Review

Some progress in sexual reproduction and sex determination of economic algae

Chen Peng1, 4, Chen Qi-Jie2, Chu Li-Ye3 and Shao Hong-Bo1,3*

[1]The CAS/Shandong Provincial Key Laboratory of Coastal Environmental Process, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences (CAS), Yantai 264003, China.
[2]College of Life Science and Technology, Harbin Normal University, Harbin 150025, China.
[3]Institute of Life Sciences, Qingdao University of Science and Technology (QUST), Qingdao 266042, China.
[4]The Graduate University of Chinese Academy of Sciences, Beijing 100049, China.

With the rapid development of marine industry, algae as an important economic plant as well as an extremely promising energy source, is now attracting more and more attention. As a result of this, elucidating the basic physiological mechanisms of algae becomes even more urgent. Of all the fields, sexual reproduction and sex determination are basic and essential aspects. In this review, we summarized the advances of sex in several typical algae which are of great economic importance and often used as model organism for basic research.

Key words: Sexual reproduction, sex determination, sex-related genes, algae, life cycle.

INTRODUCTION

Algae, with extremely rich diversity, appeared on earth in the ancient age. Many algae have life cycles involving quite different stages such as gametophytes, carpos-porophytes and tetrasporophytes (Chen et al., 2009). Even though the phases and sexes of many algae look identical before sexual maturation, there are physical differences between them (Bird and McLanchlan, 1984), such as growth rate in phases (Tseng and Xia, 1999), levels of polyamines in sexes and phases (Santelices and Doty, 1989) and lipid composition among different developmental stages. Owing to their particular life cycle, differentiation of phase and sex in algae has already attracted researchers' attention. Researchers have been engaged in the study of the mechanisms of phase formation since early time (Fernandez et al., 1989). How-ever, no satisfactory results were obtained due to the limitation of applicable methods in the past. With the development of molecular biology and other developed technology, great progress has been made recently.

Studies on these topics may play important roles in understanding algae physiological mechanisms and will provide clues for algal evolution pathways (Zhang et al., 2005).

EUKARYOTIC MICROALGAE

They are unicellular species which exist individually, or in chains or groups. Depending on the species, their sizes can range from a few micrometers to a few hundreds of micrometers. Unlike higher plants, microalgae do not have roots, stems or leaves. Microalgae, capable of performing photosynthesis, are important for life on earth. They produce approximately half of the atmospheric oxygen and use simultaneously the greenhouse gas carbon dioxide to grow photoautotrophically.

Chlamydomonas

The unicellular green chlorophyte algae *Chlamydomonas*, sometimes called the 'green yeast', has achieved recog-nition as a model system for the study of photosynthesis, organelle biogenesis, transgene and many other aspects of cell and genetic biology. It is the first species in which

*Corresponding author. E-mail: shaohongbochu@126.com (Shao HB); chenqij@yahoo.com.cn (Chen QJ). Tel: +86-0532-84023984.
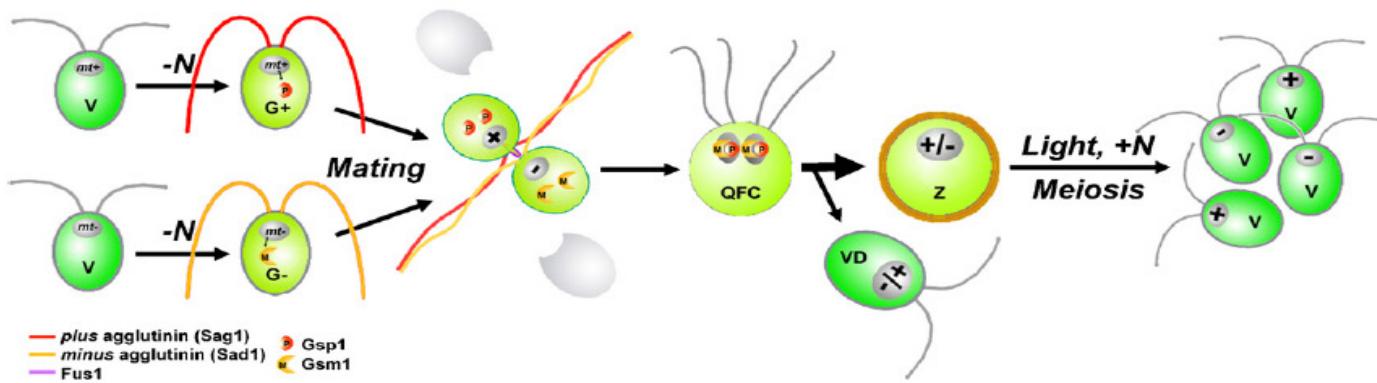
Figure 1. The life cycle of *C. reinhardtii* (modified from Goodenough et al., 2007).

genetic transformations of all the three genomes (nucleus genome, chloroplast genome and mitochondria genome) have been successfully carried out (Zhang et al., 2005).

