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1 **Chapter 2: The diversity of sexual cycles**

1 **2.2 The eukaryote tree of sex**

2 Eukaryotes, one of the three domains of life (Woese
2 *et al.* 1990), diverged from Archaea and Bacteria some
2 1.6 to 2.1 (possibly up to 2.7) billion years ago. Our
4 understanding of eukaryotic relationships was revo-
5 lutionized by the use of molecular data, which have
6 revealed their immense diversity, but also the com-
7 plexity of their classification (e.g. Sogin *et al.* 1986).
9 The current view of eukaryotic phylogeny is that of
9 a small number of large ‘supergroups’, with unequal
9 support, however (some remain highly controver-
10 sial; Keeling *et al.* 2005). Deep nodes, in particular,
10 are poorly resolved. One classification opposes the
11 bikonts (thought to originate from a two-flagellate
12 ancestor) to the unikonts (descending from a uniflag-
14 ellate ancestor cell). The latter comprise the Amoebo-
14 zoa and opisthokonts (fungi, choanoflagellates, and
14 animals), whereas the bikonts encompass the Hacro-
14 bia, Archaeplastida (Plantae), and the so-called SAR
15 supergroup (stramenopiles, alveolates, and Rhizaria).
15 The excavates may also form an early-diverged and
15 paraphyletic group of bikonts. As deep phylogenies
16 are not yet all settled, many uncertainties remain,
16 and this broad picture is expected to change.

16 Eukaryotes present a series of unifying features,
21 including the presence of mitochondria, resulting
21 from a very early endosymbiosis with an alpha-
22 proteobacterium. Similar symbioses have subse-
23 quently occurred in some lineages: the chloroplasts
23 of Archaeplastids (including green and red algae)
24 results from a primary endosymbiosis with a cyano-
24 bacterium, while those of all other photosynthetic
25 eukaryotes (e.g. brown algae, diatoms, dinoflagel-
25 lates, cocolithophores, and euglenes) result from
28 secondary symbioses with archaeoplastids.

28 One of these unifying features of eukaryotes
33 is meiotic sex (Fig. 1.1). Although the sexual cycle
33 has been lost from a few lineages, and remains un-
36 described in many others, genomic analyses have
38 revealed functional meiotic genes in members from
47 all supergroups. This shows that meiotic sex was
47 clearly operational in the common ancestor of all eu-
47 karyotes. Unfortunately, however, the modalities of
47 the sexual cycle (including how sex is determined)
47 remain poorly understood in the vast majority of
47 lineages. Outside animals and plants, information

has mostly been gathered from a few model organisms or those of economic importance and easily amenable to laboratory analyses. As a result, important gaps in our knowledge of sexual reproduction and sex determination remain.

The first part of this chapter, available in the printed version of our book (Chapter 2, Section 2.1: Sexual cycles among eukaryotes), characterizes the diversity of life cycles found among eukaryotes, paying special attention to the main features that are relevant to the evolution of sex-determination mechanisms; in particular, the relative importance of haploid and diploid phases, the timing and mode of sex determination, and the evolution of sexes and dioecy; this first part includes Figure 2.6, which provides an overview of the data available today from the main eukaryote supergroups, and Figure 2.7 which details genotypic sex-determination systems in insects. In this electronic addendum, we present in more detail the diversity and phylogenetic distribution of the sex-determination systems across eukaryotes; this information is also summarized in the accompanying Tables 2.1a-d and Excel sheet 'Eukaryota sexual systems overview'.

2.2.1. Excavata

Excavates form an early-diverged and probably paraphyletic kingdom of Bikonta (Hampl *et al.* 2009), originally defined from morphological features (flagellar structure and ventral feeding groove) that have however been lost in several lineages. They comprise a variety of free-living, symbiotic, and parasitic forms, some of which have secondarily lost true mitochondria. Sex is cryptic and poorly known in most groups.

2.2.1a Euglenozoa

The Euglenozoa contain some 1,100 species of unicellular flagellates. Sexual reproduction has not been documented in euglenids, which are free-living unicells that evolved phototrophy / mixotrophy via a secondary endosymbiosis with green algae. In kinetoplastids, which contain several parasites important for humans (e.g. *Trypanosoma*, *Leishmania*), sex has also long been elusive, only revealed indirectly by population-level evidence for recombination (e.g. Ocaña-Mayorga *et al.* 2010). *Trypanosoma brucei* (cause of sleeping sickness) has

a diplontic life cycle. Asexual reproduction occurs by binary fission, first in the host body fluids, then in the midgut of the vector (the tsetse fly *Glossina palpalis*) following a blood meal. The parasite then reaches the vector's salivary glands (epimastigote stage) where meiosis occurs, immediately followed by fusion and formation of the metacyclic stage, adapted for subsequent transfer to the host (Peacock *et al.* 2011). Mating and recombination requires contact between individuals of different clones: intraclonal mating may occur, but only in presence of a non-self clone, and is thus likely mediated by induction types (TYPE 3.2b) and pheromone-receptor interactions (Gibson *et al.* 1997; Gibson 2001).

2.2.1b Heterolobosea

Heterolobosea (or Percolozoa) form a small group (ca. 140 described species) of heterotrophs, either free living (e.g. feeding on bacteria) or parasites. The typical life cycle alternates between amoeboid, flagellate, and resting stages, some of which can be absent depending on lineage. Acrasid slime moulds have developed an additional multicellular stage: when starving, individual amoebae aggregate to form a simple multicellular fruiting body or sorocarp (Bonner 2003). However, unlike the dictyostelid slime moulds (which belong to a totally different lineage, the Amoebozoa), the stalk-forming cells do not lose their viability. Amoebae were suggested to represent the diploid stage, and flagellates the haploid and gametic stage (Droop 1962; Fulton 1993), but confirmation is needed. Evidence for sexuality comes from studies on *Naegleria*, a genus comprising several parasites (e.g. *N. fowleri* causes primary amoebic meningoencephalitis). Natural populations of *N. lovaniensis* provide evidence for segregation and recombination (Pernin *et al.* 1992). Functional meiotic genes are found in *N. gruberi* (Fritz-Laylin *et al.* 2010), which also shows the level of heterozygosity typical for sexual reproduction.

2.2.1c Metamonada

Metamonads are unicellular heterotrophic flagellates, free-living, parasitic, or symbiotic (some digest cellulose in the gut of termites). All are anaerobic and lack some organelles, in particular typical mitochondria. Losses are secondary, however, as shown by reduced mitochondria-related

Table 2.1a Phylogenetic overview of sex determination systems among non-Metazoan eukaryotes

Taxon		Approx. number described (estimated) species	CYCLE					
			Haplontic	Haplodiplontic	Diplontic	Sex determination TYPE		
			1.1 Haplo-genotypic	1.2 Epigenetic	2.1 Haplo-genotypic	2.2 Epigenetic	3.1 Diplo-genotypic	3.2 Epigenetic
Excavata	Euglenozoa	1,100					✓	✓
	Heterolobosea	140			?			
	Metamonada	??					?	
Stramenopiles	Synurophyceae	250	✓					
	Chrysophyceae (Golden algae)	1,000	✓					
	Xanthophyceae (Yellow-green algae)	600	✓	✓				
	Diatoms (Diatoms)	10,000 (100,000)				✓	✓	
	Phaeophyceae (Brown algae)	2,000			✓	✓	✓	✓
	Slopalinida	200					✓	
	Oomycota (Water moulds)	500					✓	✓

Table 2.1a *continued*

Taxon		Approx. number described (estimated) species	CYCLE								
			Haplontic	Haplodiplontic	Diplontic	Sex determination TYPE					
Alveolates	Apicomplexa (Sporozoa)	5,000	✓	✓			2.1 Haplo-genotypic	2.2 Epigenetic	3.1 Diplo-genotypic	3.2 Epigenetic	3.3 Secondary mating types
	Dinoflagellates	2,000	✓	✓							
Rhizaria	Ciliates	8,000							✓	✓	
	Cercozoa	??			?						
Hacrobia	Radiolaria	10,000									
	Foraminifera	4,000			✓	✓					
	Haptophyta	500			?						
	Cryptophyta	150			?						

organelles (mitosomes). Diplomonads comprise important parasitic species (e.g. *Giardia intestinalis*). Life cycles are diplontic, alternating between a bi-nucleated flagellated trophozoite and a four-nucleated cyst (resting stage, allowing host-to-host transmission and survival of unfavourable conditions). Upon germination, the cell undergoes one cell division followed by one nuclear and cellular division, producing four binucleate trophozoites. Sex was only recently revealed by identification of

meiosis-specific genes (Ramesh *et al.* 2005; Birky 2005), expressed during the cyst stage, where karyogamy and genetic exchange occur (Poxleitner *et al.* 2008). Population genetic analyses also show between-individuals genetic exchange and recombination (Cooper *et al.* 2007; Andersson 2012).

2.2.2. Stramenopiles

The stramenopiles (or heterokonts, or Chromista), which comprise more than 14,000 described

Table 2.1a *continued*

Taxon		Approx. number described (estimated) species	CYCLE					
			Haplontic	Haplodiplontic	Diplontic	Sex determination TYPE		
			1.1 Haplo-genotypic	1.2 Epigenetic	2.1 Haplo-genotypic	2.2 Epigenetic	3.1 Diplo-genotypic	3.2 Epigenetic
Archaeplastida	Rhodophyta (Red algae)	5,000			✓			
	Chlorophyta (Green algae)	4,300	✓	✓	✓	✓	✓	✓
	Streptophyta (Charophyta + land plants)	300,000	✓	✓	✓	✓	✓	✓
	Amoebozoa	??	✓	✓	✓			
Fungi	Chytridiomycota	1,000	✓	✓	✓	✓		
	Zygomycota	1,060	✓	✓				
	Microsporidia	1,300						
	Glomeromycota	200						
	Dikarya	95,000			✓	✓		

species, are often united with Alveolata into the Chromalveolata (or Halvaria), which, together with the Rhizaria, form the SAR supergroup. Ancestral stramenopiles acquired a photosynthetic ability via a secondary endosymbiosis with archaeplastid algae. This ability was maintained in several photosynthetic or mixotrophic lineages (Synurophyceae, Chrysophyceae, Xanthophyceae, diatoms,

and Phaeophyceae) but secondarily lost in Oomy-cota and Slopalinida, which evolved saprophytic, commensal, or parasitic habits.

2.2.2a *Synurophyceae*, *Chrysophyceae* and *Xanthophyceae*

The Synurophyceae (e.g. *Synura*) and Chrysophyceae (golden algae; e.g. *Dinobryon*) are mixotrophic

heterokonts, mostly found in freshwater. They are mainly unicellular, with some filamentous, branched (*Dinobryon*), or spherical colonial forms (*Synura*). The main life stage is haploid (zygotic meiosis). Sexual reproduction, rarely documented, is heterothallic in *Dinobryon* (Ochromonadales) and *Synura* (Synurales), with anisomotile gametes (Bell & Hemsley 2000), suggesting haplo-genotypic sex determination (TYPE 1.1a). The Xanthophyceae (yellow-green algae) comprise little more than 600 sessile or free-living species, found primarily in freshwater, though some live in marine waters, in damp soil, or on tree trunks. About two-thirds are flagellated unicells, and most of the remainder are filamentous or siphonous (coenocytic), such as the water-felt *Vaucheria*. Life cycles are haplontic. Sexual reproduction is only known from three genera: the filamentous *Tribonema* show isogamy with anisomotility (one gamete settling down before the other unites with it; Scherffel 1901). The siphonous *Botrydium* can be either isogamous or anisogamous (e.g. *B. granulatum*; Miller 1927), and heterothallic or homothallic; unfused gametes can develop in a new thallus. *Vaucheria* are oogamous, with heterothallic species in marine environments and homothallic in freshwater (fusion taking place between gametes from the same filament TYPE 1.2a; Bell & Hemsley 2000). In *Vaucheria sessilis*, the antheridia and oogonia are formed close to each other. Antherozoids actively penetrate oogonia and fertilize the oospheres, after which the zygote enters a resting period of several months before meiosis and germination occurs. Temperature affects sex expression: in *Vaucheria repens*, both male and female organs are produced at 12°C, but only antheridia at 25°C (Klebs 1896 in Moewus 1957).

2.2.2b Diatoms

The diatoms, differentiated since ~250 Mya, form a highly successful group of unicellular photosynthetic heterokonts, with an estimated more than 100,000 living species. Pelagic species constitute a major component of marine phytoplankton, responsible for algal blooms, and account for some 20% of the net primary production on Earth. Life cycles are diplontic, a rare feature among algae, shared with a few other heterokonts (e.g. *Fucus*). The diploid vegetative cell is protected by a siliceous cell wall

(the frustule) made of two valves unequal in size: the bigger epivalve fits onto the smaller hypovalve like a lid onto its box. During vegetative division, each daughter cell inherits a maternal valve as the epivalve and synthesizes a new hypovalve. Hence, one daughter cell is as large as its mother, while the other is smaller. The average size of the population thus decreases with successive divisions (the McDonald-Pfizer rule). Sexual reproduction allows restoration of initial cell sizes: during gametogenesis, the old frustule is discarded, and the zygote matures into an auxospore that expands in size. A new cell is then formed inside the auxospore envelope, which begins a new round of vegetative multiplication. Sexual reproduction and cell size restitution are thus uniquely linked in diatoms. Another feature is that only cells below a specific size threshold can initiate gametogenesis (i.e. sex induction is size-dependent). Chepurnov *et al.* (2004) review experimental studies on sexual reproduction in diatoms.

Based on cell wall morphology, diatoms are divided into the basal and mostly planktonic Centrales (centrics), and the derived and mostly benthic Pennales (pennates). With respect to sexual reproduction, the centrics are both oogamous (producing eggs and sperm) and 'homothallic' (clones derived from the same zygote can develop into either males or females; TYPE 3.2c). Given suitable environmental conditions, gamete formation occurs spontaneously in cells that have passed below the critical size threshold. This threshold is often higher for the production of eggs than for sperm, implying that sex determination is partly size-dependent. Vegetative cells differentiate into 'gametangia' (though 'gamonts' would seem a more appropriate term) and undergo meiosis. All haploid nuclei survive during spermatogenesis, and undergo further mitoses to generate many small uniflagellate spermatozoids (4–128 per gametangium). During oogenesis, in contrast, one or two large eggs (depending on groups) remain per gametangium (other meiotic products degenerate). Plastid inheritance is maternal.

In pennates, reproduction involves gamontogamy, i.e. the meeting of two diploid gametangia, followed by meiosis and exchange of gametes. Fragilariphycaceae (= araphids) often display some gametic asymmetry, which may only be

behavioural (e.g. anisomotility in *Pseudo-nitzschia*) but is more often also morphological (anisogamy, with smaller motile male gametes and larger sessile female gametes; e.g. *Tabularia*). Oogamy is rare (e.g. *Rhabdonema*, *Grammatophora*), and may represent a transitional stage between the ancestral oogamy found in centrics and the isogamy of diverged pennates (Davidovich *et al.* 2010). Clones are differentiated into males and females (Chepurnov *et al.* 2004), normally found in equal sex ratios (Davidovich *et al.* 2010). Sex determination is probably diplo-genotypic (e.g. Mann *et al.* 2003), with, for example, male heterogamety (TYPE 3.1a) suggested in *Tabularia tabulata* (Davidovich & Davidovich 2011). Once within the sexually inducible size range, gametogenesis is triggered by cell-cell interaction between gametangia of compatible mating types or sexes (mediated by pheromone/receptor interactions in *Pseudostaurosira*; Sato *et al.* 2011). Each partner produces one or two gametes depending on lineage (the other meiotic products degenerate). The gametes from the male partner then reach the female stationary gametes and fuse to produce one or two auxospores. Although heterothallism is the rule, homothallism may occasionally occur, either in male clones only (*Tabularia fasciculata*; Davidovich *et al.* 2010) or in both sexes (*T. tabulata*; Davidovich & Davidovich 2011). Plastid inheritance may be independent of mating types or sexes: in *Pseudo-nitzschia*, both gametes contribute two plastids each. Out of the four, two are randomly eliminated from the zygote, independent of parental origin (Levialdi Ghiron *et al.* 2008).

