æ

# 10. THE CELL LIFE CYCLE

20 February 2022

Having covered the general features of cell growth, we now focus on a number of key mechanistic and temporal aspects of cell life histories. Just as the soma of multicellular organisms undergo developmental changes, single cells progress through several stages from birth to division, some of which are very tightly defined and regulated. It is, for example, vitally important for cells to have properly duplicated their genomes and oriented each complement to their appropriate destinations at the time of division. This is a particular challenge for eukaryotic cells with multiple chromosomes. During mitosis, each chromosome must be replicated once, and only once, and parallel sets of chromosomes must be transmitted to each daughter cell.

Although most unicellular eukaryotes and all prokaryotic species reproduce in an effectively clonal manner, indefinite rounds of such propagation in the former are often punctuated by phases of sexual reproduction during which pairs of individuals exchange chromosomal segments by recombination. During such sexual phases, eukaryotic cells switch from mitotic to meiotic genome division, wherein a diploid phase is reduced to the haploid life-cycle stage. To return to the diploid state, haploid individuals must locate partners of the appropriate mating type and then undergo fusion with them. Whereas for most multicellular species, the predominant growth stage is diploid, for a wide range of unicellular species, the primary (vegetative) phase is haploid, while the diploid stage is simply a transient moment between the initiation of cell fusion and meiosis.

Sexual reproduction raises a number of functional and evolutionary issues, not all of which are fully understood. How and why did the complex process of meiosis, which includes organized modes of chromosomal segregation and recombination, evolve out of the already detailed orchestrations of mitosis? How do cells make "decisions" to fuse only with appropriate partners? How are mating types determined, and why is the typical number of mating types within a species just two? Many of the proteins involved in various stages of sexual reproduction appear to diverge at unusually high rates, begging the question as to whether such evolution is the product of drive-like processes associated with the relentless operation of selection for successful gene transmission.

A secondary goal here is to introduce a breadth of comparative observations on the molecular basis of cellular features at a deeper level than in previous chapters. Although this initial exploration is restricted to the diversification of life-history mechanisms, many of the underlying themes will reappear in subsequent chapters on other cellular features. Two key issues concern the evolution of complexity at the molecular and network levels. Proteins often consist of organized multimeric

aggregations of subunits. Sometimes the subunits are all encoded by the same locus (homomers), and other times they derive from different genetic loci (heteromers, often derived by gene duplication). Commonly, but not always, eukaryotic proteins take the second route, although there is little evidence (if any) that this increase in molecular complexity is driven by adaptive processes, a point already raised on prior pages (e.g., ATP synthase and ribosomes). However, as such transitions elicit sustained, coordinated molecular coevolution at the binding interfaces of interacting partners, in the long-run such molecular remodeling can passively lead to species reproductive-isolating barriers, as incompatibilities arise between the component parts residing in different lineages.

The regulatory networks of the cell cycle and the stages of mitosis and meiosis amount to communication pathways involving gene-product interactions. Such systems are often endowed with seemingly excessive and arcane structures, the origins of which raise central evolutionary questions in themselves. Equally significant, however, is the repeated observation that even when a network structure remains constant, changes can occur in the underlying participating proteins. Recall that other types of cellular systems with highly conserved functions, e.g., ribosome biogenesis and division-time determination, encountered in the previous chapter exhibit high levels of divergence of underlying control mechanisms. An analogy is the legendary Ship of Theseus, whereby over time the Athenians gradually replaced every wooden plank, until none of the original components remained, raising the question as to whether the new construction is still equivalent to the original ship. In cell biology, the replacement planks are sometimes not even made from the original materials.

## The Eukaryotic Cell Cycle

Broadly speaking, the life cycles of eukaryotic cells can be subdivided into three phases, defined with respect to the genomic state: 1) a growth phase in which all cell contents other than the genome expand in number; 2) a period of genome replication in preparation for division (during which growth might continue); and 3) cell fission (cytokinesis) accompanied by transmission of separate genomes to each daughter cell. In practice, however, most cell biologists partition the eukaryotic cell cycle more finely into four or five genome-focused phases (Figure 10.1), the textbook model being: a brief (and sometimes undetectable) $G_0$ resting phase immediately following division; followed by a prolonged interphase, which is further divided into three phases – the $G_1$ (gap 1) phase during which cell size increases, the S (synthesis) phase during which the genome is replicated, and the $G_2$ (gap 2) phase during which the cell continues to grow while containing a duplicated genome; and finally culminating in the M (mitotic) phase during which chromosomes are separated and cell division proceeds. As discussed below, the M phase is traditionally further subdivided into four or five subsections defined by chromosomal states.

Regulated checkpoints ensure that cells do not progress from one stage to the next unless all is in order. For example, a $G_1$/S checkpoint ensures that DNA synthesis does not initiate in the absence of sufficient cellular resources; a $G_2$/M checkpoint ensures that mitosis does not proceed until all chromosomes have been

fully replicated; and an additional checkpoint within the M phase ensures that any problems in chromosome replication have been removed prior to segregation.

This traditional scheme for classifying cell-cycle steps can be confusing, as the absolute and relative lengths of the cell-cycle phases vary greatly among organisms and cell types. The $G_0$ phase is often negligible in unicellular organisms (and consequently ignored in the overall scheme), but can be effectively indefinite in terminally differentiated cells of multicellular species. The $G_1$ and $G_2$ phases can be essentially absent in rapidly dividing cells in early metazoan development (as cells simply get progressively smaller); and this can also transiently occur in some unicellular species (such as the green alga *Chlamydomonas*) that undergo multiple rounds of S/M cycles, without growth, prior to release of multiple progeny (e.g., 4, 8, or 16, following 2, 3, or 4 internal divisions).

Many overviews have been written on the complex web of interactions that constitute the eukaryotic cell cycle (e.g., Morgan 2007), but the notation for the large number of participating proteins is often opaque and inconsistent across lineages, and this is not the place to recite the details. Suffice it to say that advancement through cell-cycle stages is generally governed by a multiplicity of cyclin proteins and their cyclin-dependent kinase (CDK) partners. Cyclins vary in concentration throughout the cell cycle, as they are actively degraded once deployed and then progressively resynthesized in the next cycle. Their functional role is to activate specific client CDKs, which then phosphorylate downstream target proteins to direct entry into the next stage of cellular development. The following two sections highlight some surprising features of the underlying mechanisms, most notably that at the molecular level there is no standard eukaryotic cell-cycle machinery.

**Phylogenetic diversity.** Despite the centrality of the cell cycle to all eukaryotes, the evolved diversity in the underlying regulatory machinery is striking. Among phylogenetic lineages, unrelated genes may carry out the same functions, and network topologies can change.

First, nonorthologous gene replacements of the proteins participating in the cell cycle are common (Jensen et al. 2006). For example, whereas most (but not all) cell-cycle proteins in land plants and animals appear to be of common descent (Doonan and Kitsios 2009; Harashima et al. 2013), numerous fungi harbor key cell-cycle genes with no obvious relationship to those in the same network positions in plants and animals (Rhind and Russell 2000; Cross et al. 2011; Medina et al. 2016).

As one example, a fungal protein called E2F, which operates in the same position as SBF in plants and animals (Figure 10.2), appears to be related to a viral protein acquired by horizontal transfer. The proteins with which E2F and SBF immediately interact are unrelated as well. The fact that the genomes of some basal fungal lineages harbor both E2F and SBF suggests the presence of a redundant regulatory system in basal fungi, with certain sublineages having lost SBF subsequently. Among the well-studied yeasts *Saccharomyces, Schizosaccharomyces,* and *Candida,* there are substantial nonorthologies in additional players in the cell cycle and their downstream regulated genes (Côte et al. 2009). Even among species within the genus *Saccharomyces,* differences exist in the interacting proteins at the $G_1$/S checkpoint (Drury and Diffley 2009).

Second, there are numerous examples in which the cell-cycle network topology

itself has changed. For example, *Saccharomyces* deploys just a single CDK, whereas metazoan cells deploy at least four, while those of plants use two (Criqui and Genschik 2002). Substantial differences in the numbers of cyclins deployed in the cell cycle also exist among phylogenetic groups, e.g., three to four in yeasts, up to ten in plants and metazoans (Criqui and Genschik 2002; Cross and Umen 2015), and as many as two dozen in diatoms (Huysman et al. 2010) and ciliates (Stover and Rice 2011). In many cases, the additional genes have originated via duplication. On the other hand, a broad phylogenetic survey suggests that many of the cell-cycle proteins observed across the eukaryotic phylogeny (and hence by extrapolation were present in LECA) exhibit lineage-specific losses (Medina et al. 2016). For example, *Giardia intestinalis,* a single-celled parasite with two nuclei, has no anaphase-promoting complex (often used in targeting cyclins for degradation) and no checkpoint mechanism for mitotic entry (Gourguechon et al. 2013; Markova et al. 2016). Given that the cell-cycle network has been ascertained in only a few model organisms, and even then generally just partially so, many more variants are likely to be found.

A third key observation about the molecular basis of the cell cycle concerns the frequent redundancy in the underlying mechanisms. For example, during the DNA synthesis phase in yeast, at least three simultaneously acting mechanisms prevent secondary replication events (which would lead to chromosomal imbalance in progeny cells). The first of these involves proteolysis of the replication-initiation proteins; the second involves nuclear exclusion of key proteins; and the third involves direct binding at origins of replication. If deleted singly, none of these lead to inviability, implying that the three systems effectively back each other up (Drury and Diffley 2009).

A potential connection between such redundancy and the phylogenetic turnover of cell-cycle participants noted above can be seen as follows (with a more formal presentation appearing in Chapter 20). Imagine three layers of surveillance with error rates $e_1$, $e_2$, and $e_3$, operating in parallel so that the system fails only if all three layers fail to error-correct. The overall failure rate associated with the first mechanism alone is $e_1$, with the first and second is $e_1e_2$, and for all three is $e_1e_2e_3$. Because $e_1$, $e_2$, and $e_3$ are all $< 1$, this shows how multiple surveillance layers can greatly reduce the overall error rate. However, because natural selection operates on the cumulative error rate, $E = e_1e_2e_3$, and likely can only reduce it to some level defined by the power of random genetic drift (Chapter 8), there will typically be multiple degrees of freedom by which the overall minimum error rate can be achieved (Lynch 2012), i.e., a low value of $e_1$ can compensate for a high value of $e_2$ or $e_3$. This further implies that provided one or two components can together accomplish the target sum $E$, one (or even two) components are potentially free to be lost in individual lineages. In the long run, this may lead to a phylogenetic repatterning of the molecular mechanisms underlying a pathway through evolutionary cycles of emergence of redundancy followed by random loss of individual components (Figure 10.3).

Of further relevance to the repatterning of the underlying participants in the cell cycle is the observation that many such proteins can have additional cellular functions, including roles in transcription regulation and development in multicellular species. Multifunctional genes may be difficult to completely nonfunctionalize over evolutionary time, while still being subject to loss of individual subfunctions

(such as participation in the cell cycle, when other redundant mechanisms remain) (Chapter 6). Under the latter scenario, loss of connectivity of a particular gene with the cell cycle in a phylogenetic lineage might be followed by a regain in connectivity at a later point in time.

The key point here is that the cell-cycle, one of the most central features of eukaryotic cells, provides a dramatic example of regulatory rewiring underlying a constant cellular attribute. Many more cases of this nature involving other aspects of cell biology will be encountered in subsequent chapters. Although horizontal transfer (as implicated in yeast) can play a role in such evolutionary repatterning, the combination of gene duplication, transient redundancy, and multifunctionality of underlying participants further facilitates the opportunities for rewiring in an effectively neutral manner. Such neutral evolution apparently extends to key amino-acid residues in the final clients of the CDKs themselves, as even the phosphorylation sites appear to change locations among closely related taxa, while the regional clustering of sites within proteins is generally preserved (Moses et al. 2007; Holt et al. 2009).

**Network complexity.** It remains unclear why the cell cycle of most eukaryotes engages such a large number of proteins with various promoter and/or inhibitor activities (often on the order of 20 or more). There is no evidence that such massive genomic investment is essential to an ordered cell-cycle progression, and all other things being equal, larger networks of proteins potentially impose a greater energetic burden on the cell, while also providing a larger target for mutational disruption.

Bacteria have loosely defined cell cycles governed by simple kinase-receptor systems that enable a central response protein to cyclically dictate progression through growth, replication, and division stages (Biondi et al. 2006; Garcia-Garcia et al. 2016; Osella et al. 2017; Mann and Shapiro 2018). However, prokaryotes do not have the elaborate mitotic cycles (below) that are the hallmarks of eukaryotic genome replication, nor do they harbor any obvious orthologs of cyclins and CDKs. Notably, the cell cycle of fission yeast (*S. pombe*) can be engineered to run with an extremely simplified control mechanism relying on just one CDK fused to a single cyclin (Coudreuse and Nurse 2010). If nothing else, this demonstrates the feasibility of an ancestral cell cycle driven by something as simple as a single self-oscillating module, and requiring no differential expression, interaction, and degradation of multiple participants.

The evolutionary mechanisms leading to the growth of network complexity, and how this can emerge by effectively neutral processes, have been touched upon in Chapter 6. For now, we simply consider an observation from *S. cerevisiae,* a member of a yeast lineage that experienced an ancestral genome duplication event, possibly resulting from interspecific hybridization (Wolfe et al. 1997; Marcet-Houben and Gabaldón 2015). Although only a small fraction of duplicated gene pairs still survive in this species, a specific pair of genes involved in the mitotic cell cycle is informative. In a number of eukaryotes (including some other yeasts and metazoans), a single gene encodes for a key cell-cycle protein having two substantially different functions: binding to the kinetochore to ensure the proper segregation of sister chromosomes during mitosis, and regulating the spindle checkpoint. However, in *S. cerevisiae,* the duplicate versions of this gene partition up these tasks. Without a comparative

perspective, one might conclude that one of these two duplicates evolved a new function, but instead this represents a clear example of subfunctionalization of the joint properties of an ancestral gene.

Remarkably, very similar patterns of subfunction partitioning have occurred in parallel in multiple lineages following independent duplications of the same ancestral gene (Murray 2012; Suijkerbuijk et al. 2012) (Figure 10.4). In each of these cases of convergent evolution, complementary degenerative mutations permanently preserve the sister copies. Support for the idea that this increase in complexity of the underlying mitotic machinery does not involve the establishment of novel and/or beneficial functions is provided by an experimental replacement of the two *S. cerevisiae* genes by the single copy from a distantly related yeast species *Lachancea kluyveri,* which yielded negligible fitness consequences (Nguyen Ba et al. 2017). Taken together, these observations constitute a clear example of how the growth of network complexity can occur in the complete absence of any intrinsic selective advantages.

## Mitosis

Critical to the success of any cell lineage is the reliable production of progeny containing a full complement of the parental genome. We focus first on issues related to chromosomal transmission under mitosis, the process by which parental chromosomes are replicated and evenly transmitted to asexually produced daughter cells. In most bacterial species, mitosis is stereotypical – duplication of the genome (generally a single circular chromosome) starts from a single origin of replication, with DNA polymerases proceeding simultaneously down both sides of the circle until meeting at the single terminus. During this process, the newly emerging chromosomes begin to move towards opposite ends of the cell, and cell division is completed as a constricting furrow pinches off the two daughter cells near the parental midpoint.

Eukaryotic mitosis is much more complicated. It always involves linear chromosomes, up to several dozens in number, each often longer than entire bacterial genomes (Lynch 2007). The choreographed process is viewed as proceeding through five temporal stages familiar to all biology students (Figures 10.1 and 10.5): 1) interphase, wherein chromosomes duplicate into sisters; 2) prophase, wherein chromosomes condense and microtubule arrays begin to assemble; 3) metaphase, wherein the sister chromosomes, connected at centromeres and attached to kinetochore microtubules, line up in the middle of the spindle; 4) anaphase, wherein sister chromosomes detach from each other and move to opposite poles; and 5) telophase, wherein chromosomes decondense into their new nuclear homes and the cell divides.

The evolutionary establishment of these sequential stages required numerous innovations not generally found in prokaryotes: 1) enclosure of the genome within a nuclear membrane perforated with nuclear-pore complexes to allow export of mRNA to the cytoplasm for translation and import of key proteins into the nuclear environment; 2) the expanded use of nucleosomes (octomers involving four unique histone proteins, in contrast to tetrameric homomers in archaea) for spooling DNA; 3) expanded numbers of origins of replication per chromosome and their parallel firing to reduce the time for chromosome duplication; 4) capping of linear chromosomes with repeat-based telomeres and devoting an enzyme (telomerase) to their maintenance

to prevent end loss; 5) deployment of molecules for sister-chromatid cohesion prior to anaphase; 6) a switch from a membrane-based to a microtubule-based mechanism for segregating sister chromosomes; 7) establishment of centromeres for spindle attachment; and 8) the insertion of mitotic-checkpoint mechanisms to ensure simultaneous and equitable migration of chromosomes to daughter cells.