In nature, the sexual cycle is a responsive to stress environment. In the laboratory, gametogenesis is controlled by nitrogen deprivation and a blue light-responsive signal transduction pathway (Gloeckner and Beck, 1997; Pan et al., 1997). The sex-determination system of *Chlamydomonas* is governed by genes in the mating-type locus (*mt* locus, the *mt* locus is mapped to the left arm of Linkage Group VI) and entails additional genes located in autosomes (Ferris et al., 2002; Abe et al., 2005; Goodenough et al., 2007).

The life cycle of *Chlamydomonas reinhardtii* is simple (Figure 1). Two of the haploid products of each meiosis inherit a linkage group VI carrying the *mt*+ locus and two inherit a linkage group VI carrying the allelic *mt*- locus. Each product divides mitotically to produce clones of vegetative cells. Gene expression is initiated by nitrogen starvation, and cells differentiate into plus or minus gametes. When environmental nitrogen levels fall below threshold (Fraser et al., 2004), vegetative cells carrying the *mt*+ locus express genes that allow them to mate as plus gametes, and cells carrying the *mt*- locus express a different set of genes that allow them to mate as minus gametes. Gametes express mating type-specific glycol-proteins (agglutinins) on their flagellar surfaces (Goodenough et al., 1995). Flagellar pairing between a plus and a minus gamete initiates a cascade of process, including lysis of the gametic cell walls by a specific enzyme distinct from the vegetative cell lysine, contact of mating type-specific structures at the cell apices and fusion of the gametic cytoplasms (Ferris et al., 1996; Wilson et al., 1997). All of the above produce a binucleate zygote. During the next hour, the two nuclei fuse and a novel set of zygote-specific genes is expressed, many of which self-assemble as a zygote-specific cell wall that renders the zygote resistant to freezing, desiccation and other environmental insults. When conditions improve,

restoration of nitrogen, moisture and light induce the germination process. The dormant zygote initiates meiosis, sometimes followed by a mitotic division, and the zygospore wall bursts to release the four progeny. These nuclear genes are inherited in a Mendelian fashion and segregate 2:2 among the tetrad products. Chloroplast genes are inherited uniparentally from the *mt*+ parent in most zygotes (>95% under the usual laboratory conditions), whereas mitochondrial DNA in *C. reinhardtii* is transmitted uniparentally from the *mt*- parent.

Diatom

Diatom, another unicellular alga, is one of the important primary producers and acts have an extremely important role in maintaining the ecological balance of water. Diatoms are autotrophic photosynthesizing algae, which mean that they are able to produce their own sugars, lipids and amino acids. They attract researchers' attention not only for the large number, but also for their importance as a wide range biological resource.

A characteristic feature of diatom cells is that they are encased within a unique cell wall made of silica (hydrated silicon dioxide) called a frustule. These frustules show a wide diversity in form, but usually consist of two asymmetrical sides with a split between them, just like a culture dish.

Reproduction of diatoms is primarily asexual by binary fission (Figure 2). When a cell divides, each new cell takes a valve of the parent frustule as its epitheca (or larger frustule), into which its own hypotheca (or smaller frustule) is constructed within ten to twenty minutes. This process may occur one to eight times per day. Availability of dissolved silica limits the rate of vegetative reproduction (Zhang et al., 2005).

This form of division results in a progressive size reduction of the offspring and therefore the average cell size of a diatom population decreases. It has been
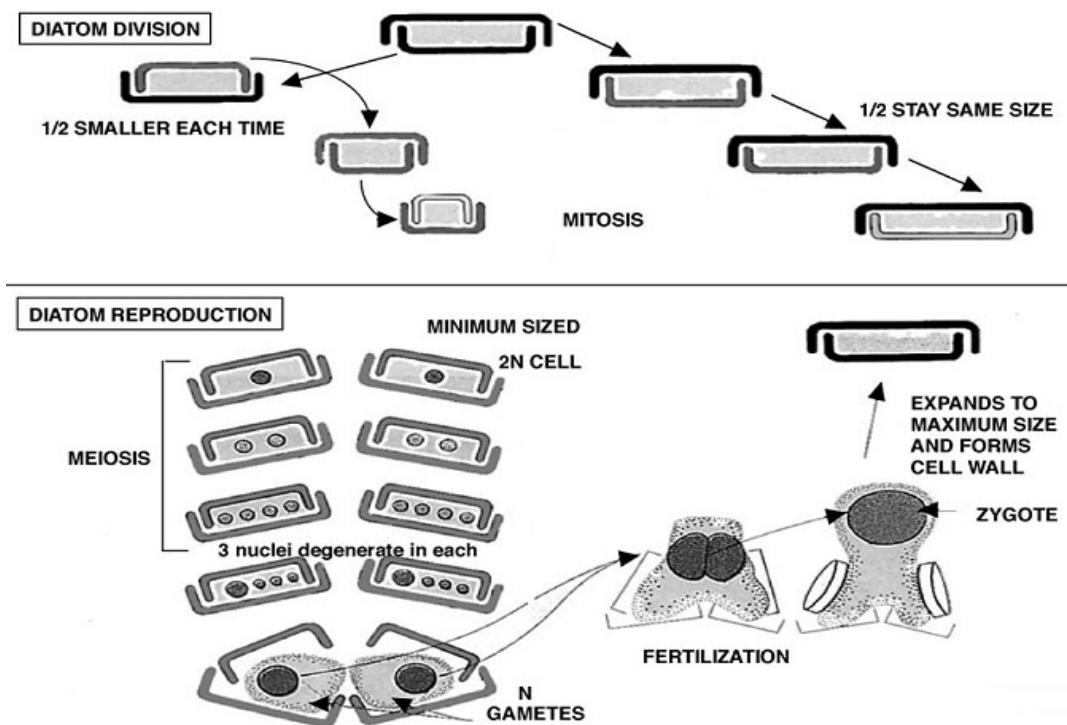
Figure 2. The life cycle of diatom for economic algae.