Bacillariophyceae (= raphids), which outnumber both centrics and araphids (from which they diverged), present a large diversity of ecologies and mating systems. Obligate dioecy (heterothallism; TYPE 3.1) is found in several members, and might represent the ancestral state. In *Haslea*, *Eunotia*, or *Seminavis*, behaviourally and morphologically identical gametes fuse following the pairing of compatible gametangia (two zygotes being produced by paired gametangia; e.g. Mann *et al.* 2003; Davidovich *et al.* 2009). The two mating types of *Seminavis robusta* are determined by a single locus on linkage group (LG)6, which is heterozygous in MT+ and homozygous in MT- (Vanstechelman *et al.* 2013). Experiments in *Seminavis* have shown

high tolerance to inbreeding: mating between sibling clones of opposite mating types is highly successful, even for clones derived from inbred parents (Chepurnov *et al.* 2008). Although morphological anisogamy was only described from *Nitzschia longissima* (with male and female clones), isogamy with anisomotility occurs in several genera. Anisomotility may be cis-behavioural, with both motile gametes produced by the same partner (as in araphids, and consistent with diplo-genotypic sex determination; TYPE 3.1). It may also be trans-behavioural, however, with both partners producing one migrating and one stationary gamete each (similar to Ciliates; TYPE 3.2b), as observed in several species of *Cymbella*, *Gomphonema*, *Placoneis*, *Lyrella*, *Nitzschia*, or *Neidium*. Homothallism is not rare: in *Nitzschia lanceolata*, each of the two mating types is capable of limited intraclonal reproduction. In *Seminavis robusta*, it only occurs in the heterogametic (MT+) mating type. In *Sellaphora bisexualis*, cells of the same diploid clone can differentiate into either males or females (TYPE 3.2c as in centrics; Mann *et al.* 2009). *Achnanthes longipes* presents unisexual clones of either sex (which are obligate outbreeders) together with bisexual clones that can mate with either of the unisexual clones, and may also self (homothallism). Some raphids (*Cymbella*, *Gomphonema*, *Nitzschia*) may also reproduce by paedogamy (fusion of the two gametes from the same gametangium, similar to the autogamy in ciliates; TYPE 3.2a).

The evolutionary trend in diatoms from oogamy to isogamy seems a remarkable exception to the rule that oogamy normally derives from isogamy. The point must be made, however, that copulation in pennates occurs between gametangia, not gametes (possibly linked to their high-density benthic habitats; Mann 1993). There is thus no advantage in making more gametes than necessary to produce the final number of zygotes (Cox & Sethian 1984, 1985).

2.2.2c Phaeophyceae

With some 2000 mostly marine species, the brown algae are one of the few eukaryotic lineages that evolved large multicellular forms with differentiated tissues (e.g. holdfast, stipe, thallus, and receptacles). An ancestral isomorphic haplo-diplontic cycle (TYPE 2.1a) is found in early-diverged lineages,

while later-diverged groups often evolved an expansion of the sporophytic stage and concomitant reduction of the gametophyte. This parallels an evolution from isogamy to anisogamy to oogamy, and from haploid mating types to diploid sexes.

Ectocarpales exhibit a variety of haplo-diplontic cycles, ranging from isomorphy to strong heteromorphy, where either the gametophyte or the sporophyte (e.g. *Scytophion*) is reduced. Both stages may reproduce asexually through **microspores**. In *Ectocarpus*, male and female gametes are produced in plurilocular gametangia formed by morphologically similar male and female gametophytes. Gametes only differ in behaviour: after release, female gametes quickly fix on the substrate and produce pheromones to attract actively swimming male gametes. The zygote develops into a small sporophyte morphologically similar to the gametophyte, which reproduces either vegetatively (via diploid spores produced by plurilocular sporangia), or sexually (through haploid meiospores produced in unilocular sporangia). The four spores resulting from a single meiosis develop into two males and two female gametophytes, sex being determined by allelic segregation at the MAT locus (TYPE 2.1a).

Anisogamy and oogamy are found in Sphaerelariales and Dictyotales. *Sphaerelaria rigidula* presents a slightly heteromorphic haplo-diplontic cycle (gametophytes being slender than sporophytes) with haplo-genotypic sex determination (TYPE 2.1a; half of meiospores develop into male gametophytes, the other half in female gametophytes) and anisogamy. Unfertilized **macrogametes** can develop into female gametophytes or haploid sporophytes. *Dictyota dichotoma* has a haplo-diplontic cycle with isomorphic gametophytes and sporophytes. Sex determination is haplo-genotypic (TYPE 2.1a): the four meiospores produced in the **tetrasporangia** of sporophytes develop into two male and two female gametophytes, producing antheridia and oogonia respectively. Oogonia produce a single egg each, which is released in seawater, where it attracts male gametes via a pheromone (dictyotene).

Life cycles are strongly heteromorphic in the oogamous Laminariales. Egg cells, released by microscopic female gametophytes, use pheromones to attract the flagellated swimming sperm produced by similar-sized (though slightly differentiated)

male gametophytes. The zygote develops into a macroscopic sporophyte, which can reach several metres in *Laminaria*, and up to 40–50 m in giant kelps (e.g. *Macrocystis*, *Nereocystis*). Haploid meiospores are produced in sporangia (two male and two female zoospores per meiosis), sex being also determined by allelic segregation at the MAT locus (TYPE 2.1a; e.g. Shan & Pang 2010). Sex chromosomes (U, V) have been proposed to occur in Laminariales (Evans 1963, 1965; Fang 1983), but with no definitive support. Temperature-dependent sex ratios have been found in *Laminaria religiosa* (Funano 1983) and *Lessonia variegata* (Nelson 2005), suggesting an epigenetic (environmental) contribution to sex determination. Monoecy (implying epigenetic sex differentiation; TYPE 2.2a) evolved in some Laminariales (*Chorda*), as well as in some Desmarestiales (*Desmarestia*) and Sporochnales (*Carpomitra*), all of which are oogamous (refs in Bell 1997).

Diplontic and sub-diplontic life cycles (i.e. with no free-living gametophytic stage) are found in Fucales and Ascoseirales. The diploid male and female organs (antheridia and oogonia respectively), developing in ‘conceptacles’ at the apex of sporophytic fronds, directly produce haploid sperm and eggs via meiosis (TYPE 3.2a). In *Cystoseira*, the male meiosis is followed by four mitoses, producing a total of 64 sperm cells, while the female meiosis is followed by a single mitosis, producing eight haploid nuclei, of which one is retained as an egg cell. Sex differentiation is thus epigenetic, resulting from differential gametogenesis of sporophytic tissues (as also occurs in hermaphroditic plants and animals). Eggs released in seawater produce pheromones to attract sperm. The zygote then fixes to the substrate and develops into a large-sized sporophyte, which may be hermaphroditic (such as in *Fucus spiralis*, with antheridia and oogonia in the same conceptacles) or monoecious (as in *Xiphophora*, with sex organs on different conceptacles). Dioecy evolved in a few species (e.g. *Fucus vesiculosus*, *Ascophyllum nodosum*), with individual sporophytes specializing into either male or female function. Diplo-genotypic sex determination involving sex chromosomes was suggested in Fucales (Clayton 1984). Results from crosses between the hermaphroditic *F. spiralis* and the dioecious *F. vesiculosus* seem consistent with a simple male-heterogametic sex determination

system (TYPE 3.1a; Billard *et al.* 2005). These patterns parallel the evolution from hermaphroditism to dioecy via gynodioecy, as observed in some angiosperms such as *Silene* (Box 3.10).

2.2.2d Slopalinida

Slopalinida include Opalinidae and Proteromonadidae. Proteromonads (five species described) are biflagellate unicells, endo-commensals of lizards (*Proteromonas lacertae*) but also found in caudate amphibians and mammals. Cycles are poorly known. The currently 200 recognized species of Opalinidae are mostly endo-commensal in the intestine and cloaca of Anura (but may also be found in reptiles, fishes, or invertebrates). All show similar diplontic life cycles (gametic meiosis) with anisogamy. *Opalina ranarum* lives as a large, multinucleate trophont (up to 2000 nuclei) in the adult anuran cloaca, where it grows and divides asexually (with frequent nuclear divisions to maintain a large number of nuclei). As the host's breeding season approaches, the trophont undergoes a series of cell divisions but stops nuclear divisions. The resulting opalines become gradually smaller with fewer (2–12) nuclei. At some point, they encyst and are released with the faeces into the breeding pond. Once eaten by foraging tadpoles, these gametocysts hatch, producing small multinucleate gamonts, which undergo further cytokinesis and meiosis to produce haploid uninucleate micro- and macrogametes. Sex is thus likely determined epigenetically (TYPE 3.2a). Fusion produces a zygocyst, which may be released with the faeces, reach another tadpole host and yield more gamonts, or hatch in its original host and grow again into a multinucleate trophont. Life-cycle transitions seem triggered by the host hormonal cycles.

2.2.2e Oomycota

Oomycetes, or 'water moulds', originally classified as fungi, form a group of some 500 recognized species, including saprophytes and parasites (e.g. *Saprolegnia*, *Phytophthora*), some of which are devastating plant pathogens (e.g. late blight of potato, downy mildew of grape vine, sudden oak death, and root and stem rot of soybean). The nuclei of vegetative hyphae are typically diploid. There is no free haploid stage (i.e. meiosis is gametic). Anisogamy is strong, with highly differentiated antheridia

and oogonia. Syngamy requires a direct contact between male and female gametangia. The oogonium provides the cytoplasm and nutrient reserve for the zygote, while the male gamete only contributes its nucleus. The zygote is a resting stage, with a thick multilayered wall. After a period of dormancy, these oospores germinate to produce diploid hyphae, which may immediately produce a sporangium.

Many species are homothallic, oogonia being often fertilized by antheridia from the same individual (TYPE 3.2a). Homothally is prevalent in the basal clades (and thus might be the ancestral state) and in pathogenic lineages. A peculiar form of 'hormonal heterothallism' (secondary mating types) evolved in several lineages, including saprophytic ones, in which pheromones from complementary strains are required to reciprocally induce sexual development (TYPE 3.2b). Both strains produce male and female gametes, and the resulting zygotes can be either selfed or outcrossed (Judelson 2007, 2009). These 'induction types' (Ko 1988) are trans-specific (sexuality can be induced in a given *P. infestans* type by the complementary type from another *Phytophthora* species; Ko 1988), and bear some analogy with the mating types of ciliates and self-incompatibility systems of angiosperms and ascidians (which were also secondarily superimposed to pre-existing sexes).

Transitions to and from this form of heterothallism seem frequent (e.g. both homo- and heterothallic species are found in *Pythium* and *Phytophthora*), and mating type switches regularly occur in *Phytophthora* strains (Ko 1988). The mechanisms apparently rely on the alternative expression or repression of two linked pairs of genes encoding complementary pheromones and receptors (see Section 3.4.2b for details). Homothallic species (e.g. in *Phytophthora*) can synthesize and express both mating pheromones and receptors, allowing the sexual cycle to be triggered in isolation.

Homothallic lineages may also specialize into a predominant male or female role depending on the mating partner (Barksdale 1967), sometimes leading to strictly male or female individuals (such as in the 'dioecious' *Saprolegniales* *Dictyuchus monosporus*, *Achlya bisexualis* or *A. ambisexualis*). The 'female' individual secretes pheromone A (steroid), to which its partner reacts by producing branches

on which antheridia are formed and meiosis occurs. The pheromone A simultaneously inhibits the formation of oogonia by the ‘male’ partner. Meanwhile, this sexually activated male secretes a second pheromone B, to which its female partner reacts by producing oogonia and entering meiosis. Fertilization is achieved by the transfer of male nuclei through tiny tubes grown from the antheridium to the eggs. Sexes seem to only differ regarding pheromone A: males produce less of it, and are more sensitive to it. Hence sex roles are relative, not absolute. A given genotype may act either as male or female, depending on its partner (a nice example of G × E sex determination). This establishes a gradient, from strong females at one end (always producing oogonia) to strong males at the other end (always producing antheridia). The same pheromone A is active in different species, hence allowing the sexual cycle to be triggered by interspecific interactions. As *A. bisexualis* produce more of the pheromone A than *A. ambisexualis*, a male *bisexualis* will behave as a female when mated with a male *A. ambisexualis*. Dioecy is thus clearly conditional. If mates are lacking, an individual will produce both oogonia and antheridia and self-fertilize.

2.2.3 Alveolates

Alveolata, often united with stramenopiles into the chromalveolates (and with Rhizaria into the SAR supergroup), form a diverse group of unicellular eukaryotes, including mainly the Apicomplexa, Dinoflagellata, and Ciliophora.

2.2.3a Apicomplexa

Apicomplexa (or Sporozoa) comprise some 5000 species, grouped into three subclasses: Gregarinina (gregarines), Coccidiásina (Adeleorina, Eimeriorina, and Haemospororina), and Piroplasmáina. All are exclusively parasites of animals. Life cycles are haplontic (the diploid stage being reduced to a resting zygote, or oocyst) and may be complex. The sporozoites, produced by meiosis, infect host tissues, where they may undergo one or more cycles of asexual reproduction. The resulting merozoites enter the sexual cycle by differentiating into gametocytes, which are sexually dimorphic in Coccidiásina.

Fertilization in gregarines, piroplasms, and adeleorins occurs through a process called syzygy, which seems ancestral to Apicomplexa: the male and female gametocytes (called gamonts) unite pairwise and encapsulate. Both partners then produce multiple gametes, which may be isogamous or anisogamous. Multiple oocysts are subsequently formed by the pairwise fusion of male and female gametes.

Gregarines have single hosts (monoxenous), which are always invertebrates. Diploid oocysts, ingested with the food, divide meiotically to form four or more sporozoites (depending on species), which penetrate the host cells and develop into larger trophozoites. Sporozoites and trophozoites may undergo asexual replication in some species. Two mature trophozoites (gamonts) eventually pair up in syzygy, and undergo multiple fissions to produce male and female gametes (often anisogamous). The oocysts formed by the fusion of two gametes are then released in faeces and later ingested by a new host. As male and female gamonts are not differentiated morphologically, sex ratios are unknown. The exact timing and mode of sex determination is also unknown. If genetic (TYPE 1.1a), then the mode of transmission (ingestion of diploid oocysts rather than post-meiotic sporozoites) should ensure an equal sex ratio. In piroplasms, similarly, male and female gamonts are not differentiated morphologically; sex ratios and sex determination are thus poorly known. In *Babesia*, the gametocytes are transferred from a mammal to a tick (the definitive host), mating occurs in the tick gut, and sporozoites then settle in salivary glands to be later re-injected. Adeleorina (haemogregarines) are also mostly heteroxenous, with a vertebrate as intermediate host (often an amphibian or a reptile), and an invertebrate as definitive host and vector. Many species present two morphologically distinct types of meronts, some producing large merozoites which initiate a further round of asexual replication; the other producing smaller merozoites, which are the progenitors of the gamonts. Gamonts may also show sexual dimorphism, with macrogamonts and microgamonts uniting in syzygy within the definitive host, then producing male and female gametes. This **anisogamy** allows measurements of sex ratios, which seem equal (West *et al.* 2001). Either oocysts or sporozoites may be the units of transmission to the intermediate host.

Syzygy does not occur in the more derived Eimeriorina and Haemosporidia. Male and female gametocytes are transferred to the definitive host (e.g. a cat in the case of *Toxoplasma*; a mosquito in the case of the malaria parasite *Plasmodium*) before producing free male and female gametes, which then fuse to produce oocysts. Haemosporidian cycles involve two hosts (heteroxenous), usually a vertebrate and an invertebrate. In *Plasmodium falciparum*, the sporozoites localized in the mosquito salivary glands are injected during a blood meal, and reach liver cells where they undergo asexual reproduction (schizogony). The resulting merozoites invade erythrocytes where they grow into 'ring forms' (early trophozoites). Each trophozoite then undergoes multiple rounds of nuclear division without cytokinesis, resulting in a multinucleate schizont. Merozoites budding from the mature schizont are then released to infect other erythrocytes. Some ring forms develop into micro- or macro gametocytes, which are then taken up in the mosquito blood meal. Gametogenesis occurs within 10–15 min in the insect gut. The macro gametocyte differentiates into a single female gamete, while the micro gametocyte undergoes exflagellation to produce male gametes (2–8 in *Plasmodium*). After fertilization, the zygote transforms into a mobile ookinete, which encysts into the stomach wall of the mosquito. Meiosis then produces several hundreds of haploid sporozoites that invade salivary glands, to be injected into a new vertebrate host.

Infection by a single haploid clone results in development of gametocytes of both sexes (homothallism). Sex determination is thus not genetic, but triggered by environmental cues at some point during the life cycle (TYPE 1.2b). All merozoites stemming from a single schizont are committed to either an additional asexual cycle, or to differentiation into gametocytes. In the latter case they all form gametocytes of the same sex, suggesting that sex determination occurs during the trophozoite stage which develops into a gametocyte-producing schizont (even though this stage is not differentiated morphologically). Gametocyte sex ratio is often female biased. The proportion of males increases with host erythropoiesis, itself induced in response to increased anaemia (Paul *et al.* 2002). Sex ratio also changes in response to the presence of unrelated

conspecifics, though exact cues are unknown (Reece *et al.* 2008).

Eimeriorins are also homothallic, with gametocysts of both sexes produced by a single clone (TYPE 1.2b). The gamontoblasts committed to either asexual or sexual reproduction show early morphological differentiation, as do male and female gametocysts. Sex ratios among gametocysts are extremely female biased (West *et al.* 2001), and each micro gametocyst may produce many male gametes (up to 1000).