Although these features are shared by all of today's eukaryotes, the evolutionary order in which they appeared remains unknown. Moreover, as in the case of cell-cycle regulation, the molecular and cellular details of many aspects of eukaryotic mitosis have diverged so much among phylogenetic lineages that it is difficult to even specify the ancestral state of the underlying machinery. Comparative phylogenetic analysis implies that at least 43 proteins involved in genome replication were present in LECA, only 23 of which, found in all modern lineages, may be indispensable (Aves et al. 2012). The following paragraphs attempt to highlight the diversity of mitotic mechanisms, albeit in a nontechnical manner.

A number of the proteins involved in eukaryotic mitosis have orthologs in archaea, with many of these experiencing duplication and functional divergence in eukaryotes (Aves et al. 2012; Lindås and Berlander 2013). To start the discussion, three key complexes involved in the initiation and progression of eukaryotic chromosome replication merit special attention (Figure 10.6): 1) PCNA (proliferating cell nuclear antigen), a trimeric ring that serves as a clamp to recruit DNA polymerase to single-stranded DNA; 2) RFC (replication factor, also known as the clamp loader), a pentamer consisting of a chain of four similar subunits anchored to a larger component, which together endow the DNA polymerase with processivity; and 3) MCM (minichromosome maintenance complex), a hexameric ring that unwinds DNA at the replication fork.

All three of these complexes exhibit substantial phylogenetic variation in terms of their underlying components (Chia et al. 2010). For example, in archaea, the trimeric PCNA can be a homomer (all three subunits encoded by the same locus) or a heteromer constructed from two or three distinct proteins, whereas it is homomeric in eukaryotes, with no evidence of functional superiority of one form over another (Fang et al. 2014). The RFC chain consists of one or two protein types in archaea, whereas each of the four subunits is encoded by a different gene in eukaryotes. The MCM has one to five subunit types in archaea, whereas all six subunits are encoded by different genes in eukaryotes. In all cases, the divergent eukaryotic components arose by gene duplication of ancestral components, independent from the duplication events in archaea, all occurring on the path from FECA to LECA (Liu et al. 2009). Finally, another complex (GINS) that interacts with the MCM at origins of replication is generally a homotetramer in archaea but a heterotetramer in eukaryotes (Onesti and MacNeill 2013).

This collection of observations provides a first illustration of what will become a recurrent theme of multimeric eukaryotic proteins often (but not always) being more complex (in terms of number of gene products involved) than orthologous prokaryotic complexes (Chapter 13). The stochastic coevolution of interface residues among the partners in heteromeric complexes can initiate and sharpen species boundaries, as the gene products from sister taxa diverge to the point of failing to interact (Zamir et al. 2012).

A second set of examples involving variation in the complexity of the components

of the mitotic machinery involves the SMC (structural maintenance of chromosome) proteins, which are ubiquitous across the Tree of Life. All SMC dimers of coiled-coil proteins, with one end of the two members joining to make a flexible hinge, and the other ends providing an opening that can be closed in certain contexts. In bacteria, the molecules are involved in chromosome maintenance and compaction and are homodimeric. In eukaryotes, the complexes are heterodimeric, and there are multiple copies with more diverse roles: SMC1/3 dimers form cohesins, which hold sister chromatids together during S phase; SMC2/4 dimers are part of the complex that condenses chromosomes to their metaphase state; and SMC5/6 dimers are recruited in some forms of DNA repair. Although five gene duplications account for the six SMC proteins in eukaryotes, the components of individual complexes are not consistently each others' closest relatives. For example, SMC1 and 4 are sister genes, as are SMC2 and 3 (Cobbe and Heck 2004). All three heterodimers were established prior to LECA, again reflecting the deep roots of the components of the eukaryotic mitotic machinery.

Finally, although most readers will be familiar with a basic textbook version of mitosis (as illustrated in Figure 10.5), as with the broader cell cycle, the mechanisms of eukaryotic chromosome segregation have diversified to an enormous extent from the standard model. For example, in most taxa, DNA-attachment factors (known as kinetochores) assemble onto the centromeres of sister chromosomes, connecting them to long polymeric proteins (the spindle microtubules) that guide chromosomes into daughter cells. Kinetochore complexes consist of $\sim 50$ different proteins, many of which appear to have arisen by duplication (Tromer et al. 2019), but although the structure is thought to be relatively conserved, there are significant differences in component compositions among mammals, insects, and yeasts (Drinnenberg et al. 2016). In the kinetoplastids (which include the parasitic trypanosomes), kinetochores are constructed out of 19 lineage-specific proteins (Akiyoshi and Gull 2014). Generally, the ends of spindle microtubules are anchored to cytoplasmic centrosomes during cell division, but the centrosome is absent in some groups such as planarians (Azimzadeh et al. 2012) and replaced by a nonhomologous spindle pole body in budding yeast (Winey and Bloom 2012).

The most remarkable and visually obvious forms of variation in mitosis involve the behavior of the nuclear envelope and the locations of the microtubule organizing centers from which the spindles emerge (Sazer et al. 2014). In some lineages, mitosis is open, with the nuclear envelope disappearing prior to metaphase, whereas in others the nuclear envelope remains intact throughout mitosis. Across the phylogeny of eukaryotes, however, is a complete continuum of intermediate forms of partially open mitosis. In addition, under closed mitosis, spindles can initiate inside or outside of the nuclear envelope (in the latter case penetrating the membrane). The numbers of microtubules per kinetochore vary among lineages, as do the ways in which these are bundled. Most species have point centromeres on each chromosome, but numerous cases exist in which chromosomes are holocentric (with microtubules attaching along their full lengths).

Summaries of these and numerous other mitotic features across a wide range of phylogenetic lineages are provided in Kubai (1975), Heath (1980), and Raikov (1982). In one of the few studies to ever analyze genetic variation in internal cellular features, Farhadifar et al. (2015, 2020) revealed substantial levels of within- and

between-species variation in spindle lengths, elongation rates, and centrosome sizes in *Caenorhabditis* nematodes, illustrating how mitosis-related traits can evolve both qualitatively and quantitatively.

In the Darwinian tradition, here we would be expected to speculate on, if not celebrate, the adaptive basis of the substantial lineage-specific diversification of mitosis across eukaryotes. However, there is no evidence that the emergence of mitosis or the downstream divergence of chromosome assortment mechanisms endowed their bearers with adaptive superiority. Moreover, the widespread existence (and success) of prokaryotes with consistently simple means of chromosome segregation, despite having had billions of years and thousands of lineages to have evolved alternative procedures further challenges the view of eukaryotic cell-division mechanisms are intrinsically advantageous. This being said, however, once mitosis had become established in LECA, it opened up novel pathways for further diversification by descent with modification, the primary innovation being the subject of the following section.

### Meiosis

The most unique aspect of eukaryotic genome inheritance is meiotic cell division. Combined with the use of separate sexes, meiosis endows diploid eukaryotic cells with an organized mode of sexual reproduction and a capacity for generating genetic variation among progeny. Via two nuclear divisions, only the first of which involves replication, meiosis reduces each pair of chromosomes in a diploid cell to single chromosomes in each of four haploid progeny cells (or gametes) (Figure 10.5). Diploidy is subsequently restored by gamete fusion.

Although meiosis shares some physical processes with mitosis, two differences have major genetic consequences: 1) copies of homologous chromosomes in the diploid parent cell segregate independently into haploid progeny; and 2) prior to doing so, most chromosomes experience at least one crossover (with homologous parental chromosomes swapping segments by recombination). Thus, unlike the situation in mitotic cell division, the products of meiosis are almost never identical to each other. The subsequent union of haploid gametes (generally from different parents) into diploids creates still more genetic diversity.

Almost certainly an evolutionary derivative of mitosis, the establishment of meiosis required four innovations (Figure 10.5): 1) physical pairing of homologous chromosomes during first-division prophase; 2) recombination between homologs (nonsisters) initiated by enzymatically induced double-strand breaks; 3) suppression of sister-chromatid separation (connected by centromeres) in the first division; and 4) the absence of chromosome replication during the second division. In effect, these modifications convert one-step mitosis into a two-step process by inserting the first meiotic division (and its associated peculiarities) into the mitotic cycle (Gerton and Hawley 2005; Wilkins and Holliday 2009). During the first division, the genome is duplicated and rearranged, with the first two daughter cells being effectively genetically haploid (homozygous) for all DNA residing between the centromere and the proximal crossover, but potentially heterozygous for sites distal to the last crossover. For this reason, the first division is referred to as reductional. Cells enter the second meiotic division in the same way as in mitosis, with replicated

chromosomes, which leads to complete haploidy for all chromosomal regions and is referred to as the equational division.

**Origin and evolutionary modifications of meiosis.** There is no shortage of speculation on the order of events leading from mitosis to the more complex meiotic program (e.g., Maguire 1992; Kleckner 1996; Solari 2002; Egel and Penny 2007; Niklas et al. 2014). As with mitosis, many of the molecular components of the meiotic machinery appear to have arisen by gene duplication on the phylogenetic path from FECA to LECA. One such pair, Rad51/Dmc1, with the respective copies being used in mitotically and meiotically dividing cells, will be further discussed below. Two pairs of proteins involved in mismatch repair and the processing of recombinant molecules resulting from single-strand invasion (Pms1 and Mlh2, and Mlh1 and Mlh3) also arose by duplication (Ramesh et al. 2005). In addition, Spo11 (which, as described below, is involved in creating double-strand breaks during meiosis) gave rise to two new genes by duplications prior to LECA (Malik et al. 2007).

Although meiosis is consistently associated with the production of variation in today's eukaryotes, it need not follow that the earliest evolutionary steps towards meiosis had anything to do with generating variation. Assuming a haploid ancestral state, as in prokaryotes, the diploid phase may have started as a simple form of endoreplication without cell division. If this were the case, the subsequent addition of meiotic mechanisms for restoring haploidy (and producing four progeny) would have evolved prior to sexual reproduction and simply involved closed diploid homozygous lineages (Cleveland 1947).

An alternative starting point for diploidy would be the fusion of two compatible haploid cells, a necessary condition for sexual reproduction. Without recombination between homologs, restoration of haploidy would only generate variation by independent segregation of chromosomes (in which case, there would be no effect if the ancestral species had a single chromosome). However, FECA likely had a capacity for recombination, as most prokaryotes harbor systems for repairing broken chromosomes off of homologous sequence in another chromosomal copy or segment (Haldenby et al. 2009). In such processes, bacterial RecA protein forms a helical filament that coats single-stranded DNA and plays a central role in searching and transferring the strand to homologous double-stranded sequence. Eukaryotic Rad51 is related to bacterial RecA, and like the latter, coats single-stranded DNA and guides the initial stages of repair by homology search in mitotically dividing cells. Moreover, the duplicate version of Rad51, called Dmc1, is specifically involved in inter-homolog pairing during meiosis (Ramesh et al. 2005). Thus, the physical mechanism of recombination is highly conserved, and this further implies that meiosis has never been a requirement for recombination.

Although it has been argued that meiotic recombination provides an organized mechanism for the repair of double-strand breaks (Bengtsson 1985; Bernstein et al. 1988; Hurst and Nurse 1991), the selective processes driving such selection may have extended beyond physical / chemical processes of DNA damage. For example, Hickey and Rose (1988) suggested a scenario in which cell fusion might have been forced upon an ancestral eukaryote by a selfish DNA element as a means for the latter's transmission among host cells. Without such transmission, a mobile element is essentially confined to a single host-cell lineage, possibly driving the host to ex-

tinction by generating deleterious insertions if overly aggressive or itself succumbing to mutation load. This general idea is made plausible by the fact that some bacteria engage in a sort of sexual reproduction guided by the activities of plasmids. In *Enterococcus faecalis,* for example, strains not containing a particular plasmid produce sex pheromones that attract plasmid-carrying strains, resulting in conjugation and transfer of the plasmid to the naive strain (Wirth 1994).

Finally, despite the canonical view of meiosis outlined in Figure 10.5, as with mitosis, the process has diversified in numerous ways across the eukaryotic domain (Loidl 2016; Zickler and Kleckner 2016). Textbook descriptions of meiosis are virtually always based on mammalian cells and the budding yeast *S. cerevisiae,* but almost every aspect of meiosis common to these organisms has been found to vary among other phylogenetic lineages. For example, several species of *Candida* are lacking multiple genes thought to be essential to meiosis, and yet still engage in the process, complete with Spo11-dependent recombination (Butler et al. 2009; Reedy et al. 2009); the missing components include members of the synaptonemal complex (a chromosome-length assemblage of polymeric proteins used to bind homologs together during meiosis I), the crossover-resolution pathway, and Dmc1 (the meiosis-specific cohesion protein). In the ciliate *Tetrahymena pyriformis,* chromosomes pair within a tube-like nucleus nearly twice the length of the cell, with all of the telomeres grouped at one end and all of the centromeres at the other, again without a synaptonemal complex. Whereas most species deploy point centromeres, many cases exist in which the chromosomes are holocentric (tightly paired over the entire length, as in the nematode *C. elegans*). Cases also exist in which no recombination occurs during meiosis despite chromosome pairing, as in male *Drosophila.* In addition, certain proteins involved in determining the fates of meiotic double-strand breaks (crossovers vs. no crossovers, as described below) differ among phylogenetic groups (Zetka 2017). All of these observations raise obvious questions as to whether genomic scans for key meiosis genes can provide unambiguous evidence on the absence of meiotic potential in an individual lineage.

**Rapid evolution of meiosis-associated proteins.** Despite their conserved functions and structural features across eukaryotes, some meiosis-associated proteins have remarkably rapid rates of evolution at the protein-sequence level (Bogdanov et al. 2007; Bomblies et al. 2015; Bonner and Hawley 2019). Particularly notable is the synaptonemal complex (SC), which consists of lateral elements bound to each homolog, a central parallel element, and a series of transverse filaments connecting the lateral and central elements. The SC exhibits dramatically different protein sequences across metazoan lineages, to the point of there being questions as to homology (Fraune et al. 2012). Just within the genus *Drosophila,* the amino-acid sequences of several of the component proteins of the SC evolve at rates on the order of at least 40% that expected under neutrality (Anderson et al. 2009; Hemmer and Blumenstiel 2016), with a number of sites putatively being under positive selection for change. In the yeast *S. pombe,* the SC has been replaced by thread-like structures called linear elements, which are structurally different from conventional lateral elements and do not appear to engage with transverse filaments (Lorenz et al. 2004).

Although the iconic view of the SC invokes a rigid ladder-like structure, the

underlying elements appear to be movable, with the overall structure behaving as a liquid crystal (Rog et al. 2017). This is of interest because liquid crystals are known to be highly sensitive to temperature variation (and for this reason, are often used as temperature sensors in industrial applications). Meiotic processes tend to be highly sensitive to temperature, with the optimum temperature varying substantially among taxa, and the within-species temperature range for the faithful operation of meiosis often being only $\sim 5^{\circ}$C (Bomblies et al. 2015; Lloyd et al. 2018). However, temperature variation alone cannot fully explain the extreme situation with the SC, as temperature influences the entire proteome. It has been suggested that such rapid evolution is a consequence of a coevolutionary dance between interacting partners – with a slight modification of one member of the pair being met with a compensatory change in another (Bomblies et al. 2015), but as touched upon in Chapter 6, numerous factors determine whether coevolution between interacting molecules accelerate vs. decelerate rates of sequence evolution.

One potential reason for high rates of evolution of the meiotic machinery involves the relentless selection that must operate on parental chromosomes competing for successful transmission to gametes (Lindholm et al. 2016). Normally, one expects meiosis to give rise to four gametic products, all of which are free to contribute to the next generation. However, post-meiotic conflict or competition can lead to situations in which one allelic type exhibits superiority with respect to another. Pre-meiotic interactions can have similar consequences, e.g., when one parental haplotype somehow prevents the successful inheritance of another into gametes. Examples of such a meiotic-drive process are the spore-killer genes (often called sister killers) in a number of fungi, which increase their relative rates of transmission by killing haploid products that do not contain them (Turner and Perkins 1991; Vogan et al. 2019; López Hernández et al. 2021; Svedberg et al. 2021). Under such scenarios, parental cells will produce fewer than four haploid gametes, but the driving allele can still be brought to high frequency provided its success during meiosis exceeds the reduced production of successful progeny.

