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Algal Sex Determination and the Evolution of Anisogamy

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Abstract

Algae are photosynthetic eukaryotes whose taxonomic breadth covers a range of life histories, degrees of cellular and developmental complexity, and diverse patterns of sexual reproduction. These patterns include haploid- and diploid-phase sex determination, isogamous mating systems, and dimorphic sexes. Despite the ubiquity of sexual reproduction in algae, their mating-type-determination and sex-determination mechanisms have been investigated in only a limited number of representatives. These include volvocine green algae, where sexual cycles and sex-determining mechanisms have shed light on the transition from mating types to sexes, and brown algae, which are a model for UV sex chromosome evolution in the context of a complex haplodiplontic life cycle. Recent advances in genomics have aided progress in understanding sexual cycles in less-studied taxa including ulvophyte, charophyte, and prasinophyte green algae, as well as in diatoms.

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Contents

INTRODUCTION	268
Origins and Diversity of Algae	268
Evolution of Sex, Multicellularity, and the Emergence of Anisogamy	270
VOLVOCINE ALGAE	271
Life Cycles and Emergence of Anisogamy/Oogamy from Ancestral Isogamy	271
Additional Dimorphic Traits of Volvocine Algal Gametes and Gametogenesis	273
Volvocine Algal Mating-Type or Sex Determination Overview	273
Genetic Control of Mating Types and Sex Determination in Volvocine Algae	274
OTHER CHLOROPHYTE ALGAE	277
<i>Ulva</i>	277
<i>Ostreococcus</i>	278
CHAROPHYTE ALGAE	278
<i>Closterium</i>	279
RED ALGAE	279
DIATOMS	280
BROWN ALGAE	281
Evolution of Sexual Dimorphism in Brown Algae	281
Sex Determination in <i>Ectocarpus</i>	283
Evolution of <i>Ectocarpus</i> and Other Brown Algal Sex-Determining Regions	283

INTRODUCTION

Origins and Diversity of Algae

The theme of this review is sex determination and sexual dimorphism in algae. It is important to appreciate that the terms algae and eukaryotic algae refer to informal and paraphyletic groupings of essentially all photosynthetic eukaryotes excluding land plants (**Figure 1**). Algae are also informally subcategorized as microalgae (unicellular species or colonial species with a few cells) and macroalgae (e.g., kelps, seaweed, sea lettuce), but these subcategories also do not describe taxonomic groupings. While the signature organelles of nearly all eukaryotic algae, plastids, are thought to be monophyletic in origin, their hosts have a complex history and distribution across the tree of life, starting with a single primary endosymbiosis event at the root of the Archaeplastida (glaucohyte algae, red algae, green algae/land plants), followed by secondary and tertiary endosymbioses that occurred in distantly related and independently derived branches of the eukaryotic tree and that gave rise to algae within the Heterokonta (e.g., diatoms, brown algae), Alveolata (e.g., dinoflagellates), Rhizaria (e.g., chlorarachniophytes), Hacrobia (haptophytes and cryptophytes), and Excavata (e.g., euglenids) (76). Their paraphyly and deep divergence at the root of the eukaryotic tree make comparative studies of sex-related traits across all algal taxa problematic; but within specific monophyletic subgroupings algae show a wonderful propensity for evolutionary experimentation on sex determination, sexual dimorphism, multicellularity, and/or life cycle architecture. As such, eukaryotic algae represent a relatively understudied collection of independent evolutionary vignettes and stories that may enable empirical investigations and testing of theoretical models for the evolution of sex and sex-related traits (6, 26, 134). On a practical level, algae have important biotechnological and aquacultural applications as potential

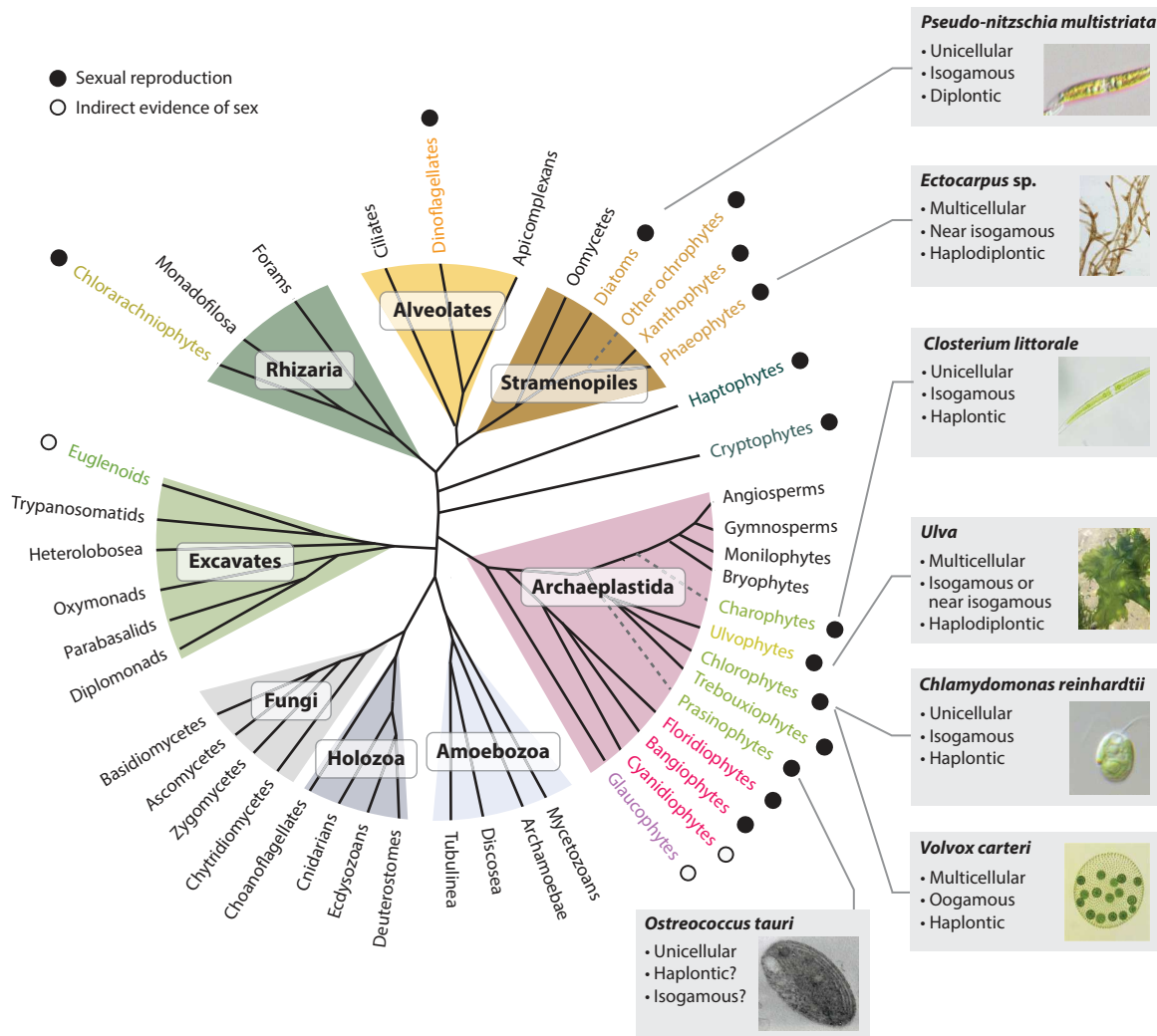


Figure 1

Distribution and life cycles of eukaryotic algae. Cladogram of eukaryotes adapted from Reference 25 highlighting groups containing algae, which are indicated by colored text at branch tips. Gray dashed branches are polyphyletic taxa that are grouped together for simplicity. Solid black circles next to each algal clade indicate that there is direct observational evidence of sex for one or more clade members, and open circles indicate indirect evidence based on presence of sex-related genes in one or more clade members. For less-studied algal groups, references for sex or sex-related genes are as follows: chlorarachniophytes, 9, 82; cryptophytes, 67, 81; cyanidiophytes, 94; dinoflagellates, 115; euglenoids, 42; glaucophytes, 128; haptophytes, 151; prasinophytes, 132; and trebouxiophytes, 45. Panels on the right show summaries of model algae discussed in more detail in this article. For *Ostreococcus tauri*, mating has not been directly observed so assignment as isogamous and haplontic is provisional. Photomicrographs provided by Gavriel Matt (*Chlamydomonas reinhardtii*), Yuki Tsuchikane and Hiroyuki Sekimoto (*Closterium littorale*), Mariella Ferrante (*Pseudo-nitzschia multistriata*), Kevin Cascella (*Ulva*), Gwenael Piganeau (*Ostreococcus tauri*), and Susana Coelho (*Ectocarpus*). The *Volvox carteri* photomicrograph was reproduced from Reference 96 (CC-BY 4.0 license).

Anisogamy: condition of having gametes that differ in size

Oogamy: condition of having large nonmotile female gametes fertilized by small motile male gametes

Isogamy: condition of having gametes that do not differ in size

food crops and feedstocks for biomass/biofuel or other high-value products. Understanding their sex-determination mechanisms and controlling their sexual cycles are prerequisites for exploiting natural diversity of algae and generating advanced strains through breeding (15). Sex has been described in many groups of algae and generating advanced strains through breeding (15). Sex has been described in many groups of algae (50) (**Figure 1**), and evidence of a meiotic tool kit has been uncovered even in lineages where sex has not been directly observed, such as euglenoids (42), glaucophytes (128), and cyanidiophytes (single-celled red algae) (94) (**Figure 1**). However, there are only a few representative clades or species where any molecular characterization of sexual cycles has been done. This review focuses on those species/clades where the most progress has been made in understanding sexual cycles and those that are on the cusp of new discoveries (**Figure 1**).

Evolution of Sex, Multicellularity, and the Emergence of Anisogamy

The evolution of eukaryotic sex and sex-related phenomena has engaged and puzzled biologists since Darwin (36) first described his theories of sexual selection. The core set of molecular processes that define meiotic sex are thought to have emerged in the last eukaryotic common ancestor, which was a single-celled organism, and have been largely conserved over the more than one billion years during which eukaryotes underwent spectacular diversification (8, 48, 128). Eukaryotic sex (aka meiotic sex) is a cyclical process that involves alternation of ploidy between 1N haploid gametic cells and 2N diploid zygotes. The transition from 1N to 2N (i.e., mating) involves differentiation of haploid gametes into specific types that must be different for fertilization (gamete fusion) and syngamy to occur. The reverse transition, from 2N to 1N, involves meiotic recombination and genome reduction (**Figure 2**). Intercalated into the haploid and/or diploid stages of sexual cycles are extended phases of mitotic proliferation that allow organismal or lineage growth in between sexually controlled ploidy transitions (12, 27, 66) (**Figure 2**). Life cycles and sexual cycles of algae are highly diverse, and each taxonomic group has some specific nomenclature that is defined in appropriate sections along with general terms and concepts.

Sex-determining or mating-type-determining systems govern the production and differentiation of gamete types and dictate whether this differentiation occurs in the diploid or haploid stage of the life cycle (**Figure 2**). By convention, the different gametic or conjugating cell types are described as mating types when they are morphologically similar (isogamy), and they are referred to as male/female when they are different in size (anisogamy and oogamy). A positive correlation between anisogamy and organismal size/complexity helps to support the most widely cited theory on the evolution of anisogamy, originally developed by Parker, Baker, and Smith (abbreviated PBS) (114), with further refinements of PBS described in subsequent studies (16, 112, 113). The key element of PBS is the role of disruptive selection in the evolution of gamete size when survival of the zygote and its development into a functioning diploid-stage individual are related to its size in a nonlinear manner. Additional selection for anisogamy as a stable reproductive strategy may derive from selection on fertilization kinetics (optimization of finding a mating partner in an aquatic environment) (41, 73, 134) and from selection for uniparental organellar DNA transmission that is thought to mitigate intergenomic conflicts between nuclear and organellar genes (32, 69, 71); but a generally accepted theory for the origins of anisogamy has not yet emerged (84, 117). Eukaryotic algae encompass a full range of sexual dimorphism, from isogamy to moderate anisogamy to oogamy, with some monophyletic subgroups containing both isogamous and anisogamous or oogamous members. Moreover, the occurrence of anisogamy does not always follow a unicellular to multicellular progression, as expected from PBS theory (6, 135). Thus, algae provide multiple opportunities for exploring theories of sexual dimorphism and for understanding its evolution across the tree of life.