2.2.3b Dinoflagellata

Dinoflagellates form a group of highly successful alveolates, comprising heterotrophic, mixotrophic, or photosynthetic forms, some of which are involved in algal blooms ('red tides'). They are among the most important primary producers in oceans (second only to diatoms). Zooxanthellae, which are endosymbionts of animals, play an important role in coral reef biology. Most of the 2000 or so known living species are marine, while some 220 live in freshwater (Taylor *et al.* 2008). Dinoflagellates exist as biflagellated cells, coccoid stages, or plasmodia (i.e. multinucleate organisms). A small group forms cell arrays that approach multicellularity.

Most dinoflagellates (grouped into Dinokaryota, as opposed to Syndiniales) possess a particular form of nucleus (dinokaryon) in which chromosomes are attached to the nuclear membrane, lack histones, and remain condensed during interphase (not only during mitosis). Typical genomes are huge (larger than most eukaryotes examined to date), and have undergone a series of reorganizations including massive amplification and recombination, with multiple copies of each gene, and gene fragments linked in numerous combinations (Lin 2011).

Cycles are haplontic, with a few exceptions (*Noctiluca* is diplontic). Reproduction is asexual during the proliferation phase (cell fission), the sexual cycle being switched on when conditions worsen. Gametes resemble regular motile cells, and may resume asexual reproduction if unmated. The first meiotic division directly follows nuclear fusion. The mobile zygote (planozygote) grows in size, and may then either finalize meiosis and restore the vegetative stage, or form a resting hypnozygote (dinocyst). The second meiotic division eventually occurs, all

but one of the meiotic products disappear, and a single haploid vegetative cell emerges from the cyst (sex is thus totally decoupled from reproduction).

Gametes may be isogamous or anisogamous, sometimes within the same species (e.g. *Alexandrium tamarensense*). Similarly, both homothallic and heterothallic strains are known, sometimes within the same species. Heterothallism may be simple (with two complementary mating types such as in *Lingulodinium polyedrum*) or complex, with more than two sexual types (e.g. *Alexandrium minutum*, Figueroa *et al.* 2007; *Gymnodinium nollerii*, Figueroa & Bravo 2005), and possibly several loci involved. Different mating systems can apparently occur within the same species, with, for example, a continuum between homothally and heterothally (such as in *Gymnodinium catenatum*; Figueroa *et al.* 2010) or a continuum of mating affinities (e.g. *Alexandrium excavatum*; Destombe & Cembella 1990). However, the zygotes resulting from self-fertilization seem unable to germinate or to complete meiosis, suggesting that gametic recognition, meiosis, and zygote germination are controlled by independent loci (Destombe & Cembella 1990). Mating affinities may also change with age, akin to the epigenetic processes (maturation) found in ciliates.

2.2.3c Ciliates

Despite being mostly mobile single-cell heterotrophs (with a few branching colonies among sessile species), ciliates present a large diversity of body sizes, forms, and feeding ecologies, including filter feeding, predation, mutualisms, or parasitism. Some are photosynthetic, sequestering functional chloroplasts from their prey, which contribute to their carbon and energy budget. Although unicellular, ciliates evolved a separation of soma (controlled by a highly polyploid macronucleus) and germ line (represented by one or more diploid micronuclei). The micronucleus genome is transcriptionally silent in vegetative cells, while the macronucleus genome is the primary source of gene transcripts, and directly determines cell phenotype (including its mating type). Macronuclei undergo a form of senescence through successive divisions and must be regularly rejuvenated via a sexual cycle. A period of maturation is also observed before a newly produced clone can enter a sexual cycle.

Life cycles are strictly diplontic. Sexuality, which is decoupled from reproduction, consists in an exchange of micronuclei between two morphologically identical gamonts of compatible mating types (secondary mating types; TYPE 3.2b). Recognition and pairing occurs through mating-type specific molecules, for example, pheromones and receptors (see Section 3.4.2a for molecular mechanisms). At conjugation, the micronucleus of each partner enters meiosis. One daughter nucleus disintegrates after each maturation division, and the only remaining haploid nucleus is then duplicated by mitosis, resulting in two genetically identical products. One of these, the migratory (male) nucleus, is then reciprocally exchanged, and fuses with the partner's stationary (female) nucleus. Hence, both partners are simultaneously donor (male) and receiver (female). As the two nuclei from any partner are genetically identical, the ex-conjugants also inherit identical diploid micronuclei. Mitochondria differ, however, being inherited from the 'female' parent (thus, mating types play no role in controlling mtDNA transmission).

In each ex-conjugant, the new micronucleus then enters mitoses, which numbers may differ among groups. In *Tetrahymena thermophila*, for instance, two mitotic divisions produce four identical diploid nuclei (Orias 1981). Two of them move to the posterior cytoplasm and remain diploid micronuclei, while the two others move to the anterior cytoplasm and differentiate into macronuclei. This differentiation involves a drastic reorganization of the genetic material, with excisions of many sequences, including transposable elements and other repeated or unique sequences. In *T. thermophila*, 15% of DNA sequences are lost in the process. In addition, the genome is cleaved in many small fragments ('chromosomes'), each comprising a few hundred genes in *T. thermophila*, but as few as a single gene in other clades (Orias *et al.* 2011). These fragments are differentially amplified, some being found in hundreds or thousands of copies. Depending on species, this reorganization may be controlled by the new diploid micronucleus, the old maternal macronucleus, or by random and environmental factors (e.g. temperature; Preer 2000). These different mechanisms determine three main categories of mating type inheritance (see below).

Following this process, the old maternal macronucleus disintegrates, and the two conjugants separate. Ex-conjugants undergo their first post-zygotic cell division, forming ‘caryonids’ with identical micronuclei, but independently derived (and thus possibly different) macronuclei. These caryonids then enter asexual reproduction, which occurs through cell division (rarely budding or multiple fission). Micronuclei go through a proper mitosis, while macronuclei divide amitotically. The genetic material is thereby randomly allocated between daughter cells, so that drift induces a progressive segregation of alleles: cells stemming from the same caryonid may have different macronuclei, and thus display different phenotypes. In particular, heterozygosity decreases with successive rounds of fission, which may affect mating type expression (Orias 2000). Maturation also induces a form of senescence, the longevity of a macronucleus being determined by telomere lengths, which sets a maximum for the number of fissions. A new round of conjugation will be necessary to generate a new macronucleus from the germ line.

The sexual cycle can be achieved by autogamy in some lineages (TYPE 3.2a; e.g. *Euploites*, *Paramecium*). Meiosis occurs like in a normal conjugation, three haploid products being discarded and the only one left being duplicated by mitosis. These two identical haploid nuclei subsequently fuse to produce a diploid micronucleus, which then divides by mitosis and produces a new macronucleus. The new genome is thus necessarily homozygous (which might induce a switch of mating type).

Anisogamonty evolved in a few groups, in particular the sessile Chonotrichia, Suctoria, and Peritrichia (e.g. *Vorticella*), which also divide by budding. Unequal cell division results in a macrogamont (which remains sessile) and a microgamont, which detaches from its pedicel and swims freely to reach a macrogamont. Both partners enter meiosis, followed by a mitosis. All nuclear products but one disappear in each conjugant. The microgamont is then absorbed, resulting in nuclear and cytoplasmic fusion.

Mating types have long been known in ciliates (e.g. Sonneborn 1937), and found in almost all species investigated, in numbers varying from two to 12 or more (up to 100 in *Stylonychia mytilus*; Ammermann

1982). A binary system seems ancestral (Phadke & Zufall 2009). Mating is sometimes possible within a single clone, as a result of either mating type switch or homothallism (homotypic mating).

As for other phenotypic traits, mating type expression depends on the macronucleus, so that mating-type inheritance directly depends on the way macronuclei are formed from the initial micronucleus. When macronucleus differentiation is entirely controlled by the newly formed micronucleus, all caryonids resulting from a conjugant pair inherit the same mating type (genetic determination of secondary mating types). This ‘synclonal’ inheritance, seemingly the ancestral state, is found, for example, in *Paramecium caudatum* and *P. bursaria*. The two mating types (O and E for Odd and Even) are determined by a bi-allelic mating-type locus Mt (see Section 3.4.2a), O being homozygous recessive (*mm*) and E heterozygous dominant (*Mm*). As conjugation occurs between *Mm* and *mm* individuals, all resulting caryonides will be of the same type, either E (*Mm*) or O (*mm*) depending on which allele (*M* or *m*) is left and duplicated in conjugant E after the last meiotic division. Note that a type E clone can switch to O by losing its *M* allele during successive macronuclear fissions or during autogamy, but the reverse switch (from O to E) cannot happen, for obvious reasons. Spirotrichs also have synclonal inheritance, with multiple mating types. *Euploites patella*, for instance, has ten mating types, determined by one locus with four co-dominant alleles (four mating types being homozygous, and six heterozygous; Akada 1985).

When the differentiation of the new macronucleus is controlled by the old one, mating type will show ‘cytoplasmic’ inheritance, resulting from maternally inherited epigenetic effects (Duharcourt *et al.* 1995; Preer 2000). In such a case, all caryonids stemming from one ex-conjugant display the same mating type (inherited from their mother cell), while those born to the partner express a complementary mating type (despite having identical germ line). This mode was only described from some *Paramecium* species (e.g. *P. biaurelia*, *P. tetraurelia*). In that case all mating types possess the dominant *M* allele, but whether it is expressed or silenced is controlled epigenetically (epigenetic determination of secondary mating types).

Finally, when random or environmental factors affect macronucleus differentiation, the caryonids stemming from the same ex-conjugant may all present different mating types. This form of ‘caryonal’ inheritance is found, for example, in *Paramecium primaurelia* or *P. triaurelia*, where epigenetic silencing of the *M* allele is random (and also depend on temperature; Sonneborn 1975). In *T. thermophila*, alleles at the MAT locus determine the distribution of probabilities with which one of the seven mating types will be expressed. Cells homozygous for *Mat-B* produce mating types II, III, IV, V, VI, or VII, at frequencies that depend on temperature. Cells homozygous for *Mat-A*, *Mat-C* or *Mat-D* may all produce mating types I, II, III, V, and VI, but at different frequencies, and also as a function of temperature (Orias 1981; Orias *et al.* 2011).

2.2.4 Rhizaria

Rhizaria, which are often united with chromalveolates (stramenopiles and alveolates) into the SAR supergroup, consist mostly of amoeboid and flagellate unicells. They comprise the Cercozoa and Radiolaria (which include Radiolaria and Foraminifera).

2.2.4a Cercozoa

Cercozoa form a very diverse, species-rich group of amoeboid and flagellate protozoa, abundant in soils, freshwaters, and marine habitats. They are mostly free-living and heterotrophic, feeding on bacteria, fungi, algae, other protozoa, or even small invertebrates by means of filose pseudopods. Some are parasitic, others (Chlorarachniophyta) benefit from the photosynthetic activity of endosymbiotic algae. Cercozoa are typically unicellular and uninucleate, and undergo binary fission. Some can form multinucleate plasmodia and undergo multiple fission. Cysts are widespread. Sex has only been documented with certainty in plasmodiophorids, which are obligate parasites of economic importance. The resting haploid meiospores of *Plasmodiophora brassicae* (cause of cabbage clubroot disease) germinate in a dead host cell. Large groups of germinating protoplasts coalesce to form multinucleate (possibly heterokaryotic) primary plasmodia. Following mitotic divisions and cytoplasm cleavage, zoosporangia are formed within the plasmodium, releasing biflagellate gametes that pair by

two (isogamy). Whether mating types exist is unknown. Karyogamy does not follow plasmogamy. The binucleate plasmodium invades a new host cell, where it develops by internal mitoses into a multinucleate secondary plasmodium. At each host cell division, the plasmodium also divides, infecting the two daughter cells. Karyogamy only occurs at host cell death, haploid nuclei fusing by pairs within the plasmodium. Meiosis follows immediately, and haploid resting meiospores are produced by cytoplasm cleavage (Tommerup & Ingram 1971).

2.2.4b Radiolaria

Radiolaria are amoeboid unicells with reticulose pseudopods, protected by a siliceous test that shows a radial symmetry. Planktonic and strictly marine, they feed on organic particles and bacteria as well as phyto- and zooplankton. Some have symbiotic algae. Reproduction is poorly known, mostly due to problems with keeping them alive in the laboratory. Asexual reproduction may occur through binary fission, multiple fission, or budding. The only evidence for sexual reproduction is the occasional production of numerous gamete-like biflagellated swimmers.

2.2.4c Foraminifera

With an estimate of 4000 living species, foraminiferans constitute a very important component of marine benthos, where they display a variety of feeding strategies. The calcareous test that typically protects the amoeboid cell can be quite elaborate in structure, with multiple chambers. Pseudopods are used for locomotion and capture of food (often diatoms or bacteria). A number of forms host unicellular algae from a diversity of lineages as endosymbionts, or sequester functional chloroplasts from ingested algae. The life cycle is typically haplo-diplontic and isogamous. The uninucleate haploid gamont divides to produce gametes, and the diploid zygote resulting from fertilization develops into a multinucleate diploid agamont, usually isomorphic to the gamont, but often slightly larger. Agamonts may show nuclear dimorphism in some lineages, similar to the one found in ciliates, with one large polyploid somatic nucleus, involved in cell metabolism, which degenerates at the onset of meiosis, and one to five generative diploid nuclei that remain inactive until

meiosis. After meiotic divisions, multiple fissions of the agamont produce new haploid cells, which may develop directly into new gamonts, or into schizonts that undergo an additional round of asexual reproduction. Such trimorphism is not uncommon among benthic forms. Some planktonic forms, on the other hand, have dropped the agamont stage and display a haplontic cycle with zygotic meiosis.

In the typical (and presumably ancestral; Goldstein 1997) gametogamous cycle, gamonts release up to thousands of biflagellate gametes directly in the seawater. Whether mating types exist is not known. In gamontogamous species, the gamonts associate in pairs or groups before releasing gametes, which are usually triflagellated or amoeboid. During plastogamy (similar to the syzygy found in Apicomplexa), two gamonts of complementary mating types attach to each other, and release gametes within the shared space. In species that associate in groups, both mating types must also be present for reproduction to occur (TYPE 2.1a). A few species show autogamy, where a single gamont produces amoeboid gametes that are not released, fertilization occurring within the parental test (TYPE 2.2a).

2.2.5 Hacrobia

Hacrobia form a large and diverse group of unicellular flagellates and amoebae, primarily found in marine and freshwater environments. Cryptomonads and haptophytes are important primary producers, while katablepharids, centrohelid Heliozoa, and telonemids are heterotrophs. Though molecular data suggest that all Hacrobia derived from a common ancestor that gained photosynthetic capacity via secondary endosymbiosis with a red alga (Archibald & Keeling 2002; Bhattacharya *et al.* 2004; Gould *et al.* 2008), they have no single morphological character in common and might be polyphyletic. Exact genetic relationships are likely obscured by the massive gene transfer from endosymbionts.

2.2.5a Haptophyta

Haptophytes comprise some 500 species of unicellular algae, an important component of the marine plankton. The Coccolithophora (Prymnesiophyceae) present haplo-diplontic cycles with heteromorphic stages (Billard 1994). Generations alternate seasonally between a planktonic diploid stage

(non-motile cells covered by large calcified scales, or 'heterococcoliths'), and a benthic haploid stage (biflagellate motile cells, which may be naked or protected by smaller scales, 'holococcoliths'). The two stages have often been described as different species. In *Pleurochrysis pseudoroscoffensis*, four non-calcified motile haploid cells (meiospores) are formed within a heterococcospore, and remain motile for some time before settling and dividing mitotically. Syngamy has rarely been directly observed. In *Coccolithus pelagicus*, gametes are isogamous and morphologically indistinguishable from vegetative haploid cells (Houdan *et al.* 2004). The two haploid nuclei seem to fuse immediately following cytoplasmic fusion (Gayral & Fresnel 1983). How sex is determined and whether mating types occur is unknown. Alternation of generations has not yet been demonstrated in the other Haptophyte class (Pavlovophyceae) but transitions from non-motile to motile cells are common, which may also correspond to haplo-diplontic cycles (Billard 1994).

2.2.5b Cryptomonads

The cryptomonads (or cryptophytes) are mostly photosynthetic biflagellate unicells living in marine and freshwater environments. Together with the chlorarachniophytes (Cercozoa, Rhizaria), the cryptomonads are unusual in having retained the vestigial nucleus of their endosymbiotic alga. This small 'nucleomorph' (three chromosomes in *Guillardia theta*, 551 kb total) is gene-poor (as a result of massive gene transfer) and highly compact, with very little non-coding DNA. Cycles are poorly known, and might also be haplo-diplontic (Hill & Wheterbee 1986; Hoef-Emden *et al.* 2002), with dimorphic haploid and diploid stages originally described as different taxa (e.g. the genus *Campylomonas* represents the alternate morph of *Cryptomonas*; Hoef-Emden & Melkonian 2003). Sexual reproduction has only been documented in one *Cryptomonas* (Kugrens & Lee 1988). The isolate used in this study was bisexual, with vegetative cells acting as isogametes.