One cytological feature in particular naturally invites exploitation by driving chromosomes, while incurring no fertility costs. For reasons that remain unclear, numerous phylogenetic groups exhibit a form of meiosis in females in which only one of the four meiotic products matures to a successful gamete, the other three being discarded. This so-called female meiosis has apparently evolved independently multiple times, being present in metazoans, land plants, ciliates, and a number of diatoms (Chepurnov et al. 2004), and naturally sets up a situation in which the four meiotic products compete for transmission to the one successful haploid egg. A drive-like process might then arise if centromere variants differ in their ability to successfully navigate to a particular location in the final meiotic tetrad (Figure 10.7). For example, an expansion of centromeric repeats leads to larger centromeres, which in principle can attract more kinetochores and spindle microtubules, and centromere location can also have effects (Chmátal et al. 2014; Iwata-Otsubo et al. 2017; Bracewell et al. 2019). As discussed in Chapter 8, even a $10^{-5}$ or so fitness advantage (on a scale of 1.0) would be adequate to drive such a centromere to high frequency. It is this that motivates the centromere-drive hypothesis (Henikoff et al. 2001; Malik and Henikoff 2001).

Although there may be few side effects in female meiosis other than determining

which homolog is promoted to the single egg cell, collateral problems may ensue in male meiosis (or even in mitosis), where there is an expected balanced outcome of cell division. This might then impose counter-selection for modifier mutations in centromeric proteins that restore normal meiotic segregation, thereby driving the rapid evolution of other genes involved in meiosis (Figure 10.7). In principle, once a such a modifier is driven to fixation, a new opportunity might then arise for another driving chromosome of a different nature to emerge, encouraging still another establishment of a suppressor mutation.

Whereas the centromere-drive hypothesis provides a potentially simple explanation for the rapid evolution of the meiotic machinery, there are several reasons for caution in accepting the validity of the verbal model. First, the key requirement for a coevolutionary drive process is the maintenance of functionally significant polymorphisms in centromeric regions for a sufficiently long time to enable the centromeric proteins to respond by counter-selection. If a highly aggressive centromere rapidly goes to fixation, this will thwart the selective promotion of modifier mutations, as homozygotes for driving centromeres do not experience problems with meiotic imbalance. Likewise, if the deleterious effects of a driving centromere on male fitness sufficiently exceed the power of the drive process, the driving centromere will simply be eliminated from the population too rapidly for the arrival of modifier mutations. Small population size might facilitate stochastic increases in the frequencies of mildly deleterious centromeres, but this will also reduce the rate of mutational origin of modifiers and the ability of natural selection to promote them. Thus, there must be a narrow range of population-genetic parameters conducive to centromeric drive.

A second concern with the centromere-drive hypothesis is that centromeric proteins must recognize the full set of centromere sequences across all chromosomes – there are no known chromosome-specific centromeric proteins. This means that in order to be successful, any modifier that restores parity at the problematical chromosome would have to do so without generating new difficulties with nonhomologous chromosomes.

Finally, whereas the numerous examples noted above suggest a high rate of molecular evolution and turnover of components of the meiotic machinery, it is not entirely clear whether such rapid evolution is a general feature of biology, let alone a consequence of driving centromeres. Of particular interest is the centromeric variant of the histone H3 protein found in nucleosomes (CENP-A), which interacts with kinetochores and has been argued to evolve at an exceptionally high rate in *Drosophila* (Zwick et al. 1999; Malik and Henikoff 2001). In contrast, very distantly related plant species, which also have female meiosis, are able to accept transformations of CENP-A from each other, implying a relatively low level of functional divergence (Rosin and Mellone 2017). Moreover, the yeast *Saccharomyces,* which does not have female meiosis, nonetheless exhibits relatively high rates of centromere-sequence evolution, possibly as a simple consequence of a localized increase in the mutation rate (Bensasson et al. 2008; Bensasson 2011). These observations raise the additional caveat that the cellular environments in which centromere drive (or any other meiotic-drive like process) can lead to long-term acceleration in rates of evolution of the participating genes are also rather limited.

One observation of potential relevance has been made in the ciliate *Tetrahymena,* where male meiosis is entirely absent – both members of a conjugating pair

undergo female meiosis, duplicate their single remaining haploid nucleus, and then pass one copy on to the other member. If rapid evolution of centromeric proteins in species with female meiosis is normally driven by deleterious side effects on male meiosis, accelerated rates of evolution of modifiers are expected to be absent in species without male meiosis. The observation of relative evolutionary stability of CENP-A in *Tetrahymena* is consistent with this idea (Elde et al. 2011), although this still leaves unexplained the counterexamples in the prior paragraph.

**Recombination mechanisms.** From the standpoint of genetics, the key features of meiosis are the independent segregation of nonhomologous chromosomes and the production of chimeric daughter chromosomes by recombination between parental homologs. To avoid losses of chromosomal segments by deletion and/or duplication gains, recombination must be strictly confined to homologous chromosome regions, which requires proper chromosome alignment. In organisms that have been well characterized, the initial search space for homology during early meiosis I prophase is usually greatly reduced by the clustering of telomeres near the nuclear periphery into a chromosomal bouquet (Scherthan 2001). Once juxtaposed, homologs are generally then held together by the SC, in combination with physical intercalations of single strands of DNA from one chromosome into the homologous regions of another (known as chiasmata), and perhaps pairing of noncoding RNAs (Barzel and Kupiec 2008; Ding et al. 2012). However, some species use one mechanism to the exclusion of another, or use entirely different mechanisms for chromosome pairing (Gerton and Hawley 2005).

Meiotic recombination events are not simple consequences of accidental chromosome breakage. Rather, they are specifically induced by the creation of double-strand breaks by Spo11, an enzyme that appears to be related to a topoisomerase used in archaea to relieve supercoiling or untangling intertwined chromosomal regions (Robert et al. 2016; Vrielynck et al. 2016). Following a double-strand break, the ends of each fragment are partially digested, leaving single-stranded DNA overhangs, which then seek out, invade, and hybridize with the homologous region on the matching homolog. One or both members of the broken strand can invade the homolog, sometimes only transiently, and the ways in which the conjoined strands separate have consequences for the nature of the recombination event (Chapter 4). In some cases, a crossover occurs, yielding complete reciprocal exchange between homologs distal to the break (Figure 4.6).

Typically, no more than one double-strand break is resolved as a crossover per chromosome arm during an individual meiotic event (Chapter 4). However, the numbers of non-crossover events are tens to hundreds of times higher (De Muyt et al. 2009; de Massy 2013), and these transiently conjoined chromosomal regions keep the parental chromosomes in parallel during metaphase I. Meiosis-specific cohesins hold sister chromatids together, with removal distal to a crossover allowing homologs to separate at meiosis I, and maintenance of cohesion proximal to the centromere keeping sisters joined until meiosis II (Watanabe 2012). Notably, the ciliate *Tetrahymena,* appears to utilize the same hinge during mitosis and meiosis (Howard-Till et al. 2013), and other species may use an entirely unrelated protein (Watanabe 2005).

As discussed in the following section, the typical adaptive view of homolog

pairing is that such juxtaposition helps ensure a steady supply of recombinant chromosomes, providing useful variation upon which natural selection can act. A more structural view is that the primary role of homolog pairing is the inhibition of nonhomologous recombination, which would lead to deleterious ectopic insertions, deletions, and chromosomal rearrangements (Wilkins and Holliday 2009). This being said, however, meiosis is far from a perfect process. For example, in humans on the order of 25% of female meiotic products are aneuploid (Wang et al. 2017), and separation of sister rather than homologous chromosomes at meiosis I is not uncommon (Ottolini et al. 2015). With 23 chromosomes per human genome, this implies an error rate of $\sim 1\%$ per chromosome. The rate of nondisjunction for the X chromosome in *Drosophila* is estimated to be $\simeq 0.5\%$ (Zeng et al. 2010).

**The evolutionary consequences of sexual reproduction.** The myriad of novel cellular features in LECA opened up numerous avenues for further evolutionary elaborations by descent with modification. However, the onset of meiosis was unique in that it dictated a new mechanism for the inheritance of the genetic machinery itself, potentially defining new paths by which general evolutionary genetic processes could proceed. For example, meiosis combined with conjugation (syngamy) ensures that, except in the case of self-fertilization, sexually produced progeny genomes are mixtures from two individual parents. Although the generation of variation may be viewed as beneficial from the standpoint of natural selection, it also means that, once obtained, an optimal parental genotype will generally not be perfectly transmitted to offspring. Sexual reproduction is also costly in other ways: cells of different mating types must locate each other and then fuse; cell fusion provides a vehicle for pathogen transmission; and in species with separate sexes, females often contribute the bulk of the energetic investment in offspring, and the average number of progeny produced by individual is typically reduced by a factor of two. The fact that asexual organisms pay none of these costs inspires the search for explanations for the evolution of sexual reproduction.

Obligate asexuality does not appear to be difficult to evolve from sexual reproduction, especially in organisms with mixed life cycles where phases of clonal reproduction alternate with sexual episodes (the usual situation for unicellular eukaryotes). Nearly every major phylogenetic group of eukaryotes harbors at least one obligately asexual lineage (Bell 1982), although obligate asexuality is thought to be rare. However, the latter view is largely derived from observations of multicellular organisms, where the mating system is fixed and easily observed, and the situation might be quite different in microbes, where induction of the sexual phase is often nonobvious. Nonetheless, with a typical focus on multicellular species, most evolutionary biologists assume that the rarity of reversions to asexuality (called parthenogenesis in animals, and apomixis in land plants) implies that there must be an intrinsic advantage of sexual reproduction large enough to offset the significant disadvantages just noted, and the usual conclusion is that this must be associated with the production of genetically variable offspring.

There are numerous ways in which the production of genetic variation by meiotic segregation and recombination might be advantageous (Maynard Smith 1971; Williams 1975; Kondrashov 1993; Barton and Charlesworth 1998). For example, outcrossing provides a means for promoting beneficial combinations of alleles from

different genetic loci – instead of waiting for two complementary mutations to sequentially arise in a single asexual lineage, single mutations contained within two different lineages can be combined, potentially reducing the waiting time for the emergence of a complex adaptation (Chapter 6). In addition, sexual reproduction can facilitate the purging of deleterious alleles (which constitute the bulk of spontaneously arising mutations) – by expanding the range of variation in the numbers of deleterious mutations in offspring (some inheriting more and others less than the average parental number), sexual reproduction provides a more efficient route to reducing harmful mutation load by natural selection.

One concern with all of these arguments for the evolutionary maintenance of sex is their dependence on group-selection arguments – the inferred advantages are viewed through a long-term lens of the population. The fact that selection at the individual level is much more immediate raises the question as to why, once established, sexual reproduction is resistant to invasion and eventual displacement by derived asexuals. One potential mechanism is purely genetic. With no known exceptions, diploid asexual cells are still capable of mitotic recombination, and use this capacity to repair double-strand breaks off a homolog. However, because recombination generates local patches of homozygosity via gene conversion (Chapter 4), purely asexual lineages can be expected to experience progressive loss of heterozygosity, and hence a relentlessly increasing exposure of deleterious recessive alleles carried in the original founder of the asexual lineage (as well as those subsequently arising), eventually leading to extinction. Thus, the capacity for complementation after each round of outcrossing may be a primary factor favoring at least periodic sexual reproduction (Archetti 2004, 2005), although this argument seems of minor significance for species with predominantly haploid life stages.

Meiotically reproducing species necessarily go through both of these phases and have the option for either to be the state in the vegetative segment of the life cycle. In most multicellular species, diploidy is the rule, and the imagined genetic advantages include the masking of deleterious recessive alleles, the ability to exploit any instances of heterozygote advantage, and the provisioning of secondary templates for double-strand break repair in mitotically reproducing cells. However, many unicellular eukaryotes are predominantly haploid, which reduces the investment in DNA, while also enhancing the exposure of recessive alleles to natural selection. If meiosis arose in a predominantly haploid organism, there is less justification for invoking diploid-specific genetic arguments (e.g., heterozygote superiority, or deleterious-mutation masking) for the origin of sexual reproduction.

Finally, to put things in a broader context, it should be noted that ameiotically reproducing prokaryotes are not strictly asexual, owing to the availability of multiple forms of gene transmission (i.e., the incorporation of exogenous DNA by direct uptake, plasmid transformation, or viral transfection). The archaea, in particular, are capable of cell fusion and bidirectional exchange of genomic material (Naor and Gophna 2013; van Wolferen et al. 2016; Wagner et al. 2017). Thus, strictly speaking, sexual reproduction (and any evolutionary advantages that come with it) is not unique to eukaryotes, further raising doubts as to whether meiosis arose as a means for generating variation.

In summary, although an enormous amount of evolutionary theory has been devoted to trying to understand the adaptive significance of sexual reproduction,

the preceding observations raise questions about the traditional assumption that meiotic recombination originated and continues to be maintained by selection as a variance-generating mechanism. Rather, recombination in eukaryotes may be an inevitable structural consequence of the mechanisms of meiosis, with any variation generated being an indirect by-product, just as variance produced by mutation is a consequence of the inability of natural selection to reduce the replication-error rate to zero (Chapter 4). The near-constancy of one crossover per chromosome arm among all eukaryotes (regardless of chromosome size; Chapter 4) does not inspire confidence in the idea that natural selection favors crossing over. Rather, it raises the possibility that selection reduces the latter to a near absolute minimum.

### Mating Types

Aside from matters of genome processing, sexual reproduction introduces novel evolutionary challenges that are absent from asexual lineages. Most notably, the necessity of cell fusion raises the issue of how cells avoid nonproductive interactions with inappropriate mating types and/or foreign species, and equally importantly, how cells efficiently attract conspecifics. The origin of mating types themselves and the factors that govern the numbers of such types within populations are also of key interest here.

In unicellular species, most sexual communication systems rely on pheromones, with contact between appropriate mating types initiating a cascade of effects from cell fusion to downstream meiotic control. The genetic bases for such systems are generally quite simple, usually involving just one or two linkage groups of a small number of genes, each segregating effectively as a single supergene. The norm is just two mating types per species, although exceptions exist. Notably, despite their centrality to organismal fitness, the components of mating-type systems sometimes evolve quite rapidly. Indeed, the phylogenetic breadth of mate-recognition systems implies that existing mechanisms are frequently taken over by entirely new processes. As already noted for the cell cycle and meiosis, this again poses the question as to how such shifts occur without causing massive internal incompatibilities in the lineages involved.

**Mating-type determination.** The mere existence of mating types raises an evolutionary challenge, as each individual can only mate with a fraction of the members of the population. The situation is most extreme in the case of two mating types, where only half of the population is available to each individual (assuming a 1:1 sex ratio). Yet most sexual eukaryotic species have just two self-incompatible mating types.

Chemical recognition does not impose an absolute need for mating types, as all members of the population could in principle encode for the same signal and receptor proteins, a mutual recognition system defined as bipolar (Figure 10.8). However, an obvious limitation of such a system is the potential for an individual's receptors to be overwhelmed by its own pheromone molecules, removing the chemical gradients necessary to localize other members of the population. This might then endow a selective advantage to a genotype that loses the ability to either signal or receive.

In a sea of bipolar cells, a mutant cell defective for pheromone production might also gain a selective advantage owing to the absence of expenditure on biosynthesis of the attractant.

In this sense, a bipolar recognition system is expected to be vulnerable to the emergence of a unipolar system (two unique mating types) by subfunctionalization (Chapter 6) – with one cell lineage retaining the signal-producing gene but losing the receptor, and vice versa for the second cell lineage. Once established, such a system might then be further refined by secondary novel gene acquisitions such that both mating types produce unique pheromones and receptors (Figure 10.8). Maintenance of a unipolar mating system requires that the receptor and signal genes be tightly linked chromosomally, as recombination would assort inappropriate mixes into the same gamete, thereby leading to nonfunctional mating capacities (Nei 1969; Hoekstra 1980).

Unfortunately, biology's descriptive language for mating systems is nonstandardized, with different terms often used for functionally equivalent systems in fungi (and other unicellular species), land plants, and animals. Homothallism, equivalent to self-compatibility, refers to situations in which specific genotypes are capable of mating with other members of the same genotype. Heterothallism refers to self-incompatible systems requiring separate mating types (in land plants, systems with separate sexes are denoted as dioecious). These terms get blurred in organisms such as some yeast with internal mechanisms for switching mating types through genetic modifications; such species are homothallic, but could also be termed sequential hermaphrodites. In multicellular organisms, simultaneous hermaphroditism is possible, as in monoecious plants in which individuals produce male and female floral parts, but this is not known for unicellular species. Finally, the terms isogamous and anisogamous are used to refer to situations in which gamete types are morphologically indistinguishable vs. distinct (as in eggs and sperm in land plants and animals); even these terms can be a bit misleading, as isogamous species generally have different mating types and hence underlying molecular differences.

A broader array of mating systems has been described in the fungi than in any other major eukaryotic lineage, although this could be a simple consequence of the magnitude of research focused on this group. In *S. cerevisiae, S. pombe,* and several other yeasts, there are two distinct mating types, each with unique pheromones and receptors, but these are achieved by mating-type switching (Hanson and Wolfe 2017), whereby casettes of genes are swapped into a particular site by recombination. In this sense, mating-type determination involves a single tightly linked region (multigenic, but effectively segregating as a single locus) – individual genotypes are genetically hermaphrodites, but at the phenoytpic level, individual cells mate in a unipolar manner. Once two complementary types are attracted to each other, the production of mating-type specific agglutinins (coagulants) is induced, and heterodimeric transcription factors constructed from components from each pair member elicit downstream meiotic activities.