observed, however, that certain taxa has the ability to divide without causing a reduction in cell size (Drebes, 1977). There is a certain threshold of the cell size, which is about one-third of their maximum size (Hasle et al., 1997). In order to restore the cell size of a diatom population, sexual reproduction and auxospore formation occur (Hasle et al., 1997). Vegetative cells of diatoms are diploid and so meiosis can take place, producing male and female gametes which then fuse to form the zygote. The zygote sheds its silica theca and grows into a large sphere covered by an organic membrane. Auxospores are then produced, which are cells that posses a different wall structure lacking the siliceous frustule and swell to the maximum frustule size. The auxospore then forms an initial cell which forms a new frustule of maximum size within itself. Then, a new generation begins. Resting spores may also be formed as a response to un-favourable environmental conditions with germination occurring when conditions improve (Horner, 2002).

In centric diatoms, the small male gametes have one flagellum, while the female gametes are larger and non-motile (oogamous). Conversely, in pinnate diatoms, both gametes lack flagella (isogamous) (Hasle et al., 1997). Certain araphid species have been documented as anisogamous and are, therefore, considered to represent a transitional stage between centric and pinnate diatoms. Many neritic planktonic diatoms alternate between a vegetative reproductive phase and a thicker walled

resting cyst or statospore stage. The siliceous resting spore commonly forms after a period of active vegetative reproduction when nutrient levels have been depleted. Statospores may remain entirely within the parent cell, partially within the parent cell or be isolated from it. An increase in nutrition levels or length of daylight causes the statospore to germinate and return to its normal vegetative state.

The diatom reproduction mode of auxospore is unique. Besides the requirements of frustule size restoration, changes of environmental conditions such as light, salinity and temperature may also induce sexual repro-duction. Armbust (1999) found a new gene family expressed during the onset of sexual reproduction in the centric diatom. Researchers speculate that there is a unique secretion signal sequence as well as a cysteine rich domain among the amino-terminal peptide of its members. The polypeptides may be related to the identified process of sexual reproduction.

Chlorophyta

Chlorophyta (green algae) includes about 7000 species of mostly aquatic photosynthetic eukaryotic organisms. Green algae contain chlorophylls a and b, giving them a bright green color (as well as the accessory pigmentsbeta carotene and xanthophylls). Green algae contain both
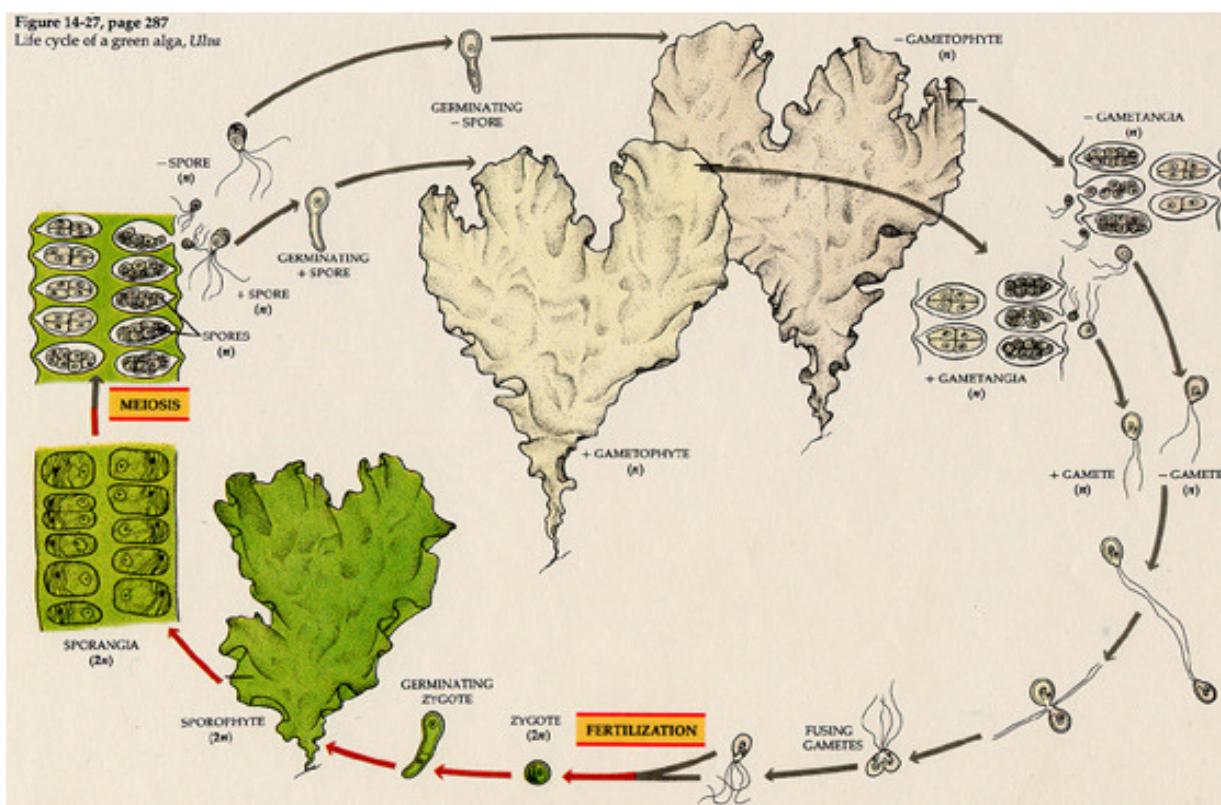
Figure 3. The life cycle of *Ulva.*