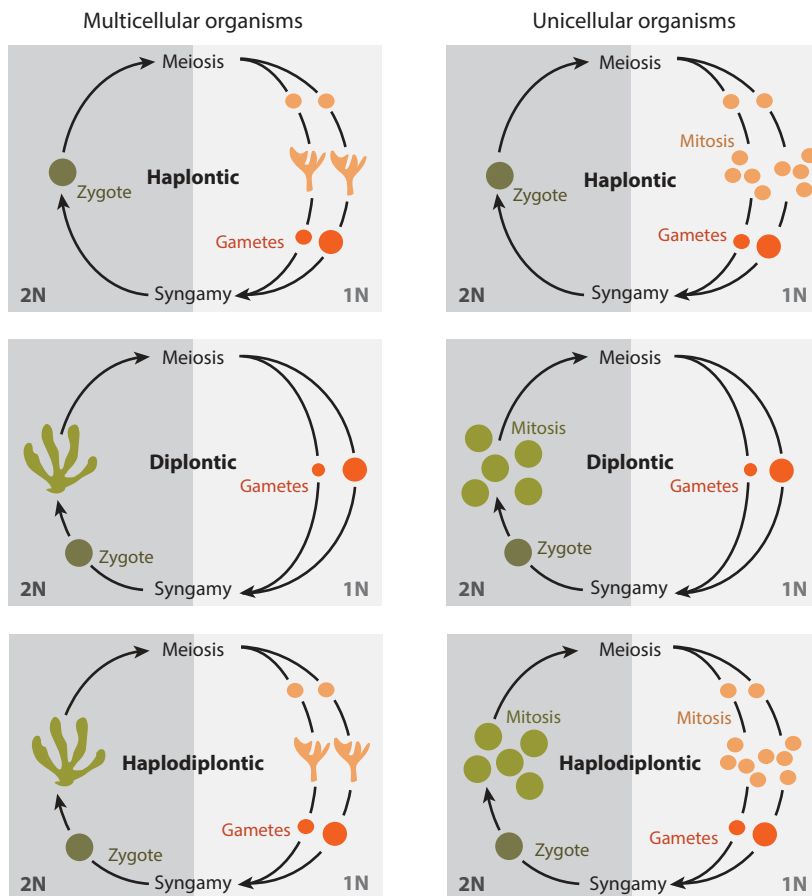


Figure 2

Algal life cycle architecture. Main types of sexual life cycles found in multicellular (*left panels*) and unicellular (*right panels*) algae. Life cycles involve alternation between a haploid phase (1N) and a diploid phase (2N), separated by meiosis and syngamy (i.e., fertilization). Differences between life cycles depend principally on the extent of mitotic divisions and/or development that occurs in each of the two phases. Organisms that spend the majority of their life cycle in the haploid phase are haplontic (*upper panels*) and those that spend the majority of their life cycle in the diploid phase are diplontic (*middle panels*). Haplodiplontic organisms spend significant portions of their life cycle in both phases (*lower panels*). Sex determination occurs in the haploid phase for haplontic species and diploid phase for diplontic species. It may occur in either phase for haplodiplontic species. Anisogamous gametes are shown in this figure, but any of the life cycles could have a range of gamete dimorphism from isogamy to oogamy as described in the main text.

VOLVOCINE ALGAE

Life Cycles and Emergence of Anisogamy/Oogamy from Ancestral Isogamy

Volvocine green algae are an informal grouping that includes a monophyletic clade comprising several multicellular or colonial genera plus the unicellular species *Chlamydomonas reinhardtii* that is a relatively close outgroup (**Figure 3a**). While multicellularity is a monophyletic trait in the volvocines, there has been extensive convergent evolution within the clade leading to some traditionally designated genera such as *Eudorina*, *Pleodorina*, and *Volvox* turning out to be

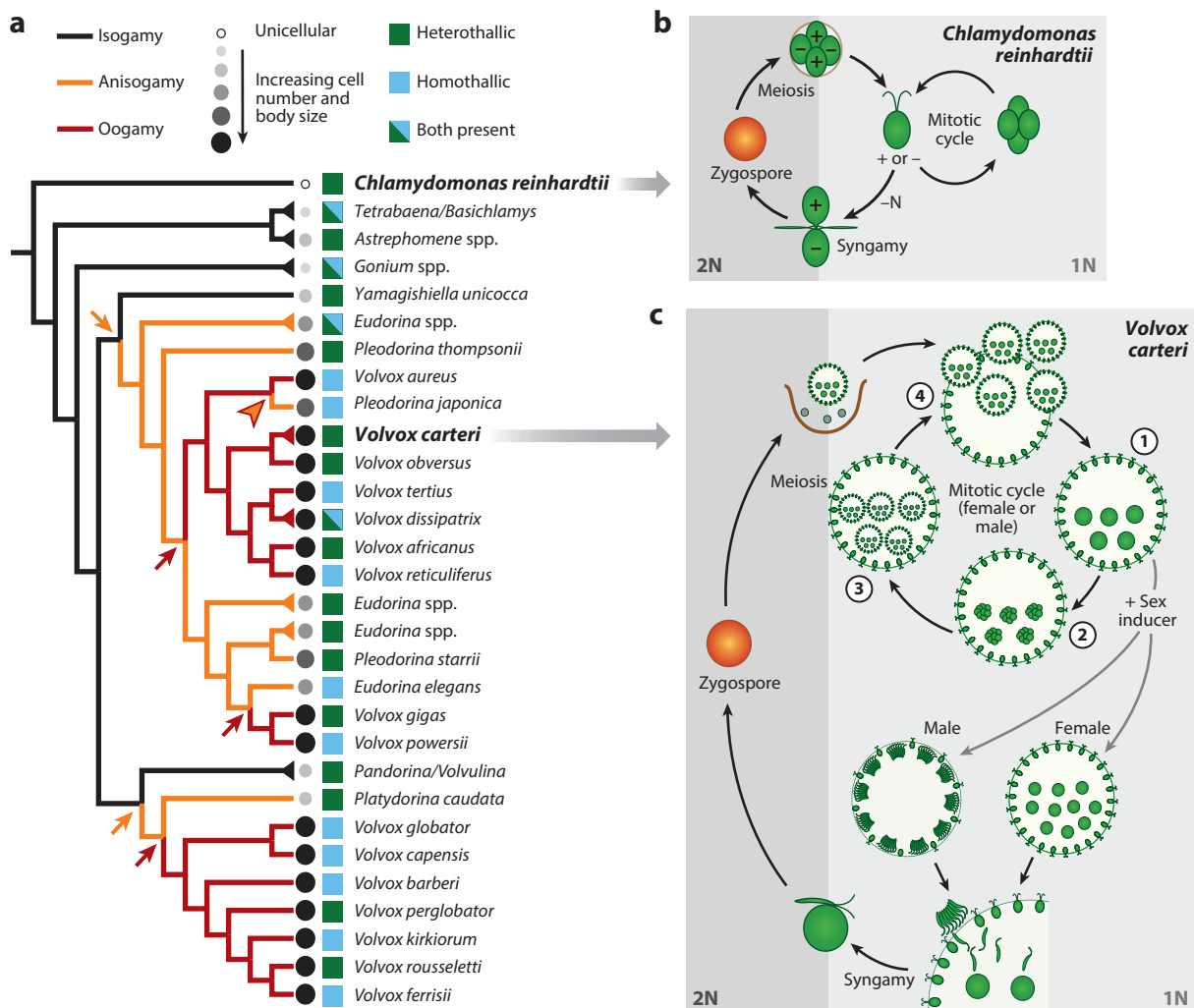


Figure 3

Volvocine algal sexual dimorphism and life cycles. (a) Cladogram of selected volvocine algal taxa adapted from References 59 and 60 depicting transitions in sexual dimorphism and distribution of heterothallism or homothallism. Note that several volvocine genera, including *Volvox*, *Eudorina*, and *Pleodorina*, are polyphyletic. The key above the cladogram shows meanings of symbols and colors. Orange and red arrows show nodes where anisogamy and oogamy evolved, respectively. Arrowhead shows predicted reversal from oogamy to anisogamy. Branches terminating in triangles contain multiple species or genera. Note that *Volvox carteri* has three forms (*f. nagariensis*, *f. weissmannia*, *f. kawasakiensis*) that are biological species, and *V. carteri f. nagariensis* is the one described in this review. (b) Haplontic life cycle of *Chlamydomonas reinhardtii*. Transitions between life cycle stages are depicted similarly to the depiction in **Figure 2**. Plus and minus mating types are indicated by + and - symbols. Gametogenesis is induced by the absence of nitrogen (-N). Isogametes fuse and differentiate into a dormant diploid zygospore. Meiosis occurs during zygospore germination and produces a tetrad with 2 MT+ and 2 MT- recombinant progeny. (c) Haplontic life cycle of *Volvox carteri*. Males and females undergo a similar vegetative/mitotic reproductive cycle, diagrammed in four phases in the top-right part of the panel. Mature parental spheroids (1) contain small somatic cells on their periphery and large reproductive cells (gonidia) in the interior, and they are filled with clear extracellular matrix (ECM). During embryogenesis (2) gonidia cleave and eventually form new spheroids (3) that grow and hatch from their parent (4) and continue to grow and mature to complete the vegetative cycle. When exposed to sex inducer, gonidia undergo sexually dimorphic cleavage programs and maturation (not shown) to produce adult male or females (bottom). Males contain sperm packets, while females contain eggs. Sperm packets are released and swim to a sexual female, where they break apart, enter the female ECM (bottom, expanded view), and complete fertilization. Meiosis occurs during zygospore germination as in *Chlamydomonas*, but only one viable haploid meiotic progeny is formed.

polyphyletic based on molecular data (65) (**Figure 3a**). All volvocine algal life cycles are haplontic with unlimited potential for vegetative reproduction in the haploid phase. In most genera vegetative cell division occurs via a multiple-fission cell cycle (33, 142) (**Figure 3b**). Gametogenesis and mating are triggered by either nitrogen starvation (–N) or diffusible hormonal cues (28, 29). After mating and fertilization, a zygotic gene expression program is activated that leads to immediate development of a dormant and environmentally resistant diploid zygospore cell (29, 49, 74, 89). Upon encountering a favorable environment, zygospores undergo meiosis and germinate to release recombinant haploid progeny (**Figure 3b**).

Their phylogenetic coherence and experimental tractability make volvocines an ideal group in which to investigate the evolution of anisogamy and oogamy. Sexual dimorphism within the volvocines roughly follows a progression from isogamy in the smaller genera (*Chlamydomonas*, *Gonium*, *Pandorina*, *Yamagishiella*) through anisogamy in intermediate-sized genera (*Eudorina*, *Pleodorina*) to oogamy in the genus *Volvox* (34, 60, 105) (**Figure 3c**). It has been noted that this pattern of larger and more complex species showing greater gamete dimorphism and larger zygote sizes fits with predictions of the PBS theory described above (6, 7, 34, 77, 116) (**Figure 3a**). A recent study using an expanded volvocine data set confirmed the ancestral state of isogamy in the lineage and estimated that anisogamy may have evolved two times with no reversions back to isogamy (60) (**Figure 3a**). Overall, these data strengthen support for the PBS theory as they document not just a general trend but multiple independent instances of increased degrees of sexual dimorphism accompanying increased organismal size and complexity within the volvocine clade.

Additional Dimorphic Traits of Volvocine Algal Gametes and Gametogenesis

In *Chlamydomonas*, *Tetrabaena*, and *Gonium* a complete meiotic tetrad with four viable members is formed upon zygote germination, while in the remaining genera that have larger, more complex body plans only one of the four meiotic progeny emerges during zygospore germination (28, 60). This trait of reduced meiotic products in larger species, like anisogamy, may have evolved under selection for partitioning more resources into fewer progeny to ensure higher net viability (**Figure 3c**). While all anisogamous and oogamous volvocine algae have reduced meiotic products, some isogamous multicellular genera (*Yamagishiella*, *Pandorina*, *Astrephomene*) also show this trait, whose evolution likely preceded that of anisogamy (60). Reduced meiotic products are also a feature of female reproductive development in many plants (121) and metazoans (92). Another derived trait found in the anisogamous and oogamous volvocine algae is terminal differentiation of sperm cells, which have highly specialized morphology and behavior compared with other volvocine cell types (10). The bases for terminal differentiation of volvocine sperm are unknown.