In the smuts, a group of plant pathogens within the mushroom family, the mating system sometimes involves two unlinked loci, although again each locus actually consists of linked blocks of genes (Bakkeren et al. 2008). In this case, one locus typically encodes for linked pheromones and receptors, while the second encodes for a transcription factor that governs downstream cellular events associated with

syngamy and meiosis. As in the yeasts, different mating types recognize different pheromones, but in smuts four possible outcomes are possible, from fully compatible to fully incompatible, depending upon the allelic status at the two loci.

To further emphasize the diversity of evolved systems, just a few other examples of unicellular mating systems will be noted here. Diatoms are known for their diversity of mating systems and sometimes rapid rates of evolution of underlying components (Armbrust and Galindo 2001; Chepurnov et al. 2004). Some diatoms have homothallic mating systems (capable of selfing), whereas others are heterothallic, and among these are cases of both isogamy and anisogamy. The diatom *Seminavis* has two mating types whose activities are coordinated by a two-step signaling system, the first involving a chemo-attractant that acts on a global basis, and the second operating only after the perception of a mating partner and stimulating entry into cell-cycle arrest and gametogenesis (Moeys et al. 2016).

Not all species use mate-attraction pheromones. Although some ciliates, such as *Euplotes* (below) do use pheromones, others such as *Paramecium* simply deploy mating-type specific agglutinins upon contact. In *Paramecium tetraurelia,* which has a transcriptionally silent germline nucleus (the micronucleus) and a "somatic" macronucleus (Figure 10.9), epigenetic events are involved in the maintenance of the two mating types (E and O, for even and odd), such that the latter are determined entirely by the maternal cytoplasm. In E-type cells, the mating-type gene (*mtA,* residing in the micronucleus) is passed on intact to the macronucleus, whereas in O-type cells, the promoter region is spliced out, rendering the macronuclear variant nonfunctional (Singh et al. 2014). In a related species, *P. septaurelia, mtA* is not differentially processed, but instead another gene (*mtB,* a transcription factor that regulates *mtA*) experiences a nonfunctionalizing deletion in the macronucleus of O-type cells. Thus, even members of the same genus can have substantially different mechanisms of mating-type determination.

In *Chlamydomonas,* two mating types (+ and −) produce unique agglutinins on their flagella, which cross-react as recognition and adhesion mechanisms, leading to a cascade of events, again including the formation of a heterodimeric transcription factor composed of subunits derived from each mating type (Goodenough et al. 2007). The mating-type locus consists of a moderate-sized ($\sim$ 300 kb) nonrecombining linkage region. The agglutinins are very large ($>$ 3300 amino acids in length), and the two types within a species are almost completely divergent in sequence, despite having very similar overall structures. The level of sequence divergence between *Chlamydomonas* species for orthologous agglutinins is high, $\sim 2\times$ that for proteins used in cell wall construction, which themselves are quite divergent (Lee et al. 2007).

**Mating-type number.** Given the relative simplicity of mating-type determination in most species, the establishment of more than two mating types is feasible, and as discussed below, rare mating types can sometimes have a strong selective advantage. However, unless a newly emergent mating type has a very high affinity towards the existing two, a two-type system can be very difficult to invade (Hadjivasiliou and Pomiankowski 2016), and indeed in multicellular species, there are virtually always just two distinct sexes.

Nonetheless, there are well-documented cases of unicellular species with more

than two mating types. As one extreme example, two Basidiomycetes, *Schizophyllum commune* and *Coprinus cinereus,* have thousands of mating types (Kothe 1999; Riquelme et al. 2005). Some members of the green-algal genus *Closterium* have up to 15 mating types (Sekimoto et al. 2012). Three mating types are known in the slime mold *Dictyostelium discoideum,* and the number can be higher in other congeners, although there are also cases of homothallism (Bloomfield 2011). Two of the mating types in *D. discoideum* are specified by completely unrelated genes (and unknown to be related to those in any other species).

Although *Paramecium* species typically have two mating types, a number of ciliates (including *Euplotes, Tetrahymena, Glaucoma,* and *Stylonychia*) have up to twelve types (Phadke and Zufall 2009). In *Tetrahymena thermophila,* seven mating-type genes are tandemly arrayed in the germline micronucleus, but stochastic deletion events result in the macronucleus of progeny cells having only one complete gene (Cervantes et al. 2013), as in yeast mating-type switching. The overall commonality of multiple mating-type systems is unclear, as there is a likely reporting bias for multiple mating types, and for most unicellular species there is no information at all.

**Cell fusion.** Once appropriate mating types have encountered each other, the final climax of the sexual life cycle requires gamete fusion, which necessitates merging of the lipid bilayers of two cells that otherwise would be repellent. For such purposes, a wide variety of unicellular eukaryotes utilize an integral membrane protein known as HAP2 (Wong and Johnson 2010; Speijer et al. 2015; Okamoto et al. 2016), although different, lineage-specific proteins appear to be involved in initial adhesion (Liu et al. 2015). Fungi and a number of animal species do not encode for HAP2 at all (although land plants do), and utilize alternative mechanisms for cell fusion.

Although quite divergent sequence-wise, HAP2 is highly similar in structure to the fusogen proteins used by lipid-bound viruses as an entry mechanism into host cells (Fédry et al. 2017; Pinello et al. 2017), implying either an extraordinary example of convergent evolution or an outcome of horizontal transfer. If eukaryotic HAP2 is derived from a virus (rather than the other way around), this would be compatible with sexual reproduction having arisen via the guidance of an ancient mobile element, as proposed by Hickey and Rose (1988).

HAP2 is generally expressed in both mating types of isogamous species such as the slime mold *Physarum* and the green algae *Chlamydomonas* and *Gonium* (reviewed in Cole et al. 2014), whereas in anisogamous species (e.g., animals and land plants), HAP2 is typically expressed by just one gamete type. In ciliates such as *Paramecium* and *Tetrahymena,* both members of a conjugating pair express HAP2, exchange meiotic products, and then disconnect (Cole et al. 2014; Orias 2014); this process is functionally equivalent to isogamy, but requires a mechanism for separation as well as merger.

**Coevolution of pheromones and their receptors.** It has been argued that below a critical cell size, active searching (swimming motility) for mates is energetically less costly than the recurrent production of released pheromones (Cox and Sethian 1985; Dusenbery and Snell 1995), but empirical work makes clear that chemical

pheromones are widely used throughout unicellular eukaryotes. Mating pheromones may be simply attached to the cell surface, serving as final checkpoints in the decision to mate, or they may be released to the environment, with the resultant plume increasing the effective target size of a cell. However, given the expense of biosynthesizing and exporting pheromones into the surrounding medium, and the potentially catastrophic outcome of mating with the wrong species, pheromone receptors can be expected to have a high specificity for their cognate pheromones.

The best understood mate-recognition systems in unicellular eukaryotes have been described in yeast species (Michaelis and Barrowman 2012; Hanson and Wolfe 2017). In *S. cerevisiae,* each of the two mating types, $\alpha$ and **a**, produces a unique pheromone that attracts and elicits a developmental cascade in the other. That is, **a**-type cells secrete **a** pheromone while expressing the $\alpha$-pheromone receptor, and vice versa. A similar system exists in *S. pombe.* Known pheromones in yeast and other fungi are small peptides, on the order of a dozen amino acids in length (Urban et al. 1996), although the precursor molecules from which they are proteolytically cleaved prior to excretion are substantially larger (typically 40 to 160 amino acids). There are on the order of 8,000 receptors on the surface of *S. cerevisiae* cells ($\sim 80$ per $\mu m^2$ of surface area), and these have a dissociation constant of $6 \times 10^{-9}$ M (Jenness et al. 1986). The latter quantity is approximately equal to the substrate concentration at which the receptor is operating at 50% of its maximum rate (Chapters 18 and 19). For intracellular enzymes, dissociation constants for substrates are typically in the neighborhood of $10^{-4}$ M, indicating the exceptionally strong affinity of pheromone receptors for their signal molecules.

In species of the ciliate *Euplotes,* the pheromone and receptor are both encoded by the same gene, and alternative splicing results in one variant (the pheromone) being excreted into the environment and the other variant being a trans-membrane receptor (Luporini et al. 1996, 2005). The initial (pre-cleavage) protein is 70 to 140 amino acids in length, with the pheromone itself being reduced to 40 to 90 residues and excreted at a level of $\sim 2$ to 20 pg per cell per day (equivalent to $\sim 10^8$ to $10^9$ proteins). There are 20 to 50 million receptors per cell surface, equivalent to $\sim 1700$ per $\mu m^2$ of surface area, and dissociation constants have been estimated to be in the range of 0.6 to $10^{-8}$ M.

As in yeasts and *Euplotes,* the green alga *Closterium* deploys small peptides as pheromones (in this case, glycoproteins with $\sim 150$ amino acids), and these exhibit mensurable activity down to $\sim 10^{-10}$ M (Sekimoto et al. 2012). The pheromone of *Volvox carteri,* also a glycoprotein, is one of the most potent effector molecules known, thought to operate with full effectiveness at $6 \times 10^{-17}$ M, a sensitivity that may be made possible by secondary amplification involving the extracellular matrix (Hallmann et al. 1998; Hallmann 2008).

Not all pheromones are proteins. Those in the diatom *Seminavis* are small metabolites (Moeys et al. 2016), and those in the brown alga *Ectocarpus* consist of a blend of simple organic compounds derived from fatty acids and often containing pentane or hexane rings, which can be highly effective down to concentrations of $5 \times 10^{-10}$ M (Boland 1995; Pohnert and Boland 2002).

These kinds of observations make clear that mating pheromones have independently evolved multiple times. However, once established simple one-to-one signal-receptor systems are also subject to passive divergence by random genetic drift.

This can happen if there are latent degrees of freedom in the signal and the receptor, as the sequence of the signaling molecule drifts slightly from its current state, opening up an opportunity for the receptor to change to a better match (or vice versa) (Lynch and Hagner 2015). Over a time scale sufficient for multiple mutations to accumulate, the basic mode of communication may then remain the same, while the communication language (i.e., the sequence motifs underlying the pheromone and its receptor) diverges (Figure 10.10). The end result is the passive development of incompatibilities between the receptor of one species and the signal molecules of related species.

Coevolutionary drift in pheromones and their receptors can be expected to become so extreme in some cases as to lead to complete absence of interspecific recognition. A clear indication of this potential is provided by the engineering of a reproductively isolated strain (effectively creating a new species) of *S. pombe* with just a few amino-acid changes (Seike et al. 2015). However, although comparative analysis suggests a combination of relaxed and positive selection in generating pheromone diversity among yeast species (Martin et al. 2011), there may be constraints on such systems, as cross-talk is still possible between members of quite distant phylogenetic lineages.

For example, *S. cerevisiae* still recognizes pheromones from species that have been separated for 100s of millions of years (Rogers et al. 2015), and similar observations have been made for distantly related species of the yeast genus *Candida* (Lin et al. 2011). In addition, cases exist in smuts where pheromone-receptor systems are cross-compatible between species that have been separated for 100s of millions of years (Kellner et al. 2011; Xu et al. 2016), although again single amino-acid changes imposed on the pheromones and/or receptors can elicit large changes in specificity in other mushrooms (Fowler et al. 2001).

The limited studies that have been pursued in other phylogenetic groups provide indirect support for the widespread occurrence of considerable divergence in communication systems. In the ciliate *Euplotes,* the amino-acid sequences of excreted pheromones are far more variable among species than the peptides contained within the cleaved portions of the precursor molecules, to the point of being nearly completely divergent among congeners, despite having very similar structures (Luporini et al. 1996, 2005). Within the genus *Closterium,* some species do not respond to the pheromones of others (Tsuchikane et al. 2008).

Taken together, these observations suggest that mating pheromone-receptor systems are capable of rapid interspecific divergence within at least some phylogenetic lineages. However, this is clearly an area in need of more mechanistically informative work. Whereas new receptors (with only single amino-acid changes) can be manufactured to discriminate against foreign pheromones, such modifications for specificity may often come at the expense of efficiency of mating with conspecifics, reducing their likelihood of accumulating in nature.

### Sexual Systems in Unicellular vs. Multicellular Organisms

The vast majority of our knowledge of sex-determination systems and their genetic bases derives from studies on multicellular organisms. This gives a quite biased view

of the general condition across the bulk of eukaryotic phylogeny. Three substantial differences, noted below, emphasize the fact that similar selective challenges lead to radically different evolutionary responses in multicellular vs. unicellular species, possibly as a consequence of the dramatic shifts in the population-genetic environment.

**Isogamy vs. anisogamy.** First, as noted above, sexual reproduction in most unicellular species involves isogamy, with the morphologically identical gamete types being denoted as mating types. In contrast, virtually all complex multicellular organisms (animals and land plants) exhibit anisogamy, the operating definition being that females produce larger gametes than males. Notably, among freshwater phytoplankton, there is a moderate tendency for larger-celled species to be anisogamous (Madsen and Waller 1983). Once established, anisogamy is thought to secondarily facilitate the evolution of numerous other sexually dimorphic traits, as females with high investments per egg are selected to be choosy, and males with more numerous and individually cheap sperm are selected to be more indiscriminate and to acquire traits that enhance access to females (Maynard Smith 1978).

One explanation for the evolution of anisogamy invokes the concept of disruptive selection owing to an inherent evolutionary tradeoff (Kalmus and Smith 1960; Parker et al. 1972; Bell 1978; Charlesworth 1978; Parker 1978; Bulmer and Parker 2002; Iyer and Roughgarden 2008). With a fixed amount of resources at the time of gametogenesis ($R$), there is an inverse relationship between gamete size and gamete number – one large gamete of size $R$ could be produced, or two of size $R/2$, four of size $R/4$, and so on. All other things being equal, larger numbers of gametes are advantageous, as they cumulatively have the potential to encounter more recipient partner gametes. However, if zygote survival increases with zygote size (the sum of the sizes of the two fusing gametes) at least to a point, there can also be a premium on producing a few large gametes. For the overall productivity of such a system to be elevated above that of isogamy, the increase in zygote survival with size need not be much greater than linear (Schuster and Sigmund 1982; Cox and Sethian 1984). Under this view, once two gamete sizes are established, strong preferential fusion between large and small gametes is expected to evolve secondarily, as the combination of two small gametes would have disproportionately low fitness, and the combination of two large gametes may also diminish fitness (owing to an inappropriately large size). Close linkage between a mating-type locus and a gamete-size locus can facilitate the maintenance of this kind of disassortative-mating system (Charlesworth 1978; Matsuda and Abrams 1999).

Given the universality of anisogamy in multicellular species, how can the opposite – the ubiquity of isogamy within unicellular species – be explained? Perhaps the most fundamental issue is that in multicellular organisms, there are no strict limits on the numbers of gametes that can be produced by the two sexes, as complex gonads can produce up to millions of meiotic products. In contrast, under unicellularity and isogamy, all mating types produce the same number (four) of meiotic products, removing much of the selective pressure for alternative sexually selected traits. A second relevant issue, ignored by the models noted above, is stabilizing selection on the size of haploid cells themselves, a plausible scenario given that the predominant life stage for many unicellular organisms is haploid. As all mating

types will typically be exposed to the same ecological conditions, and potentially so for long periods of clonal expansion, they will also typically be under the same size-selective forces.

**Sex ratio.** Simple population-genetic models suggest that separate sexes (or mating types) will typically lead to a stable 1:1 sex ratio as a consequence of frequency-dependent selection (Charlesworth and Charlesworth 2010). If + mating types are rare, then many − mating types will go unsatisfied, imposing selection in favor of genotypes producing more of the former to take advantage of this open resource (Figure 10.11). The opposite is expected if − mating types are disproportionately rare. For large populations with $n$ unique mating types, assuming equal access to each other, all types would be expected to have equilibrium frequencies of $\sim 1/n$. The well-known 1:1 sex ratio seen in most animals with separate sexes is in general accordance with this hypothesis, as are the limited data for mating-type frequencies in unicellular species.

In *Tetrahymena thermophila,* which as noted above has a mating-type switching mechanism, all seven mating types typically coexist at roughly equal frequencies in the same ponds (Doerder et al. 1995). A study of another ciliate, *Paramecium bursaria,* which has a genetic sex-determination mechanism, revealed approximately equal frequencies of all four possible mating types (Kosaka 1991). The three mating types in the slime mold *D. discoideum* are also found in approximately equal frequencies in nature (Douglas et al. 2016). Likewise, natural populations of the ascomycete *Stagonospora nodorum* harbor approximately equal frequencies of the two possible mating types (Sommerhalder et al. 2006), and the same is true for the wheat blotch fungus *Mycosphaerella graminicola* (Gurung et al. 2011).