unicellular and multicellular species (Hoek et al., 1995). Green algae follow a reproduction cycle called alternation of generations. Reproduction varies from fusion of identical cells (isogamy) to fertilization of a large non-motile cell by a smaller motile one (oogamy).

Ulva

Ulva is a genus of Chlorophyta living primarily in marine environments. They can also be found in brackish water, particularly estuaries. They live attached to rocks in the middle to low intertidal zone, and as deep as 10 m in calm, protected harbors. *Ulva* are usually seen in dense groups. The shapes of *Ulva* are quite varied, ranging in size from microscopic to tens of centimeters. The delicate blades of *Ulva* are usually only 40 microns thick.

Green algae such as *Ulva* have two life phases (Figure 3). These two phases are called the sporophyte and gametophyte phases. During the sporophyte phase, the algae produce spores by meiosis. During the gameto-phyte phase, the algae produce gametes by mitosis. During the sporophyte phase, the membranous thallus, or body of the plant, creates a holdfast attachment to the rock (Han et al., 2003). The haploid spores (zoospore) produced by meiosis during the sporophyte phase are

released into the water, where they settle and grow into the following generation. This next generation is referred to as the gametophyte phase. During the gametophyte phase, after the zoospore develops into sexual gametophyte, it produces anisogametes (mt^+ or mt^-) by mitosis (Zhang et al., 2005). The biflagellate gametes are produced at the margin of a thallus in a zone 5 to 15 mm broad, of different colors from vegetative portion and a zone in which every cell forms gametes. The gametes are formed by repeated bipartition of the protoplast of a cell. Cleavage continues until 32 to 64 daughter protoplasts are formed. Each daughter protoplast metamorphoses into a biflagellate gamete. Just before the cleavage of the protoplast, each cell develops a beak like outgrowth as its outer face and it expends to the thallus surface. Later on, a pore is formed at the tip of this beak, through which the gametes are liberated. The gametes are smaller than zoospores. These anisogametes fuse together in pairs to create zygotes (Miyamura, 2004). After fusion of the gametes, quadriflagellate zygote is formed. It swims for a short time and then comes to rest, withdraws its flagella and secretes a wall around it. Within a day or two, the germination of zygote takes place. The division of the zygote nucleus is mitotic. The two daughter cells are formed by means of division of the zygote. One of the two daughter cells develops into a rhizoid and the other
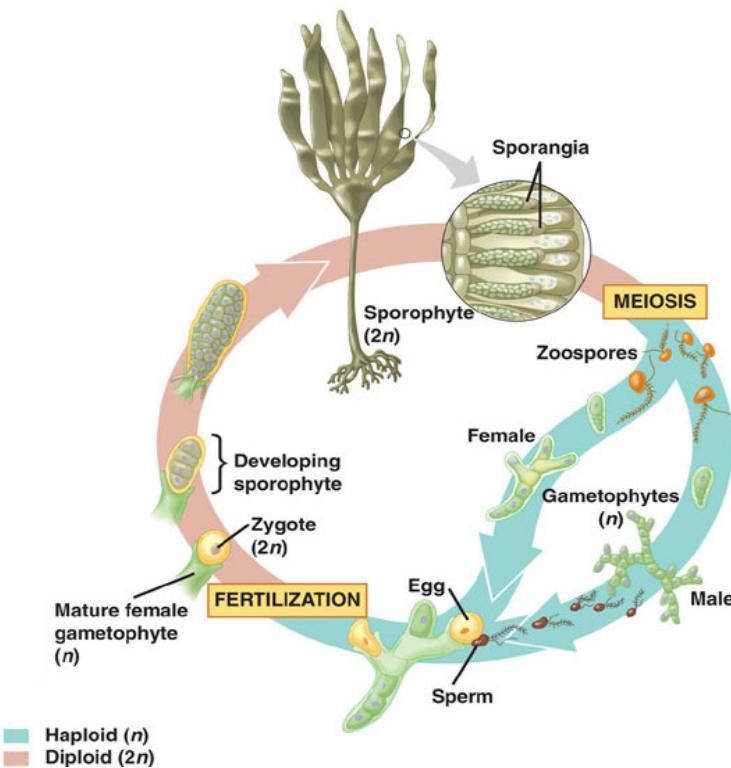
Figure 4. The life cycle of *Laminaria*.