2.2.6 Archaeplastida (Plantae)

Plants originated from an endosymbiosis, formed some 1 to 1.5 billion years ago (Hedges *et al.* 2004; Yoon *et al.* 2004), between a heterotrophic eukaryote and a cyanobacterium that became integrated

as a chloroplast (Archibald 2009; Keeling 2010). The subsequent diversification of this plastid-containing eukaryote gave rise to the glaucophytes (freshwater unicellular algae with no sexual cycle described), the rhodophytes (red algae), and the Viridiplantae (green lineage). The two latter groups independently evolved multicellularity and cell differentiation, particularly developed among the higher plants (embryophytes).

2.2.6a Rhodophyta

No sexual cycles have been observed in the unicellular, early-diverging lineages of rhodophytes, but population genetic analyses have shown that recombination occurs in *Galdieria* (Cyanidiales), an extremophile living in hot and acidic springs (Yoon *et al.* 2006). Multicellularity independently evolved in Bangiophyceae and Florideophyceae (Eurhodophytina). Bangiophyceae (e.g. *Porphyra*) are unusual in having bisexual gametophytes despite haplo-genotypic sex determination (pseudo-homothallism; TYPE 2.1b). The cycle is haplo-diplontic, with strong predominance of the haploid gametophyte. Diploid spores produced by a microscopic filamentous sporophyte ('conchocelis') undergo meiosis during germination. The whole tetrad contributes to the resulting gametophyte, with the two lower cells giving rise to the rhizoidal holdfast, and the two upper cells to the thallus blade. The gametophyte is thus a genetic chimera, comprising two cell lineages that differ at the sex-determination locus (Mitman & van der Meer 1994). Upon sexual differentiation, the two cell lineages produce male and female tissue respectively. Male gametes ('spermatia'), released from the blade margins, fertilize the trichogynes of carpogonia (female gametes), formed some way back from the margin. The 'carposporangium' that develops mitotically from the zygote then releases diploid **carpospores**, which germinate to form diploid conchocelis filaments.

Florideophyceae, which comprise the majority of large red algae, present triphasic life cycles ('*Poly-siphonia*' type), with haplo-genotypic sex determination (TYPE 2.1c). Isomorphic male and female gametophytes produce spermatia and carpogonia respectively. The zygote, retained on and fed by the female gametophyte, develops into a diploid carposporophyte that releases numerous diploid

mitospores. The resulting tetrasporophytes then produce meiospores, the four meiotic products developing into two male and two female gametophytes. Gametophytes and tetrasporophytes are usually isomorphic. Interestingly, Florideophyceae occasionally present 'mixed phases', with sexual organs (spermatia and carpogonia) developing directly from diploid tetrasporophytic tissues, together with tetrasporangia. As shown in *Gracilaria*, this results from asymmetric allelic segregation during the differentiation of reproductive structures, stemming from mitotic recombination (van der Meer & Todd 1977) or random segregation of chromosomes during depolyploidization (Goff & Coleman 1986; Haig 1993). Tetrasporophytic tissues that are recombinant for mating type (and thus homozygous mt^m/mt^m or mt^f/mt^f) enter the male or female gametophytic programme respectively. The resulting diploid spermatia (mt^m/mt^m) can fertilize haploid carpogonia (mt^f), but the resulting triploid tetrasporophytes are not fertile, due to the problems inherent to triploid meiosis. In *Griffithsia*, however, mixed phases seem to occur on haploid thalli: male or female gametophytes sometimes also produce tetrasporangia (Lee *et al.* 1995).

2.2.6b Viridiplantae

The Viridiplantae, or 'green lineage', comprises the Chlorophyta (most green algae) and the Streptophyta (charophytes and land plants). Life cycles seem originally haplontic (zygotic meiosis), but haplo-diplontic and diplontic life cycles independently evolved in the Ulvophyceae, Bryopsidophyceae, and Embryophyta.

Among **Chlorophyta**, the paraphyletic Prasinophyceae comprise a group of early-diverged classes of unicellular chlorophytes, predominantly marine and planktonic, with haplontic life cycles. Sexual reproduction has only been documented in a few species. In *Nephroselmis olivacea*, morphologically similar gametes of opposite mating type meet to form a resting zygote with a cell wall (TYPE 1.1a). The two cells emerging at meiosis further divide to produce four haploid vegetative individuals (Suda *et al.* 1989, 2004). Resistant cysts with two chloroplasts have been documented in other species, suggestive of sexual reproduction (Moesstrup *et al.* 2003). Sexual reproduction has also been implied in *Micromonas*

and *Ostreococcus* based on the presence of meiosis-specific and sex-related genes (Derelle *et al.* 2006; Worden *et al.* 2009; Grimsley *et al.* 2010).

The Prasinophyceae gave rise to the core Chlorophyta, including the Ulvophyceae, Trebouxiophyceae, Bryopsidophyceae, and Chlorophyceae. The Ulvophyceae are predominantly marine, but several transitions to freshwater or terrestrial habitats have occurred independently (Shimada *et al.* 2008; Ichihara *et al.* 2009; Suutari *et al.* 2010). The Ulotrichales, with both marine and freshwater species, are haplontic, heterothallic, and isogamous (TYPE 1.1a). In freshwater species (e.g. *Ulothrix*), the diploid stage is reduced to a thick-walled zygote, attached to the substrate. After a resting period, meiosis occurs, followed by several mitoses producing many haploid spores of complementary mating types (*Codiolum* stage). Once released, they develop into isomorphic filamentous gametophytes, which later produce morphologically similar gametes. The Cladophorales (e.g. *Cladophora*), which also colonized both marine and freshwater environment, are also heterothallic and isogamous (the flagellated gametes, produced by + and - gametophytes, are identical in morphology and mobility), but cycles are haplo-diplontic (TYPE 2.1a), with isomorphic gametophytes and sporophytes. The Ulvales, which are mostly marine, also present haplo-diplontic heterothallic cycles (TYPE 2.1a). Gametophytes and sporophytes are isomorphic in *Ulva* and *Enteromorpha*. Meiospores produced by the sporophyte develop into morphologically similar male or female gametophytes, mating types being determined by a bi-allelic locus (Fjeld & Løvlie 1976). Gametes are slightly anisogamous, with a larger, but still mobile, female gamete. Dasycladales (*Acetabularia mediterranea*, *A. acetabulum*) present pseudo-diplontic cycles, with haplo-genotypic sex determination but no free gametophyte (TYPE 2.1d). The siphonous thalli are diploid and monoecious. Haploid isogametes of both mating types are produced in walled cysts, meiosis occurring before cyst formation. A single haploid nucleus enters a cyst and undergoes several rounds of mitoses, so that all gametes produced by one cyst belong to the same mating type (Green 1976).

Prasiola stipitata (Trebouxiophyceae) has a very similar pseudo-diplontic cycle, with haplo-

genotypic sex determination but no free gametophyte (TYPE 2.1d): meiosis occurring in the developing sporophyte is followed by haploid mitoses, producing a mosaic of male and female tissues. The small and motile spermatia fertilize the large and immobile eggs (Friedmann 1959).

Haplo-diplontic cycles are also found in many Bryopsidophyceae. In *Derbesia* (*D. marina*, *D. tenuissima*), the diploid sporophyte is dominant and the much-reduced haploid gametophyte was originally described as a different genus (*Halicystis*). Sex determination is likely haplo-genotypic, with isomorphic male and female gametophytes (dioicy, TYPE 2.1a), but larger female gametes (anisogamy; van den Hoek *et al.* 1995). *Bryopsis* is also haplo-diplontic, with a dominant gametophyte and epigenetic sex determination: male and female gametangia are produced on the same gametophyte (monoicy, TYPE 2.2a), with larger female gametes (anisogamy; e.g. Brück & Schnetter 1997). Diplontic cycles evolved in a few lineages. In *Codium*, strongly differentiated male and female gametes are directly formed in gametangia on the diploid sporophyte (gametic meiosis). Sporophytes are monoecious in *C. isthmocladum* and some populations of *C. fragile* (TYPE 3.2a) but dioecious in other species (e.g. *C. coactum*) potentially with diplo-genotypic sex determination (TYPE 3.1), similar to the situation found in some Phaeophyceae (e.g. *Fucus*) and some land plants (e.g. *Silene*; Prince & Trowbridge 2004; Miravalles *et al.* 2011, 2012).

The Chlorophyceae are mainly found in freshwater, but also occur in terrestrial habitats. Most of them are haplontic, with a single-celled and often dormant zygote as the diploid stage. The Volvocales present an interesting trend of increased organismic complexity, from small biflagellate cells (*Chlamydomonas*) to multicellular colonies with a full germ-soma division of labour (Kirk 2005; Hallmann 2011). The gametophytes of *Gonium*, *Pandorina*, and *Eudorina* consist of small colonies of 8, 16, or 32 biflagellate cells, while *Pleodorina* and *Volvox* evolved towards much larger sizes, with a division of labour between few large non-motile reproductive cells, and numerous somatic biflagellate cells (up to 128 in *Pleodorina* and more than 2000 in *Volvox*).

Unicellular and small colonial species are isogamous. The unicellular haploid cells of *Chlamydomonas reinhardtii* reproduce vegetatively as long as conditions

are favourable. In response to nitrogen depletion, they differentiate into morphologically similar *Mt+* or *Mt-* gametes, mating type being determined by a transcription factor (MID, for minus dominance) encoded by the *Mt-* allele of the MAT locus (TYPE 1.1a; molecular mechanisms are detailed in Box 3.5; see Goodenough *et al.* 2007 for a review).

Larger multicellular lineages evolved oogamy (possibly several times independently). Anisogamy (measured as the ratio of egg to sperm volume) increases with adult size (Knowlton 1974), as otherwise expected from theory (Parker *et al.* 1972). Interestingly, the same master sex-determination gene (*Mid*) is found on the MAT locus of male gametes in the oogamous multicellular *Pleodorina starrii* (Nozaki *et al.* 2006; Kirk 2006) and *Volvox carteri* (Ferris *et al.* 2010), which diverged from *Chlamydomonas* ~200 Mya, showing that sexes directly derived from mating types in this lineage. The MAT locus also contains genes coding for anisogamy (Ferris *et al.* 2010), as also expected from theory (if sexes evolved from preexisting mating types, then genes that affect gamete size can only spread if they are linked to the mating type; Charlesworth & Charlesworth 2010). Interestingly, some *Volvox* species also evolved homothallism (i.e. epigenetic sex determination; TYPE 1.2), both eggs and sperm being produced by the same clones; Starr 1968). *V. aureus* is dioicous (with genetically identical male and female gametophytes; TYPE 1.2b) while *V. barbieri* is monoicous (male and female gametes produced by the same gametophyte; TYPE 1.2a).

Oedogoniales are also haplontic. The resting unicellular zygote eventually divides into four motile meiospores that germinate to form filamentous gametophytes. Anisogamy is strong: the antheridia found on male gametophytes produce tiny multi-flagellate sperm cells, which fertilize the oogonia (single large non-flagellate egg cells) of female gametophytes. In some species the male gametophytes produce motile androspores (larger than normal antherozoids) that reach the female gametophytes and attach to an oogonium or close to it. After germination they produce a 2–4-cell filament ('dwarf males'). The first cell forms a holdfast, while the other cells become an antheridium liberating two antherozoids each. The male and female gametophytes of heterothallic lineages do not differ in

chromosome numbers and morphology, suggesting that sexual differentiation is controlled by a bi-allelic MAT locus (rather than by sex chromosomes; TYPE 1.1a). Some lineages evolved homothallism, oogonia, and antheridia being produced by the same gametophytes (TYPE 1.2a).

Streptophyta, which diverged from Chlorophyta one billion years ago or more, comprise the Charophyta (a group of mostly freshwater, haplontic green algae) and the Embryophyta (land plants), which colonized terrestrial habitat and evolved haplo-diplontic and sub-diplontic life cycles. The Streptophyta are not only species rich, but also represent a large range of structural diversity, from unicellular flagellates to branched filaments in Charophyta, to the complex three-dimensional architecture of Embryophyta.

In **Charophyta**, which are paraphyletic to land plants, sexual reproduction is absent or unknown among the early diverging groups, but widespread among the more evolved Zygnematophyceae, Coleochaetophyceae, and Charophyceae (McCourt *et al.* 2004). Cycles are commonly described as haplontic (though the 'haploid' stage might sometimes harbour more than one set of chromosomes; Haig 2010). Meiosis is zygotic, but the zygote typically disperses before releasing spores.

In Zygnematophyceae, sexual reproduction occurs by conjugation, involving the fusion of non-motile cells. Among unicellular Desmidiales, species may be heterothallic (e.g. *Closterium ehrenbergii*), homothallic (e.g. *Closterium siliqua*), or may present both homothallic and heterothallic strains (e.g. *Cosmarium botrytis*). In heterothallic species, conjugation occurs between morphologically identical cells of complementary mating types (TYPE 1.1a), forming an encysted zygote (resting stage). Meiosis produces two daughter cells of complementary mating types (the two other products abort; Starr 1959; Brandham & Godward 1965).

Zygnematales (e.g. *Spirogyra*, *Mougeotia*, *Zygnema*, *Sirogonium*) are filamentous. 'Scalariform' conjugation starts with the alignment of two filaments of opposite mating types (respectively male and female). Cells that come into contact establish a conjugation tube, through which the male protoplast

passes to fuse with the female protoplast. The male chloroplast is then resorbed, and the zygote develops into a resting stage (zygospore). Meiosis occurs at germination, producing a single new filament (the three other meiotic products are discarded). In some *Spirogyra*, conjugation may occur between cells from the same filament, through lateral conjugation (homothallism; TYPE 1.2a). Most *Zygnema* species are homothallic, but one species at least (*Z. circumcarinatum*) is heterothallic with haplo-genotypic sex determination (TYPE 1.1a; Miller 1973). Anisogamy evolved in *Sirogonium* species, which are homothallic.

Anisogamy is more pronounced in the oogamous Coleochaetophyceae, with antheridia and oogonia formed on either the same gametophyte in homothallic species (TYPE 1.2a; e.g. *Coleochaete pulvinata*, *C. nitellarum*), or different gametophytes in heterothallic species (TYPE 1.1a; e.g. *C. scutata*). The zygote, which is provisioned by the female gametophyte, is also a resting stage. The Charophyceae (e.g. *Chara*) also display a marked oogamy, with small mobile sperm cells produced by antheridia, and large female gametes produced in oogonia. Many species are homothallic (monoicous; TYPE 1.2a), but some have antheridia and oogonia produced by distinct gametophytes (e.g. *Chara tomentosa*). Sex determination in this case presumably involves mating type loci (TYPE 1.1a; there are no differentiated sex chromosomes; Kunachowicz *et al.* 2001).

Embryophyta evolved from a charophyte-like ancestor and colonized terrestrial environments, with far-reaching consequences on terrestrial ecosystems, atmospheric chemistry, and climate (Berner 1997; Kenrick & Crane 1997; Steemans *et al.* 2009). This colonization was accompanied by a major shift in life history, including gametophytic retention of the zygote and matrotrophy of the developing embryo. Non-vascular groups (bryophytes) are restricted to small sizes and moist habitats, but vascular plants (tracheophytes) may colonize dry habitats and reach gigantic sizes. The cycle is basically haplo-diplontic, with a predominant haplontic phase in basal lineages (bryophytes), and predominant diplontic phase in vascular plants. Oogamy is the rule. The sporophytes are originally homosporous (a single type of spore is produced), but

heterospory evolved several times independently, with mega- and microspores developing into female and male gametophytes respectively (Stewart & Rothwell 1993).

Bryophytes originated over 400 Mya and have changed very little since then. The group is paraphyletic and comprises Hepatophyta (liverworts), Bryophyta s.s. (mosses), and Anthocerophyta (hornworts). The gametophytes produce flagellate sperm and eggs in specialized sex organs (antheridia and archegonia respectively). The fertilized zygote develops into a small and short-lived sporophyte that remains fixed to the gametophyte. Sporophytic development progressively increases from the earlier derived Hepatophyta, to the most recently derived Anthocerophyta. A single type of spore is produced (homospory), which grow into either dioicous (unisexual) or monoicous (bisexual) gametophytes. Male and female gametophytes may be strongly dimorphic: in *Micromitrium*, the dwarf male gametophytes grow on the leaves of larger female gametophytes (Hedenäs & Bisang 2011). Haplo-genotypic sex determination seems the rule among dioicous species (TYPE 2.1a; e.g. Okada *et al.* 2001; Yamato *et al.* 2007). In *Sphaerocarpos*, for instance, the four spores resulting from a meiosis produce two male and two female gametophytes. Sex chromosomes (U/V) have been identified in both liverworts and mosses (the only known groups of homosporous land plants with non-recombining sex chromosomes). Epigenetic triggers may include environmental and social cues: in the monoicous *Tetraphis pellucida*, for instance, gametophytes tend to develop into males as density increases (Kimmerer 1991). Dioicy is considered primitive, with independently evolved monoicy in several lines (Longton & Schuster 1983). However, recent phylogenetic analyses in mosses suggest a very high lability and frequent transitions between these two states, with a higher rate of speciation in hermaphrodites and more frequent transitions to dioicy than the reverse (McDaniel *et al.* 2013).