One nuance with respect to the predicted equilibration of mating-type frequencies is the assumption of equal costs of producing alternative mating types, which is likely usually met under isogamy and unicellularity. More generally, theory predicts an equal total expenditure on different mating types (sexes) – if the production of individual daughters is energetically more expensive than that of sons, the equilibrium sex ratio is expected to be male biased (Charnov 1981; Charlesworth and Charlesworth 2010). It is, therefore, of interest that in the diatom *Cyclotella meneghiniana,* which is anisogamous (producing eggs and sperm) and has female meiosis, the sex ratio tends to be male biased (Shirokawa and Shimada 2013).

**Sex chromosomes.** The third major distinction between the genetics of mating systems in unicellular vs. multicellular species concerns the nature of the genetic regions involved. Numerous animal and land-plant species have sex-determination systems based on fully differentiated sex chromosomes. In contrast, no unicellular species is known to harbor sex chromosomes. Instead, unicellular species rely on cassettes of a small number of tightly linked genes embedded within an otherwise freely recombining region. It is often argued that the somewhat expanded sex-determination regions in some fungi and algae represent "incipient sex chromosomes" in early stages of development towards full-fledged sex chromosomes. However, there is no obvious reason why sex-chromosome evolution should have been delayed in unicellular species, nor is there any reason why sex chromosomes should be viewed

as an evolutionary advance. In short, the idea that organisms with separate sexes are destined to ultimately acquire such specialized chromosomes is less than compelling.

An alternative view is that the evolution of sex chromosomes is a pathological consequence of the low rates of recombination and high rates of random genetic drift experienced by multicellular species (Lynch 2007). Complete differentiation of sex chromosomes requires an outward expansion of recombination suppression around the primordial sex-determination (mating-type) locus, so as to allow the differential establishment and silencing of appropriate genes involved in sexual differentiation. Owing to their much higher rates of recombination per physical distance on chromosomes and to the diminished sensitivity to random genetic drift (Chapter 4), unicellular lineages may simply not provide the appropriate population-genetic environment for full sex-chromosome differentiation by degenerative mutation, regardless of the available time span. Although the isogamous mating systems of unicellular species also mitigate the opportunities for the evolution of differentiated sex chromosomes, a case can also be made for the opposite causal connection, i.e., that the origin of sex chromosomes facilitates the evolution of phenotypic sexual differentiation (Rice 1984).

**Summary**

- Cell biologists commonly subdivide the life of a cell into stages based on the state of growth and genome replication, the so-called cell cycle, although the precise recipe and relative durations of stages varies greatly among phylogenetic lineages. In eukaryotes, check-points dictating the progression between stages are usually directed by elaborate networks of interacting proteins that cycle in expression levels.

- Despite the centrality of a well-coordinated cell cycle to all eukaryotes, there is remarkable evolutionary fluidity in the structure of the regulatory network, the nature of the participating proteins, and the positions of active sites in such proteins. In other words, there is no "text-book" molecular description of the eukaryotic cell cycle.

- Variation in cell-cycle complexity and evolutionary rewiring of the overall network appear to be facilitated by duplication of ancestral component genes followed by subfunctionalization, providing striking examples of passive increases in network complexity in the absence of any intrinsic selective advantage of such structure.

- Establishment of the eukaryotic mitotic mechanism for replicating and evenly apportioning chromosomes to asexually produced daughter cells involved the introduction of at least eight modifications not found in prokaryotes. The participating proteins typically assemble into multimers, which are commonly homomeric in archaea, but heteromeric in eukaryotes. Again, there is enormous phylogenetic

variation in the structural features of mitosis among eukaryotes, but as yet no compelling evidence that the emergence of such variation, or of mitosis itself, was driven by adaptive processes.

- Unique to eukaryotes is meiosis, a two-stage modification of mitosis, which reduces diploid genomes to haploids that subsequently fuse as gametes to restore the diploid stage. Meiosis creates genetic variation via independent segregation and the exchange of sequence among homologous parental chromosomes, although it remains unclear that this was the driving force for the origin of meiosis. As in the case of mitosis, much of the meiotic machinery appears to have arisen by gene duplication in the path from FECA to LECA, and a good deal of phylogenetic diversification of the underlying mechanisms has subsequently developed.

- Many of the proteins associated with meiosis appear to undergo relatively rapid sequence evolution. One popular argument for this, the centromere-drive hypothesis, postulates that meiosis sets up opportunities for centromeres to evolve so as to enhance their probability of appearing in haploid products, which induces secondary selective pressures on the meiotic machinery to eliminate negative cytogenetic effects of the drive process. However, empirical support for this idea remains mixed.

- It is commonly believed that meiosis increases the efficiency of natural selection in promoting beneficial mutations and purging deleterious load. However, it remains unclear whether this adaptive explanation for the maintenance of sexual reproduction is relevant to the question of why meiosis arose in the first place.

- Because it relies on the fusion of two haploid cells to produce the diploid substrate necessary for meiosis, sexual reproduction promoted the evolution of pheromone/receptor-based mating types to enhance the likelihood of mate acquisition. Most species have two mating types, presumably because a single mating type leads to inefficient tracking of mates, as a cell's own plume of pheromone would overwhelm the gradient from foreign cells. However, the mating-type determination systems of different phylogenetic lineages are highly diverse, often independently evolved, and including multiple mating types.

- The simple one-to-one signal-receptor interactions in most mating-type recognition systems appear to be highly susceptible to coevolutionary drift, which over long time scales can lead to the passive emergence of reproductively isolated lineages.

- The sexual reproductive systems of unicellular species are substantially different from those in animals and land plants. Unlike the latter, the former generally

have morphologically indistinguishable mating types, isogamous gamete types (as opposed to size-differentiated eggs and sperm), and short chromosomal segments involved in sex determination (as opposed to fully differentiated sex chromosomes). All of these features appear to be natural outcomes of the altered population-genetic environments of unicellular species.

## Literature Cited

Akiyoshi, B., and K. Gull. 2014. Discovery of unconventional kinetochores in kinetoplastids. Cell 156: 1247-1258.

Anderson, J. A., W. D. Gilliland, and C. H. Langley. 2009. Molecular population genetics and evolution of *Drosophila* meiosis genes. Genetics 181: 177-185.

Archetti, M. 2004. Loss of complementation and the logic of two-step meiosis. J. Evol. Biol. 17: 1098-1105.

Archetti, M. 2005. Recombination and loss of complementation: a more than two-fold cost for parthenogenesis. J. Evol. Biol. 17: 1084-1097.

Armbrust, E. V., and H. M. Galindo. 2001. Rapid evolution of a sexual reproduction gene in centric diatoms of the genus *Thalassiosira.* Appl. Environ. Microbiol. 67: 3501-3513.

Aves, S. J., Y. Liu, T. A. Richards. 2012. Evolutionary diversification of eukaryotic DNA replication machinery. Subcell. Biochem. 62: 19-35.

Azimzadeh, J., M. L. Wong, D. M. Downhour, A. Sánchez Alvarado, and W. F. Marshall. 2012. Centrosome loss in the evolution of planarians. Science 335: 461-463.

Bakkeren, G., Kämper, and J. Schirawski. 2008. Sex in smut fungi: structure, function and evolution of mating-type complexes. Fungal Genet. Biol. 45 Suppl 1: S15-S21.

Barton, N. H., and B. Charlesworth. 1998. Why sex and recombination? Science 281: 1986-1990.

Barzel, A., M. Kupiec. 2008. Finding a match: how do homologous sequences get together for recombination? Nat. Rev. Genet. 9: 27-37.

Bell, G. 1978. The evolution of anisogamy. J. Theor. Biol. 73: 247-270.

Bell, G. 1982. The Masterpiece of Nature: The Evolution and Genetics of Sexuality. Univ. Calif. Press, Berkeley, CA.

Bengtsson, B. O. 1985. Biased conversion as the primary function of recombination. Genet. Res. 47: 77-80.

Bensasson, D. 2011. Evidence for a high mutation rate at rapidly evolving yeast centromeres. BMC Evol. Biol. 11: 211.

Bensasson, D., M. Zarowiecki, A. Burt, and V. Koufopanou. 2008. Rapid evolution of yeast centromeres in the absence of drive. Genetics 178: 2161-2167.

Bernstein, H., F. A. Hopf, and R. E. Michod. 1988. Is meiotic recombination an adaptation for repairing DNA, producing genetic variation, or both?, pp. 139-160. In H. Bernstein, F. Hopf, R. Michod, and B. R. Levin (eds.) The Evolution of Sex. Sinauer Assocs., Sunderland, MA.

Biondi, E. G., S. J. Reisinger, J. M. Skerker, M. Arif, B. S. Perchuk, K. R. Ryan, and M. T. Laub. 2006. Regulation of the bacterial cell cycle by an integrated genetic circuit. Nature 444: 899-904.

Bloomfield, G. 2011. Genetics of sex determination in the social amoebae. Dev. Growth Differ. 53: 608-616.

Bogdanov, Y. F., T. M. Grishaeva, and S. Y. Dadashev. 2007. Similarity of the domain structure of proteins as a basis for the conservation of meiosis. Int. Rev. Cytol. 257: 83-142.

Boland, W. 1995. The chemistry of gamete attraction: chemical structures, biosynthesis, and (a)biotic degradation of algal pheromones. Proc. Natl. Acad. Sci. USA 92: 37-43.

Bomblies, K., J. D. Higgins, and L. Yant. 2015. Meiosis evolves: adaptation to external and internal environments. New Phytol. 208: 306-323.

Bonner, A. M., and R. S. Hawley. 2019. Functional consequences of the evolution of Matrimony, a meiosis-specific inhibitor of polo kinase. Mol. Biol. Evol. 36: 69-83.

Bracewell, R., K. Chatla, M. J. Nalley, and D. Bachtrog. 2019. Dynamic turnover of centromeres drives karyotype evolution in *Drosophila.* eLife 8: e49002.

Bulmer, M. G., and G. A. Parker. 2002. The evolution of anisogamy: a game-theoretic approach. Proc. Biol. Sci. 269: 2381-2388.

Butler, G., M. D. Rasmussen, M. F. Lin, M. A. Santos, S. Sakthikumar, C. A. Munro, E. Rheinbay, M. Grabherr, A. Forche, J. L. Reedy, et al. 2009. Evolution of pathogenicity and sexual reproduction in eight *Candida genomes.* Nature 459: 657-662.

Cervantes, M. D., E. P. Hamilton, J. Xiong, M. J. Lawson, D. Yuan, M. Hadjithomas, W. Miao, and E. Orias. 2013. Selecting one of several mating types through gene segment joining and deletion in *Tetrahymena thermophila.* PLoS Biol. 11: e1001518.

Charlesworth, B. 1978. The population genetics of anisogamy. J. Theor. Biol. 73: 347-357.

Charlesworth, B., and D. Charlesworth. 2010. Elements of Evolutionary Genetics. Roberts and Co. Publ., Greenwood Village, CO.

Charnov. E. L. 1981. The Theory of Sex Allocation. Princeton Univ. Press, Princeton, NJ.

Chepurnov, V. A., D. G. Mann, K. Sabbe, and W. Vyverman. 2004. Experimental studies on sexual reproduction in diatoms. Int. Rev. Cytol. 237: 91-154.

Chia, N., I. Cann, and G. J. Olsen. 2010. Evolution of DNA replication protein complexes in eukaryotes and Archaea. PLoS One 5: e10866.

Chmátal, L., S. I. Gabriel, G. P. Mitsainas, J. Martinez-Vargas, J. Ventura, J. B. Searle, R. M. Schultz, and M. A. Lampson. 2014. Centromere strength provides the cell biological basis for meiotic drive and karyotype evolution in mice. Curr. Biol. 24: 2295-2300.

Cleveland, L. R. 1947. The origin and evolution of meiosis. Science 105: 287-289.

Cobbe, N., and M. M. Heck. 2004. The evolution of SMC proteins: phylogenetic analysis and structural implications. Mol. Biol. Evol. 21: 332-347.

Cole, E. S., D. Cassidy-Hanley, J. Fricke Pinello, H. Zeng, M. Hsueh, D. Kolbin, C. Ozzello, T. Giddings, Jr., M. Winey, and T. G. Clark. 2014. Function of the male-gamete-specific fusion protein HAP2 in a seven-sexed ciliate. Curr Biol. 24: 2168-2173.

Côte, P., H. Hogues, and M. Whiteway. 2009. Transcriptional analysis of the *Candida albicans* cell cycle. Mol. Biol. Cell 20: 3363-3373.

Coudreuse, D., and P. Nurse. 2010. Driving the cell cycle with a minimal CDK control network. Nature 468: 1074-1079.

Cox, P. A., and J. A. Sethian. 1984. Search, encounter rates, and the evolution of anisogamy. Proc. Natl. Acad. Sci. USA 81: 6078-6079.

Criqui, M. C., and P. Genschik. 2002. Mitosis in plants: how far we have come at the molecular level? Curr. Opin. Plant Biol. 5: 487-493.

Cross, F. R., N. E. Buchler, and J. M. Skotheim. 2011. Evolution of networks and sequences in eukaryotic cell cycle control. Philos. Trans. R. Soc. Lond. B Biol. Sci. 366: 3532-3544.

Cross, F. R., and J. G. Umen. 2015. The *Chlamydomonas* cell cycle. Plant J. 82: 370-392.

De Muyt, A., R. Mercier, C. Mézard, and M. Grelon. 2009. Meiotic recombination and crossovers in plants. Genome Dyn. 5: 14-25.

Ding, D. Q., K. Okamasa, M. Yamane, C. Tsutsumi, T. Haraguchi, M. Yamamoto, and Y. Hiraoka. 2012. Meiosis-specific noncoding RNA mediates robust pairing of homologous chromosomes in meiosis. Science 336: 732-736.

Doerder, F. P., M. A. Gates, F. P. Eberhardt, and M. Arslanyolu. 1995. High frequency of sex and equal frequencies of mating types in natural populations of the ciliate *Tetrahymena thermophila.* Proc. Natl. Acad. Sci. USA 92: 8715-8718.

Doonan, J. H., and G. Kitsios. 2009. Functional evolution of cyclin-dependent kinases. Mol. Biotechnol. 42: 14-29.

Douglas, T. E., J. E. Strassmann, and D. C. Queller. 2016. Sex ratio and gamete size across eastern North America in *Dictyostelium discoideum,* a social amoeba with three sexes. J. Evol. Biol. 29: 1298-1306.

Drinnenberg, I. A., S. Henikoff, and H. S. Malik. 2016. Evolutionary turnover of kinetochore proteins: a ship of Theseus? Trends Cell Biol. 26: 498-510.

Drury, L. S., and J. F. Diffley. 2009. Factors affecting the diversity of DNA replication licensing control in eukaryotes. Curr. Biol. 19: 530-535.

Dusenbery, D. B., and T. W. Snell. 1995. A critical body size for use of pheromones in mate location. J. Chem. Ecol. 21: 427-438.

Egel, R., and D. Penny. 2007. On the origin of meiosis in eukaryotic evolution: coevolution of meiosis and mitosis from feeble beginnings. Genome Dynamics Stability 3: 249-288.

Elde, N. C., K. C. Roach, M. C. Yao, and H. S. Malik. 2011. Absence of positive selection on centromeric histones in *Tetrahymena* suggests unsuppressed centromere: drive in lineages lacking male meiosis. J. Mol. Evol. 72: 510-520.

Fang, J., P. Nevin, V. Kairys, Č. Venclovas, J. R. Engen, and P. J. Beuning. 2014. Conformational analysis of processivity clamps in solution demonstrates that tertiary structure does not correlate with protein dynamics. Structure 22: 572-581.

Farhadifar, R., C. F. Baer, A. C. Valfort, E. C. Andersen, T. Müller-Reichert, M. Delattre, and D. J. Needleman. 2015. Scaling, selection, and evolutionary dynamics of the mitotic spindle. Curr. Biol. 25: 732-740.

Farhadifar, R., C. H. Yu, G. Fabig, H. Y. Wu, D. B. Stein, M. Rockman, T. Müller-Reichert, M. J. Shelley, and D. J. Needleman. 2020. Stoichiometric interactions explain spindle dynamics and scaling across 100 million years of nematode evolution. eLife 9: e55877.

Fédry, J., Y. Liu, G. Péhau-Arnaudet, J. Pei, W. Li, M. A. Tortorici, F. Traincard, A. Meola, G. Bricogne, N. V. Grishin, et al. 2017. The ancient gamete fusogen HAP2 is a eukaryotic Class II fusion protein. Cell 168: 904-915.

Fowler, T. J., M. F. Mitton, L. J. Vaillancourt, and C. A. Raper. 2001. Changes in mate recognition through alterations of pheromones and receptors in the multisexual mushroom fungus *Schizophyllum commune.* Genetics 158: 1491-1503.