eventually develops into a blade. Then, it grows to enter the next sporophyte generation. *Ulva* looks the same in the gametophyte phase as it does in the sporophyte phase. Due to this, it is considered to undergo isomorphic alternation of generations, gametophyte and sporophyte are indistinguishable by external anatomy.

In 1996, a research showed that differentiation of *Ulva mutabilis* gametangia and gamete release are controlled by extracellular inhibitors, and maybe glycoprotein (Stratmann et al., 1996).

Phaeophyta

The Phaeophyta (brown algae) is a large group of mostly marine multicellular algae, including many seaweeds from colder northern hemisphere waters. They play an important role in marine environments as well as food. For instance, *Macrocystis* may reach 60 m in length, and form prominent underwater forests. Another example is *Sargassum*, which creates unique habitats in the tropical waters. Worldwide, there are about 1500 to 2000 species of brown algae (Hoek et al., 1995). Some species are of sufficient commercial importance, they have become subjects of extensive research (Senn, 1987).

Most brown algae have a sexual alternation of generations between two different multicellular stages.

The differences in life cycle define a number of orders, some with a dominant diploid phase (heteromorphic alternation of generations), and some with isomorphic phases (isomorphic alternation of generations). Some others have no free-living gametophyte stage at all. The largest kelps are diploid, and release flagellated swimming sperm into the water to find egg cells. It has been shown that chemical signals called pheromones aid the sperm in their quest in at least some phaeophytes.

Laminaria

Laminaria is a genus of 31 species of Phaeophyceae, whch is a temperate brown algae belonging to the group known as kelps. It is found in the northern Atlantic Ocean and the northern Pacific Ocean at depths of 8 to 30 m (exceptionally to 120 m in the warmer waters of the Mediterranean Sea and off Brazil). The greater proportion of commercial cultivation is for algin, iodine and mannitol, which are used in a wide range of industrial applications. The largest producer of kelp products is China.

Laminaria exhibits a typical heteromorphic alternation of generations, which means that the sporophyte generation alternates with the gametophyte generation (Figure 4). *Laminaria* exhibits heterothallism. Male and female gametophytes are morphologically dissimilar, the

male gametophyte having smaller cells and being more branched than the female gametophyte (Zhang et al., 2007).

The asexual sporophyte generation produces motile zoospores which develop into male and female gametophytes, whereas the sexual gametophyte generation produces male and female gametes. The male gametophyte produces male gametes called spermatozoids or antherozoids. The female gametophyte plant produces female gametes (eggs). The sperm is released, whereas the eggs remain attached to the gametophyte. The sperm reaches the female gametophyte by a chemical signal secreted by the eggs that attract sperm of the same species (Mizuta et al., 2007). At fertilization, male and female gametes fuse to form the zygote which subsequently develops into a young sporeling at the beginning of the sporophyte generation.

Zoospores are produced on the fronds of mature sporophyte in sporangial sori. Sporangial sori are cup-like structures where cells divide through meiosis to produce haploid male and female zoospores. The pelagic zoospores are motile, having two flagellas. When released from the sporangial sori, they drift and swim around in the water for 5 to 10 min at 15 to 20°C and up to 48 h at 5°C, then settle and adhere to the substratum where they develop into male and female gametophytes (Yang et al., 2009).

After a number of cell divisions, the microscopic male gametophyte plant develops several spermatangia (also called antheridia), each spermatangium producing a single motile biflagellate spermatozoid which is released into the seawater. The female gametophyte develops a single large oogonium which produces an egg. The egg is extruded during ovulation but remains attached to the apical lip of the oogonium. Here, the egg is fertilized by motile spermatozoids with fusion of male and female gametes producing the fertilized zygote. The zygote germinates and develops into a 'young sporeling', also called a 'young seedling', which subsequently develops into a 'young sporophyte' (Nimura and Mizuta, 2002).