Seedless vascular plants (**pteridophytes**) are also often limited to moist habitats, being dependent on water for external fertilization (motile sperm) and for the development of the free-living gametophytes. Unlike many bryophytes, sex is determined by epigenetic activation of distinct developmental

programmes (sometimes under environmental control), at the level of either the gametophyte (homosporous lineages) or the sporophyte (heterosporous lineages). The earliest lycophytes and extant members of the Lycopodiales are homosporous, with small and short-lived monoicous gametophytes bearing both antheridia and archegonia simultaneously (TYPE 2.2a). In contrast, Selaginellales and Isoetales evolved heterospory. The hermaphroditic sporophytes simultaneously produce megaspores and microspores (TYPE 2.2c), which give rise to small free-living female and male gametophytes respectively (the latter usually consists of little more than one antheridium).

The monilophytes include the Psilotopsida, Equisetopsida, as well as the euporangiatae (Marattiopsida) and leptosporangiatae ferns (Polypodiopsida), as a monophyletic clade sister to the seed plants (Pryer *et al.* 2001). Most monilophytes are homosporous, except for Salviniales (e.g. *Azolla*, *Salvinia*, *Marsilea*) and some Polypodiales (*Platyzoma*), which evolved heterospory (TYPE 2.2c): the nutrients stored in large female spores allow rapid embryonic development of the female gametophyte, while smaller male spores are more widely dispersed. The large spores of *Platyzoma*, however, may also subsequently produce antheridia. Homosporous lineages may be monoicous (TYPE 2.2a), dioicous, or androdioicous (TYPE 2.2b; males and females, or males and hermaphrodites). In *Equisetum*, about half the gametophytes emerging from a mass sowing remain small and produce only antheridia. If isolated, some continue to grow and produce archegonia. The other half are larger, longer lived (up to 2 years), and first produce archegonia. If not fertilized, they start producing antheridia, followed by another set of archegonia. Sowing density and other cultural conditions affect the proportions of the two kinds of gametophytes (Duckett 1977; Guillot & Fievet 2003). Similar patterns are found in the leptosporangiatae homosporous ferns, such as *Pteridium aquilinum* and *Ceratopteris richardii*, where gametophytic gender (male or hermaphroditic) is determined epigenetically by the pheromone antheridiogen (ACE; Döpp 1950; Näf 1979; Näf *et al.* 1975; Banks *et al.* 1993), a gibberellin-related compound (Yamane 1998). Isolated spores develop into hermaphrodites and produce the pheromone, which induces the

development into males of incoming spores settling in the neighbourhood (epigenetic andro dioicy; TYPE 2.2b). Culture experiment with *Woodwardia radicans* have shown that antheridiogen inhibits the growth of gametophytes, and that slow growth favours maleness, whereas fast growth favours femaleness, irrespective of the presence or absence of antheridiogen (Quintanilla *et al.* 2007), supporting the suggestion that antheridiogen effect is mediated by its effect on growth (Korpelainen 1994, 1998).

All **Spermatophyta** (seed plants) are heterosporous: the haploid microspore, produced within anthers by the **microsporangium**, develops into a male gametophyte (the pollen grain). The **megaspore**, produced within the ovule by the **macroporangium**, develops into a female gametophyte (or mega-gametophyte). The ovule is only protected by integuments in gymnosperms, but by an additional ovary wall in angiosperms. These cycles may be considered sub-diplontic (no free gametophyte).

Among gymnosperms, Cycadophyta and Gingkophyta are dioecious, but with little sexual dimorphism (except for sex organs). Male sporophytes produce motile flagellate sperm that swim directly to the egg inside the ovule produced by female sporophytes. Heteromorphic sex chromosomes have been described in some cycads, such as the male-heterogametic *Cycas revoluta* (XY; TYPE 3.1a). The single living gingkophyte, *Ginkgo biloba*, is female heterogametic (ZW; TYPE 3.1b) with slightly differentiated sex chromosomes. Gnethophyta are also dioecious, but Coniferophyta are mainly monoecious (producing unisexual male and female cones on the same sporophyte; TYPE 3.2a). Sex chromosomes have been described in dioecious Podocarpaceae (a male-heterogametic X_1X_2Y sex-determination system is found in *Podocarpus macrophyllus* and related species; TYPE 3.1a).

Most angiosperms are hermaphroditic (TYPES 3.2a and b), certainly the original state. The so-called 'perfect' flower comprises four kinds of structures, arranged in concentric whorls (Box 3.6): from outermost to innermost are the calyx (sepals), the corolla (petals), the androecium (stamens), and the gynoecium (carpels). Unisexual flowers arise from the developmental arrest, at different stages, of male or female organs (Section 3.3.1). Some 5% of species are monoecious (unisexual flowers of

different sexes on the same sporophyte), including gynomonoecy and andromonoecy (female and hermaphroditic, respectively male and hermaphroditic flowers on the same sporophyte). An additional 5% are dioecious (unisexual flowers on different sporophytes), including gynodioecious (female and hermaphroditic individuals), and rare androdioecious lineages (males and hermaphrodites). Monoecy and dioecy evolved several times independently in different lineages (Lebel-Hardenack & Grant 1997; Charlesworth 2002), with some returns to hermaphroditism. Dioecy, for instance, evolved in the common ancestor of Begoniaceae, Daticacea, Cucurbitaceae, and Tetrameleaceae, and was secondarily lost in some species (Zhang *et al.* 2006). Comparative analyses suggest that dioecious lineages have short evolutionary lives (Heilbuth 2000).

Monoecious species may show labile sex expression: the epiphytic orchid *Catasetum viridiflavum*, for instance, shows a strongly bimodal gender distribution, producing few hermaphrodites, but mostly male or female individuals depending on local conditions (and thus may switch sex from year to year). The female phenotype is triggered by better light exposure, while shadow conditions favour male development (Zimmermann 1991). Plant size may also affect gender, though to a smaller extent (smaller plants are more likely to be male).

Diplo-genotypic sex determination (TYPE 3.1) evolved in several lineages. Sex chromosomes have been identified in some 40 species (21 genera, 15 families). Female heterogamety is rare (TYPE 3.1b), having been found only in *Fragaria*, *Populus*, *Datisca*, and *Silene*. Male heterogamety is by far more widespread, mostly among recent sex chromosomes (TYPE 3.1a). It evolved, for example, twice in *Silene*, twice in *Schiedia* (Caryophyllaceae), and twice in *Rumex*, all within the past 10–15 My. Sex is usually determined by a dominant masculinizing gene on the Y, but by X to autosome ratio in *Humulus lupulus* and *Rumex acetosa* (Löve 1969; Navajas-Perez *et al.* 2005). Recombination is often suppressed only in a small region around the sex-determination genes. In some cases, no markers seem fully sex linked, despite dense mapping, and YY individuals are viable (Lloyd 1974). Heteromorphic sex chromosomes have only been found in some 20 species

from six genera (*Cannabis*, *Humulus*, *Silene*, *Coccinia*, *Trichosanthes*, and *Rumex*).

Many hermaphroditic angiosperms have evolved self-incompatibility (SI) systems (secondary mating types; TYPE 3.2b) as an alternative to dioecy to avoid inbreeding. These systems are often controlled by a single di- or multi-allelic locus (S) encoding two proteins, one expressed in the pistil (the female determinant), the other in the anther and/or pollen (the male determinant). Interaction between proteins from the same haplotype prevents pollen germination and/or arrests pollen tube elongation. SI is said gametophytic (GSI) if the male determinant is determined by the haploid genotype of the pollen (e.g. in Solanaceae or Rosaceae), and sporophytic (SSI) if it is determined by the diploid genotype of the anther (e.g. in Brassicaceae; Fig. 3.10). Molecular mechanisms are detailed in Section 3.4.1a. Other SI mechanisms exist which rely, for example, on flower heteromorphy. Distly is normally determined by a single S locus with two alleles, one recessive s and one dominant S, generating two self-incompatible genotypes (ss and Ss) at 1:1 ratio within populations. Tristly involves two di-allelic loci and a larger number of genotypes, also maintained at equilibrium frequencies by frequency-dependent selection (Ganders 1979).

2.2.7. Amoebozoa

Amoebozoa form a sister group to the Opisthokonta within the Unikonta. Most are unicellular (e.g. Gymnamoebia), feeding on bacteria in water or in the soil. When food becomes scarce, amoeboid cells form cysts, which may disperse to new environments. Some are parasitic (e.g. *Entamoeba*). Many species that seem to reproduce only asexually still possess the full complement of genes required for meiosis (e.g. *Entamoeba histolytica*), so that sex is certainly more widespread than previously thought (Lahr *et al.* 2011). Multicellularity evolved in Mycetozoa (slime moulds), where sex is well documented in Dictyostelia (cellular slime moulds) and Myxogastria (plasmodial slime moulds).

2.2.7a *Dictyostelia*

Dictyostelia have a haploid life cycle (meiosis is zygotic). Though also known as ‘social’ amoebae, they spent most of their lives as single amoeboid cells,

feeding on bacteria and yeasts. When food becomes scarce, individual cells aggregate (coordinated by cyclic AMP) to produce a mass of cells surrounded by an extracellular matrix, resembling a slug and capable of movement. At some point the slug settles down and produces fruiting bodies. The cells at the tips of the fruiting body develop into haploid resting mitospores, which, after dispersal, germinate when conditions become favourable. Hence, unlike plasmodial slime moulds, the fruiting body is haploid and not involved in the sexual cycle. Sexuality occurs when two amoeboid cells, usually of complementary mating types (TYPE 1.1a), merge to form a resting zygote. This zygote secretes cAMP to attract surrounding cells (Abe *et al.* 1984), as well as other factors to prevent their fusion into zygotes (O'Day *et al.* 1981). The outer cells of the aggregate lay down a protective cellulose wall, while the zygote digests all other cells trapped within this macrocyst. After a period of dormancy, the cyst germinates, releasing haploid progeny that arise after one meiotic division and several rounds of mitoses.

Social amoebae may be homo- or heterothallic (Bloomfield 2011). Heterothallic lineages (TYPE 1.1a) commonly have more than two mating types, determined by one multi-allelic locus. *Dictyostelium discoideum*, one of the best-studied species, possesses three mating types (which can pair with each other but not with self), as well as rare self-fertile homothallic strains (TYPE 1.2a; Bloomfield *et al.* 2010; Bloomfield 2011; see Section 3.2.2d for mechanisms). In *Polysphondylium pallidum*, mtDNA transmission has been shown to be uniparental and under control of the MAT locus (Mirfakhrai *et al.* 1990).

2.2.7b Myxogastria

Myxogastria (= myxomycetes) constitute the syncytial (or plasmodial) slime moulds. The feeding stage is a diploid multinucleate plasmodium, formed by repeated mitotic divisions of the zygote without cytoplasmic division. When conditions become harsh, the plasmodium forms a stalked fruiting body (sporangium). Meiosis takes place within the bulbous tip of the sporangium, producing resistant dispersing spores. Haploid spores germinate when conditions are favourable, developing into active cells that may be either amoeboid

or flagellate depending on environmental moisture (the two forms can readily convert into each other), and reproduce by cell division. Two cells of complementary mating types (either both flagellate or both amoeboid) mate to form a zygote, which then divides mitotically to produce a new plasmodium. Mating types are determined by one or more multi-allelic loci (TYPE 2.1a). As populations increase in density, cells produce a mating factor that acts as a pheromone, inducing conversion from the vegetative to a gametic state and, later, plasmodial formation. In *Physarum polycephalum*, cell fusion is decided by the MAT-B locus, with 15 alleles known (the two haploid cells must differ at this locus to be able to mate). After cell fusion, nuclear fusion only occurs if the two nuclei also differ at their MAT-A allele (16 alleles known); otherwise, cells separate again and go their own way. If (and only if) nuclear fusion occurs, all mitochondria from one parent will be selectively digested. Which parent transmits its mitochondria to the plasmodium is also decided by alleles at the MAT-A locus, which establish a linear hierarchy ('pecking order') among haplotypes. The exact mechanisms, however, are unknown (Iwanaga & Sasaki 2004), and apparently not perfect, resulting in paternal leakage or biparental transmission in some crosses (Moriyama & Kawano 2010). In *Didymium iridis*, mtDNA transmission, though uniparental, is random regarding mating types (Silliker *et al.* 2002; Scheer & Silliker 2006).

Sometimes, amoeboflagellates derived from the same spore can mate and successfully develop into a plasmodium, and some clones can develop into plasmodia without mating. Most of these non-heterothallic lines have been shown to result from a meiotic failure producing diploid spores (Clark & Haskins 2010). When two compatible diploid clones are crossed, the resulting tetraploid plasmodium produces diploid spores. Half of them are heterozygous for mating type, and thus capable of developing into plasmodia without crossing. At sporulation, normal haploid lines are restored, which have to mate to form plasmodia. Homothallic clones have also been reported, where haploid mitosis produces a binucleate amoeba, then fusion into a diploid nucleus triggers plasmodial development (Anderson *et al.* 1976).

2.2.8. Fungi

Together with Filozoa (Choanoflagellata and Metazoa), fungi form the domain of Opisthokonta. Fungi comprise several basal phyla, including the paraphyletic Chytridiomycota and Zygomycota, the Microsporidia, and the Glomeromycota. Ascomycota and Basidiomycota diverged later, and belong to a monophyletic group known as Dikarya. Some 100,000 species in total have been described, but the real number is estimated to exceed 1.5 million. Most fungi reproduce sexually, even if sometimes cryptically (Kück & Pöggeler 2009). Cycles are haplontic or haplo-diplontic, with vegetative growth and reproduction both as haploid and diploid, either as single cells (yeasts) or multicellular hyphae (mycelium). In some Ascomycota and in the Basidiomycota, the fusion of haploid cells is followed by a long-lived dikaryotic stage. Nuclear fusion only occurs in special fruiting bodies and is immediately followed by meiosis. The sexual cycle is controlled by one or more MAT loci, with complementary alleles found either in different individuals (heterothallism; TYPES 1.1a, 2.1a, 2.1e) or in the same individual (homothallism; TYPES 1.2a, 2.2a).

2.2.8a Chytridiomycota

Although sometimes also referred to as ‘water moulds’, due to their aquatic habits, chytridiomycetes are not to be confused with oomycetes (which are not fungi). This paraphyletic phylum, which constitutes < 1% of described fungi, occupies a basal place in the kingdom, retaining ancestral traits (such as flagellate zoospores, lost in other fungi but occurring in other opisthokonts). Approximately 1000 species are described, mostly saprophytic or parasitic (e.g. *Batrachochytrium dendrobatidis*, responsible for amphibian chytridiomycosis). They are characterized by coenocytic or irregularly septate hyphae. Asexual reproduction occurs via thin-walled sporangia. Sexual reproduction always produces thick-walled resting sporangia, which may also be produced asexually. Life cycles are diverse and vary with classes.

Chitridiales have haplontic cycles with zygotic meiosis (though the parasitic *B. dendrobatidis*, possibly resulting from an hybrid cross, is diploid and largely asexual, in a way similar to another invading

parasitic fungus, the ascomycete *Candida albicans*). They seem generally heterothallic (TYPE 1.1a), but details of mating types are largely unknown. Fertilization may occur by the fusion of zoospores, gametangia, or somatic structures. *Chytriumyces hyalinus* is a freshwater unicellular saprobe that typically feeds on moults of may flies or other insects, via a rhizomycelium. Sex, which is induced by nutrient depletion, occurs by somatogamy: the rhizoids of two compatible thalli come into contact and anastomose, forming a thick-walled resting body that receives cytoplasm and nuclei from the two contributing thalli (Miller & Dylewski 1981; Mehrotra & Aneja 1990). The two nuclei fuse within the resting body, and meiosis occurs at germination. In *Dictyomorpha dioica*, an obligate endoparasite of oomycetes, fertilization occurs by pairing of two flagellate isogametes, stemming from thalli with complementary mating types (TYPE 1.1a; Mullins 1961). *Rhizophlyctis rosea*, widespread in soils where it decomposes cellulose, also seems heterothallic (Couch 1939). The algal parasite *Zygorhizidium planktonicum* is dioicous and reproduces sexually by gametangial conjugation: the donor cytoplasm migrates through a conjugation tube towards the recipient thallus, where a resting zygote is formed. Meiosis occurs at germination. Autogamy has been documented (mating between male and female individuals from the same sporangium), but the details of sex determination are unknown (Doggett & Porter 1996).