Fraune, J., M. Alsheimer, J. N. Volff, K. Busch, S. Fraune, T. C. Bosch, and R. Benavente. 2012. *Hydra* meiosis reveals unexpected conservation of structural synaptonemal complex proteins across metazoans. Proc. Natl. Acad. Sci. USA 109: 16588-16593.

Garcia-Garcia, T., S. Poncet, A. Derouiche, L. Shi, I. Mijakovic, and M. F. Noirot-Gros. 2016. Role of protein phosphorylation in the regulation of cell cycle and DNA-related processes in bacteria. Front Microbiol. 7: 184.

Gerton, J. L., and R. S. Hawley. 2005. Homologous chromosome interactions in meiosis: diversity amidst conservation. Nat. Rev. Genet. 6: 477-487.

Goodenough, U., H. Lin, and J. H. Lee. 2007. Sex determination in *Chlamydomonas.* Semin. Cell Dev. Biol. 18: 350-361.

Gourguechon, S., L. J. Holt, and W. Z. Cande. 2013. The *Giardia* cell cycle progresses independently of the anaphase-promoting complex. J. Cell Sci. 126: 2246-2255.

Gurung, S., S. B. Goodwin, M. Kabbage, W. W. Bockus, and T. B. Adhikari. 2011. Genetic differentiation at microsatellite loci among populations of *Mycosphaerella graminicola* from California, Indiana, Kansas, and North Dakota. Phytopath. 101: 1251-1259.

Hadjivasiliou, Z., and A. Pomiankowski. 2016. Gamete signalling underlies the evolution of mating types and their number. Philos. Trans. R. Soc. Lond. B Biol. Sci. 371(1706).

Haldenby, S., M. F. White, and T. Allers. 2009. RecA family proteins in archaea: RadA and its cousins. Biochem. Soc. Trans. 37: 102-107.

Hallmann, A. 2008. VCRPs, small cysteine-rich proteins, might be involved in extracellular signaling in the green alga *Volvox.* Plant Signal Behav. 3: 124-127.

Hallmann, A., K. Godl, S. Wenzl, and M. Sumper. 1998. The highly efficient sex-inducing pheromone system of *Volvox.* Trends Microbiol. 6: 185-189.

Hanson, S. J., and K. H. Wolfe. 2017. An evolutionary perspective on yeast mating-type switching. Genetics 206: 9-32.

Harashima, H., N. Dissmeyer, and A. Schnittger. 2013. Cell cycle control across the eukaryotic kingdom. Trends Cell Biol. 23: 345-356.

Heath, I. B. 1980. Variant mitoses in lower eukaryotes: indicators of the evolution of mitosis. Int. Rev. Cytol. 64: 1-80.

Hemmer, L. W., and J. P. Blumenstiel. 2016. Holding it together: rapid evolution and positive selection in the synaptonemal complex of *Drosophila.* BMC Evol. Biol. 16: 91.

Henikoff, S., K. Ahmad, and H. S. Malik. 2001. The centromere paradox: stable inheritance with rapidly evolving DNA. Science 293: 1098-1102.

Hickey, D. A., and M. R. Rose. 1988. The role of gene transfer in the evolution of eukaryotic sex, pp. 161-175. In H. Bernstein, F. Hopf, R. Michod, and B. R. Levin (eds.) The Evolution of Sex. Sinauer Assocs., Sunderland, MA.

Hoekstra, R. F. 1980. Why do organisms produce gametes of only two different sizes? Some

theoretical aspects of the evolution of anisogamy. J. Theor. Biol. 87: 785-793.

Holt, L. J., B. B. Tuch, J. Villén, A. D. Johnson, S. P. Gygi, and D. O. Morgan. 2009. Global analysis of Cdk1 substrate phosphorylation sites provides insights into evolution. Science 325: 1682-1686.

Howard-Till, R. A., A. Lukaszewicz, M. Novatchkova, and J. Loidl. 2013. A single cohesin complex performs mitotic and meiotic functions in the protist *Tetrahymena*. PLoS Genet. 9: e1003418.

Hurst, L. D., and P. Nurse. 1991. A note on the evolution of meiosis. J. Theor. Biol. 150: 561-563.

Huysman, M. J., C. Martens, K. Vandepoele, J. Gillard, E. Rayko, M. Heijde, C. Bowler, D. Inzé, Y. Van de Peer, L. De Veylder, et al. 2010. Genome-wide analysis of the diatom cell cycle unveils a novel type of cyclins involved in environmental signaling. Genome Biol. 11: R17.

Iwata-Otsubo, A., J. M. Dawicki-McKenna, T. Akera, S. J. Falk, L. Chmátal, K. Yang, B. A. Sullivan, R. M. Schultz, M. A. Lampson, and B. E. Black. 2017. Expanded satellite repeats amplify a discrete CENP-A nucleosome assembly site on chromosomes that drive in female meiosis. Curr. Biol. 27: 2365-2373.

Iyer, P., and J. Roughgarden. 2008. Gametic conflict versus contact in the evolution of anisogamy. Theor. Popul. Biol. 73: 461-472.

Jenness, D. D., A. C. Burkholder, and L. H. Hartwell. 1986. Binding of alpha-factor pheromone to *Saccharomyces cerevisiae* a cells: dissociation constant and number of binding sites. Mol. Cell Biol. 6: 318-320.

Jensen, L. J., T. S. Jensen, U. de Lichtenberg, S. Brunak, and P. Bork. 2006. Co-evolution of transcriptional and post-translational cell-cycle regulation. Nature 443: 594-597.

Kalmus, H., and C. A. Smith. 1960. Evolutionary origin of sexual differentiation and the sex-ratio. Nature 186: 1004-1006.

Kellner, R., E. Vollmeister, M. Feldbrügge, and D. Begerow. 2011. Interspecific sex in grass smuts and the genetic diversity of their pheromone-receptor system. PLoS Genet. 7: e1002436.

Kleckner, N. 1996. Meiosis: how could it work? Proc. Natl. Acad. Sci. USA 93: 8167-8174.

Kondrashov, A. S. 1993. Classification of hypotheses on the advantage of amphimixis. J. Hered. 84: 372-387.

Kosaka, T. 1991. Life cycle of *Paramecium bursaria* syngen 1 in nature. J. Protozool. 38: 140-148.

Kothe, E. 1999. Mating types and pheromone recognition in the Homobasidiomycete *Schizophyllum commune*. Fungal Genet. Biol. 27: 146-152.

Kubai, D. F. 1975. The evolution of the mitotic spindle. Int. Rev. Cytol. 43: 167-227.

Krylov, D. M., K. Nasmyth, and E. V. Koonin. 2003. Evolution of eukaryotic cell cycle regulation: stepwise addition of regulatory kinases and late advent of the CDKs. Curr. Biol. 13: 173-177.

Lee, J. H., S. Waffenschmidt, L. Small, and U. Goodenough. 2007. Between-species analysis of short-repeat modules in cell wall and sex-related hydroxyproline-rich glycoproteins of *Chlamydomonas*. Plant Physiol. 144: 1813-1826.

Lin, C. H., A. Choi, and R. J. Bennett. 2011. Defining pheromone-receptor signaling in *Candida albicans* and related asexual *Candida species*. Mol. Biol. Cell 22: 4918-4930.

Lindås, A. C.,and R. Bernander. 2013. The cell cycle of archaea. Nat. Rev. Microbiol. 11: 627-638.

Lindholm, A. K., K. A. Dyer, R. C. Firman, L. Fishman, W. Forstmeier, L. Holman, H. Johannesson, U. Knief, H. Kokko, A. M. Larracuente, et al. 2016. The ecology and evolutionary dynamics of meiotic drive. Trends Ecol. Evol. 31: 315-326.

Liu, Y., J. Pei, N. Grishin, and W. J. Snell. 2015. The cytoplasmic domain of the gamete membrane fusion protein HAP2 targets the protein to the fusion site in *Chlamydomonas* and regulates the fusion reaction. Development 142: 962-971.

Liu, Y., T. A. Richards, and S. J. Aves. 2009. Ancient diversification of eukaryotic MCM DNA replication proteins. BMC Evol. Biol. 9: 60.

Lloyd, A., C. Morgan, F. C. Franklin, and K. Bomblies. 2018. Plasticity of meiotic recombination rates in response to temperature in *Arabidopsis*. Genetics 208: 1409-1420.

Loidl, J. 2016. Conservation and variability of meiosis across the eukaryotes. Annu. Rev. Genet. 50: 293-316.

López Hernández, J. F., R. M. Helston, J. J. Lange, R. B. Billmyre, S. H. Schaffner, M. T. Eickbush, S. McCroskey, and S. E. Zanders. 2021. Diverse mating phenotypes impact the spread of wtf meiotic drivers in *Schizosaccharomyces pombe*. eLife 10: e70812.

Lorenz, A., J. L. Wells, D. W. Pryce, M. Novatchkova, F. Eisenhaber, R. J. McFarlane, and J. Loidl. 2004. *S. pombe* meiotic linear elements contain proteins related to synaptonemal complex components. J. Cell Sci. 117: 3343-3351.

Luporini, P., C. Alilmenti, C. Ortenzi, and A. Vallesi. 2005. Ciliate mating types and their specific protein pheromones. Acta Protozool. 44: 89-101.

Luporini, P., C. Miceli, C. Ortenzi, and A. Vallesi. 1996. Ciliate pheromones. Prog. Mol. Subcell. Biol. 17: 80-104.

Lynch, M. 2007. The Origins of Genome Architecture. Sinauer Assocs., Inc., Sunderland, MA.

Lynch, M. 2012. Evolutionary layering and the limits to cellular perfection. Proc. Natl. Acad. Sci. USA 109: 18851-18856.

Lynch, M., and K. Hagner. 2015. Evolutionary meandering of intermolecular interactions along the drift barrier. Proc. Natl. Acad. Sci. USA 112: E30-E38.

Madsen, J. D., and D. M. Waller. 1983. A note on the evolution of gamete dimorphism in algae. Amer. Natur. 121: 443-447.

Maguire, M. P. 1992. The evolution of meiosis. J. Theor. Biol. 154: 43-55.

Malik, H. S., and S. Henikoff. 2001. Adaptive evolution of Cid, a centromere-specific histone in *Drosophila*. Genetics 157: 1293-1298.

Malik, S. B., M. A. Ramesh, A. M. Hulstrand, and J. M. Logsdon, Jr. 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: 2827-2841.

Mann, T. H., and L. Shapiro. 2018. Integration of cell cycle signals by multi-PAS domain kinases. Proc. Natl. Acad. Sci. USA 115: E7166-E7173.

Marcet-Houben, M., and T. Gabaldón. 2015. Beyond the whole-genome duplication: phylogenetic evidence for an ancient interspecies hybridization in the baker's yeast lineage. PLoS Biol. 13: e1002220.

Markova, K., M. Uzlikova, P. Tumova, K. Jirakova, G. Hagen, J. Kulda, and E. Nohynkova. 2016. Absence of a conventional spindle mitotic checkpoint in the binucleated single-celled parasite *Giardia* intestinalis. Eur. J. Cell. Biol. 95: 355-367.

Martin, S. H., B. D. Wingfield, M. J. Wingfield, and E. T. Steenkamp. 2011. Causes and consequences of variability in peptide mating pheromones of ascomycete fungi. Mol. Biol. Evol. 28: 1987-2003.

Matsuda, H., and P. A. Abrams. 1999. Why are equally sized gametes so rare? The instability of isogamy and the cost of anisogamy. Evol. Ecol. Res. 1: 769-784.

Maynard Smith, J. 1978. The Evolution of Sex. Cambridge Univ. Press, Cambridge, UK.

Medina, E. M., J. J. Turner, R. Gordân, J. M. Skotheim, and N. E. Buchler. 2016. Punctuated evolution and transitional hybrid network in an ancestral cell cycle of fungi. eLife 5: e09492.

Michaelis, S., and J. Barrowman. 2012. Biogenesis of the *Saccharomyces cerevisiae* pheromone a-factor, from yeast mating to human disease. Microbiol. Mol. Biol. Rev. 76: 626-651.

Moeys, S., J. Frenkel, C. Lembke, J. T. F. Gillard, V. Devos, K. Van den Berge, B. Bouillon, M. J. J. Huysman, S. De Decker, J. Scharf, et al. 2016. A sex-inducing pheromone triggers cell cycle arrest and mate attraction in the diatom *Seminavis robusta.* Sci. Rep. 6: 19252.

Morgan, D. O. 2007. The Cell Cycle: Principles of Control. Oxford Univ. Press, Oxford, UK.

Moses, A. M., M. E. Liku, J. J. Li, and R. Durbin. 2007. Regulatory evolution in proteins by turnover and lineage-specific changes of cyclin-dependent kinase consensus sites. Proc. Natl. Acad. Sci. USA 104: 17713-17718.

Murray, A. W. 2012. Don't make me mad, Bub! Dev. Cell 22: 1123-1125.

Naor, A., and U. Gophna. 2013. Cell fusion and hybrids in Archaea: prospects for genome shuffling and accelerated strain development for biotechnology. Bioengineered 4: 126-129.

Nei, M. 1969. Linkage modifications and sex difference in recombination. Genetics 63: 681-699.

Nguyen Ba, A. N., B. Strome, S. Osman, E. A. Legere, T. Zarin, and A. M. Moses. 2017. Parallel reorganization of protein function in the spindle checkpoint pathway through evolutionary paths in the fitness landscape that appear neutral in laboratory experiments. PLoS Genet. 13: e1006735.

Niklas, K. J., E. D. Cobb, and U. Kutschera. 2014. Did meiosis evolve before sex and the evolution of eukaryotic life cycles? Bioessays 36: 1091-1101.

Okamoto, M., L. Yamada, Y. Fujisaki, G. Bloomfield, K. Yoshida, H. Kuwayama, H. Sawada, T. Mori, and H. Urushihara. 2016. Two HAP2-GCS1 homologs responsible for gamete interactions in the cellular slime mold with multiple mating types: implication for common mechanisms of sexual reproduction shared by plants and protozoa and for male-female differentiation. Dev. Biol. 415: 6-13.

Onesti, S., and S. A. MacNeill. 2013. Structure and evolutionary origins of the CMG complex. Chromosoma 122: 47-53.

Orias, E. 2014. Membrane fusion: HAP2 protein on a short leash. Curr. Biol. 24: R831-R833.

Osella, M., S. J. Tans, and M. C. Lagomarsino. 2017. Step by step, cell by cell: quantification of the bacterial cell cycle. Trends Microbiol. 25: 250-256.

Ottolini, C. S., L. Newnham, A. Capalbo, S. A. Natesan, H. A. Joshi, D. Cimadomo, D. K. Griffin, K. Sage, M. C. Summers, A. R. Thornhill, et al. 2015. Genome-wide maps of recombination and chromosome segregation in human oocytes and embryos show selection for maternal recombination rates. Nat. Genet. 47: 727-735.

Parker, G. A. 1978. Selection on non-random fusion of gametes during the evolution of anisogamy. J. Theor. Biol. 73: 1-28.

Parker, G. A., R. R. Baker, and V. G. Smith. 1972. The origin and evolution of gamete dimorphism and the male-female phenomenon. J. Theor. Biol. 36: 529-553.

Phadke, S. S., and R. A. Zufall. 2009. Rapid diversification of mating systems in ciliates. Biol. J. Linnean Soc. 98: 187-197.

Pinello, J. F., A. L. Lai, J. K. Millet, D. Cassidy-Hanley, J. H. Freed, and T. G. Clark. 2017. Structure-function studies link Class II viral fusogens with the ancestral gamete fusion protein HAP2. Curr. Biol. 27: 651-660.

Pohnert, G., and W. Boland. 2002. The oxylipin chemistry of attraction and defense in brown algae and diatoms. Nat. Prod. Rep. 19: 108-122.

Raikov, I. B. 1982. Morphology of Eukaryotic Protozoan Nuclei. Cell Biol. Monographs, Volume 9. Springer-Verlag, Vienna, Austria.

Ramesh, M. A., S. B. Malik, J. M. Logsdon, Jr. 2005. A phylogenomic inventory of meiotic genes; evidence for sex in *Giardia* and an early eukaryotic origin of meiosis. Curr. Biol. 15: 185-191.

Reedy, J. L., A. M. Floyd, and J. Heitman. 2009. Mechanistic plasticity of sexual reproduction and meiosis in the *Candida* pathogenic species complex. Curr. Biol. 19: 891-899.

Rhind, N., and P. Russell. 2000. Chk1 and Cds1: linchpins of the DNA damage and replication checkpoint pathways. J. Cell Sci. 113: 3889-3896.

Rice, W. R. 1984. Sex chromosomes and the evolution of sexual dimorphism. Evolution 38: 735-742.

Riquelme, M., M. P. Challen, L. A. Casselton, and A. J. Brown. 2005. The origin of multiple B mating specificities in *Coprinus cinereus*. Genetics 170: 1105-1119.