Volvocine Algal Mating-Type or Sex Determination Overview

In volvocine algae the terms heterothallic and homothallic are used to distinguish genetic and epigenetic sex-determination mechanisms, respectively, while dioecy and monoecy (terms usually reserved for diploid sex determination in plants) are used to distinguish two subcategories of homothallism: In homothallic dioecious species, individual sexual colonies derived from a parental clone contain gametes that are exclusively male or exclusively female. In homothallic monoecious species individual sexual colonies contain a mixture of both eggs and sperm packets. Heterothallism is likely the ancestral state in volvocine algae and is observed in the two best-studied members—*C. reinhardtii* and *Volvox carteri*—but homothallism has arisen multiple times with likely transitions back and forth between hetero- and homothallism (59) (**Figure 3a**). The control of

Haplontic life cycle: life cycle with no or highly reduced diploid mitosis; zygote may be only representative of diploid stage

Dioecy: condition of having separate genetically determined male and female individuals in the diploid (sporophyte) phase; equivalent to gonochorism in animals

Monoecy: condition of a diploid individual (sporophyte) that produces both male and female gametes

Homothallism: refers to self-mating compatibility between genetically identical individuals

Heterothallism: refers to mating incompatibility between genetically identical individuals

Pseudoautosomal region (PAR): portion of a sex chromosome or mating type chromosome that still recombines with its homolog

Gametolog: Allele of a gene whose copies reside in the nonrecombining regions of UV chromosomes or mating-type loci

Supplemental Material >

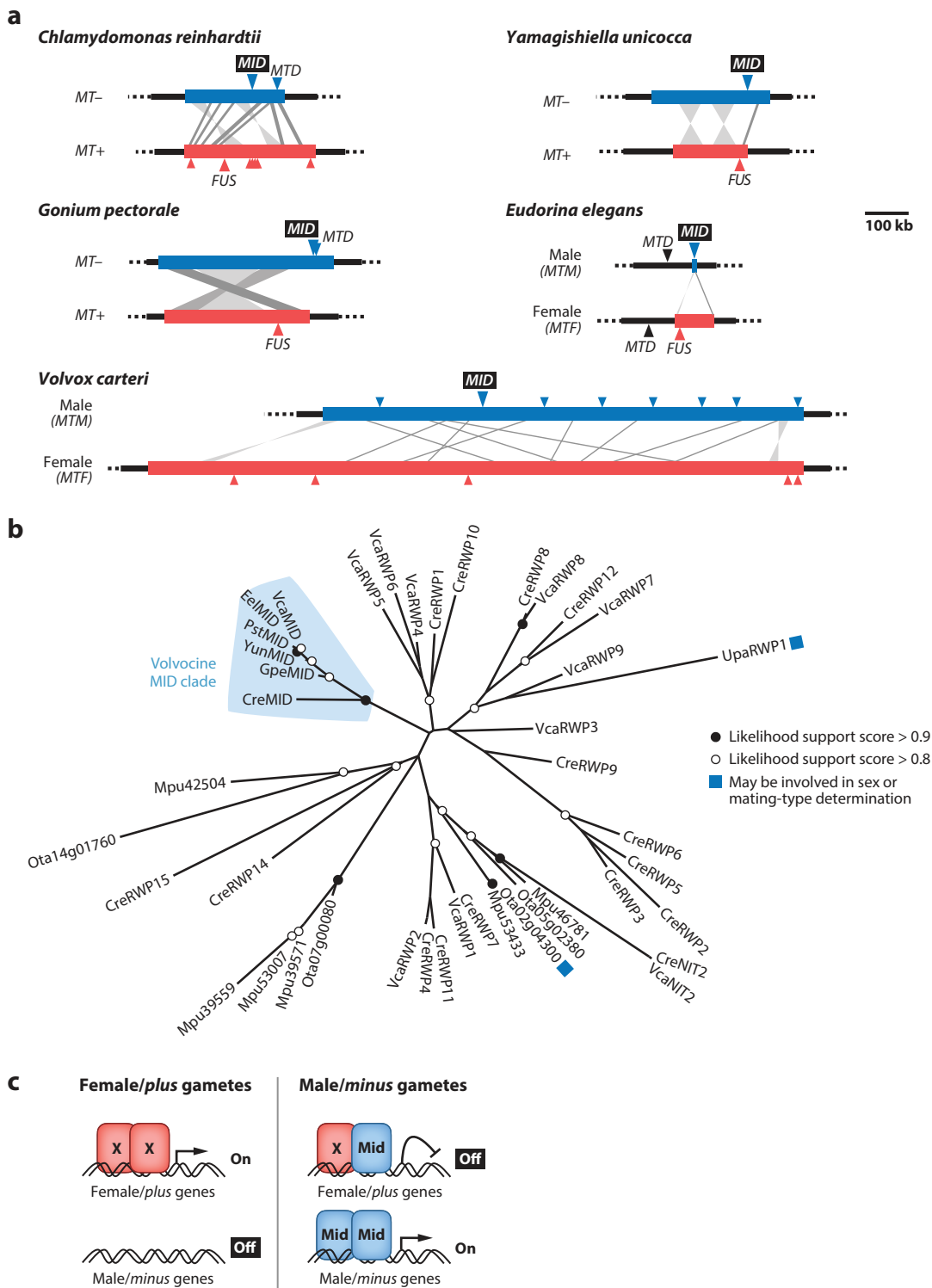
homothallic sexual differentiation and of dioecy versus monoecy may be related to the timing of sexual differentiation under the control of the *MID* gene, as speculated previously (44, 46, 147).

Genetic Control of Mating Types and Sex Determination in Volvocine Algae

The single locus that determines mating type or sex in heterothallic volvocine algae (designated the *MT* locus) is a multigenic region with two haplotypes (one for each mating type or sex) characterized by various sequence rearrangements and suppressed recombination across the *MT* region R (rearranged) domain (140) (**Figure 4a**). The genetic contents of *MT* include mating-type or sex-related genes as well as genes that are not sex related but were presumably trapped in the R domain during its formation. Currently, *MT* sequences are available for five species: *C. reinhardtii* (isogamous), *Gonium pectorale* (isogamous), *Yamagishiella unicocca* (isogamous), *Eudorina elegans* (anisogamous), and *V. carteri* (oogamous) (38, 43, 57, 58) (**Figure 4a**) (**Supplemental Table 1**). Comparison of pseudoautosomal gene contents of the two most distantly related volvocine species in this list, *C. reinhardtii* and *V. carteri*, indicates that *MT* has remained on the same chromosome since the two lineages diverged up to 200 Mya (43, 64). Despite this apparent continuity of genomic location, there is little evidence of long-lived or shared strata in the R domains or pseudoautosomal regions (PARs) of the above species (57). On the contrary, the *MT* loci that have been sequenced so far show no shared syntenic blocs between species and appear to have arisen through periodic destruction/reformation of the *MT* region. The oldest-appearing and largest *MT* locus is from *V. carteri*, where gametologs show high divergence across most of the locus (43) (**Figure 4a**). The idea that more complex and differentiated *MT* loci might be associated with the transition to anisogamy or oogamy appears to have been ruled out by the lack of correlation between *MT* size or genetic complexity and the presence of sexual dimorphism in *E. elegans*, whose *MT* region is the smallest and simplest identified to date among volvocine algae (57) (**Figure 4a**).

MT loci control multiple aspects of the sexual cycle in volvocine algae: (a) gamete differentiation, (b) organelle DNA inheritance, and (c) production of zygotic differentiation factors. Additional traits that are encoded by *MT* locus genes but not universally shared across species are (d) gamete recognition and fertilization factors and (e) specification of sexually dimorphic colony development. Aspects a, b, and c are covered in other reviews (49, 99, 101, 123). We focus here on gamete differentiation and dimorphic development in *C. reinhardtii* and *V. carteri*, as they are the two best-studied volvocine species.

Gamete differentiation in volvocine algae is largely governed by a single transcription factor (TF) called Mid (*minus* dominance), which is a member of the RWP-RK (aka RKD) family (17). A *MID* ortholog is found in the *minus* or male *MT* loci of all heterothallic volvocine algae identified to date (43, 44, 56, 57, 106), and *MID* has also now been identified in a homothallic volvocine species where its expression may be controlled epigenetically (147) (**Figure 4a,b**). These data and others described below show the genetic continuity between isogamous mating types and sexes in volvocine algae. Some volvocine species have a second *MT*— or male *MT* gene, *MTD1*, whose *C. reinhardtii* ortholog contributes to *minus* gametic differentiation but is not essential for it (86) (**Figure 4a**). In *C. reinhardtii*, and in other volvocine genera where $-N$ is the cue for gametogenesis, *MID* expression is induced by $-N$ (44, 55, 57, 86) (**Figure 3b**). *C. reinhardtii* *mid* mutants have a pseudo *plus* phenotype where they differentiate and agglutinate as *plus* gametes but cannot complete fertilization due to absence of the *FUS1* gene (**Figure 4a**), whose expression as a transgene can fully rescue fertility in a *mid* mutant. Conversely, ectopic expression of a *CrMID* transgene in a *MT+* strain causes differentiation into functional *minus* gametes (44). Intriguingly, RWP-RK protein-coding genes have also been implicated in sexual development or mating-type determination in other green algal taxa (see below) (**Figure 4b**) and in land plants (78, 79, 119, 133),



(Caption appears on following page)

Figure 4 (Figure appears on preceding page)

Volvocine algal mating-type and sex determination. (a) Diagrams of volvocine sex-determining regions or mating-type-determining regions adapted from Reference 57 (CC-BY 4.0 license). The heteromorphic regions (R domains) are depicted in blue (*MT*− or male) and red (*MT*+ or female) for each species with colinear flanking regions in solid and dashed black lines. Rearranged genic or syntenic regions between haplotypes are shown in gray. Blue and red triangles show locations of the *MID* gene as well as *MTD* and *FUS*. Unlabeled triangles are sex-limited or mating-type-limited genes that are species specific. (b) Unrooted maximum likelihood tree of selected RWP-RK domains from different green algae constructed using PhyML (53) and based on previous alignments (119). Prefixes indicate species as follows: Cre, *Chlamydomonas reinhardtii*; Gpe, *Gonium pectorale*; Eel, *Eudorina elegans*; Mpu, *Micromonas pusilla* CCMP1545; Ota, *Ostreococcus tauri*; Pst, *Pleodorina starrii*; Upa, *Ulva partita*; Vca, *Volvox carteri*; and Yun, *Yamagishiella unicocca*. Nodes with likelihood support scores of >0.9 are marked by black circles and with scores >0.8 by open circles. The volvocine *MID* clade is shaded light blue. Blue squares are shown next to *Ulva* and *Ostreococcus* RWP proteins that may be involved in sex or mating-type determination. (c) Speculative model for sex and mating-type determination in volvocine algae. *MID* protein (blue rounded rectangle) is a transcription factor that may form homodimers or heterodimers with a hypothetical partner protein X in red that is expressed in gametes of both sexes or mating types. *MID* is produced in *MT*− or male gametes, where *MID* homodimers activate expression of male or *minus* genes, while *MID*-X heterodimers repress expression of female or *plus* gamete genes. In the absence of *MID* (female or *plus* gametes), X homodimerizes or interacts with other factors to activate female or *plus* genes, while male or *minus* genes have no activator.

suggesting a possible ancestral role in sex determination for this class of transcription factors within the green lineage (Viridiplantae).

In *V. carteri* and other species of *Volvox* exposure of vegetative-phase spheroids to a diffusible, species-specific glycoprotein hormone called sex-inducer (SI) triggers dimorphic sexual development leading to production of sperm-packet-bearing males and egg-bearing females (28, 54) (Figure 3c). SI is highly potent, acting at concentrations as low as 10^{-16} M, and also triggers an auto-amplification loop that leads SI-exposed males to produce additional SI (129). While the SI-encoding gene and its protein product from *V. carteri* have been known for decades (93, 136), the receptor(s) for SI and its mode of action remain unknown.

MID is the only conserved *MT* gene between *V. carteri* and other volvocine algae (Figure 4a), and its ectopic expression in *V. carteri* females leads to a pseudomale sexual phenotype where gametic precursor cells that would normally be destined to differentiate as egg cells are converted to functional sperm packets. Knockdown of *VcMID* in males leads to a reciprocal conversion of what should be sperm packet precursor cells called androgonidia into functional eggs (46). Partial knockdown of *VcMID* in males led to a self-fertile homothallic dioecious phenotype and suggested that epigenetic modulation of *MID* expression could underlie homothallism in naturally homothallic volvocine species (46). The *VcMID* gene showed constitutive expression in males with evidence for posttranscriptional and posttranslational control restricting its function to the sexual phase of the life cycle (46).

The finding that manipulating expression of a single conserved mating-type gene, *VcMID*, could alter gamete differentiation in *V. carteri* sexes suggests that anisogamy or oogamy may have evolved without the incorporation of separate gamete size-control genes into an ancestral *MT* region (18, 43, 68); this finding is also supported by the discovery of *MID* as the only *MT*-locus-specific gene in males of the anisogamous species *E. elegans* (57). Cross-species testing of *CrMID* and *VcMID* in *V. carteri* and *C. reinhardtii*, respectively, showed that the two genes could not function interchangeably, leading to the hypothesis that *MID* protein function may have evolved to control the transition to anisogamy/oogamy (46). However, a subsequent study revealed that *GpMID* from the isogamous species *G. pectorale* was able to partially substitute for *VcMID* in *V. carteri* spermatogenesis, indicating that additional components of the *MID* network must also be involved in specifying anisogamous gamete differentiation (47). These other components could be *MID*-interacting proteins and/or *MID* target genes that are specific to anisogamous and oogamous gamete differentiation. Although *plus* or female gamete differentiation is the default state

when MID is not expressed in gametes, it is likely that other TFs interact with MID to modulate its roles as both activator of *minus*/male genes and repressor of *plus*/female genes (**Figure 4c**).