In 1896, a research on chromosome number of *Ascophyllum nodudum* was carried out by Farmer and Willaims, which started the chromosome research on Phaeophyta (Zhang et al., 2005). Until now, more than 200 researches on Phaeophyta chromosome have been reported (Liu et al., 2009). It is difficult to study the chromosome morphology or caryotype of Phaeophyta for it is too small in size. That is why researches on Phaeophyta chromosome were mainly focus on comparison of chromosome number and size in the beginning. Gametophytes of Phaeophyta are also divided into male and female ones with a ratio of 1:1, which fully indicates the existence of sex determination and sex differentiation in Phaeophyta. In the early 1990s, Schreiber observed that the ratio of male and female gametophytes produced by sporophytes is 1:1, according to which he speculated

that sex determination of *Laminaria* was governed by a pair of sex chromosomes or sex-determining genes (Schreiber, 1930). Evans believes that to some extent, there is a certain universality of XY chromosome sex determination in Phaeophyta through his researches on *Saccorhiza*, *Laminaria* and *Kombu* (Evans, 1965). Parthenogenesis of *Laminaria* provides evidence to this speculation. In parthenogenesis, spores produced by mature sporophytes all develop into female gametophytes, which indicates that the sex of *Laminaria* is determined by X and Y chromosomes. X chromosome determines the female gametophyte, while Y chromosome determines male gametophyte. Normal chromosome composition of sporophyte is XY, resulting in same number of male and female gametophytes after meiosis. However, the chromosome composition of spores in parthenogenesis is XX, so gametophytes are all female. Further research also ruled out the possibility that the sex determination of *Laminaria* is governed by a pair of alleles (Zhang et al., 2005).

Undaria

Studies on another important economic brown algae *Undaria* reach similar conclusions with *Laminaria*. But specially, it was reported that the male parent affected the genetic characters of its filial generation, which is quite different from the general sense of sex-linked inheritance (Pang and Wu, 1996; Pang et al., 1997). Due to this, for most species, the egg cytoplasm contain a large number of cytoplasmic genes while the sperm generally contains little, and the offspring characters are determined by female parent (Pang, 1998). Until now, the only observed male parental sex-linked inheritance is in drosophila (Zhang et al., 2005). However, more evidence is required to confirm this conclusion.

Rhodophyta

The Rhodophyta (red algae) is one of the oldest groups of eukaryotic algae (Lee, 2008) and also one of the largest. The red algae form a distinct group and are characterized by the following attributes: eukaryotic cells without flagella and centrioles, using floridean starch as food reserve, with phycobiliproteins as accessory pigments (characterized by the accessory photosynthetic pigments phycoerythrin, phycocyanin and allophycocyanins arranged in phycobilisomes), and with chloroplasts lacking external endoplasmic reticulum and containing unstacked thylakoids (Woelkerling, 1990; Ragan et al., 1994). Most red algae are also multicellular, macroscopic, marine, and have sexual reproduction (Figure 5).

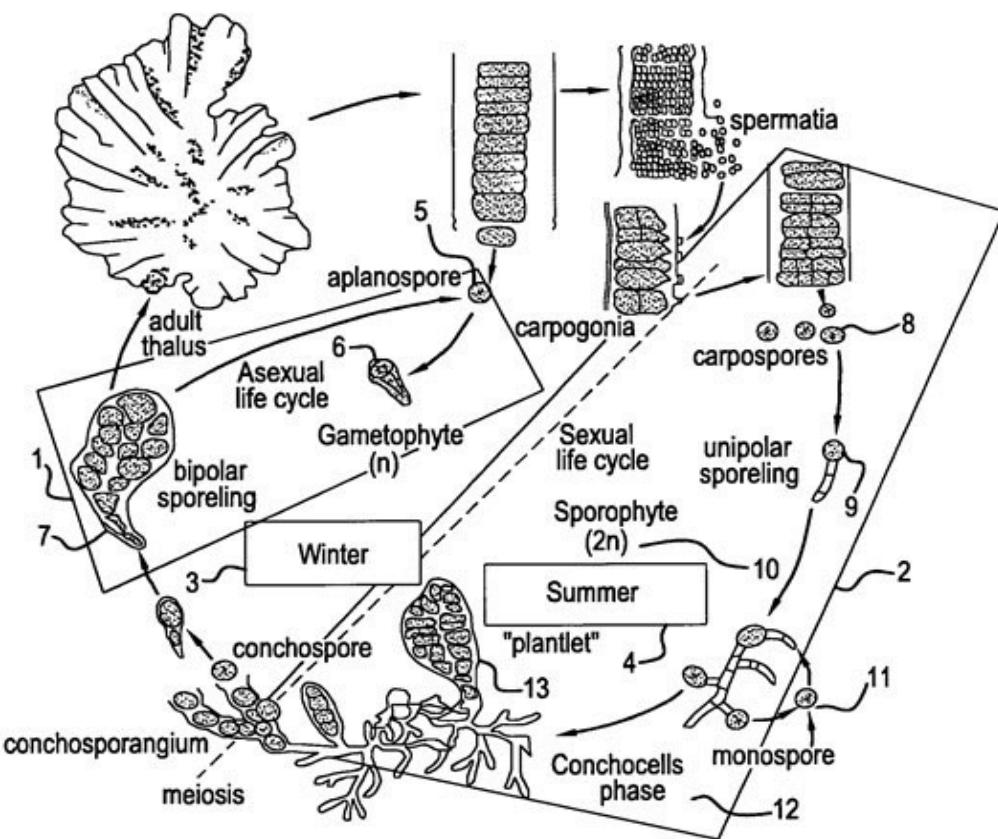Within the red algae, a triphasic life history is pretty