Robert, T., A. Nore, C. Brun, C. Maffre, B. Crimi, H. M. Bourbon, and B. de Massy. 2016. The TopoVIB-Like protein family is required for meiotic DNA double-strand break formation. Science 351: 943-949.

Rog, O., S. Köhler, and A. F. Dernburg. 2017. The synaptonemal complex has liquid crystalline properties and spatially regulates meiotic recombination factors. eLife 6: e21455.

Rogers, D. W., J. A. Denton, E. McConnell, and D. Greig. 2015. Experimental evolution of species recognition. Curr. Biol. 25: 1753-1758.

Rosin, L. F., and B. G. Mellone. 2017. Centromeres drive a hard bargain. Trends Genet. 33: 101-117.

Sazer, S., M. Lynch, and D. Needleman. 2014. Deciphering the evolutionary history of open and closed mitosis. Curr. Biol. 24: R1099-R1103.

Scherthan, H. 2001. A bouquet makes ends meet. Nat. Rev. Mol. Cell Biol. 2: 621-627.

Schuster, P., and K. Sigmund. 1982. A note on the evolution of sexual dimorphism. J. Theor. Biol. 94: 107-110.

Seike, T., T. Nakamura, and C. Shimoda. 2015. Molecular coevolution of a sex pheromone and its receptor triggers reproductive isolation in *Schizosaccharomyces pombe.* Proc. Natl. Acad. Sci. USA 112: 4405-4410.

Sekimoto, H., J. Abe, and Y. Tsuchikane. 2012. New insights into the regulation of sexual reproduction in *Closterium.* Int. Rev. Cell. Mol. Biol. 297: 309-338.

Shirokawa, Y., and M. Shimada. 2013. Sex allocation pattern of the diatom *Cyclotella meneghiniana.* Proc. Biol. Sci. 280: 20130503.

Singh, D. P., B. Saudemont, G. Guglielmi, O. Arnaiz, J.-F. Gout, M. Prajer, A. Potekhin, E. Przybòs, A. Aubusson-Fleury, S. Bhullar, et al. 2014. Genome-defence small RNAs exapted for epigenetic mating-type inheritance. Nature 509: 447-452.

Solari, A. J. 2002. Primitive forms of meiosis: the possible evolution of meiosis. Biocell 26: 1-13.

Sommerhalder, R. J., B. A. McDonald, and J. Zhan. 2006. The frequencies and spatial distribution of mating types in *Stagonospora nodorum* are consistent with recurring sexual reproduction. Phytopath. 96: 234-239.

Speijer, D., J. Lukes, and M. Eliás. 2015. Sex is a ubiquitous, ancient, and inherent attribute of eukaryotic life. Proc. Natl. Acad. Sci. USA 112: 8827-8834.

Stover, N. A., and J. D. Rice. 2011. Distinct cyclin genes define each stage of ciliate conjugation. Cell Cycle 10: 1699-1701.

Suijkerbuijk, S. J., T. J. P. van Dam, G. E. Karagöz, E. von Castelmur, N. C. Hubner, A. M. S. Duarte, M. Vleugel, A. Perrakis, S. G. D. Rüdiger, B. Snel, et al. 2012. The vertebrate mitotic checkpoint protein BUBR1 is an unusual pseudokinase. Dev. Cell 22: 1321-1329.

Svedberg, J., A. A. Vogan, N. A. Rhoades, D. Sarmarajeewa, D. J. Jacobson, M. Lascoux, T. M. Hammond, and H. Johannesson. 2021. An introgressed gene causes meiotic drive in *Neurospora sitophila*. Proc. Natl. Acad. Sci. USA 118: e2026605118.

Tromer, E. C., J. J. E. van Hooff, G. J. P. L. Kops, and B. Snel. 2019. Mosaic origin of the eukaryotic kinetochore. Proc. Natl. Acad. Sci. USA 116: 12873-12882.

Tsuchikane, Y., M. Ito, and H. Sekimoto. 2008. Reproductive isolation by sex pheromones in the *Closterium lobsterium peracerosum-strigosum-litrorale* complex (Zygnematales, Charophycea). J. Phycol. 44: 1197-1203.

Turner, B. C., and D. D. Perkins. 1979. Meiotic drive in *Neurospora* and other fungi. Amer. Natur. 137: 416-429.

Urban, M., R. Kahmann, and M. Bölker. 1996. The biallelic a mating type locus of *Ustilago maydis:* remnants of an additional pheromone gene indicate evolution from a multiallelic ancestor. Mol. Gen. Genet. 250: 414-420.

van Wolferen, M., A. Wagner, C. van der Does, and S. V. Albers. 2016. The archaeal Ced system imports DNA. Proc. Natl. Acad. Sci. USA 113: 2496-2501.

Vogan, A. A., S. L. Ament-Velásquez, A. Granger-Farbos, J. Svedberg, E. Bastiaans, A. J. Debets, V. Coustou, H. Yvanne, C. Clavé, S. J. Saupe, et al. 2019. Combinations of *Spok* genes create multiple meiotic drivers in *Podospora*. eLife 8: e46454.

Vrielynck, N., A. Chambon, D. Vezon, L. Pereira, L. Chelysheva, A. De Muyt, C. Mézard, C. Mayer, and M. Grelon. 2016. A DNA topoisomerase VI-like complex initiates meiotic recombination.

Science 351: 939-943.

Wagner, A., R. J. Whitaker, D. J. Krause, J. H. Heilers, M. van Wolferen, C. van der Does, and S. V. Albers. 2017. Mechanisms of gene flow in archaea. Nat. Rev. Microbiol. 15: 492-501.

Wang, S., N. Kleckner, and L. Zhang. 2017. Crossover maturation inefficiency and aneuploidy in human female meiosis. Cell Cycle 4: 1-3.

Watanabe, Y. 2005. Shugoshin: guardian spirit at the centromere. Curr. Opin. Cell. Biol. 17: 590-595.

Watanabe, Y. 2012. Geometry and force behind kinetochore orientation: lessons from meiosis. Nat. Rev. Mol. Cell Biol. 13: 370-382.

Wilkins, A. S., and R. Holliday. 2009. The evolution of meiosis from mitosis. Genetics 181: 3-12.

Williams, G. C. 1975. Sex and Evolution. Princeton Univ. Press, Princeton, NJ.

Winey, M., and K. Bloom. 2012. Mitotic spindle form and function. Genetics 190: 1197-1224.

Wirth, R. 1994. The sex pheromone system of *Enterococcus faecalis.* More than just a plasmid-collection mechanism? Eur. J. Biochem. 222: 235-246.

Wolfe, K. H., and D. C. Shields. 1997. Molecular evidence for an ancient duplication of the entire yeast genome. Nature 387: 708-713.

Wong, J. L., and M. A. Johnson. 2010. Is HAP2-GCS1 an ancestral gamete fusogen? Trends Cell Biol. 20: 134-141.

Xu, L., E. Petit, and M. E. Hood. 2016. Variation in mate-recognition pheromones of the fungal genus *Microbotryum.* Heredity 116: 44-51.

Zamir, L., M. Zaretsky, Y. Fridman, H. Ner-Gaon, E. Rubin, and A. Aharoni. 2012. Tight coevolution of proliferating cell nuclear antigen (PCNA)-partner interaction networks in fungi leads to interspecies network incompatibility. Proc. Natl. Acad. Sci. USA 109: E406-E414.

Zeng, Y., H. Li, N. M. Schweppe, R. S. Hawley, and W. D. Gilliland. 2010. Statistical analysis of nondisjunction assays in *Drosophila.* Genetics 186: 505-513.

Zetka, M. 2017. When degradation spurs segregation. Science 355: 349-350.

Zickler, D., and N. Kleckner. 2016. A few of our favorite things: pairing, the bouquet, crossover interference and evolution of meiosis. Semin. Cell Dev. Biol. 54: 135-148.

Zwick, M. E., J. L. Salstrom, and C. H. Langley. 1999. Genetic variation in rates of nondisjunction: association of two naturally occurring polymorphisms in the chromokinesin nod with increased rates of nondisjunction in *Drosophila melanogaster.* Genetics 152: 1605-1614.

**Figure 10.1.** An idealized depiction of the eukaryotic cell cycle, divided into different phases (the absolute and relative lengths of which can vary dramatically among cell types). Checkpoints are denoted by the large black arrows.

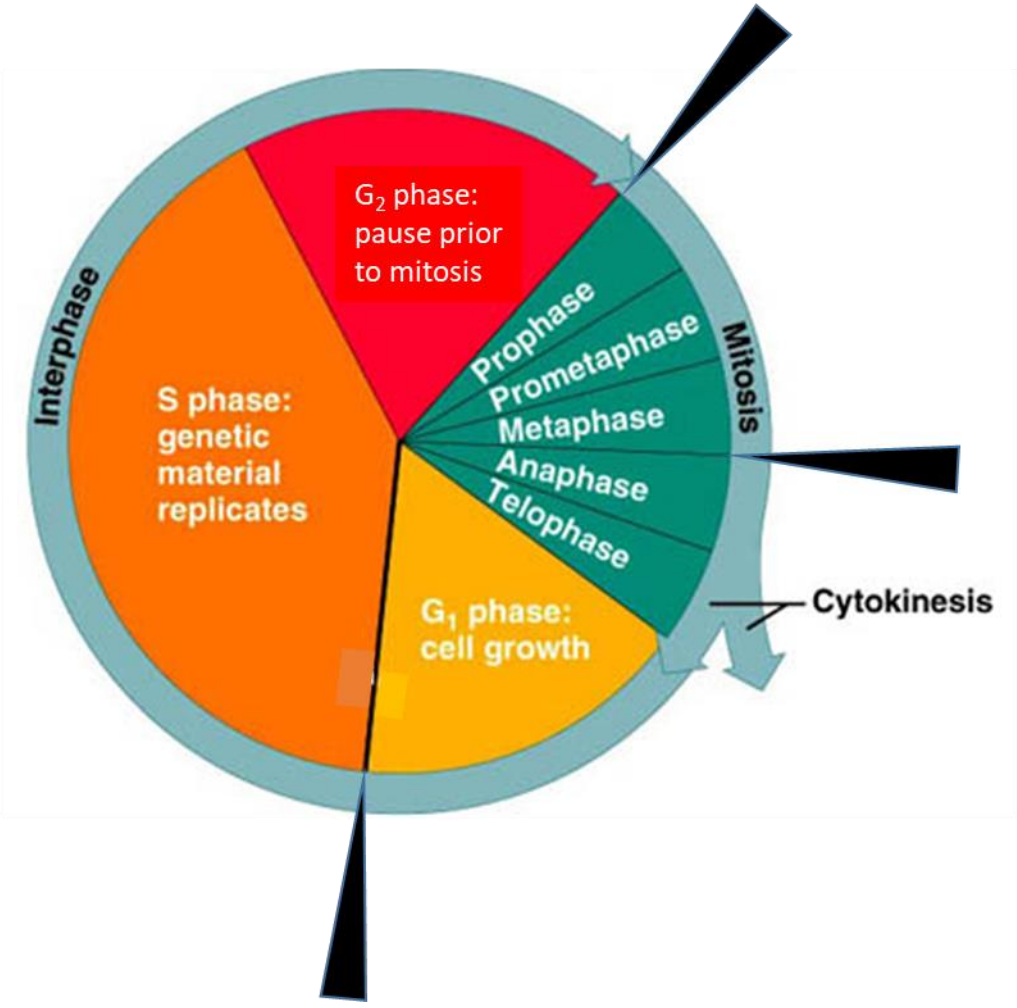


**Figure 10.2.** Nearly constant cell-cycle topology in the face of dramatic re-patterning of the underlying components. The upper and lower panels denote the networks for mammal and budding yeast *S. cerevisiae,* respectively. Triangles denote CDKs, and circles cyclins. Green arrows denote activation steps; red lines with blunt ends denote inhibitory interactions. Proteins with colored enclosures have orthologous sequences in other eukaryotes, and those with the same color in mammal and yeast are orthologous to each other, whereas those in white are unrelated. The genes discussed in the text E2F/SBF appear as trapezoids at the top. From Cross et al. (2011).

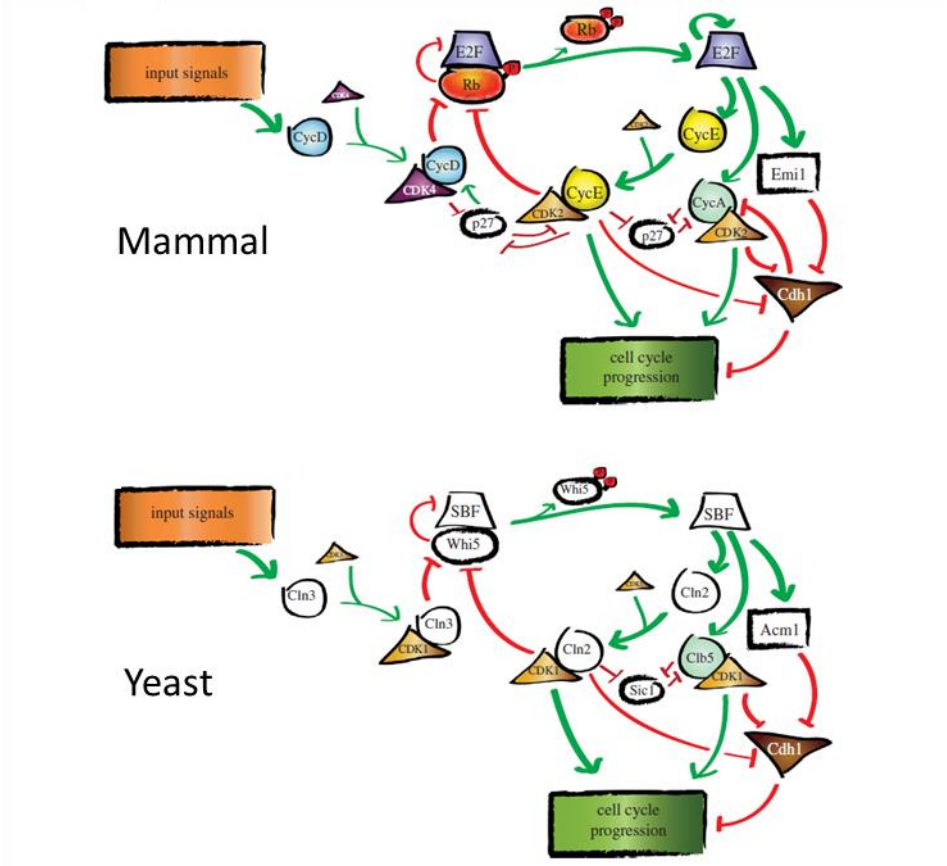


**Figure 10.3.** Three alternative surveillance pathway architectures for perfecting a final outcome. There are three progressive steps in the black pathway. Under the red pathway, which has lost the first step, the same final outcome is accomplished by employing a highly efficient surveillance in step 2, whereas the blue pathways deploy only a single, highly efficient step. The graph on the right illustrates the progressive removal of errors by successive steps. As shown on the left, the black pathway with three progressive steps can be simplified to three different one-step pathways, rendering nonoverlapping mechanisms (with no shared molecular steps) in the resultant lineages.

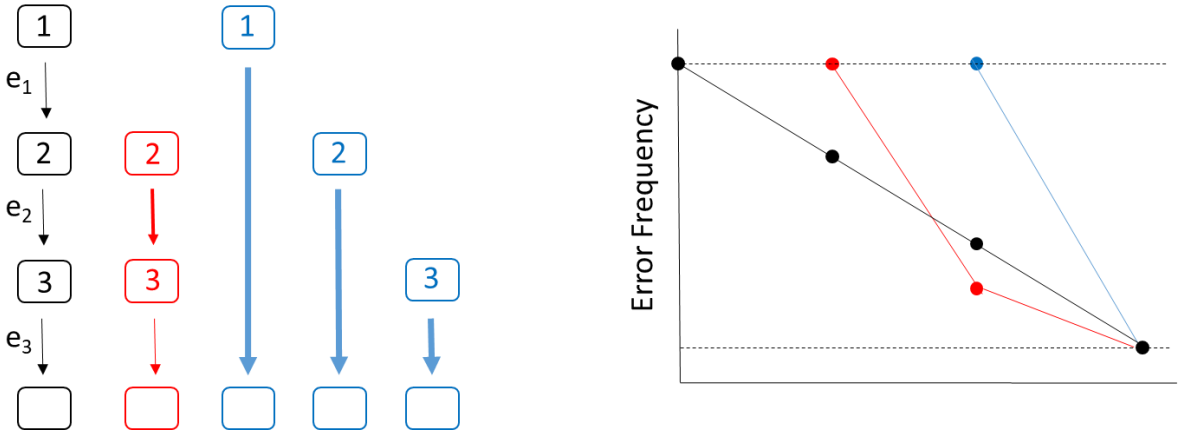


**Figure 10.4.** The phylogenetic distribution of genes related to two spindle-checkpoint genes in the yeast *S. cerevisiae*, Bub1 (blue) and Mad3 (red). In several species, e.g., *L. kluyveri* and *N. crassa*, a single gene is endowed with both the Bub1 and Mad3 subfunctions, but in four species (denoted by the red vertical bars on the phylogenetic tree on the left), including the reference *S. cerevisiae*, the bifunctional gene was duplicated, with each descendent copy then losing a complementary subfunction. The figure is simplified from Nguyen Ba et al. (2017).