Dimorphic sexual development in the genus *Volvox* involves more than just egg cell and sperm cell formation. Under the influence of SI males and females undergo modified embryogenesis programs that differ from each other and from vegetative embryogenesis in terms of numbers of somatic cells and reproductive cell precursors, as well as organismal size, which in some cases manifests as dwarf sexual males (29, 60, 107, 140) (**Figure 3c**). In the case of *V. carteri* at least some of its sexual development traits are governed by male or female *MT* genes independent of the MID pathway, as revealed from transgenic experiments described above where early embryogenic cleavage patterning retained male or female characteristics in strains where gamete differentiation was reversed by manipulation of *VcMID* expression (46). Identification of these additional *MT*-encoded sexual development and fitness genes (**Figure 4a**) may lead to further insight into how sexually antagonistic functions are added to a sex chromosome to promote male-specific or female-specific traits.

OTHER CHLOROPHYTE ALGAE

Sexual differentiation and mating-type control are best understood in volvocine algae, but most groups of green algae are known to have sexual cycles and/or possess meiotic genes (45, 50, 94) (**Figure 1**). Mechanistic insights into algal sexual cycles and sex determination are possible when one or more of the following advances have been made: (a) identification of mating-type loci or sex chromosomes for heterothallic or dioicous/dioecious species [see **Supplemental Figure 1** for examples of methods used to identify sex-determining regions (SDRs) and mating-type-determining region (MDRs)]; (b) reliable control of gametogenesis, mating, and meiosis; and/or (c) some form of forward or reverse genetics to enable functional testing of regulatory systems. At least some of these criteria have been met for several species of green algae (**Supplemental Table 1**).

Ulva

The green seaweed (or sea lettuce) *Ulva* belongs to the Ulvophyceae, one of several major classes of green algae (85) (**Figure 1**). Most ulvophytes are macroalgae with either multicellular body plans or single-celled syncytial body plans (23). Although little is known about sex determination in most ulvophytes, they are of particular interest in the study of anisogamy and oogamy, as members of this group exhibit different degrees of gamete dimorphism (75, 134, 135). Unlike volvocines and most other chlorophyte algae outside of the Ulvophyceae, *Ulva* has a haplodiplontic life cycle with separate isomorphic haploid (gametophyte) and diploid (sporophyte) generations, both of which produce multicellular blade-like thalli (146) (**Figure 2**). *Ulva* is either isogamous or slightly anisogamous (75, 134, 135) and dioicous (heterothallic) with two mating types, *plus* (*MT*+) and *minus* (*MT*-), determined by a UV chromosome system (70, 148). Cells within the gametophyte thalli, usually near blade margins, may undergo sporogenesis to produce motile biflagellate gametes that, upon fertilization, produce a diploid zygote that develops into a sporophyte. Chloroplast DNA (cpDNA) inheritance in *Ulva* is uniparental and controlled by mating type, with cpDNA being derived from the *MT*+ parent (75) while mitochondrial DNA is inherited either from the *MT*- parent or biparentally (99). Cells within the thalli of the sporophyte can undergo meiosis to produce haploid tetraflagellate spores that develop into gametophytes. The sexual cycles of several *Ulva* species can be controlled, allowing them to serve as models for further study of sex determination (145, 146, 148). An interesting feature of *Ulva* is its production

Sex-determining region (SDR):

genomic region that differs between males and females and determines the sex of anisogamous/oogamous individuals

Mating-type-determining region (MDR):

genomic region that determines the mating type of haploid isogametes

Gametophyte:

haploid multicellular generation of a life cycle that produces gametes

Sporophyte:

diploid multicellular generation of a life cycle that produces spores and where meiosis occurs

Supplemental Material >

of autoinhibitory substances that block sporulation and thereby regulate the extent and timing of gamete production (131). The molecular identities of these inhibitors remain unknown.

A full genome sequence of ~98 Mb has been reported for a *MT*[−] gametophyte of the species *Ulva mutabilis* (37) (though the *MT* locus was not identified), while the mating locus of both *MT*⁺ and *MT*[−] mating types has recently been reported for the slightly anisogamous species *Ulva partita* (72, 148). The *U. partita* *MT* locus occupies 1–1.5 Mb of a heteromorphic region between the *MT*⁺ and *MT*[−] haplotypes (**Supplemental Table 1**). Twenty-three *MT* genes are gametologs that have undergone extensive rearrangements between the two haplotypes and show little or no evidence of syntenic blocs or strata. The remaining genes (23 in *MT*[−], 44 in *MT*⁺) are specific to each mating haplotype and presumably include those controlling mating-type differentiation, uniparental organelle inheritance (99), and/or production of factors needed for the haploid-to-diploid transition in zygotes. Intriguingly, the male (*MT*[−]) locus contains an RWP-RK-related gene, *RWPI*, whose predicted protein is in the same TF family as the volvocine MID protein, though its phylogenetic relationship to the Mid family versus other RWP-RK domain TFs remains unresolved (148) (**Figure 4b**). Expression of *RWPI* and several other *MT* genes was up-regulated during gametogenesis, making *RWPI* a candidate sex-determining gene. While transformation methods have not been reported for *U. partita*, they were developed for another species of *Ulva* (108), and forward genetics are also possible for members of this genus (146), raising the possibility of functional studies to test the roles of *MT* genes in sex determination and life cycle transitions.

Ostreococcus

The unicellular marine picoalga *Ostreococcus tauri* belongs to the paraphyletic assemblage of basal chlorophyte algae termed prasinophytes (85) (**Figure 1**). *Ostreococcus* cells are tiny (~1 μm diameter) and have relatively compact haploid genomes of ~13 Mb (39, 111). Sex has not been directly observed in this genus [though it has in at least one prasinophyte (132)], but strong evidence of sexual exchange comes from testing polymorphic markers in population studies (11, 51) and is further supported by the retention of key meiotic genes in *Ostreococcus* genomes (39, 94, 111). A heteromorphic and nonrecombining region with two haplotypes of 450 and 650 kb on chromosome 2 has hallmarks of a potential mating-type locus that appears to be a well-established UV chromosome system (11, 25) (**Supplemental Table 1**). Because gametogenesis and mating have not been directly observed for *Ostreococcus*, it is unknown whether it has isogamous or anisogamous mating. Intriguingly, like the case for *Ulva*, one of the haplotype-specific genes in the *O. tauri* putative mating locus is in the RWP-RK family (11), though its phylogenetic relationships to the volvocine Mid family are somewhat unclear (119) (**Figure 4b**). *Ostreococcus* is a promising model with some molecular genetic tools, such as transformation and homologous recombination (90, 143). If the cues that induce gametogenesis and mating were identified, then this system could be further exploited to investigate sex in one of the earlier-diverging groups of chlorophyte algae.

CHAROPHYTE ALGAE

Charophyte algae are a paraphyletic grade within the streptophyte lineage (charophyte algae and embryophytes) (**Figure 1**) and are the closest living relatives of land plants (85). There are six major groups of charophytes that range from unicellular (e.g., *Mesostigma*) to filamentous or simple colonial (e.g., *Klebsormidium*, *Chlorokybus*) to those having more complex body plans and cell-type differentiation (e.g., *Chara*, *Nitella*, *Coleochaete*) (97, 141). Sex has not been observed in

the earlier-branching charophytes but is routinely seen in Zygnematales such as *Closterium* (see below) and in the other more derived multicellular charophytes such as *Chara* and *Coleochaete*, where sex is oogamous and species are monoicous with separate male and female reproductive structures produced within the same multicellular individual (50, 97). Very little is known about sex determination in charophytes other than in the model species *Closterium*, described below.

Closterium

Within the zygnematophytes are multiple species of the genus *Closterium* that have well-characterized sexual cycles (reviewed in 124, 139). *Closterium* is unicellular and isogamous with a haplontic life cycle (**Figure 2**), much like volvocine algae. Both heterothallic and homothallic isolates exist (138), though the ancestral state and transitions between heterothallism and homothallism within this genus remain unresolved. While many species of green algae including volvocines and *Ulva* species produce motile flagellated gametes, gametes and vegetative cells of *Closterium* and other zygnematophytes are aflagellate, and sexual interactions occur through conjugation where adjacent cells of opposite mating type signal to each other to induce the process of mating. Mating involves a round of sexual cell division induced by nitrogen starvation, and the release of pheromones by opposite mating partners to induce production of mating-competent daughter cells. *mt*[−] cells produce a hormone called protoplast-release-inducing protein (PR-IP) inducer that stimulates sexual cell division and stimulates *mt*⁺ cells to release PR-IP, which induces *mt*[−] sexual cell divisions and causes gametangia to be released from their mother walls, allowing protoplast fusion. Sexual cells of opposite mating type pair side-by-side and then release gametangial protoplasts from their mother cell walls. The two released protoplasts undergo fertilization (fusion) to form diploid zygotes that mature into dormant zygospores. Upon germination, the zygospores undergo meiosis and produce two haploid meiotic progeny of opposite mating types (for heterothallic strains) while the other two meiotic nuclei are discarded, as occurs in some modified fungal meiosis under nutrient limitation (102, 118). The signaling pheromones of the *Closterium peracerosum-strigosum-littorale* (*C. psl*) species complex have been purified and the genes encoding them cloned (104, 125, 126, 137). Genes encoding PR-IP inducer and PR-IP were found to encode glycoproteins that are not related to each other or to known pheromones or hormones in other taxa (125, 126, 137). Their receptors and modes of action remain undetermined.

The mating type loci of *Closterium* species have not yet been described, but an experiment to test mating locus dominance was performed using artificially generated *mt*[−]/*mt*[−] diploids that were mated to an *mt*⁺ haploid. The mating type ratios of resulting progeny were used to infer that *mt*[−] is dominant to *mt*⁺, an idea that will require direct testing. Identification of mating-type-linked genes in *C. psl* has been reported, though details of these findings remain to be described (139). When it becomes available, a full *mt* locus sequence in *Closterium* should lead to the identification of its mating-type regulators, whose functions may be tested or confirmed using transgenic approaches.

RED ALGAE

Sexuality has never been observed in unicellular red algae (cyanidiophytes), but evidence exists for genetic exchange in populations (150) and for meiotic tool kit genes (94) (**Figure 1**). Multicellular red algae inhabit marine environments and exhibit several different types of sexual cycles (62). In Bangiophyceae (e.g., *Porphyra*), the diploid sporophyte is microscopic and meiosis occurs during germination of the spores. The postmeiotic tetrads stay together, forming a mosaic gametophyte thallus (98, 110). Multicellular red algae may reproduce asexually through production of

Diplontic life cycle:

no or highly reduced haploid mitosis after meiosis; nonmitotic gametes may be only haploid stage representatives

unicellular mitospores or sexually via dimorphic gametes, which may be either female macrogametes (oocytes) or male microgametes (spermatia) (66, 122). Although spermatia lack flagella and other means of active motility, the sexual system is considered oogamous because male and female gamete sizes are so different from each other. Syngamy occurs while the egg is still attached to the female gametophyte (61). The resulting zygotes divide to produce mitospores (or conchospores) that are released into the seawater. It is unknown how sexes are determined in red algae, though the genome of a haploid *Porphyra* strain of unknown sex has been fully sequenced (13).

The Florideophyceae constitute the second large multicellular red algal group and include *Chondrus crispus*, whose genome has been sequenced (30). In this group fertilization occurs on the female gametophyte, as it does in *Porphyra*, and involves the formation of a carposporophyte as an epiphyte attached to the female gametophyte. Molecular genetic analyses indicate that both self-fertilization and outcrossing occur in *C. crispus* (80). At maturity, the carposporophyte releases diploid spores that develop as multicellular tetrasporophytes where meiosis may occur. Both monoicous and dioicous species occur in florideophytes, for which, like bangiophytes, there is no information about sex-determining mechanisms. However, RAPD and SCAR molecular markers linked to sex determination in *Gracilaria chilensis* and *Gracilaria gracilis* have been identified and may be a route toward cloning the SDRs (52, 95) (**Supplemental Figure 1**). Overall, the red algae are an important taxonomic group and hold potential for understanding sex determination in the context of complex life cycles (66). Species within the genera *Porphyra* and *Gracilaria* have potential for development into model organisms. Their genomes are relatively small (14, 30), their life cycles can be controlled in the laboratory, and progeny from genetic crosses can be obtained. Pigmentation mutants in the model species *Porphyra yezoensis* have been described (103, 109, 149), so in theory, developmental mutants and developmental genetics would also be possible to develop for this and other genetically tractable species of red algae.

DIATOMS

Diatoms are an ecologically important and extremely diverse group of microalgae, belonging to the stramenopiles, one of the largest groups of eukaryotes (**Figure 1**). All diatoms exhibit a diplontic life cycle (**Figure 2**), involving a size-dependent transition from mitotically dividing cells to sexual reproduction (20, 40). The vegetative cells are diploid, and the only haploid cells are the postmeiotic gametes, which exist transiently and do not undergo any mitotic proliferation. With each round of vegetative mitosis, cell size decreases due to constraints of enclosure of each cell within a rigid bipartite silica cell wall or frustule that is incapable of expansion. Gametogenesis and sexual development are initiated when mitotic cells fall below a certain size, called the sexual size threshold. After mating (described below) the specialized zygotes, called auxospores, grow and elongate to reconstitute the initial cell size, after which they differentiate back into vegetative cells to restart the mitotic cycle. Thus, periodic sexual reproduction is an essential part of the diatom life cycle.