Figure 5. The life cycle of *Porphyra*.

common. Red algae lack motile sperm, hence they rely on water currents to transport their gametes to the female organs, although their sperm are capable of 'gliding' to a carpogonium's trichogyne (Lee, 2008).

Porphyra

Life history of *Porphyra* has been studied intensely throughout time, especially because of its high economic value. *Porphyra* displays a heteromorphic alternation of generations which is temperature dependent and seasonal in nature (Figure 5). The thallus we see is the haploid generation, it can reproduce asexually by forming spores which grow to replicate the original thallus. It can also reproduce sexually. Both male and female gametes are formed on one thallus. The female gametes which are still on the thallus are fertilized by the released male gametes. The fertilized, now diploid, carposporangia after mitosis produce spores (carpospores) which settle, then bore into shells, germinate and form a filamentous stage. This stage was originally thought to be a different species of algae, and was referred to as *Conchocelis rosea*. The fact that *Conchocelis* was the diploid stage of *Porphyra* was discovered in 1949 for the European species

Porphyra umbilicalis (Drew and Kathleen, 1949). It was later shown for species from other regions as well (Brodie and Irvine, 2003; Thomas, 2002).

The differentiation of male and female thallus of *Porphyra* is complicated. *Porphyra yezoensis, Porphyra suborbiculata* are monoecious, while *Porphyra dentate* is dioecious. Yang et al. (2009) carried out expressed sequence tags (EST) analysis of *P. yezoensis*, obtained 170 ESTs, of which 73 were new. These new tags may be specific genes of *P. yezoensis* filamentous sporophyte (Chen et al., 2009). Abe et al. (2005) performed comparative analysis between 10625 ESTs of *P. yezoensis* sporophyte and 10154 ESTs of *P. yezoensis* gametophyte and found that 1940 of them were gametophyte-specific, while 1543 of them were sporophyte-specific, still 1013 tags were two generations shared. Studies on *Porphyra purpurea* by Mitman et al. (1994) deepen the understanding of the sex determination mechanisms, but the diversity and complexity of *Porphyra* sex differentiation make it extremely complicated mainly in the following three aspects: first, time relation between gender mechanisms and meiosis; second, algae of monoecious type may have different mechanism of sex determination; third, the evolution of *Porphyra* gender mechanisms (Mitman and van der meer, 1994; Liu et al., 1996). Eight
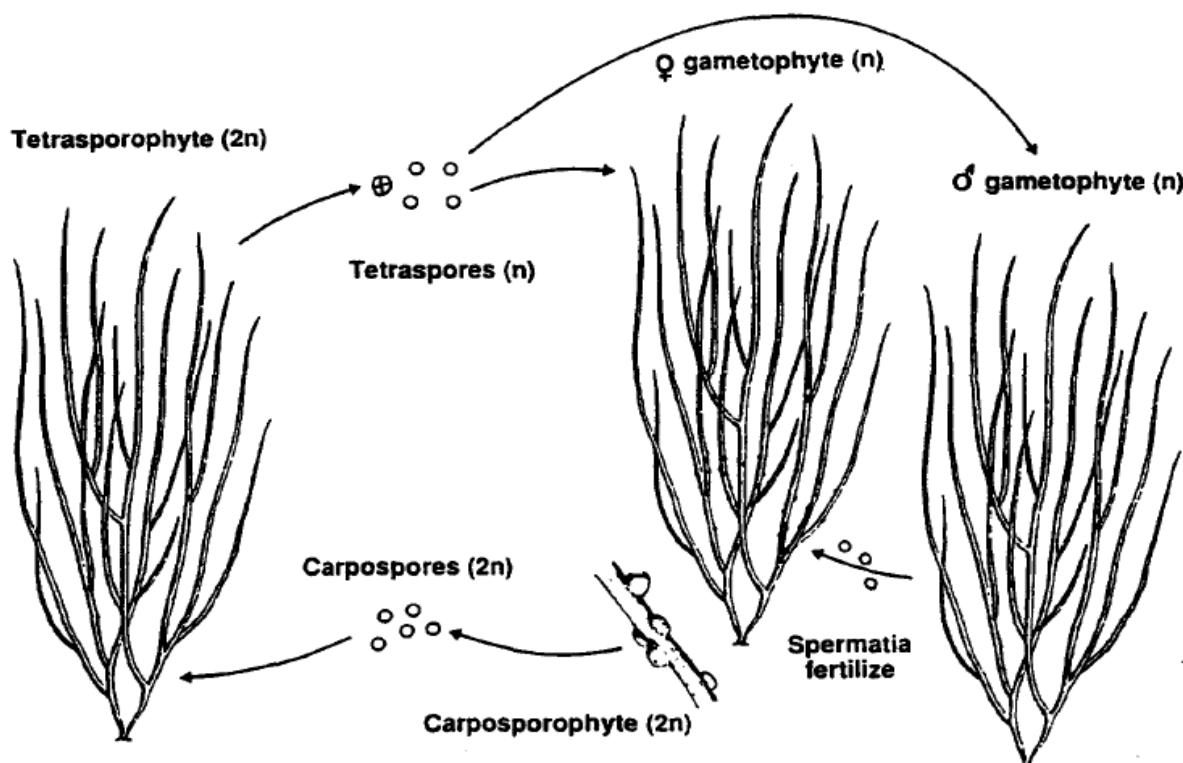
Figure 6. The life cycle of *Gaillardia* (modified from Kain and Destombe, 1995).