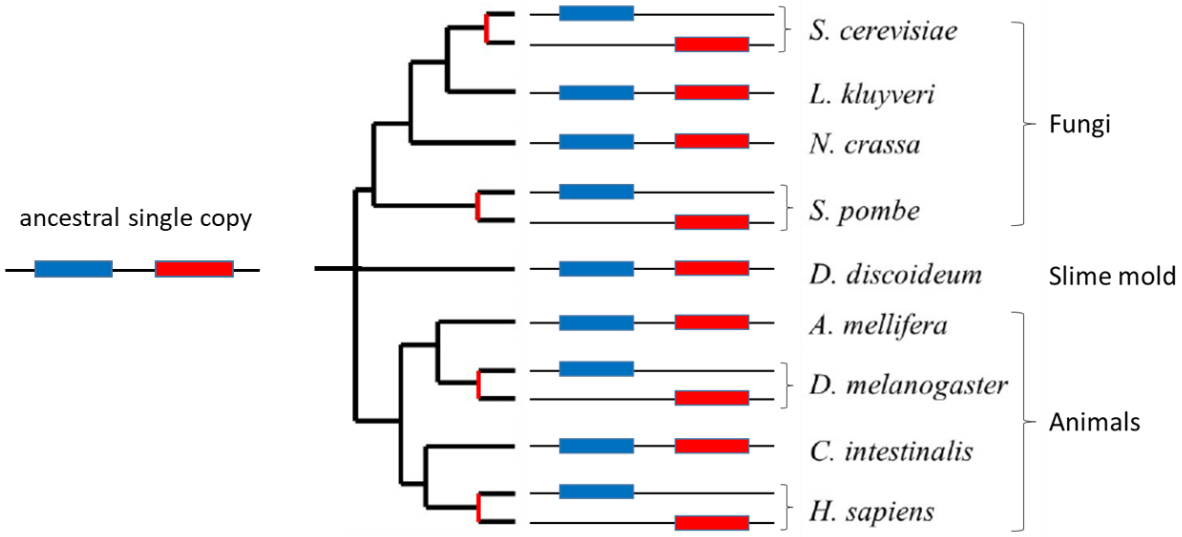


**Figure 10.5.** The contrast between the basic steps in eukaryotic mitosis and meiosis. Depicted are the assortment of three pairs of homologous chromosomes (red and blue denote chromosomes derived from paternal and maternal sources), which after duplication appear at metaphase as six pairs of sister chromatids in mitosis and as three pairs of tetrads in meiosis.

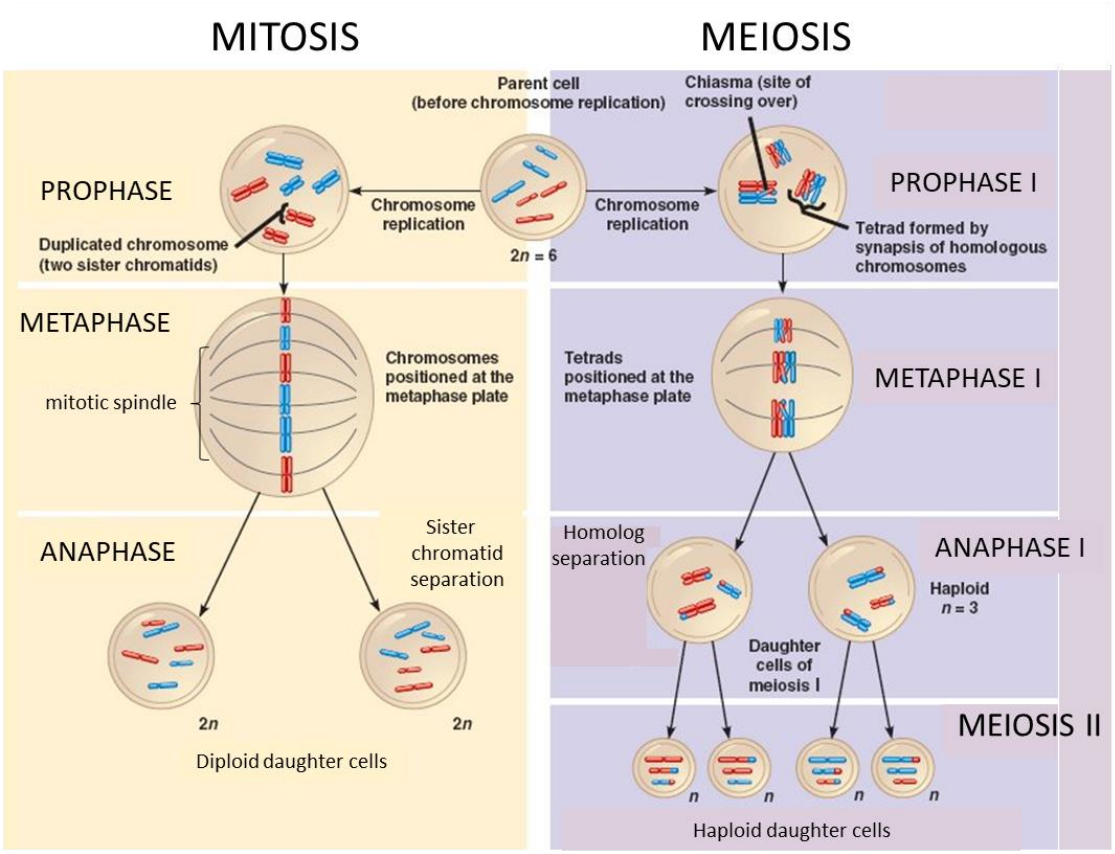
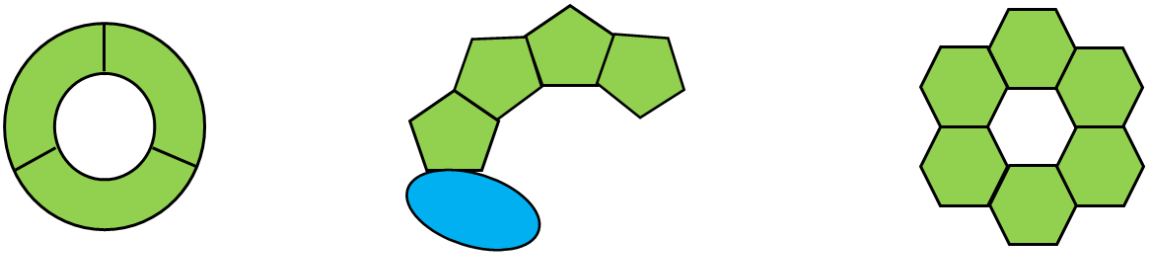


**Figure 10.6.** Simplified depictions of three of the multimeric proteins involved in DNA replication. The green subunits may all be encoded by the same genetic locus (homomers) or by two or more proteins (up to the number of subunits; heteromers).



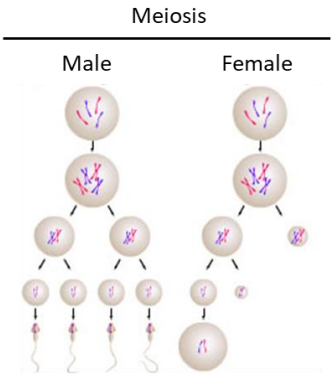
PCNA
trimeric ring

RFC
tetrameric hinge

MCM
hexameric ring

**Figure 10.7.** The centomere-drive hypothesis. **Upper right:** male and female meiosis in diploid cells initially containing two pairs of homologous chromosomes, with the former leading to four haploid products and the latter to just one surviving gamete. **Lower left:** a driving centromere is depicted as one with an excess number of attachments to spindle fibers during meiosis.

## The Centromere-drive Hypothesis




The challenge of female meiosis:

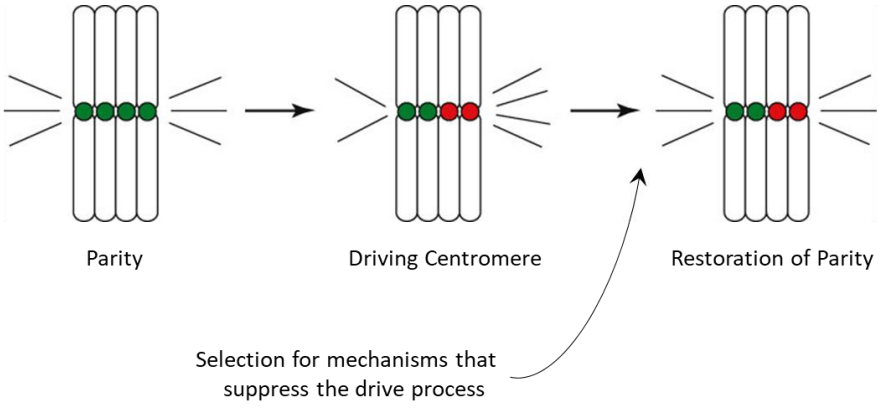


**Figure 10.8.** The evolution of mating-type determination by pheromone-receptor systems, with P and p denoting the presence and absence of a gene associated with pheromone production, and R and r denoting the presence and absence of a gene for the receptor. **Upper row:** in the simplest system, there are four potential haplotypes, one having both functions, two having just a single function, and one being nonfunctional. In a system containing only haplotype PR, all individuals are capable of interacting with each other, whereas a system containing Pr and pR haplotypes (but no PR) will consist of two mating types. **Lower row:** Over time, the latter unipolar system may experience gains of novel pheromones and receptors, such that all participants contain noninteracting pairs of pheromones and receptors, further increasing the efficiency of recognition by compatible genotypes.

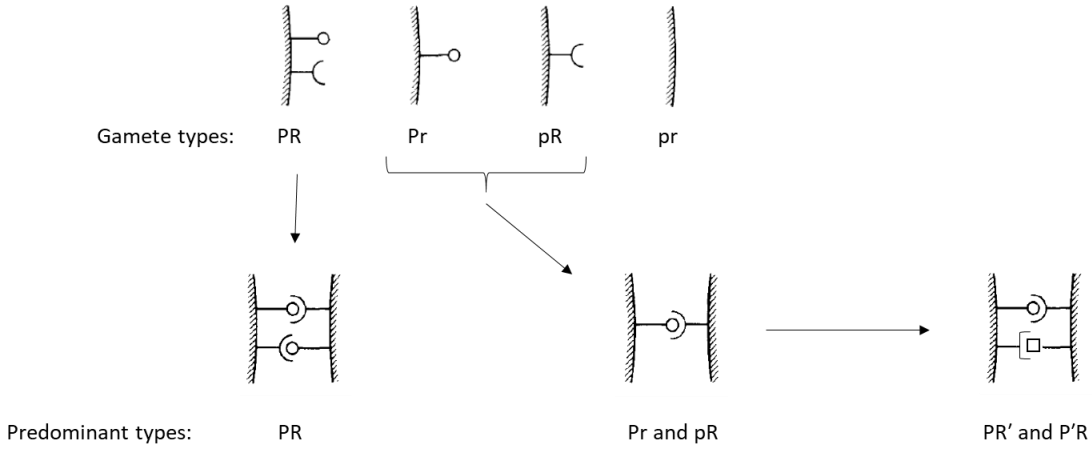


**Figure 10.9.** The binuclear endowment of a ciliate cell. Ciliates generally harbor a single, transcriptionally silent, germline micronucleus and a larger transcriptionally active macronucleus, which is comprised of hundreds of copies of micronuclear chromosomes, each with specific segments of DNA spliced out. The macronucleus is degraded after mating and replaced by a processed version of the new micronucleus. Both are transmitted intact during clonal phases of growth, the micronucleus by mitosis and the macronucleus by a less precise fission process. When two mating types conjugate with each other, their micronuclei undergo meiosis, but three of the meiotic products are discarded ("female meiosis"); the remaining haploid nucleus is duplicated, with each conjugating cell exchanging a single gamete to restore diploidy; finally, the conjugating partners separate, and the new micronuclei serve as templates for building new macronuclei.

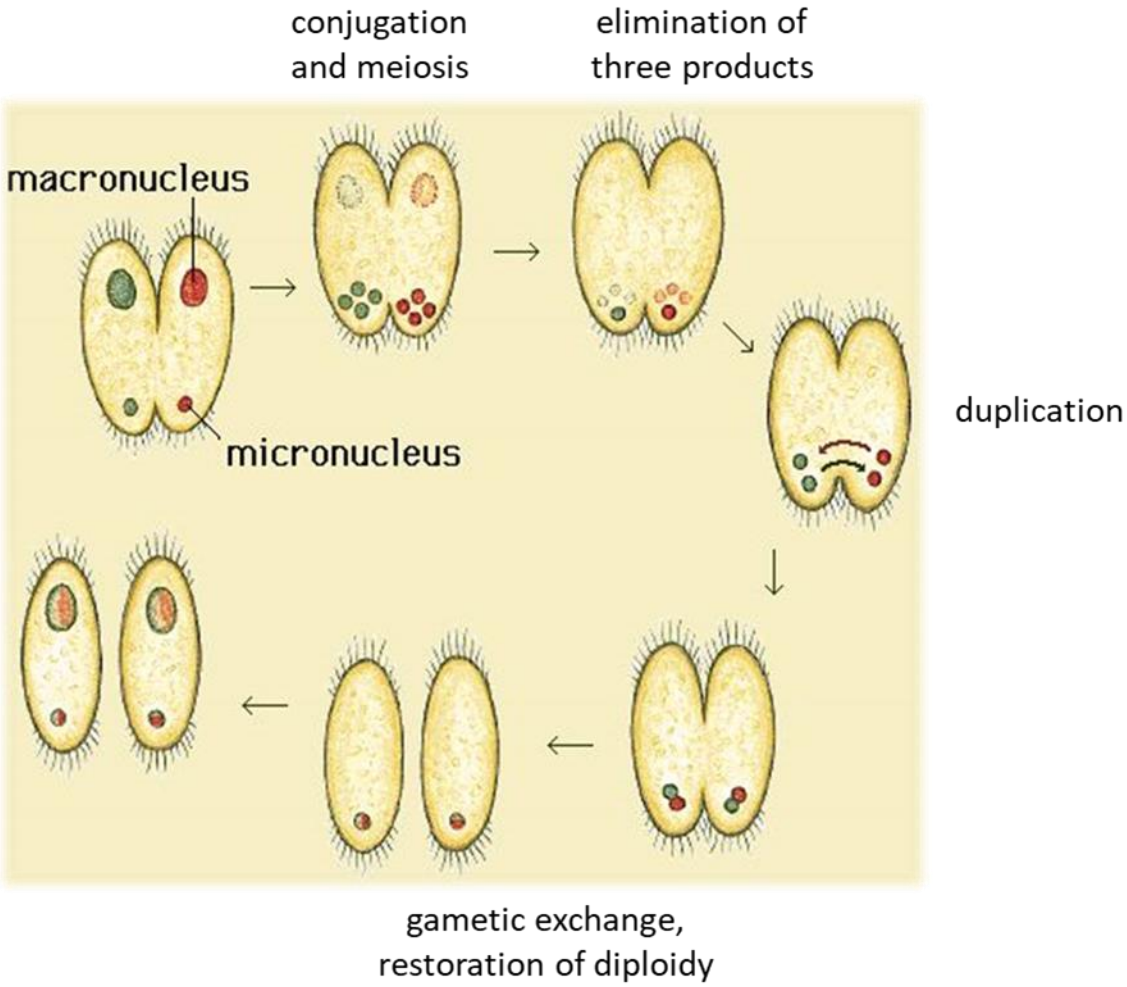


**Figure 10.10.** Gradual change in the communication system involving a receptor and its signal. Upper and lower cases of the same letter imply a match, and the only requirement of the system is that at least two of three letters match. Six incremental changes lead to completely nonoverlapping (and incompatible) words, although at each step the system is fully functional.

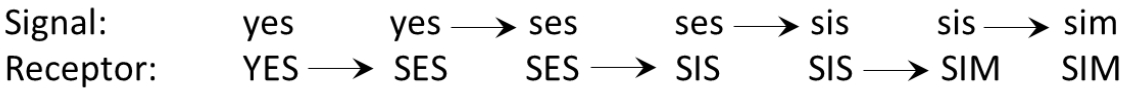


**Figure 10.11.** Frequency-dependent selection for alternative mating types. On the left, the blue type is outnumbered by reds, leading to a selective advantage for blue, as not all reds will be capable of finding an appropriate mate. The opposite situation occurs to the right. In the center, blues and reds have equal access to each other, leading to equivalent fitnesses for both types.

blue favored stable equilibrium red favored