The mechanisms of gametogenesis and sexual reproduction among diatoms are diverse. Two major groups of diatoms are the ancestral centrics, whose cells exhibit radial symmetry and which are oogamous, and a large derived clade of pennate diatoms that are generally isogamous and produce nonflagellated gametes, with sexual interactions taking place via a spatially and temporally controlled process of conjugation that bears similarity to sexual conjugation in *Closterium* (see above) (19, 40). Heterothallic reproduction is widespread in pennate diatoms, with two mating types (*MT*+ or *MT*−), but many centrics are homothallic (20). In homothallic species the size ranges in which cells differentiate as eggs or sperm are different, with oogenesis starting first,

followed by a phase when both eggs and sperm are produced; finally, when the cells are small, only sperm cells are produced (20, 130).

The raphid pennate diatom *Seminavis robusta* is particularly suitable for studies on life cycle regulation (21, 100), and construction of *MT*-specific linkage mapping has identified the *MT*+ and *MT*− SDRs, with the *MT*+ haplotype dominant to *MT*− for sexual differentiation. While no genomic information is available currently for this species, linkage mapping suggests that *MT* chromosomes in this species are homomorphic, with a small nonrecombining region and a large PAR (144). More recently, *Pseudo-nitzschia multistriata* has also emerged as a model to look at sexual cycles in diatoms. This species has a typical, controllable size reduction–restitution life cycle in which isogamous diploid cells of opposite mating type (*MT*+ and *MT*−) undergo meiosis and produce gametes when they are below the size threshold for sex (35). The diploid genome of *P. multistriata* has been sequenced and phylogenomic and transcriptomic analyses have documented gene gains and losses, horizontal gene transfers, conservation, and evolutionary divergence of sex-related genes (5). A recent study has shown that in *P. multistriata*, gamete differentiation into *MT*+ or *MT*− mating types is defined by the monoallelic expression of the gene *MRP3* in *MT*+ cells. The specific function of *MRP3* is unknown, but it appears to regulate the expression of several autosomal genes (120). *MRP3* is conserved in other *Pseudo-nitzschia* species and in the closely related *Fragilariopsis cylindrus*, but it remains to be determined whether it acts as a mating-type determinant in these relatives. Orthologs of *MRP3* were not detected in centric and other pennate diatoms, including *S. robusta*, a result that may be due to true absence of an ortholog, incomplete genome sequence, or rapid sequence evolution (120).

BROWN ALGAE

The brown algae (phaeophytes) are a clade of multicellular stramenopile eukaryotes that have been evolving independently from animals and plants for over a billion years (Figure 1). They encompass a large diversity of life cycles and reproductive systems (22, 63) (Figure 5) and have recently emerged as interesting model organisms to investigate the evolution of sexes (1, 25).

Evolution of Sexual Dimorphism in Brown Algae

All brown algae are traditionally considered to have sexes, since all species exhibit some degree of gamete size dimorphism. Indeed, contrary to the case of algae from the green lineage, no strict isogamy exists in the brown algae. While in some species the two gamete types are very close in size (mild anisogamy), they still differ in behavior and physiology: Shortly after formation and release into the water column, female gametes quickly settle onto the substrate and reabsorb their flagella. Pheromones released into the seawater by female gametes attract fast-swimming male gametes. Among brown algal species there is a wide degree of variability in gamete size dimorphism, ranging from mild anisogamy (e.g., *Ectocarpus*), to strong anisogamy and oogamy (91). Interestingly, phylogenetic reconstruction suggests that oogamy is the ancestral state in brown algae, with several independent reversals to anisogamy and mild anisogamy (63, 127). These reversals from oogamy may be especially valuable for testing ideas about the selective forces that maintain anisogamy and oogamy. As described above (see the section titled Introduction) Bulmer & Parker (16) proposed that both anisogamy and zygote size are expected to increase with organism size and complexity, and Lehtonen and Kokko (83) confirmed this expectation but also showed that evolution of anisogamy requires some level of gametic competition and limitation. Thus, brown algae might be predicted to vary according to their level of anisogamy in

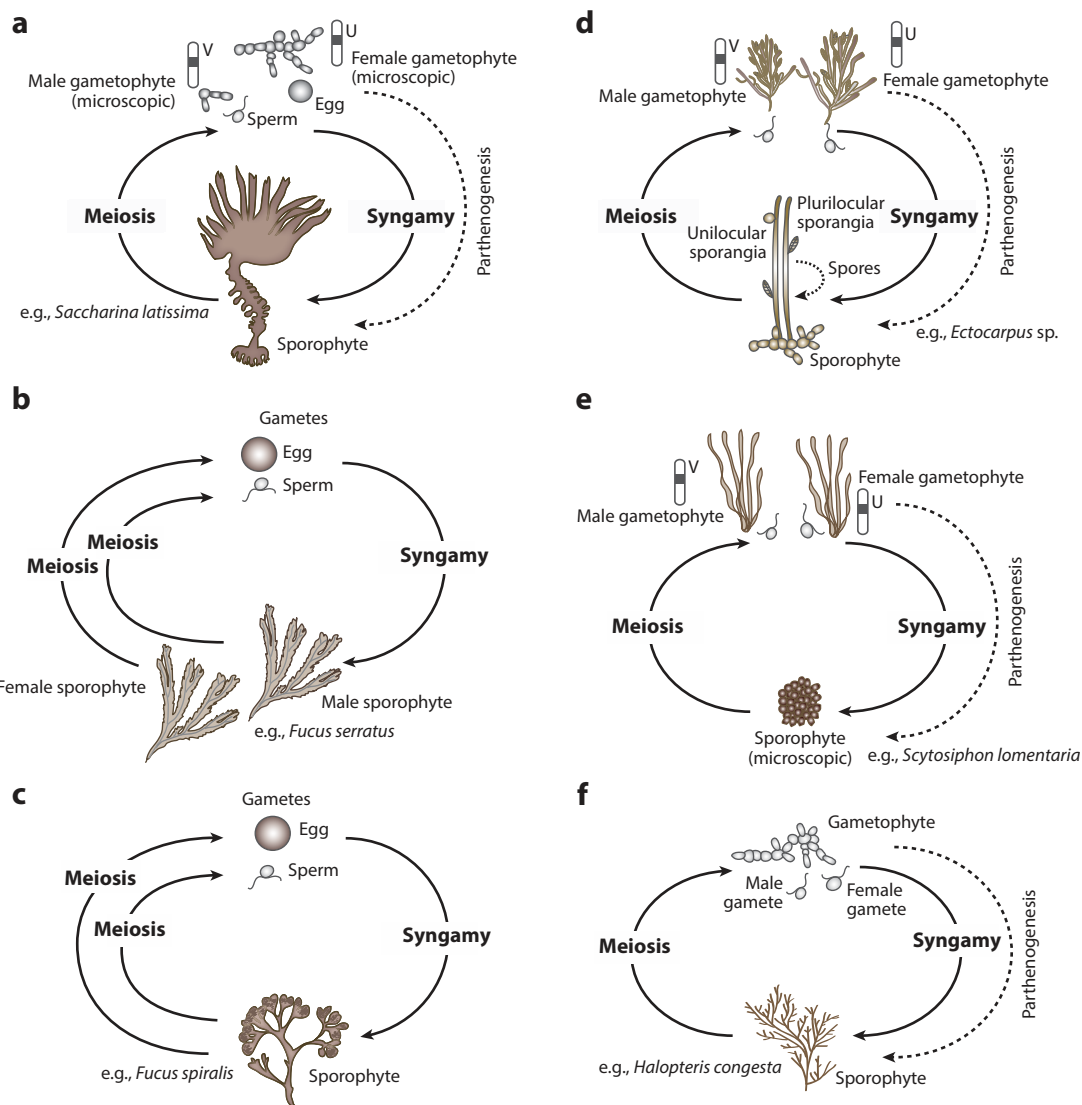


Figure 5

Brown algal life cycle and sexual cycle diversity. (a) The kelps exhibit a haplodiaplontic life cycle. The gametophyte generation is reduced but nonetheless develops independently of the sporophyte, and the male and female gametophytes are strongly anisomorphic. In *Fucus serratus* (b) and *Fucus spiralis* (c), the gametophyte generation has been lost, resulting in a diaplontic life cycle, with dioecious or monoecious individuals and strongly dimorphic male and female gametes. The Ectocarpales include species with isomorphic haplodiaplontic life cycles, but they also include species with slightly heteromorphic life cycles (such as *Ectocarpus* sp., depicted in panel d) and species with strongly heteromorphic haplodiaplontic life cycles, with either the gametophyte or the sporophyte generation being microscopic (panel e represents an example of the latter). Gamete size dimorphism in the Ectocarpales is very slight. (f) *Halopteris congesta* is an example of a monoicous haplodiaplontic brown alga. In many brown algae, a vegetative or mitotic cycle that includes parthenogenetic development of gametes is superimposed in the sexual cycle (dashed arrows). Male (V) and female (U) sex chromosomes are depicted for species that express sex during the haploid phase of the life cycle. Note that dioecious *Fucus* species such as *F. serratus* (b) have a diploid sex-determining system (XY or ZW) whose details are unknown.

terms of gamete competition, gamete limitation, and/or resource requirements for zygotic fitness. This variation in sexual dimorphism may also impact evolution of brown algal SDRs, though as described for volvocine algae (see above) (Figure 4), there may not be a strong correspondence between level of sexual dimorphism and the degree of MDR or SDR haplotype differentiation.

Sex Determination in *Ectocarpus*

The genus *Ectocarpus* has recently emerged as a model for studies on the evolution of sex determination in the brown algae, in particular evolution of UV sex chromosomes. The haplodiplontic life cycle of *Ectocarpus* consists of an alternation of generations (Figure 5) and represents what is thought to be the most frequent type of sexual system in the brown algae (dioicy), in which male and female sexes are determined during the haploid, gametophyte, generation by a pair of UV sex chromosomes, a feature that has important implications for SDR evolution (1, 4, 25). The UV sex chromosomes of *Ectocarpus* sp. have been identified, and an ~1 Mb nonrecombining region that contains the SDR has been characterized (1) (Supplemental Table 1). At the sequence level, the male and female haplotypes of the SDR in *Ectocarpus* sp. are highly divergent. The only regions found with high similarity are the gametolog exons, and large blocks of highly similar sequences are absent. Genetic crosses using life cycle mutants (2, 24) have shown that the male SDR is dominant over the female (1), and one particularly interesting male-limited gene encoding an HMG (high mobility group)-domain putative TF is predicted to be a master regulator of sex determination. This gene is strongly upregulated during fertility and consistently male linked in all the brown algal species whose sex chromosomes have been identified so far (1, 88). Although the key regulator(s) of *Ectocarpus* sex determination residing in the SDR has yet to be definitively validated, a transcriptome data set of two ontogenetic stages of male and female gametophytes has revealed the expression network involved in *Ectocarpus* sex differentiation (87). Coding regions of both male- and female-biased genes in *Ectocarpus* had a greater proportion of sites experiencing positive selection, suggesting that their accelerated divergence is at least partly driven by adaptive evolution. Interestingly, gene duplication appears to have played a significant role in the generation of sex-biased genes in *Ectocarpus*, expanding previous models that propose this mechanism for the resolution of sexual antagonism in diploid systems (31). These patterns of sex-biased gene expression in *Ectocarpus* correspond to the distinctive aspects of this organism's reproductive biology, which involves separate sexes at the haploid stage of the life cycle and overall low levels of sexual dimorphism.

Dioicy: condition of having separate genetically determined male and female individuals in the haploid (gametophyte) stage (e.g., mosses, brown algae)

Sexual antagonism: situation where optimal alleles or strategies for reproductive fitness differ between sexes

Supplemental Material >

Evolution of *Ectocarpus* and Other Brown Algal Sex-Determining Regions

Because the heteromorphic regions of U and V chromosomes (SDRs) are nonrecombining, they are expected to evolve differently compared with autosomal regions (25). *Ectocarpus* U and V SDRs exhibit about half as much neutral diversity as the autosomes, and this difference is consistent with the reduced effective population sizes of these regions compared with the rest of the genome. This suggests that the influence of additional factors such as background selection or selective sweeps is minimal (3).

The sex-specific genes of several brown algal species that have diverged over a time frame of 100 million years were compared, and this helped shed light on long-term properties of UV sex chromosomes (88). This study revealed a substantial amount of gene traffic in and out of the sex-linked region but also highlighted a group of genes that has been stably maintained on the SDR of all the brown algae studied so far, suggesting that the genes are important for sex determination and/or originated on an ancestral sex chromosome. A comparative analysis of sex chromosomes

from the kelp *Saccharina japonica* and *Ectocarpus* sp. suggested that the two species resolve sexual conflict by different strategies—with *Ectocarpus* modifying expression levels of sex-related genes (sex-biased gene expression of autosomal or PAR loci) and kelps integrating dimorphically expressed sex-related genes into the SDR.