unique cDNAs for the sporophyte and seven specific for the gametophyte, including elongation factor alpha and lipoxygenase encoding genes have been isolated from *P. purpurea*.

Gracilaria

Gracilaria lemaneiformis is a commercially important agarophyte that can be used to produce agar, a major ingredient of dairy products, surgical jellies, ointments, cosmetics and healthcare products (Chen et al., 2009). *Gracilaria*, along with *Porphyra*, *Laminaria* and *Undaria* has a bulk production by farming in China. It is not only an economically important algae species, but also a good material for genetical studies (Bird and Mc Lanchlan, 1982). Thus, it is important to pursue basic studies on *Gracilaria*.

In most populations of *Gracilaria*, the life history is like that of the *Polysiphonia* type, that is, having morphologically identical diploid tetrasporophyte and haploid gametophyte phases, the latter consisting of equal numbers of male and female plants (Figure 6). The female gamete is fertilized *in situ* and the zygote develops as a third phase, the diploid carposporophyte, a spore-producing structure entirely dependent on the female gametophyte. The resulting carpospores, the

product of a single fusion of gametes, are numerous and genetically identical. Each spore can develop into a diploid tetrasporophyte in which reproduction involves meiosis, resulting in haploid genetically variable tetraspores (Chen et al., 2009).

Gracilaria has a life history involving three distinct stages: gametophytes, carposporophytes and tetrasporophytes. Researchers have been engaged in the study of the mechanisms of phase formation since 1976 (Ren and Zhang, 2008). With the development of molecular biology, great progress has been made recently (Ye et al., 2006). Six inter simple sequence repeat (ISSR) primers, which had proved previously to be able to yield clear bands in *Gracilaria*, were used to distinguish the phases and sexes of *G. lemaneiformis* (Sun et al., 2003). Until now, several phase-specific and sex-specific genes have been identified. A heat-shock protein encoding gene, which might be involved in the differentiation of female gametophyte, has been identified in *Griffithsia japonica* (Lee et al., 1998). An ubiquitin gene of *G. lemaneiformis* during phase formation is identified and characterized (Ren et al., 2009). GlRab11, the first functional Rab-like protein identified in *G. lemaneiformis* was isolated and the cDNA full-length of *GlRab11* was obtained (Ren et al., 2008). Sun (2002) reported an analysis of 180 ESTs of the *G. lemaneiformis* tetrasporophyte cDNA library. Suppression subtractive

hybridization (SSH) was employed between RNA extracted from female gametophyte and tetrasporophyte. Fourteen cDNAs are identified, among which *SSH466* is a putative tetrasporophyte-specific gene (Ren et al., 2006). *Gracilaria* is dioecious, it is shown that the sex of *Gracilaria* is determined by a pair of alleles, mt^f and mt^m. In gametophyte generation, mt^f determines female gametophyte and mt^m determines male gametophyte. In sporophyte generation, this pair of alleles is in mt^f / mt^m heterozygous state (Zhang et al., 2005).

FUTURE PROSPECT

Algae are of great economical, ecological and social significance. They are also good research materials in biology and molecule researches. At present, however, there are still many shortages in this field, such as cloning and analysis of genes which are crucial in growth, development and sex differentiation. Biological growth, differentiation, cell cycle regulation and also response to stimulation, are all regulated by gene expression. Gene expression depends on the differentiation stage of organism, characteristics of genes and environmental factors. Whether in lower or higher organisms, gene expressions at different developmental stages and parts show significant diversity, which is a prominent feature of gene. With the explosive development of molecular biology and information technology, more and more researchers are devoted to explore the molecular mecha-nism of biological development, including sex deter-mination and differentiation. It can be easily predicted that human understanding of biological evolution and development will continue to progress. In the past few years, a series of genes involved in algae sex deter-mination have been identified and analyzed which are really helpful in elucidating sex determination mecha-nism. Progresses in this field will greatly promote the understanding of biological evolution and enable us to carry out beneficial biological modification.

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