The Monoblepharidales, also haplontic, are homothallic and oogamous, producing large non-motile female gametes (an exception among fungi). *Monoblepharis polymorpha* is monoicous, producing both male and female gametangia on the same thallus (TYPE 1.2a). The antheridium, fixed on the oogonium, produces 4–8 flagellate antherozoids that swim to the oogonium. A resting oospore is produced after fertilization, meiosis occurring at germination (Mehrotra & Aneja 1990).

In Blastocladiales (sometimes considered a separate phylum, Blastocladiomycota; Stajich *et al.* 2009), cycles alternate between a haploid gametophyte and a diploid sporophyte. Some lineages are heterothallic (TYPE 2.1a), such as the heteromorphic *Caelomomyces psorophorae*. The haploid gametophyte, which parasitizes a copepod, releases gametes that

must mate with isogametes from a complementary type (possibly stemming from the same copepod in case of multiple infections). Details of the MAT locus are unknown. The free-moving diploid zygote subsequently infects a mosquito larva, develops into a sporophyte, and produces resting sporangia. Meiospores are released in water and infect a new copepod (Whisler *et al.* 1975). In *Blastocladiella variabilis* (also isogamous and heterothallic), the *plus* and *minus* gametophytes differ in colouration (one mating type has an orange gametophyte, while the other is colourless; Couch & Whiffen 1942). In contrast, *Allomyces macrogynus* (with an isomorphic haplo-diplontic cycle) displays both anisogamy and homothallity (TYPE 2.2a). Morphologically differentiated male and female gametangia are produced on the same gametophyte (monoecy). The orange (β -carotene producing) male gametangium is terminal on the same hyphal branch as the colourless female gametangium. The larger (but still motile) female gametes attract male gametes through the pheromone sirenin (a sesquiterpene). The male gamete also produces an attractant (parisin, of unknown structure). Diploid hyphae may produce either mitosporanges (with diploid spores developing into a new sporophyte) or meiosporanges (with haploid spores developing into a gametophyte).

2.2.8b Zygomycota

Approximately 1060 species of zygomycetes are known, most of which terrestrial, living in soil or on decaying plants or animals. Some are parasites of plants or arthropods (trichomycetes), other form symbioses with plants. Life cycles are haplontic. Hyphae are generally coenocytic, allowing locally rapid growth through the migration of cytoplasm and nuclei. Asexual reproduction is achieved by bulbous sporangia formed at the tips of upright hyphae, the haploid spores being sometimes actively expelled (e.g. *Pilobolus*, Entomophthorales).

Many lineages are strictly heterothallic (TYPE 1.1a; e.g. the Mucorales *Mucor mucedo*, *Phycomyces blakesleeanus* or *Rhizopus stolonifer*, the black bread mould), with two mating types (*plus* and *minus*) determined by a bi-allelic MAT locus (details in Section 3.2.2a). Sexual reproduction is initiated by a contact between haploid hyphae of different mating types. Complementary enzymatic pathways

(see Section 3.2.2a) allow converting β -carotene into trisporic acid, which triggers hyphal differentiation into zygomycetes and the formation of multinucleate gametangia, isolated by cell walls. Upon fusion, a thick-walled resting zygosporangium is formed. Karyogamy sometimes involves many pairs of nuclei, but this number may be reduced down to one single pair as in *P. blakesleeanus*. At germination, the diploid nuclei undergo meiosis and produce vegetative hyphae. A sporangium may develop at the end of a germ tube, and disseminate haploid spores. Homothallic lineages evolved epigenetic sex determination (TYPE 1.2a), with cell signalling processes based on the same metabolic pathways (Werkman & van den Ende 1974).

2.2.8c Microsporidia

Microsporidia are highly specialized fungi, probably derived from zygomycetes (Lee *et al.* 2008). Some 1300 species are known, all being small-sized unicellular, with a compact genome (~2.6 Mb; < 2000 genes) and vestigial mitochondria (mitosomes). They are obligate intracellular parasite of animals, mostly insects (some *Nosema* are parasites of honey bees and silk worms), with both vertical and horizontal transmission. Microsporidia may directly interfere with host metabolism, causing hypertrophic growth of the invaded cell (xenomas), host castration, or sex reversal (e.g. *N. granulosus* feminizes its crustacean host, favouring its vertical transmission; Terry *et al.* 1998).

Complex dixenic cycles are found in Amblyosporidae, where life cycles alternate between a bi-nucleate diploid stage and a uninucleate haploid stage. At some point, the bi-nucleate meront, which reproduces asexually in the final host (e.g. a mosquito), undergoes meiosis and karyokinesis, developing into a multi-nucleate haploid plasmodium (e.g. Chen & Barr 1995). Haploid meiospores are produced and transmitted to the intermediate host (e.g. a copepod), where they undergo rounds of asexual reproduction (haploid mitoses). Mitospores are finally transmitted back to the definitive host, where they develop into gametes and fuse, producing new bi-nucleated meronts. Several stages might be dropped depending on species. Several lineages seem to have lost sex, apparently quite recently (Ironside 2007). Microsporidia harbour the

same MAT locus as zygomycetes (Section 3.2.2a). The locus seems functional but whether and how it affects sex determination is unknown. In particular, microsporidia only present a single allele and lack the complementary allele required for sex determination in zygomycetes. However, some *Encephalitozoon* species possess two diverged high mobility group (HMG) copies on their MAT locus, which might underlie epigenetic sex differentiation (Lee *et al.* 2010).

2.2.8d Glomeromycota

Glomeromycetes form a species poor (with ~200 species described) but ecologically important group, developing mutualisms with > 90% of plant species via arbuscular endomycorrhizae where they trade phosphate for sugars. Hyphae are coenocytic, containing many haploid nuclei, and (depending on genetic distance) may anastomose with other individuals, creating large hyphal networks that exchange nuclei. Hyphae are thus genetically heterogeneous, as are the large asexual spores, which may contain several hundreds to thousands of nuclei. Glomeromycota have long been thought to be purely asexual, but some evidence for recombination exists, even though mating and meiosis are not documented.

2.2.8e Dikarya

The Dikarya comprise > 95% of all fungal species described. Multicellularity and cell differentiation independently evolved in the two composing phyla, Ascomycota and Basidiomycota. As their name indicates, Dikarya are characterized by a dikaryont stage, marked by the multiplication of haploid nuclei from the two mating individuals before karyogamy occurs. Transient in Ascomycota, this stage may cover most of the life cycle in Basidiomycota. As a main consequence, a single mating may result in ten of thousands of independent fusions and meoses.

Ascomycota form a large group (~64% of fungal species described) containing mainly saprobic, parasitic, and mutualistic forms (including lichens and ectomycorrhizae). Saccharomycotina comprise mostly unicellular yeasts (e.g. *S. cerevisiae*), and Pezizomycotina filamentous fungi (many moulds and some mushrooms). A third basal subphylum

(Taphrinomycotina) includes fission yeasts (*Schizosaccharomyces*) and a few hyphal fungi. Both growth forms seem ancestral to ascomycetes. A majority of lineages are homothallic. Heterothallic species are bipolar, the MAT locus harbouring two highly divergent and possibly non-homologous alleles (idiosyncratic) that encode transcription factors (TYPE 2.1a; see Section 3.2.2b). Individuals of complementary mating types communicate via oligopeptide pheromones.

The baker's yeast *S. cerevisiae* is one of the most intensively studied model organisms among eukaryotes. Yeasts have haplo-diplontic single-celled life cycles, and can divide mitotically both as haploids and diploids. Sex determination is haplo-genotypic, being controlled by a bi-allelic MAT locus (TYPE 2.1a; see Box 3.1 for details). The diploid phase, which predominates in nature, is heterozygous α/α . Upon facing stressful conditions (e.g. nutrient depletion) diploid cells undergo meiosis, producing ascospores with two α and two α haploid spores, which may undergo asexual reproduction by budding. Specific pheromones and receptors ensure that mating occurs between haploid cells of complementary mating types (which may stem from the same ascus, i.e. the same meiotic event). Nuclear fusion (karyogamy) immediately follows cell fusion. Mitochondria are transmitted biparentally and undergo fusion in the zygote, followed by mtDNA recombination (Takano *et al.* 2010). *S. cerevisiae* also evolved specific gene conversion mechanisms that allow haploid cells of one mating type to produce progeny presenting the alternative mating type. Hence, a colony founded by one single haploid cell can rapidly restore the complementary mating type, and resume sexual reproduction (see Section 3.2.3a). Mating-type switching is also found in other Saccharomycotina (e.g. *Kluyveromyces lactis*), and independently evolved in Taphrinomycotina (*Schizosaccharomyces pombe*).

The human pathogen yeast *Candida albicans*, which diverged from *S. cerevisiae* some 200 Mya, was long thought to reproduce only clonally or via a form of parasexuality, during which two diploid cells (the normal state in *C. albicans*) fuse to form 4N cells. Chromosome homologues are then randomly lost during the ensuing mitoses, making cells return to their diploid state without meiosis

or recombination. However, a fully functional mating type locus similar to that in *S. cerevisiae* was recently found (Miller & Johnson 2002). Naturally occurring diploid strains are heterozygous α/α at this locus, hence unable to enter gametogenesis and mate. Sexual cycles can be triggered in the laboratory via the construction of homozygotes or hemizygotes and are thought to occasionally occur in nature.

The filamentous ascomycetes (Pezizomycotina) normally live as haploid mycelia. Asexual reproduction occurs through mitospores (microconidia). Heterothallic lineages (such as the Sordariacea *Neurospora crassa* and *Podospora anserina*, two model organisms) most often have two mating types (Mat+ and Mat-) coded by one bi-allelic locus (Box 3.3). Many filamentous ascomycetes evolved a form of anisogamy by re-assigning a role of male gamete to microconidia (sexes were thereby secondarily superimposed to primary mating types; TYPE 2.1e). Under appropriate environmental conditions (including light and starvation for specific nutrients), haploid gametophytes from complementary mating type produce both small dispersing male gametes (microconidia) and more complex and sessile female structures (ascogonia) (Coppin *et al.* 1997; Billiard *et al.* 2011). Specific pheromones and receptors ensure that mating occurs between complementary mating types. Mitochondria are inherited from the female mating partner (i.e. independent of mating types). Transmission is actually biparental, followed by active elimination of the paternal contribution.

As also observed in basidiomycetes, gametic fusion is not followed immediately by nuclear fusion (karyogamy). Instead, the two haploid nuclei divide independently, creating a short-lived multi-nucleus mycelium (the ascogenous hyphae). Binucleate ascogonial cells (croziers) are formed within the developing fruiting body, containing one nucleus of each mating type. This process clearly requires nucleus recognition, mediated by genes from the MAT locus (Box 3.3). Karyogamy finally occurs, rapidly followed by meiosis, which produces four haploid nuclei (two Mat+ and two Mat-). There is therefore no free sporophyte, so that the cycle can be considered as pseudo-haplontic (TYPE 2.1e).

In several species (e.g. *P. anserina*, *Neurospora tetrasperma*, *Gelasinospora tetrasperma*), meiosis is

followed by a post-meiotic mitosis, so that each the four ascospores receives two nuclei, one of each mating type (which also requires nuclear recognition). As a consequence, cultures from single ascospores are self-fertile heterokaryons (pseudo-homothallic). However, in contrast to true homothallic species, each haploid nucleus contains only one of the two alternative mating types.

True homothallism is common in filamentous ascomycetes: self-fertile homokaryotic haploid strains complete their sexual cycle without seeking a mate (e.g. through a fusion between microconidia and ascogonia from the same gametophyte). Its phylogenetic distribution, together with sequence similarity between mating types across the phylogeny, suggests independent evolution from an ancestral heterothallism. Homothallic lineages usually possess copies of both mating-type alleles (Section 3.2.3b). In *Aspergillus*, homothallism evolved several times independently by genic capture (possibly via unequal recombination) of the two complementary MAT loci found in heterothallic lineages. Depending on lineages, the two copies may occur in close linkage, at some distance on the same chromosome, or on different chromosomes. Homothallism is likely mediated by the differential expression of one of the two mating types in different cell lineages from the same clone (Paoletti *et al.* 2007). In homothallic Sordariaceae (e.g. *Sordaria macrospora*), where gametophytes possess copies of both the A and a mating-type, the dikaryotic ascogenous hyphae display proper nucleus segregation according to parental origin. This strongly suggests alternative expression of either mating type, depending on male or female origin, which might occur through differential parental imprinting in microconidia versus ascogonia.

Basidiomycota comprise, on the one side, the Pucciniomycotina and Ustilaginomycotina (rusts and smuts, which form budding yeasts in the haploid phase and naked basidia at meiosis; e.g. *Puccinia graminis*, *Ustilago maydis*), and on the other side Agaricomycotina (many mushrooms such as *Coprinus*), which form a filamentous mycelium in the haploid phase, and protect their basidia within a fruiting body. Basidiomycetes are mostly heterothallic, with complex mating-type systems encoded in two unlinked loci (Section 3.2.2c). One locus (known

as MAT-a in *U. maydis*, and MAT-B in mushroom fungi) encodes pheromones and receptors, while a second locus (known as MAT-b in *U. maydis* and MAT-A in mushroom fungi) encodes homeo-domain HD transcription factors. Partners must differ at both loci to complete the sexual cycle. This system is named tetrapolar because four mating types may segregate among the four basidiospores resulting from a single meiosis (i.e. one spore can only mate with one of the three others). There are no differentiated cells playing the role of gametes: sexual reproduction is usually achieved by the fusion of two haploid mycelia (Agaricomycotina) or of similar-sized cells (rusts and smuts). Cell fusion is prolonged by a characteristic long-lived (up to centuries!) dikaryotic phase in the vegetative sporophyte. The proportions of the two nuclei can vary with environment, and dikaryons seem quicker to adapt to changing environments than their haploid components. Nuclear fusion only occurs in specific tissues of the fruiting bodies, and is immediately followed by meiosis.

Ustilaginomycotina, with 1400 species recognized, are mostly parasites of grasses and sedges. *U. maydis* (saprobic as yeast and parasitic as a dikaryonic mycelium) is tetrapolar (Box 3.4). The MAT-a locus is bi-allelic, each allele encoding a pheromone and a receptor, and the MAT-b locus multi-allelic, each allele encoding a pair of HD1/HD2 transcription factors. Pheromones and receptors control the development of long mating filaments (conjugation tubes) that fuse at their tips. After cell fusion, dikaryotic filaments are only formed if the two partners also differ at the MAT-b locus. Some species (e.g. the related *Sporisorium reilianum*) have three alleles at the MAT-a locus, each coding for two pheromones and one receptor, enabling it to attract and mate with either of the two alternative partners, but not with itself. The two loci MAT-a and MAT-b might fuse, such as found in *U. hordei* where the resulting MAT locus expands up to ~500 kb, with the MAT-a locus at one end and the MAT-b locus at the other end.

Pucciniomycotina are often saprobic as yeasts, and obligate parasites as dikaryotic hyphae, mostly on plants, sometimes on insects or other fungi. *Helicobasidium* is parasitic on other rusts as haploid, and on plant roots as diploid. *Microbotryum violaceum*,

a parasite of *Silene dioica* (cause of the anther smut disease) is secondarily bipolar. Spores are produced within its host's anthers, and transmitted to a next host by pollinating insects. Female plants are sex reversed by the parasite, thereby allowing its transmission via anthers. The dispersing teliospores are diploid: meiosis only occurs once a new host has been reached, ensuring the co-occurrence of two complementary mating types. Autogamy (namely, mating between two gametophytes stemming from the same meiosis) is thus the rule (Giraud *et al.* 2008).

Agaricomycotina comprise some 22,000 saprophytic/mutualistic species. Many mushrooms form ectomycorrhizae with plants. Mating systems are mostly tetrapolar, with a MAT-B locus encoding pheromones and receptors, and a MAT-A locus encoding homeodomain HD1 and HD2 transcription factors (Section 3.2.2c). The haploid hyphal filaments resulting from the germination of a basidiospore are monokaryotic. Cell fusion will occur when encountering another monokaryon. If the two fungi turn out to be compatible (as decided from the pheromones-receptors at locus MAT-B), nuclei will be multiplied and exchanged, reciprocally migrating from donors to recipients. The septa separating cells are degraded to allow colonization, and reformed afterwards. Once two compatible nuclei are present within a hypha, all subsequent growth will be as a mycelial dikaryon, through a specific mechanism involving both A and B MAT loci. In the apical cell, a HD1/HD2 heterodimer (formed by complementary alleles from the MAT-A locus) initiates the formation of a clamp, in which one haploid nucleus is isolated. Meanwhile the complementary nucleus moves to the hyphal cell proximal extremity, where it is isolated in a sub-apical cell by formation of a septum. MAT-B then coordinates fusion of the clamp with the newly formed sub-apical cell, restoring the dikaryotic state. Dikaryons retain the potential to act as donor when encountering a monokaryon, transferring haploid nuclei of either mating type to fertilize receptive mycelia (Buller's phenomenon). Only nuclei are transmitted, no other organelles, so that the recipient mycelium keeps its mitochondrial haplotype. This dikaryotic phase is characteristically prolonged in basidiomycetes. Karyogamy eventually occurs in the fruiting body

(carpophore). The diploid state is short-lived, being immediately followed by meiosis and the formation of haploid basiodiospores.