It will be fascinating to explore the wealth of life cycles and reproductive systems in the brown algae to increase our understanding of the mechanisms underlying transitions among the different types of sexual systems, specifically the switches between developmental and genetic sex determination (i.e., from combined to separate sexes, and vice versa). The Fucales, in particular, are unique models to examine the genetic and evolutionary forces underlying transitions from haploid (UV) to diploid (XY/ZW) systems (26, 91).

SUMMARY POINTS

1. Algae are a diverse collection of photosynthetic eukaryotes with different types of life cycles and sexual cycles, including those with isogamous mating systems and those with male/female sexes.
2. Volvocine green algae have well-studied haplontic sexual cycles and sex-determination systems that include a progression from unicellular species with isogamous mating types like *Chlamydomonas reinhardtii* to multicellular species with dimorphic sexes like *Volvox carteri*.
3. *Ulva* is an emerging model genus within the major green algal clade Ulvophyceae. Sequenced genome and SDRs for *Ulva* help set the stage for understanding the origins and maintenance of its slightly anisogamous haploid mating system.
4. RWP-RK family transcription factor genes including the volvocine master regulatory *MID* gene are implicated in haploid mating-type or sex determination across the green lineage including in ulvophytes (*Ulva*) and prasinophytes (*Ostreococcus*) and in early diverging land plants (*Marchantia*).
5. The charophyte algae are the closest relatives of land plants, and haplontic *Closterium* is a model for isogamous mating-type determination with a conjugation-based mating system coordinated by intercellular pheromone signaling.
6. The diatom *Pseudo-nitzschia multistriata* is emerging as a model for studies on isogamous sexual reproduction and mating-type determination in unicellular stramenopiles. Its mating-type locus has recently been identified.
7. Brown algae display a wide diversity of life cycles and reproductive systems and have recently emerged as model organisms to investigate the evolution of sexes and sex-determination mechanisms that control gametogenesis in either the haploid or diploid phase of the life cycle.
8. Advances in genomics will continue to enable progress in understanding algal sex determination in a broader representation of algal species and taxa.

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LITERATURE CITED

1. Ahmed S, Cock JM, Pessia E, Luthringer R, Cormier A, et al. 2014. A haploid system of sex determination in the brown alga *Ectocarpus* sp. *Curr. Biol.* 24(17):1945–57
2. Arun A, Coelho SM, Peters AF, Bourdareau S, Pérès L, et al. 2019. Convergent recruitment of TALE homeodomain life cycle regulators to direct sporophyte development in land plants and brown algae. *eLife* 8:e43101
3. Avia K, Lipinska A, Mignerot L, Montecinos A, Jamy M, et al. 2018. Genetic diversity in the UV sex chromosomes of the brown alga *Ectocarpus*. *Genes* 9(6):E286
4. Bachtrog D, Kirkpatrick M, Mank JE, McDaniel SF, Pires JC, et al. 2011. Are all sex chromosomes created equal? *Trends Genet.* 27(9):350–57
5. Basu S, Patil S, Mapleson D, Russo MT, Vitale L, et al. 2017. Finding a partner in the ocean: molecular and evolutionary bases of the response to sexual cues in a planktonic diatom. *New Phytol.* 215(1):140–56
6. Bell G. 1978. The evolution of anisogamy. *J. Theor. Biol.* 73(2):247–70
7. Bell G. 1985. The origin and early evolution of germ cells as illustrated by the Volvocales. In *The Origin and Evolution of Sex*, ed. HO Halvorson, A Monroy, pp. 221–56. New York: Alan R. Liss
8. Beukeboom L, Perrin N. 2014. *The Evolution of Sex Determination*. Oxford, UK: Oxford Univ. Press
9. Beutlich A, Schnetter R. 1993. The life cycle of *Cryptochlora perforans* (Chlorarachniophyta). *Botanica Acta.* 106(5):441–47
10. Bircham R, Kochert G. 1979. Development of sperm cells of *Volvox carteri* f. *weismannia* (Chlorophyceae). *Phycologia* 18(4):409–19
11. Blanc-Mathieu R, Krasovec M, Hebrard M, Yau S, Desgranges E, et al. 2017. Population genomics of picophytoplankton unveils novel chromosome hypervariability. *Sci. Adv.* 3(7):e1700239
12. Bowman JL, Sakakibara K, Furumizu C, Dierschke T. 2016. Evolution in the cycles of life. *Annu. Rev. Genet.* 50:133–54
13. Brawley SH, Blouin NA, Ficko-Blean E, Wheeler GL, Lohr M, et al. 2017. Insights into the red algae and eukaryotic evolution from the genome of *Porphyra umbilicalis* (Bangiophyceae, Rhodophyta). *PNAS* 114(31):E6361–70
14. Brawley SH, Johnson LE. 1992. Gametogenesis, gametes and zygotes: An ecological perspective on sexual reproduction in the algae. *Br. Phycol. J.* 27(3):233–52
15. Brodie J, Chan CX, De Clerck O, Cock JM, Coelho SM, et al. 2017. The algal revolution. *Trends Plant Sci.* 22(8):726–38
16. Bulmer MG, Parker GA. 2002. The evolution of anisogamy: a game-theoretic approach. *Proc. R. Soc. B* 269(1507):2381–88
17. Chardin C, Girin T, Roudier F, Meyer C, Krapp A. 2014. The plant RWP-RK transcription factors: key regulators of nitrogen responses and of gametophyte development. *J. Exp. Bot.* 65(19):5577–87
18. Charlesworth B. 1978. The population genetics of anisogamy. *J. Theor. Biol.* 73(2):347–57
19. Chepurinov VA, Mann DG. 2004. Auxosporulation of *Licmophora communis* (Bacillariophyta) and a review of mating systems and sexual reproduction in araphid pennate diatoms. *Phycol. Res.* 52(1):1–12
20. Chepurinov VA, Mann DG, Sabbe K, Vyverman W. 2004. Experimental studies on sexual reproduction in diatoms. *Int. Rev. Cytol.* 237:91–154

1. The first description of a sex-determining locus in multicellular stramenopiles.

11. Evidence for sexual recombination and heteromorphic sex chromosomes in prasinophytes.

21. Chepurinov VA, Mann DG, von Dassow P, Vanormelingen P, Gillard J, et al. 2008. In search of new tractable diatoms for experimental biology. *BioEssays* 30(7):692–702
22. Cock JM, Godfroy O, Macaisne N, Peters AF, Coelho SM. 2014. Evolution and regulation of complex life cycles: a brown algal perspective. *Curr. Opin. Plant. Biol.* 17:1–6
23. Cocquyt E, Verbruggen H, Leliaert F, De Clerck O. 2010. Evolution and cytological diversification of the green seaweeds (Ulvophyceae). *Mol. Biol. Evol.* 27(9):2052–61
24. Coelho SM, Godfroy O, Arun A, Le Corguillé G, Peters AF, Cock JM. 2011. *OUROBOROS* is a master regulator of the gametophyte to sporophyte life cycle transition in the brown alga *Ectocarpus*. *PNAS* 108(28):11518–23
25. Coelho SM, Gueno J, Lipinska AP, Cock JM, Umen JG. 2018. UV chromosomes and haploid sexual systems. *Trends Plant Sci.* 23(9):794–807
26. Coelho SM, Mignerot L, Cock JM. 2019. Origin and evolution of sex-determination systems in the brown algae. *New Phytol.* 222(4):1751–56
27. Coelho SM, Peters AF, Charrier B, Roze D, Destombe C, et al. 2007. Complex life cycles of multicellular eukaryotes: new approaches based on the use of model organisms. *Gene* 406(1–2):152–70
28. Coleman AW. 1979. Sexuality in colonial green flagellates. In *Biochemistry and Physiology of Protozoa*, ed. M Levandowsky, SH Hutner, pp. 307–40. New York: Elsevier
29. Coleman AW. 2012. A comparative analysis of the Volvocaceae (Chlorophyta). *J. Phycol.* 48(3):491–513
30. Collén J, Porcel B, Carré W, Ball SG, Chaparro C, et al. 2013. Genome structure and metabolic features in the red seaweed *Chondrus crispus* shed light on evolution of the Archaeplastida. *PNAS* 110(13):5247–52
31. Connallon T, Clark AG. 2011. The resolution of sexual antagonism by gene duplication. *Genetics* 187(3):919–37
32. Cosmides LM, Tooby J. 1981. Cytoplasmic inheritance and intragenomic conflict. *J. Theor. Biol.* 89(1):83–129
33. Cross FR, Umen JG. 2015. The *Chlamydomonas* cell cycle. *Plant J.* 82(3):370–92
34. da Silva J. 2017. The evolution of sexes: A specific test of the disruptive selection theory. *Ecol. Evol.* 8(1):207–19
35. D'Alelio D, Amato A, Luedeking A, Montresor M. 2009. Sexual and vegetative phases in the planktonic diatom *Pseudo-nitzschia multistriata*. *Harmful Algae* 8(2):225–32
36. Darwin C. 1871. *The Descent of Man, and Selection in Relation to Sex*. London: J. Murray
37. De Clerck O, Kao S-M, Bogaert KA, Blomme J, Foflonker F, et al. 2018. Insights into the evolution of multicellularity from the sea lettuce genome. *Curr. Biol.* 28(18):2921–25
38. De Hoff PL, Ferris P, Olson BJSC, Miyagi A, Geng S, Umen JG. 2013. Species and population level molecular profiling reveals cryptic recombination and emergent asymmetry in the dimorphic mating locus of *C. reinhardtii*. *PLOS Genet.* 9(8):e1003724
39. Derelle E, Ferraz C, Rombauts S, Rouzé P, Worden AZ, et al. 2006. Genome analysis of the smallest free-living eukaryote *Ostreococcus tauri* unveils many unique features. *PNAS* 103(31):11647–52
40. Drebes G. 1977. Sexuality. In *The Biology of Diatoms*, ed. D Werner, pp. 250–83. Oxford, UK: Blackwell
41. Dusenbery DB. 2006. Selection for high gamete encounter rates explains the evolution of anisogamy using plausible assumptions about size relationships of swimming speed and duration. *J. Theor. Biol.* 241(1):33–38
42. Ebenezer TE, Zoltner M, Burrell A, Nenarokova A, Novák Vanclová AMG, et al. 2019. Transcriptome, proteome and draft genome of *Euglena gracilis*. *BMC Biol.* 17(1):11
43. Ferris P, Olson BJSC, De Hoff PL, Douglass S, Casero D, et al. 2010. Evolution of an expanded sex-determining locus in *Volvax*. *Science* 328(5976):351–54. Erratum. 2010. *Science* 329(5998):1467
44. Ferris PJ, Goodenough UW. 1997. Mating type in *Chlamydomonas* is specified by *mid*, the minus-dominance gene. *Genetics* 146(3):859–69
45. Fučíková K, Pažoutová M, Rindi F. 2015. Meiotic genes and sexual reproduction in the green algal class Trebouxiophyceae (Chlorophyta). *J. Phycol.* 51(3):419–30
46. Geng S, De Hoff P, Umen JG. 2014. Evolution of sexes from an ancestral mating-type specification pathway. *PLOS Biol.* 12(7):e1001904

