de groupes entièrement asexués. Très peu de lignées ont été décrites pour avoir persisté et s'être diversifié au cours de millions d'années en l'absence de reproduction

sexuée, et à ce jour les quelques lignées supposées avoir une asexualité ancienne (plus de 10 millions d'années) sont les acariens oribates, les ostracodes darwinulides et les bdelloïdes rotifers. Mise à part ces rares lignées, la majorité des eucaryotes asexués ont opéré une transition de la reproduction sexuée vers l'asexuée, détournant ou altérant parfois les mécanismes de méiose pour réaliser cette transition. C'est le cas des organismes dit parthénogénétiques. La parthénogenèse fait partie des nombreux types de reproduction asexuée et implique le développement d'un embryon directement à partir d'un gamète sans qu'il y ait fécondation. Les origines de ce mode de développement restent relativement diverses avec quatre mécanismes proposant une transition de certaines lignées sexuées vers un mode de reproduction asexuée. Même si beaucoup d'eucaryotes peuvent se reproduire par parthénogenèse **(Simon *et al.*, 2003)**, les connaissances sur les bases génétiques, les causes évolutives ainsi que les conséquences d'une transition vers la reproduction asexuée restent relativement incomplètes. Les quelques études s'étant intéressées aux bases génétiques régissant le processus de parthénogenèse inclus des insectes comme les phasmes ou les pucerons et des plantes.

La production d'individus génétiquement identiques à un parent présente un intérêt important notamment pour l'agriculture. En effet, chez les plantes, l'apomixie est un processus naturel de reproduction asexuée qui engendre la production de descendants génétiquement identiques au plant mère. En milieu naturel, l'apomixie peut être réalisée via une multitude de voies de développement mais seules les formes gamétophytiques de l'apomixie font intervenir le processus de parthénogenèse **(Koltunow *et al.*, 1995)**. Ainsi, l'apomixie combine trois processus fondamentaux qui sont, d'une part, la formation de gamètes non-réduits par un mécanisme d'apoméiose, puis, le développement de ces gamètes sans fécondation (parthénogenèse), et enfin, le développement spontané ou induit de l'endosperme. Dans ce contexte, la parthénogenèse représente une composante de l'apomixie qui nécessite d'autre processus pour permettre la production de graine sans fécondation. Bien que l'apomixie représente un trait d'importance majeure pour l'agriculture, les processus développementaux mis en jeu, rendent difficiles l'identification des facteurs génétiques impliqués dans ce processus. Certaines études récentes confirment que l'apoméiose est contrôlée par un seul locus **(Spillane *et al.*, 2001)**, les mécanismes

moléculaires déclenchant la parthénogenèse chez les plantes ou les animaux restent largement méconnus.

Comme la majorité des lignées eucaryotes, les algues se reproduisent par voie sexuée. Par ailleurs, l'originalité de leurs systèmes et mécanismes de reproduction est remarquablement diversifié comparé aux modèles couramment étudiés. La reproduction sexuée peut être facultative chez certaines algues et leur développement peut alors aussi être assuré par parthénogenèse. Contrairement au développement apomictique des plantes, le développement parthénogénétique des algues correspond, *sensu stricto*, à celui d'un gamète sans fécondation et ne fait intervenir aucun mécanisme d'apoméiose, simplifiant ainsi grandement l'étude de la parthénogenèse. Ce processus a été observé chez de nombreuses algues brunes qui ont évolué indépendamment des animaux et des plantes depuis plus d'un milliards d'années. L'étude des mécanismes de parthénogenèse chez ces organismes eucaryotes présentent donc un intérêt majeur pour enrichir les connaissances sur l'apparition des transitions de la reproduction sexuée vers l'asexuée.

En 2004 **(Peters *et al.*, 2004)**, l'algue brune *Ectocarpus* fut proposée comme organisme modèle pour l'étude des algues brunes. Cette petite algue filamenteuse possède plusieurs avantages pour réaliser des analyses génétiques et génomiques notamment un cycle de vie relativement court, une petite taille et une facilité de culture en laboratoire. En 2010, le génome d'*Ectocarpus* fut le premier génome d'algue brune séquencé **(Cock *et al.*, 2010)**. Outre les outils génétiques disponibles pour *Ectocarpus*, son cycle de vie incluant un cycle de reproduction sexuée et un cycle parthénogénétique en font un très bon modèle pour l'étude des mécanismes génétiques régissant le processus de parthénogenèse chez les algues brunes.

Le principal objectif de ma thèse fut, tout d'abord, d'enrichir les connaissances sur les mécanismes moléculaires, génétiques et évolutifs de la parthénogenèse chez l'algue brune modèle *Ectocarpus*. Plus spécifiquement, il s'agissait de déterminer l'architecture génétique de la parthénogenèse et la caractérisation des mécanismes cellulaires impliqués dans ce mode de reproduction asexuée (Chapitre 2). Cette thèse a également porté sur l'étude de la transmission des mitochondries chez différentes souches et espèces d'*Ectocarpus* présentant des capacités parthénogénétiques différentes (Chapitre 3). Enfin, les données générées pour l'étude des bases génétiques chez *Ectocarpus* ont permis de comparer les réarrangements

génétiques entre deux espèces proches d'*Ectocarpus* qui présentaient des capacités parthénogénétiques différentes (Chapitre 4).