In many groups, the number of mating types has been multiplied by gene duplication and functional redundancy: it is enough, for instance, that one HD1 from a gametophyte is compatible with one HD2 from its partner for the mating to succeed. In addition, all MAT loci can be multi-allelic, which further increases the likelihood that randomly met homokaryons are compatible. *Coprinus cinereus*, for instance, has three copies of locus MAT-A (each with a HD1/HD2 pair) and 160 alleles (Pardo *et al.* 1996), as well as three copies of locus MAT-B (each with a pheromone/receptor gene pair and 79 alleles (Halsall *et al.* 2000), which generates more than 12,000 mating types. *Schizophyllum commune*, with an even larger number of alleles, displays more than 20,000 mating types. In other groups, the two loci fused to form a secondary bipolar system (as described above for *U. hordei*). The two mating types **a** and **α** of the human pathogen *Cryptococcus neoformans* resulted from such a fusion, which also trapped additional genes, generating an incipient sex chromosome with a ~150 kb non-recombining region. The MAT alleles also control mitochondrial inheritance, the mtDNA being inherited from the MAT-**a** parent (the MAT-**a** type is selectively eliminated soon after fusion; Yan & Xu 2003; Xu 2005; Yan *et al.* 2007).

2.2.9 Metazoa

Metazoans (animals) form the greatest part of Filozoa, a group of opisthokonts that also comprise the choanoflagellates, with functional meiotic genes (Carr *et al.* 2010) and a recently described haplo-diplontic sexual cycle with anisogamy (Levin & King 2013). All metazoans are oogamous with a diplontic life cycle (TYPE 3), but several independent evolutions towards haplo-diploid sex-determination systems, with haploid males and diploid females (TYPE 3.1c). Table 2.1b gives an overview of the phylogenetic distribution of the main sex determination types in non-Deuterostomia Metazoa.

2.2.9a Parazoa

The **Placozoa** form a subkingdom of basal Metazoa with disputed phylogenetic position. A single

species is recognized (*Trichoplax adhaerens*), but recent genetic evidence suggests multiple, morphologically similar species (Voigt *et al.* 2004). *Trichoplax* is small (1 mm sized) and flattened. It can reproduce both asexually by fission, and sexually, as evidenced from the observation of eggs and sperm as well as genetic variation resulting from genetic recombination (Signorovitch *et al.* 2005; Srivastava *et al.* 2008; Eitel *et al.* 2011). Its mode of sex determination is unknown.

The phylum **Porifera** (sponges) contains 5000–10,000 known aquatic species that predominantly live in marine habitats worldwide. They mainly feed on bacteria and other food particles in the water. Some host photosynthetic endosymbionts that contribute food and oxygen. A few species reproduce asexually and many have strong regeneration capability. Freshwater species often produce asexual gemmules in autumn, which are dormant cell clusters that can regain development after surviving unfavourable conditions. Most sponges are hermaphroditic. True gonads are lacking, and sperm cells are released into the water (broadcast spawning) to fertilize ova that are either also released, or retained in the maternal body until hatching. Fertilized eggs develop into free-swimming larvae that settle on the substrate. Some gonochoric species seem to have retained the ability to produce both eggs and sperm (e.g. *Cinachyra tarentina*, Lepore *et al.* 2009; *Spongia officinalis*, Baldacconi *et al.* 2007), but not others (e.g. *Rhopaloeides odorabile*, Whalan *et al.* 2007; *Geodia barretti*, Spetland *et al.* 2007). Although **heteromorphic chromosomes** have been described in some freshwater species (Ishijima *et al.* 2008), sponges seem to lack sex chromosomes in general, which is consistent with epigenetic sex differentiation (TYPE 3.2a).

2.2.9b Radiata

The **Ctenophora** (comb jellies), known since the early Cambrian, constitute a small phylum of 100–150 species restricted to marine habitats. Adults range in size from a few millimetres up to 1.5 m. They move with cilia and prey upon small invertebrates. A few species are parasitic. Fertilization is generally external, although in some groups eggs are brooded. The young are planktonic and in some species juveniles are capable of reproduction before reaching the adult

Table 2.1b Phylogenetic overview of sex-determination systems among non-Deuterostomia Metazoa

Taxon			Approx. number described (estimated) species	Sex determination TYPE			GSD mechanism				
				3.2a Epigenetic hermaphrodite-simultaneous	3.2a Epigenetic hermaphrodite-sequential	3.2b Epigenetic gonochorist	3.1 Genetic gonochorist	No heteromorphic sex chromosomes	Male heterogamety (XX/XY or XX/XO)	Female heterogamety (ZW/ZZ or ZO/ZZ)	Haplodiploidy
Metazoa (Animalia)	Parazoa	Placozoa	1	?							
		Porifera (Sponges)	5,000 (10,000)	●	●	●	●	●	●	●	
	Radiata	Ctenophora (Comb jellies)	100-150	●	●	●	●				
		Cnidaria (Corals, sea anemones, box & true jelly fish, hydroids)	10,000	●	●	●	●	●	●	●	

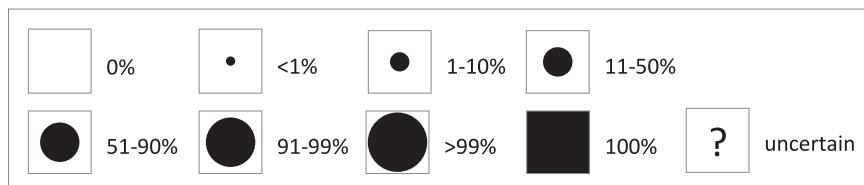


Table 2.1b *continued*

Taxon		Approx. number described (estimated) species	Sex determination TYPE				GSD mechanism			
			3.2a Epigenetic hermaphrodite - simultaneous	3.2a Epigenetic hermaphrodite - sequential	3.2b Epigenetic gonochorist	3.1 Genetic gonochorist	No heteromorphic sex chromosomes	Male heterogamety (XX/XY or XX/XO)	Female heterogamety (ZW/ZZ or ZO/ZZ)	Haplodiploidy
Lophotrochozoa	Echiura (Spoon worms)	240			●	●				?
	Annelida (Ringed worms)	>17,000	●	●	●	●	●	●	●	●
	Mollusca (Molluscs)	±120,000 (200,000)	●	●	●	●	●	●	●	●
	Brachiopoda (Lamp shells)	450			?	?				
	Entoprocta	170	●	●	●	●				
	Bryozoa (Moss animals)	±5,000	●	●						
	Phoronida (Phoronids)	10	?		?					
	Nemertea (Ribbon worms)	1,200	●		●					
	Sipuncula (Peanut worms)	144-320 (1,500)	●	●	●	●				

Table 2.1b *continued*

	Gnathostomulida (Jaw worms)	100								
	Acanthocephala (Thorny-headed worms)	1,200								
Platyzoa	Rotifera (Wheel animals)	2,180								
	Gastrotricha (Hairy backs)	790								
	Platyhelminthes (Flatworms)	30,000 (60,000)								
	Kinorhyncha (Mud dragons)	180								
Ecdysozoa	Loricifera (Loricifers)	22 (100)								
	Priapulida (Penis worms)	16								
	Nematoda (Round worms)	28,000 (500,000)								
	Nematomorpha (Horsehair worms)	350 (2,000)								
	Onychophora (Velvet worms)	180								
	Tardigrada (Water bears)	1,150								
	Arthropoda (Arthropods)	1,170,000 (10,000,000)								

Table 2.1b *continued*

Arthropoda	Chelicerata (Horsehoe crabs, scorpions, spiders, mites)	77,000 (500,000)								.
	Myriapoda (Millipedes, centipedes)	13,000 (88,000)								
	Crustacea (Crustaceans)	67,000 (150,000)								.
	Hexapoda (Hexapods)	1,023,000 (9,000,000)								

size and shape. Most species are simultaneous (and likely self-fertile) hermaphrodites. A few species are protandrous (*Platyctenids*) or gonochoric (*Ocyropsis*, *Bathocyroe fosteri*; Miller *et al.* 2000). Sex differentiation is thus predominantly epigenetic (TYPE 3.2a).

Cnidaria contain over 10,000 species restricted to aquatic environments worldwide (mostly marine), and differentiated into Anthozoa (corals and sea anemones), Scyphozoa (true jellyfish), Cubozoa (box jellyfish), and Hydrozoa. Many Anthozoa are known for their building of ecologically important coral reefs, and some box jellyfish for their extreme toxicity. Reproduction is predominantly sexual, but all known cnidarians can also reproduce through regeneration after fission or budding. Many have complex life cycles with alternations of sexual (medusa) and asexual (polyp) generations that are strongly dependent on environmental conditions (mostly temperature and photoperiod). The free-living larvae of jellyfish settle on the substrate, and become polyps that can split off juvenile free-swimming medusae (a process called strobilation). Some Hydrozoa may produce colonies composed of sexual medusae and asexual polyp-like zooids, acting as single organisms (e.g. the Portuguese man-of-war *Physalia physalis*). Some species lack the medusa (e.g. *Hydra*) or polyp stage altogether, whereas in anthozoans it is the polyp stage that reproduces sexually. Fertilization is generally external with sometimes mass spawning of ova and sperm into the water in the breeding season. Lively & Johnson (1994) documented a striking association between brooding and parthenogenesis.

Cnidarians exhibit both sequential and simultaneous (self-fertile) hermaphrodites, as well as

gonochoric species. Transitions are likely frequent: hydrozoans have hermaphroditic and gonochoric species within the same genus (Carré & Carré 2000). Hermaphroditic colonies may liberate both male and female (gonochoric) medusae, implying epigenetic sex determination, possibly mediated by temperature effects (e.g. *Clytia*, Carré & Carré 2000; *Hydra*, Littlefield 1994). Similarly, a single asexual founder of the sea anemone *Aiptasia diaphana* may produce female, male, and hermaphroditic offspring (Schlesinger *et al.* 2010). Genotypic sex determination (GSD) has been sometimes proposed based on 1:1 population sex ratios, for example, in the anthozoans *Antipathes griggi* (Wagner *et al.* 2012), *Dendronephthya castanea* (Hwang & Song 2012), *Parazoanthus axinellae* and *Savalia savaglia* (Previati *et al.* 2010), *Sarcophyton elegans* (Hellström *et al.* 2010), *Eunicella singularis* (Ribes *et al.* 2007), two species of *Flabellum* (Waller & Tyler 2011), box jellyfish *Carybdea sivickisi* (Lewis & Long 2005), and true jellyfish *Lychnorhiza lucerna* (Schiariti *et al.* 2012). However, sex chromosomes have not been reported from any cnidarian (e.g. Wijsman & Wijsman-Best 1973, Anokhin & Kuznetsova 1999; Choe *et al.* 2000; Zacharias *et al.* 2004; Flot *et al.* 2006; Stepanjants *et al.* 2006; Genikhovich & Technau 2009, Anokhin *et al.* 2010), and are believed to be absent (Fautin *et al.* 1989). Sexual dimorphism in gonochoric species is very limited: males and females are only distinguished by the structure and colour of their gonads (Fautin 1992). In general, sex determination seems very plastic and polyfactorial (Littlefield 1994) with a mixture of genetic and epigenetic effects (TYPES 3.1, 3.2a and c).

Interestingly, Miller *et al.* (2003) found a DM domain-containing protein in the coral *Acropora*

millepora with a likely role in sex differentiation, which suggests an evolutionary conservation of sex-determination pathways in Metazoa down to Cnidaria (Section 3.3.2a).

2.2.9c Lophotrochozoa

The **Echiura**, or spoon worms, are a small phylum of marine worms comprising about 240 species that live in burrows and crevices. They are closely related to the Annelida and tend now to be incorporated into this phylum. All Echiura are gonochoric with separate male and female individuals, usually with external fertilization. One of its best-known members, the green spoon worm *Bonellia viridis*, often features in textbooks as an example of environmental sex determination (ESD) (TYPE 3.2c; the first case of ESD to be proved in animals; Baltzer 1914). It has an extreme sexual dimorphism with free-living females that reach around 8 cm in length (excluding the extensible proboscis of up to one metre). The males are much smaller (1–3 mm) and live in the female uterus. The larvae are planktonic and settle as females in isolation, but as dwarf males when settling on females. Other than by exposure to females, male development can be induced by larval interaction and by unknown substances released by females (Jaccarini *et al.* 1983). However, these authors also found a proportion of male individuals whose sex is apparently determined by their genotype, as well as some intersexes. Sex determination might be polygenic and depend on the relative proportion of female- and male-determining genes, but this requires further testing. Hence, sex determination seems to result from an interaction between genetic and environmental factors ($G \times E$).

Sipunculida (peanut worms) form a small phylum of unsegmented marine worms, often incorporated into Annelida. Most sipunculans reproduce sexually, but some species multiply asexually via transverse fission followed by regeneration of vital body components. Fertilization is external and eggs hatch into larvae that develop into free-living adults. Populations of *Themiste lageniformis* have female-biased sex ratios and consist of parthenogens and sexuals. According to Pilger (1989), parthenogenesis is diploid and fertilization facultative. The vast majority of sipunculan species are gonochoric. An

exception is *Golfingia minuta* whose Scandinavian populations are protandrous, whereas other European populations are simultaneous hermaphroditic and self-fertilize. Whether this reflects a genetic or an environmental difference is unknown. In addition, nothing is known about gonochoric sex determination in this group.

The **Pogonophora**, or beard worms, comprise some 120 known species of benthic tube-dwelling worms, living in deep sea (from 100 to 10,000 m), now classified as annelids. They may reach up to 3 m long (e.g. the rift dwelling *Riftia* in Vestimentifera). Beard worms lack mouth, gut, and anus; they rely on symbiotic chemo-synthetic bacteria that produce organic compounds from dissolved nutriments. They are gonochoric with internal fertilization, but the mechanisms of sex determination are unknown.

The phylum **Annelida** (ringed worms) contains over 17,000 species that mainly live in aquatic environments. They are traditionally divided into three classes, the Polychaeta (bristle worms), the Oligochaeta (earthworms; > 3000 known species), and Hirudinae (leeches), though leeches are now viewed as a subgroup of oligochaetes, itself a subgroup of polychaetes. In addition, Pogonophora, Echiura, and Sipuncula are now also seen as subgroups of polychaetes. Some annelids are known for their important role in ecosystems, such as the terrestrial earthworms and the marine bristle worms, and from a medical perspective, such as leeches. Annelida exhibit a large variety of reproductive forms, as exemplified by the polychaete family Syllidae (Franke 1999). The predominant, and likely ancestral, mode of reproduction is sexual and gonochoristic, although parthenogenesis occurs frequently (Christensen 1980), for example, in *Tubifex*. In addition, fission and budding are widespread among polychaetes and oligochaetes, but not Hirudinae. Polyploidy and **pseudogamy** in relation with parthenogenesis has been well studied in the earthworm *Lumbricillus lineatus* (Christensen 1960). In addition, polychaetes and oligochaetes have remarkable capacities for regeneration after severe damage. In many marine groups fertilization is external; fertilized eggs develop into trophophore larvae, which live as plankton and metamorphose

into adults upon sinking to the sea floor. Fertilization is also external in earthworms, occurring within the protection of the slime tube that, after drying, forms a protective covering over the eggs (cocoon). In leeches, fertilization is internal; eggs are then transferred to the cocoon, and develop directly into adults.

Oligochaeta and Hirudinae are simultaneous hermaphrodites with obligatory outcrossing. Selfing is prevented by the morphological structure of reproductive organs. Gonochorism with diplo-genotypic sex determination (TYPE 3.1a) is common among polychaetes. For some investigated species (e.g. *Polydora brevipalpa*; Pankova & Manchenko 2003), no heteromorphic sex chromosomes have been found, despite proven sex-linked inheritance of some loci. Other species have been reported to have XY male heterogamety, such as *Polydora curiosa* (Korablev *et al.* 1999) and *Hediste japonica* (Sato & Ikeda 1992; Tosuji *et al.* 2004), in which the Y is conspicuously larger than the X chromosome. Male heterogamety of the XO-type was discovered for the first time in 1970 in the tiny polychaete *Dinophilus gyroclitatus*. This species also presents egg-size dimorphism (with large eggs developing into females and small eggs into precocious dwarf males; Traut 1970; Simonini *et al.* 2003), which implies that large female-type eggs are fertilized by X-bearing sperm and small male-type eggs by O-sperm (Martin & Traut 1987). Heritable variation of sex ratios (e.g. in *Ophryotrocha labronica*; Premoli *et al.* 1996), points to multigenic sex determination.