43. Characterization of UV sex chromosomes in *Volvax* originating from a mating-type system.

46. Revealed deep homology in volvocine MID function as a mating-type or sex-determining gene.

47. Geng S, Miyagi A, Umen JG. 2018. Evolutionary divergence of the sex-determining gene *MID* uncoupled from the transition to anisogamy in volvocine algae. *Development* 145(7):dev162537
48. Goodenough U, Heitman J. 2014. Origins of eukaryotic sexual reproduction. *Cold Spring Harb. Perspect. Biol.* 6:a016154
49. Goodenough U, Lin H, Lee J-H. 2007. Sex determination in *Chlamydomonas*. *Semin. Cell Dev. Biol.* 18(3):350–61
50. Graham LE, Graham JM, Wilcox LW. 2009. *Algae*. San Francisco: Benjamin-Cummings
51. Grimsley N, Péquin B, Bachy C, Moreau H, Piganeau G. 2010. Cryptic sex in the smallest eukaryotic marine green alga. *Mol. Biol. Evol.* 27(1):47–54
52. Guillemin M-L, Huanel OR, Martínez EA. 2012. Characterization of genetic markers linked to sex determination in the haploid-diploid red alga *Gracilaria chilensis*. *J. Phycol.* 48(2):365–72
53. Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst. Biol.* 59(3):307–21
54. Hallmann A, Godt K, Wenzl S, Sumper M. 1998. The highly efficient sex-inducing pheromone system of *Volvox*. *Trends Microbiol.* 6(5):185–89
55. Hamaji T, Ferris PJ, Coleman AW, Waffenschmidt S, Takahashi F, et al. 2008. Identification of the minus-dominance gene ortholog in the mating-type locus of *Gonium pectorale*. *Genetics* 178(1):283–94
56. Hamaji T, Ferris PJ, Nishii I, Nishimura Y, Nozaki H. 2013. Distribution of the sex-determining gene *MID* and molecular correspondence of mating types within the isogamous genus *Gonium* (Volvocales, Chlorophyta). *PLOS ONE* 8(5):e64385
57. Hamaji T, Kawai-Toyooka H, Uchimura H, Suzuki M, Noguchi H, et al. 2018. Anisogamy evolved with a reduced sex-determining region in volvocine green algae. *Commun. Biol.* 1(1):17
58. Hamaji T, Mogi Y, Ferris PJ, Mori T, Miyagishima S, et al. 2016. Sequence of the *Gonium pectorale* mating locus reveals a complex and dynamic history of changes in volvocine algal mating haplotypes. *G3* 6(5):1179–89
59. Hanschen ER, Herron MD, Wiens JJ, Nozaki H, Michod RE. 2017. Repeated evolution and reversibility of self-fertilization in the volvocine green algae. *Evolution* 19:716
60. **Hanschen ER, Herron MD, Wiens JJ, Nozaki H, Michod RE. 2018. Multicellularity drives the evolution of sexual traits. *Am. Nat.* 192(3):E93–105**
61. Hawkes MW. 1978. Sexual reproduction in *Porphyra gardneri* (Smith et Hollenberg) Hawkes (Bangiales, Rhodophyta). *Phycologia* 17(3):329–53
62. Hawkes MW. 1990. Reproductive strategies. In *Biology of the Red Algae*, ed. KM Cole, RG Sheath, pp. 455–76. Cambridge, UK: Cambridge Univ. Press
63. Heesch S, Serrano-Serrano M, Luthringer R, Peters AF, Destombe C, et al. 2019. Evolution of life cycles and reproductive traits: insights from the brown algae. bioRxiv 530477. <https://doi.org/10.1101/530477>
64. Herron MD, Hackett JD, Aylward FO, Michod RE. 2009. Triassic origin and early radiation of multicellular volvocine algae. *PNAS* 106(9):3254–58
65. Herron MD, Michod RE. 2008. Evolution of complexity in the volvocine algae: transitions in individuality through Darwin's eye. *Evolution* 62(2):436–51
66. Herron MD, Rashidi A, Shelton DE, Driscoll WW. 2013. Cellular differentiation and individuality in the “minor” multicellular taxa. *Biol. Rev. Camb. Philos. Soc.* 88(4):844–61
67. Hill DRA, Wetherbee R. 1986. *Proteomonas sulcata* gen. et sp. nov. (Cryptophyceae), a cryptomonad with two morphologically distinct and alternating forms. *Phycologia* 25(4):521–43
68. Hiraide R, Kawai-Toyooka H, Hamaji T, Matsuzaki R, Kawafune K, et al. 2013. The evolution of male-female sexual dimorphism predates the gender-based divergence of the mating locus gene *MAT3/RB*. *Mol. Biol. Evol.* 30(5):1038–40
69. Hoekstra RF. 2011. Nucleo-cytoplasmic conflict and the evolution of gamete dimorphism. In *The Evolution of Anisogamy: A Fundamental Phenomenon Underlying Sexual Selection*, ed. T Togashi, PA Cox, pp. 111–30. Cambridge, UK: Cambridge Univ. Press

60. Showed repeated evolution of sexual dimorphism in volvocine algae and strong support for PBS theory.

70. Hoxmark RC, Nordby Ø. 1974. Haploid meiosis as a regular phenomenon in the life cycle of *Ulva mutabilis*. *Hereditas* 76(2):239–49
71. Hurst LD. 1990. Parasite diversity and the evolution of diploidy, multicellularity and anisogamy. *J. Theor. Biol.* 144(4):429–43
72. Ichihara K, Suzuki R, Yamazaki T, Ota S, Mogi Y, et al. 2015. *Ulva partita* sp. nov., a novel *Enteromorpha*-like *Ulva* species from Japanese coastal areas. *Cytologia* 80(3):261–70
73. Iyer P, Roughgarden J. 2008. Gametic conflict versus contact in the evolution of anisogamy. *Theor. Popul. Biol.* 73(4):461–72
74. Joo S, Nishimura Y, Cronmiller E, Hong RH, Kariyawasam T, et al. 2017. Gene regulatory networks for the haploid-to-diploid transition of *Cblamydomonas reinhardtii*. *Plant Physiol.* 175(1):314–32
75. Kagami Y, Mogi Y, Arai T, Yamamoto M, Kuwano K, Kawano S. 2008. Sexuality and uniparental inheritance of chloroplast DNA in the isogamous green alga *Ulva compressa* (Ulvophyceae). *J. Phycol.* 44(3):691–702
76. Keeling PJ. 2013. The number, speed, and impact of plastid endosymbioses in eukaryotic evolution. *Annu. Rev. Plant. Biol.* 64(1):583–607
77. Knowlton N. 1974. A note on the evolution of gamete dimorphism. *J. Theor. Biol.* 46(1):283–85
78. Koi S, Hisanaga T, Sato K, Shimamura M, Yamato KT, et al. 2016. An evolutionarily conserved plant RKD factor controls germ cell differentiation. *Curr. Biol.* 26(13):1775–81
79. Koszegi D, Johnston AJ, Rutten T, Czihal A, Altschmied L, et al. 2011. Members of the RKD transcription factor family induce an egg cell-like gene expression program. *Plant J.* 67(2):280–91
80. Krueger-Hadfield SA, Roze D, Correa JA, Destombe C, Valero M. 2015. O father where art thou? Paternity analyses in a natural population of the haploid-diploid seaweed *Chondrus crispus*. *Heredity* 114(2):185–94
81. Kugrens P, Lee RE. 1988. Ultrastructure of fertilization in a cryptomonad. *J. Phycol.* 24(3):385–93
82. Lahr DJG, Parfrey LW, Mitchell EAD, Katz LA, Lara E. 2011. The chastity of amoebae: re-evaluating evidence for sex in amoeboid organisms. *Proc. R. Soc. B* 278:2081–90
83. Lehtonen J, Kokko H. 2010. Two roads to two sexes: unifying gamete competition and gamete limitation in a single model of anisogamy evolution. *Behav. Ecol. Sociobiol.* 65(3):445–59
84. Lehtonen J, Kokko H, Parker GA. 2016. What do isogamous organisms teach us about sex and the two sexes? *Philos. Trans. R. Soc. B* 371(1706):20150532
85. Leliaert F, Smith DR, Moreau H, Herron MD, Verbruggen H, et al. 2012. Phylogeny and molecular evolution of the green algae. *Crit. Rev. Plant Sci.* 31:1–46
86. Lin H, Goodenough UW. 2007. Gametogenesis in the *Cblamydomonas reinhardtii* minus mating type is controlled by two genes, *MID* and *MTD1*. *Genetics* 176(2):913–25
87. Lipinska A, Cormier A, Luthringer R, Peters AF, Corre E, et al. 2015. Sexual dimorphism and the evolution of sex-biased gene expression in the brown alga *Ectocarpus*. *Mol. Biol. Evol.* 32(6):1581–97
88. Lipinska AP, Toda NRT, Heesch S, Peters AF, Cock JM, Coelho SM. 2017. Multiple gene movements into and out of haploid sex chromosomes. *Genome Biol.* 18(1):104
89. Lopez D, Hamaji T, Kropat J, De Hoff P, Morselli M, et al. 2015. Dynamic changes in the transcriptome and methylome of *Cblamydomonas reinhardtii* throughout its life cycle. *Plant Physiol.* 169(4):2730–43
90. Lozano J-C, Schatt P, Botebol H, Vergé V, Lesuisse E, et al. 2014. Efficient gene targeting and removal of foreign DNA by homologous recombination in the picoeukaryote *Ostreococcus*. *Plant J.* 78(6):1073–83
91. Luthringer R, Cormier A, Ahmed S, Peters A, Cock J, Coelho SM. 2014. Sexual dimorphism in the brown algae. *Perspect. Phycol.* 1(1):11–25
92. Maddox AS, Azoury J, Dumont J. 2012. Polar body cytokinesis. *Cytoskeleton* 69(11):855–68
93. Mages HW, Tschochner H, Sumper M. 1988. The sexual inducer of *Volvox carteri*. Primary structure deduced from cDNA sequence. *FEBS Lett.* 234(2):407–10
94. Malik SB, Ramesh MA, Hulstrand AM, Logsdon JM. 2007. Protist homologs of the meiotic *Spo11* gene and topoisomerase VI reveal an evolutionary history of gene duplication and lineage-specific loss. *Mol. Biol. Evol.* 24(12):2827–41

88. Analysis of long-term gene traffic in sex chromosomes of a haploid UV system.

95. Martinez EA, Destombe C, Quillet MC, Valero M. 1999. Identification of random amplified polymorphic DNA (RAPD) markers highly linked to sex determination in the red alga *Gracilaria gracilis*. *Mol. Ecol.* 8(9):1533–38
96. Matt GY, Umen JG. 2018. Cell-type transcriptomes of the multicellular green alga *Volvox carteri* yield insights into the evolutionary origins of germ and somatic differentiation programs. *G3* 8(2):531–50
97. McCourt RM, Delwiche CF, Karol KG. 2004. Charophyte algae and land plant origins. *Trends Ecol. Evol.* 19(12):661–66
98. Mitman GG, Meer JP. 1994. Meiosis, blade development, and sex determination in *Porphyra purpurea* (Rhodophyta). *J. Phycol.* 30(1):147–59
99. Miyamura S. 2010. Cytoplasmic inheritance in green algae: patterns, mechanisms and relation to sex type. *J. Plant Res.* 123(2):171–84
100. Moeys S, Frenkel J, Lembke C, Gillard JTF, Devos V, et al. 2016. A sex-inducing pheromone triggers cell cycle arrest and mate attraction in the diatom *Seminavis robusta*. *Sci. Rep.* 6:19252
101. Mori T, Kawai-Toyooka H, Igawa T, Nozaki H. 2015. Gamete dialogs in green lineages. *Mol. Plant.* 8(10):1442–54
102. Neiman AM. 2005. Ascospore formation in the yeast *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* 69(4):565–84
103. Niwa K, Hayashi Y, Abe T, Aruga Y. 2009. Induction and isolation of pigmentation mutants of *Porphyra yezoensis* (Bangiales, Rhodophyta) by heavy-ion beam irradiation. *Phycol. Res.* 57(3):194–202
104. Nojiri T, Fujii T, Sekimoto H. 1995. Purification and characterization of a novel sex pheromone that induces the release of another sex pheromone during sexual reproduction of the heterothallic *Closterium peracerosum-strigosum-littorale* complex. *Plant Cell Physiol.* 36(1):79–84
105. Nozaki H, Misawa K, Kajita T, Kato M, Nohara S, Watanabe MM. 2000. Origin and evolution of the colonial Volvocales (Chlorophyceae) as inferred from multiple, chloroplast gene sequences. *Mol. Phylogenet. Evol.* 17(2):256–68
- 106. Nozaki H, Mori T, Misumi O, Matsunaga S, Kuroiwa T. 2006. Males evolved from the dominant isogametic mating type. *Curr. Biol.* 16(24):R1018–20**
107. Nozaki H, Ueki N, Takusagawa M, Yamashita S, Misumi O, et al. 2018. Morphology, taxonomy and mating-type loci in natural populations of *Volvox carteri* in Taiwan. *Bot. Stud.* 59(1):10
108. Oertel W, Wichard T, Weissgerber A. 2015. Transformation of *Ukva mutabilis* (Chlorophyta) by vector plasmids integrating into the genome. *J. Phycol.* 51(5):963–79
109. Ohme M, Kunifujii Y, Miura A. 1986. Cross experiments in the color mutants of *Porphyra yezoensis* Ueda. *Japanese J. Phycol.* 34:101–6
110. Ohme M, Miura A. 1988. Tetrad analysis in conchospore germlings of *Porphyra yezoensis* (Rhodophyta, Bangiales). *Plant Sci.* 57(2):135–40
111. Palenik B, Grimwood J, Aerts A, Rouzé P, Salamov A, et al. 2007. The tiny eukaryote *Ostreococcus* provides genomic insights into the paradox of plankton speciation. *PNAS* 104(18):7705–10
112. Parker GA. 1978. Selection on non-random fusion of gametes during the evolution of anisogamy. *J. Theor. Biol.* 73(1):1–28
113. Parker GA. 2011. The origin and maintenance of two sexes (anisogamy), and their gamete sizes by gamete competition. In *The Evolution of Anisogamy: A Fundamental Phenomenon Underlying Sexual Selection*, ed. T Togashi, PA Cox, pp. 17–74. Cambridge, UK: Cambridge Univ. Press
114. Parker GA, Baker RR, Smith VG. 1972. The origin and evolution of gamete dimorphism and the male-female phenomenon. *J. Theor. Biol.* 36(3):529–53
115. Pfister LA. 1989. Dinoflagellate sexuality. *Int. Rev. Cytol.* 114:249–72
116. Randerson JP, Hurst LD. 2001. A comparative test of a theory for the evolution of anisogamy. *Proc. R. Soc. B* 268(1469):879–84
117. Randerson JP, Hurst LD. 2001. The uncertain evolution of the sexes. *Trends Ecol. Evol.* 16(10):571–79
118. Reedy JL, Floyd AM, Heitman J. 2009. Mechanistic plasticity of sexual reproduction and meiosis in the *Candida* pathogenic species complex. *Curr. Biol.* 19(11):891–99

106. First evidence that MID gene is conserved between volvocine algal mating types and sexes.

120. Identified the first sex-determining gene in diatoms.

139. Up-to-date review on the molecular genetics of sex in *Closterium*.

119. Rövekamp M, Bowman JL, Grossniklaus U. 2016. *Marchantia* MpRKD regulates the gametophyte-sporophyte transition by keeping egg cells quiescent in the absence of fertilization. *Curr. Biol.* 26(13):1782–89
120. **Russo MT, Vitale L, Entrambasaguas L, Anestis K, Fattorini N, et al. 2018. *MRP3* is a sex determining gene in the diatom *Pseudo-nitzschia multistriata*. *Nat. Commun.* 9(1):5050**
121. Schmid MW, Schmidt A, Grossniklaus U. 2015. The female gametophyte: an emerging model for cell type-specific systems biology in plant development. *Front. Plant. Sci.* 6:907
122. Searles RB. 1980. The strategy of the red algal life history. *Am. Nat.* 115(1):113–20
123. Sekimoto H. 2017. Sexual reproduction and sex determination in green algae. *J. Plant Res.* 52:1676–79
124. Sekimoto H, Abe J, Tsuchikane Y. 2012. New insights into the regulation of sexual reproduction in *Closterium*. *Int. Rev. Cell Mol. Biol.* 297:309–38
125. Sekimoto H, Fukumoto R, Dohmae N, Takio K, Fujii T, Kamiya Y. 1998. Molecular cloning of a novel sex pheromone responsible for the release of a different sex pheromone in *Closterium peracerosum-strigosum-littorale* complex. *Plant Cell Physiol.* 39(11):1169
126. Sekimoto H, Sone Y, Fujii T. 1994. Regulation of expression of the genes for a sex pheromone by an inducer of the sex pheromone in the *Closterium peracerosum-strigosum-littorale* complex. *Planta* 193(1):137–44
127. Silberfeld T, Leigh JW, Verbruggen H, Cruaud C, de Reviers B, Rousseau F. 2010. A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): investigating the evolutionary nature of the “brown algal crown radiation.” *Mol. Phylogenet. Evol.* 56(2):659–74
128. Speijer D, Lukeš J, Elias M. 2015. Sex is a ubiquitous, ancient, and inherent attribute of eukaryotic life. *PNAS* 112(29):8827–34
129. Starr RC. 1970. Control of differentiation in *Volvox*. *Symp. Soc. Dev. Biol.* 29:59–100
130. Stosch HA, Drebes G. 1964. Entwicklungsgeschichtliche Untersuchungen an zentrischen Diatomeen IV. *Helgolander Wiss. Meeresunters* 11(3–4):209–57
131. Stratmann J, Paputsoglu G, Oertel W. 1996. Differentiation of *Ulva mutabilis* (Chlorophyta) gametangia and gamete release are controlled by extracellular inhibitors. *J. Phycol.* 32(6):1009–21
132. Suda S, Watanabe MM, Inouye I. 1989. Evidence for sexual reproduction in the primitive green alga *Nephroselmis olivacea* (Prasinophyceae). *J. Phycol.* 25(3):596–600
133. Tedeschi F, Rizzo P, Rutten T, Altschmied L, Bäumlein H. 2017. RWP-RK domain-containing transcription factors control cell differentiation during female gametophyte development in *Arabidopsis*. *New Phytol.* 213(4):1909–24
134. Togashi T, Bartelt JL. 2011. Evolution of anisogamy and related phenomena in marine green algae. In *The Evolution of Anisogamy: A Fundamental Phenomenon Underlying Sexual Selection*, ed. T Togashi, PA Cox, pp. 194–242. Cambridge, UK: Cambridge Univ. Press
135. Togashi T, Bartelt JL, Yoshimura J, Tainaka K-I, Cox PA. 2012. Evolutionary trajectories explain the diversified evolution of isogamy and anisogamy in marine green algae. *PNAS* 109(34):13692–97
136. Tschochner H, Lottspeich F, Sumper M. 1987. The sexual inducer of *Volvox carteri*: purification, chemical characterization and identification of its gene. *EMBO J.* 6(8):2203–7
137. Tsuchikane Y, Kokubun Y, Sekimoto H. 2010. Characterization and molecular cloning of conjugation-regulating sex pheromones in homothallic *Closterium*. *Plant Cell Physiol.* 51(9):1515–23
138. Tsuchikane Y, Sato M, Ootaki T, Kokubun Y, Nozaki H, et al. 2010. Sexual processes and phylogenetic relationships of a homothallic strain in the *Closterium peracerosum-strigosum-littorale* complex (Zygnematales, Charophyceae). *J. Phycol.* 46(2):278–84
139. **Tsuchikane Y, Sekimoto H. 2019. The genus *Closterium*, a new model organism to study sexual reproduction in streptophytes. *New Phytol.* 221:99–104**
140. Umen JG. 2011. Evolution of sex and mating loci: an expanded view from volvocine algae. *Curr. Opin. Microbiol.* 14(6):634–41
141. Umen JG. 2014. Green algae and the origins of multicellularity in the plant kingdom. *Cold Spring Harb. Perspect. Biol.* 6(11):a016170
142. Umen JG, Olson BJSC. 2012. Genomics of volvocine algae. *Adv. Bot. Res.* 64:185–243

143. van Ooijen G, Knox K, Kis K, Bouget F-Y, Millar AJ. 2012. Genomic transformation of the picoeukaryote *Ostreococcus tauri*. *J. Vis. Exp.* 2012:e4074
144. Vanstechelman I, Sabbe K, Vyverman W, Vanormelingen P, Vuylsteke M. 2013. Linkage mapping identifies the sex determining region as a single locus in the pennate diatom *Seminavis robusta*. *PLOS ONE* 8(3):e60132
145. Vesty EF, Kessler RW, Wichard T, Coates JC. 2015. Regulation of gametogenesis and zoosporogenesis in *Ulva linza* (Chlorophyta): comparison with *Ulva mutabilis* and potential for laboratory culture. *Front. Plant Sci.* 6:15
146. Wichard T, Charrier B, Mineur F, Bothwell JH, Clerck OD, Coates JC. 2015. The green seaweed *Ulva*: a model system to study morphogenesis. *Front. Plant Sci.* 6:72
147. Yamamoto K, Kawai-Toyooka H, Hamaji T, Tsuchikane Y, Mori T, et al. 2017. Molecular evolutionary analysis of a gender-limited *MID* ortholog from the homothallic species *Volvox africanus* with male and monoecious spheroids. *PLOS ONE* 12(6):e0180313
148. Yamazaki T, Ichihara K, Suzuki R, Oshima K, Miyamura S, et al. 2017. Genomic structure and evolution of the mating type locus in the green seaweed *Ulva partita*. *Sci. Rep.* 7(1):11679
149. Yan X-H, Fujita Y, Aruga Y. 2000. Induction and characterization of pigmentation mutants in *Porphyra yezoensis* (Bangiales, Rhodophyta). *J. Appl. Phycol.* 12(1):69–81
150. Yoon HS, Ciniglia C, Wu M, Comeron JM, Pinto G, et al. 2006. Establishment of endolithic populations of extremophilic Cyanidiales (Rhodophyta). *BMC Evol. Biol.* 6:78
151. Young JR, Geisen M, Probert I. 2005. A review of selected aspects of coccolithophore biology with implications for paleobiodiversity estimation. *Micropaleontology* 51(4):267–88

148. Identification and characterization of first mating-type locus from ulvophytes.



Contents

The Way It Was <i>M. J. Osborn</i>	1
Life Cycle of <i>Cryptococcus neoformans</i> <i>Youbao Zhao, Jianfeng Lin, Yumeng Fan, and Xiaorong Lin</i>	17
Posttranscription Initiation Control of Gene Expression Mediated by Bacterial RNA-Binding Proteins <i>Paul Babitzke, Ying-Jung Lai, Andrew J. Renda, and Tony Romeo</i>	43
Ecology and Evolution of Plant Microbiomes <i>Viviane Cordovez, Francisco Dini-Andreote, Víctor J. Carrión, and Jos M. Raaijmakers</i>	69
Cellular Functions and Mechanisms of Action of Small Heat Shock Proteins <i>Axel Mogk, Carmen Ruger-Herreros, and Bernd Bukau</i>	89
Protein Acetylation in Bacteria <i>Chelsey M. VanDrise and Jorge C. Escalante-Semerena</i>	111
Coordination of the Cell Cycle in Trypanosomes <i>Richard J. Wheeler, Keith Gull, and Jack D. Sunter</i>	133
Phage Therapy in the Twenty-First Century: Facing the Decline of the Antibiotic Era; Is It Finally Time for the Age of the Phage? <i>Shayla Hesse and Sankar Adhya</i>	155
Structural Basis of Response Regulator Function <i>Rong Gao, Sophie Bouillet, and Ann M. Stock</i>	175
Multikinase Networks: Two-Component Signaling Networks Integrating Multiple Stimuli <i>Vanessa I. Francis and Steven L. Porter</i>	199
Functional Regulators of Bacterial Flagella <i>Sundharraman Subramanian and Daniel B. Kearns</i>	225
Tc Toxin Complexes: Assembly, Membrane Permeation, and Protein Translocation <i>Daniel Roderer and Stefan Raunser</i>	247

Algal Sex Determination and the Evolution of Anisogamy <i>James Umen and Susana Coelho</i>	267
The Ultimate Guide to Bacterial Swarming: An Experimental Model to Study the Evolution of Cooperative Behavior <i>Jinyuan Yan, Hilary Monaco, and Joao B. Xavier</i>	293
Responses of Microorganisms to Osmotic Stress <i>Erbard Bremer and Reinhard Krämer</i>	313
Biogeography of the Oral Microbiome: The Site-Specialist Hypothesis <i>Jessica L. Mark Welch, Floyd E. Dewhirst, and Gary G. Borisy</i>	335
Bacterial Persisters and Infection: Past, Present, and Progressing <i>Bridget Gollan, Grzegorz Grabe, Charlotte Michaux, and Sophie Helaine</i>	359
Multiple Roles of c-di-GMP Signaling in Bacterial Pathogenesis <i>Martina Valentini and Alain Filloux</i>	387
Chromatic Acclimation in Cyanobacteria: A Diverse and Widespread Process for Optimizing Photosynthesis <i>Joseph E. Sanfilippo, Laurence Garczarek, Frédéric Partensky, and David M. Keboe</i>	407
Diversity, Genomics, and Distribution of Phytoplankton-Cyanobacterium Single-Cell Symbiotic Associations <i>Rachel A. Foster and Jonathan P. Zebr</i>	435
Bent Bacteria: A Comparison of Cell Shape Mechanisms in <i>Proteobacteria</i> <i>Jennifer A. Taylor, Sophie R. Sichel, and Nina R. Salama</i>	457
<i>Acinetobacter baumannii</i> : Envelope Determinants That Control Drug Resistance, Virulence, and Surface Variability <i>Edward Geisinger, Wenwen Huo, Juan Hernandez-Bird, and Ralph R. Isberg</i>	481
Two-Component Sensing and Regulation: How Do Histidine Kinases Talk with Response Regulators at the Molecular Level? <i>Alejandro Buschiazso and Felipe Trajtenberg</i>	507
Human Coronavirus: Host-Pathogen Interaction <i>To Sing Fung and Ding Xiang Liu</i>	529
Small Is Mighty—Chemical Communication Systems in <i>Pseudomonas aeruginosa</i> <i>Stephen Dela Abator and LianHui Zhang</i>	559
Signaling Cascades Governing Entry into and Exit from Host Cells by <i>Toxoplasma gondii</i> <i>Hugo Bisio and Dominique Soldati-Favre</i>	579

Metabolic Basis of Pathogenesis and Host Adaptation in Rice Blast
Yi Zhen Deng and Naweed I. Naqvi 601

Assembly and Subcellular Localization of Bacterial Type VI Secretion
Systems
Jing Wang, Maj Brodmann, and Marek Basler 621

Paleomicrobiology: Diagnosis and Evolution of Ancient Pathogens
*Kirsten I. Bos, Denise Kühnert, Alexander Herbig, Luis Roger Esquivel-Gomez,
Aida Andrades Valtueña, Rodrigo Barquera, Karen Giffin,
Aditya Kumar Lankapalli, Elizabeth A. Nelson, Susanna Sabin,
Maria A. Spyrou, and Johannes Krause* 639

Defense and Counterdefense During Plant-Pathogenic Oomycete
Infection
Yan Wang, Brett M. Tyler, and Yuanchao Wang 667

Indexes

Cumulative Index of Contributing Authors, Volumes 69–73 697

Errata

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<http://www.annualreviews.org/errata/micro>