Female heterogamety with G × E interactions has been reported from *Capitella capitata* (Petraitis 1985). Heterogametic ZW individuals always develop as females, but homogametic ZZ individuals develop into either females when at low density, or males when at high density; if females are rare, these males may then turn into hermaphrodites (Petraitis 1991). Pure environmental / social sex determination has been reported from several species, for example, two *Polydora* species (Radashevsky 1989) and the bone-eating and harem-forming *Osedax roseus* (Rouse *et al.* 2004, 2008): dispersing larvae develop into females when landing on a bone, but into paedomorphic dwarf males when landing on a female. A female may host more than ten males in her tube, and sex ratios are usually male biased.

Hermaphrodites (TYPE 3.2a) are known from many families, often with closely related gonochoric species (e.g. *Hediste limnicola* and *H. japonica*; Tosuji *et al.* 2010). Both self-fertilization and cross-fertilization occur, but there is no documentation of SI. Most cases are simultaneous hermaphrodites, but sequential hermaphroditism has been reported for several species, often with protandry (Schroeder & Hermans 1975). A well-studied example is *Ophryotrocha puerilis*, individuals of which start off as male but turn into female later when reaching a certain size (Bacci 1965). The size at which sex change occurs (in terms of number of chaetigerous segments) can evolve under selection (Bacci & Bortesi 1961). Sex determination is likely polygenic, and sequential hermaphrodites can be selected towards yielding pure females or males (Bacci 1978; Sella 1980). Populations of *Typosyllis prolifera* are partially protogynous (Franke 1986): about half of the individuals differentiate into stable primary males, whereas those that develop into females often irreversibly change sex at a later stage in the life cycle. The degree of sex change varies per population suggesting a genetic divergence between populations. Thus, a polygenic mode of sex determination, in interaction with epigenetic factors, likely applies to many annelids; sex ratios are quite variable, the expression of sexual phenotypes is very plastic, and many species contain not only males and females, but also hermaphrodites (Petraitis 1985). Premoli & Sella (1995) and Prevedelli *et al.* (2006) discuss the selective pressures on sex allocation in benthic polychaetes stemming from sex ratio selection and mating success.

With around 120,000 recognized extant species, the **Mollusca** constitute the largest animal phylum in marine habitats, and have also colonized freshwater and terrestrial environments. Including clams, squids, and cuttlefish, as well as the species-rich gastropods (snails and slugs), molluscs are highly diverse in size, shape, anatomy, behaviour, and habitat. Depending on groups, fertilization may be internal or external, and larval development direct or indirect (eggs developing into trochophore or more complex veliger larvae). Molluscs predominantly reproduce sexually, but some are parthenogenetic: populations of the snail *Potamopyrgus antipodarum* consist of varying proportions of sexuals and parthenogens (Lively 1987).

Hermaphroditism occurs in about 40% of the 5600 genera (Heller 1993): 100% of the solenogastres (Aplacophora) and Pulmonata, 99% of the Opisthobranchia, 3% of the Prosobranchia, and 9% of the Bivalvia. It is very rare in Polyplacophora and absent in Cephalopoda. Simultaneous hermaphroditism with selfing is widespread among freshwater baso-ommatophorans, amphibious stylommatophorans, and freshwater bivalves (Heller 1993). It is obligatory in some stylommatophorans with reduced or completely lost male intromission organ (aphally). Sequential hermaphroditism, only reported from gastropods and bivalves, is almost exclusively protandric as, for example, in the blacklip pearl oyster *Pinctada margaritifera*, extensively exploited for the pearl industry (Chavez-Villalba *et al.* 2011). In the slipper limpet *Crepidula fornicata* (Calyptaeidae), sex switch is socially determined. Individuals live on top of each other in stacks; the first young to settle on a substrate turns into a female after a short male phase, the next individual settles on top of it as a male, but turns into a female as soon as a third individual arrives, and so on. Gonochorism with dwarf males living upon females is common among ectoparasitic Eulimoidea snails and commensal Galeomatoidea clams. If the female determines not only the size of the male but also his sex, dwarf males should be considered as a result of social sex determination (Heller 1993).

Karyotypes have been well studied in molluscs and reveal that morphologically differentiated sex chromosomes are rare, which is to be expected when hermaphroditism is widespread. Male heterogamety is the rule among gonochorists (Nakamura 1986), including several Gastropoda (such as the knobbed whelk *Busycon carica*; Vitturi *et al.* 1998; Thiriot-Quiévreux 2003; Avise *et al.* 2004) and Bivalvia (such as the surfclam *Mulinia lateralis*; Guo & Allen 1994). Some species of the pelagic Heteropoda have multiple X or Y chromosomes (Vitturi *et al.* 1993; Thiriot-Quiévreux 2003). An XO-system has been reported for several neritid species and *Monodonta vermicularis* (Thiriot-Quiévreux 2003), and has been suggested for the clam *Mya arenaria* (Allen *et al.* 1986). A ZW-system is known from freshwater snails *Viviparus* (Barsienne *et al.* 2000). No sex chromosomes have been reported for the Ophisthobranchia and Pulmonata.

Cupped oysters (*Crassostrea*) are sequential hermaphrodites, with a mixture of unisexuals and protandric hermaphrodites. For the eastern cupped oyster *C. virginica*, Haley (1977, 1979) proposed multi-locus sex determination with environmental effects. For the Pacific oyster (*C. gigas*), Guo *et al.* (1998) proposed a single major bi-allelic sex locus with two genotypes: FM individuals develop as fixed males, while FF have labile sex determination, developing into females with a probability depending on other genetic or on environmental factors. To account for an apparent paternal control of sex ratios in controlled crosses, Hedrick & Hedgecock (2010) expanded this into a three-genotype model, with MM individuals being always male, FM individuals male or female (protandric hermaphrodites), and FF individuals always female.

In the apple snail *Pomacea canaliculata*, sex ratios also show large and heritable variation at the family level, despite being even at the population level. Using a factorial design (halfsib families), Yusa (2007) found equal contribution from both parents to sex ratio variation, with additive effects from a few (at least four) zygotic sex-determination genes.

Mitochondrial genes are involved in sex determination in dioecious species from three orders of bivalves (Mytiloidea, Unionidea, and Veneroidea) that present doubly uniparental mtDNA inheritance (apparently the sole exception to strict maternal inheritance among animals; Breton *et al.* 2007). In addition to maternal mtDNA, sons also inherit the paternal mtDNA, which then segregates to the germ line exclusively. With more than 200 My of divergence, the maternal and paternal haplotypic lines are much differentiated, particularly on a specific protein-coding gene which seems directly involved in the sex-determination pathway: the male and female sequences (M- and F-open reading frame (ORF) show > 50% amino-acid sequence divergence (Breton *et al.* 2011). The M haplotype was independently lost in several hermaphroditic species with close dioecious relatives; in parallel, the F-ORF (which typically shows a predicted transmembrane helix in the N-terminus half, with a relatively conserved 15 amino-acid motif near the C-terminus), is much diverged in the hermaphroditic lineages (H-ORF). Whether paternally transmitted mtDNA is able to colonize the germ line (and thus turn the

embryo into a male) seems controlled by maternal nuclear genes. Polymorphisms of these genes result in strong maternal effects on family sex ratios, as documented in several *Mytilus* mussels (e.g. Saavedra *et al.* 1997; Kenchington *et al.* 2002).

Brachiopods are strictly marine animals resembling bivalves, with hard shells on their upper and lower surfaces, connected at the rear end. Two groups are recognized, articulates and inarticulates. Fertilization is external: the separate sexes releasing ova and sperm into the water, but females of some species keep the embryos in brood chambers until the larvae hatch. The larvae of inarticulates are planktonic for several months, whereas the larvae of articulate species develop directly into miniature adults within a few days. Nearly all brachiopods are gonochoric but virtually nothing is known about the mechanism of sex determination (Long & Stricker 1991). Sex ratios are typically even (Meidlinger *et al.* 1998).

The phylum **Entoprocta** contains mostly sessile aquatic animals of small size (up to 7 mm). Mature individuals are goblet-shaped with long stalks and a crown of tentacles that is used to draw food particles towards the mouth. Most entoprocts are colonial and marine. Fertilization is typically external, but some species brood their eggs in a gonopore. After hatching, the larvae swim for a short time before settling on a surface where they metamorphose into adults. In addition to sexual reproduction, all colonial and solitary species can also reproduce clonally by budding. Colonial species produce new zooids from the stolon or from the stalks, and can form large colonies in this way. Sex determination is normally epigenetic. Most species are simultaneous hermaphrodites (TYPE 3.2a), but some are protandrous, switching from males to females as they mature. In *Barentsia discreta*, zooids are unisexual and start off as males during colony formation, but change into females upon maturation. A smaller proportion of species is gonochoristic, individuals remaining of the same sex all their lives, but how sex is determined in unknown.

The **Bryozoa** (or Ectoprocta), also known as moss animals, are tiny aquatic filter-feeders. About 5,000 living species are known that almost exclusively live in colonies, made of numerous zooids. All Bryozoa

are hermaphrodites, with epigenetic sex determination (TYPE 3.2a). Zooids of freshwater species are simultaneous hermaphrodites, whereas most many marine species are protandric sequential hermaphrodites. Marine colonies thus always contain a combination of zooids that are in their male and female stages. Sperm is released into the water, and fertilization can be external (in species where eggs are also released) or internal (sperm being captured by tentacles). In some groups, like the freshwater phylactolaemates, sexual reproduction alternates with asexual reproduction and produces statoblasts that are resistant to freezing and desiccation.

Phoronida are exclusively marine filter feeders. Most adult are a few centimetres in size, but some can reach 50 cm. All phoronids reproduce sexually, although *Phoronis ovalis* is known to build colonies by budding. Fertilization is external or internal, and larvae are planktonic. Both hermaphroditism and gonochorism are found among phoronids, suggesting respectively epigenetic (TYPE 3.2a) and diplogenotypic (TYPE 3.1a) sex determination, but there is no further information about the mechanisms.

Nemertea, known as ribbon worms, are colourful marine and freshwater predators. Most are of small size; some can reach several metres long but are only a few millimetres wide. Reproduction is generally sexual and egg fertilization external. Adults easily break up and some species have strong regeneration capability. Most nemerteans are gonochoric but all the freshwater forms are hermaphroditic. Females of the crab egg predator *Carcinonemertes* produce haploid larvae parthenogenetically when kept in isolation, but diploids when raised with males (Roe 1986), possibly suggesting a form of haplodiploidy (TYPE 3.1c). Whether these haploid larvae develop as males, however, is unknown. Karyotypic studies have revealed no heteromorphic sex chromosomes (Chen *et al.* 2009).

2.2.9d Platyzoa

The **Gnathostomulida**, or jaw worms, form a small phylum containing about 100 species of very small marine invertebrates that live in the sand and mud of shallow coastal waters. Sex determination is epigenetic, all gnathostomulids being simultaneous

hermaphrodites (TYPE 3.2). After fertilization, the egg ruptures through the body wall and hatches into a miniature adult without a larval stage.

Acanthocephala (thorny-headed worms), now considered Rotifera based on recent molecular information, comprise some 1200 parasitic species, with an evertable proboscis used to attach to the gut wall of their host. Many acanthocephalans have complex life cycles, involving a number of different hosts including invertebrates, fishes, amphibians, birds, and mammals. Eggs are fertilized within the body cavity where they develop into embryos that leave the body through an oviduct to end up in the host's gut and being excreted with its faeces. Some species can be highly polyploid. All Acanthocephala are gonochoric. Sex determination is diplo-genotypic (TYPE 3.1a/b) with heteromorphic sex chromosomes, consistent with equal sex ratios found in nature (Crompton 1992). Karyotypes have been described for a few species and show male heterogamety (TYPE 3.1a) of the XY-type for *Macracanthorhynchus hirudinaceus* (Robinson 1964) and of the XO-type for *Moniliformis dubius* (Robinson 1965), *Echinorhynchus truttae* (Parenti *et al.* 1965), *Neoechinorhynchus cylindratus* (Bone 1974a), *Leptorhynchoides thecatus* (Bone 1974b), *Pomphorhynchus laevis* (Fontana *et al.* 1993), and *Acanthocephalus lucii* (Špakulová *et al.* 2002).

The rotifers (phylum **Rotifera**) comprise some 2200 described species of microscopic invertebrates widespread in freshwater environments, with a few marine species. They are classically divided in three classes: Monogononta, Bdelloidea, and Seisonidea (the latter only comprising three species), but Acanthocephala are now also considered as part of this phylum. Some rotifers are free swimming and make up an important part of the freshwater zooplankton, whereas other move over a substrate, or are sessile. Some species are colonial. Rotifers reproduce sexually or by cyclical, pseudogamous or obligate parthenogenesis. Fertilization is internal, sperm being injected by the penis into the female's cloaca or through her skin. Some species are ovoviparous, retaining the eggs inside their body until they hatch. Most species hatch as miniature versions of the adult.

Bdelloid rotifers are known as anciently asexual as they apparently have reproduced without sex for millions of years. Accordingly, they have no heteromorphic sex chromosomes (Mark Welch & Melselson 1998). The two other groups are gonochoric. Monogononts have cyclical parthenogenesis, alternating rounds of asexual and sexual reproduction, often triggered by worsening environmental conditions. Non-inseminated females produce small haploid males (TYPE 3.1c), which often lack a functional digestive system and are already sexually fertile at birth. Inseminated females produce diploid resting eggs that develop as parthenogenetic females once conditions improve. Unlike haplodiploid Hymenoptera, individual females cannot control offspring sex ratios, but male-producing and resting-egg producing females seem to occur at equal frequencies during the sexual phase in populations of *Brachionus plicatilis* (Aparici *et al.* 2002). Seisonidae are obligately sexual, with similarly-sized males and females. Sex ratios seem equal in *Seison*, which might point to diplo-genotypic sex determination (TYPE 3.1a/b; Gilbert 1992), as otherwise found in the related Acanthocephala.

The **Gastrotricha**, or hairy backs, form a phylum of tiny platyzoans, with about 800 described species living in freshwater and marine environments. Fertilization is internal, the eggs are released by rupture of the body wall, and develop into small adults. Reproduction is typically sexual, but many species of the order Chaetonotida reproduce by parthenogenesis. In parthenogenetic species the male reproductive system is degenerated and non-functional, or sometimes entirely absent.

All gastrotrichs are hermaphrodites (TYPE 3.2a/b), usually simultaneous, but protandry and protogyny have been documented in Macrodastyidae (Hummon & Hummon 1992). Freshwater chaetonotids were believed for a long time to be obligately parthenogenetic, but close microscopic investigation has revealed sperm in many species (Weiss 2001). There is evidence from several species that individuals start their life as parthenogens, but mature into simultaneous hermaphrodites later in life.

The **Platyhelminthes** (flatworms) form a primitive phylum of Platyzoa comprising about 30,000

described species and an estimated 60,000 or more existing species (Špakulová and Casanova 2004) that are predominantly parasitic. They are traditionally divided in four classes: Trematoda (flukes), Cestoda (tapeworms), Monogenea, and the paraphyletic Turbellaria (flatworms). Some tapeworms and flukes cause important diseases in humans and livestock, the most devastating ones are members of the family Schistosomatidae, responsible for schistosomiasis in humans. Although sexual reproduction is the rule, many turbellarians reproduce asexually by cloning or budding, and have high regeneration capacity. Pseudogamous and polyploid parthenogenesis occurs in a number of turbellarians (Benazzi & Benazzi Lentati 1976). Fertilization is internal following copulation or sperm impregnation. Eggs typically hatch into small adults, but in some large species there is a free-swimming larval stage.

The vast majority of platyhelminths are simultaneous or sequential hermaphrodites (Benazzi 1992), normally with obligate outcrossing (TYPE 3.2b). Accordingly, the karyotypes from about 100 species of Cestoda show no heteromorphic chromosomes (Špakulová & Casanova 2004). Gonochorism apparently evolved at least eight times (Snyder & Loker 2000), for example, in trematodes (e.g. the parasitic Schistosomatidae) and some members of the freshwater Tricladida. The two sexes of the marine planarian *Sabussowia dioica* show no chromosomal difference (Charbagi-Barbarou & Tekaya 2009). *Schistosoma* have been well studied for their sexual dimorphism and heteromorphic sex chromosomes (Verjovsky-Almeida *et al.* 2003). Female heterogamety (TYPE 3.1b) has been confirmed in 13 species, including *Schistosoma japonicum* (Hu *et al.* 2003) and the bird schistosome *Trichobilharzia regenti* (Špakulová *et al.* 2001). A neo-W chromosome was found in the mammalian parasite *Heterobilharzia americana* (Short *et al.* 1987). Interestingly, in a transcriptional analysis of *Schistosoma mansoni*, Verjovski-Almeida *et al.* (2003) discovered orthologues of a number of genes that are involved in *Caenorhabditis elegans* sex determination, suggesting an ancient role in sex determination.

2.2.9e Ecdysozoa

Tardigrada, commonly known as waterbears or moss piglets, form a phylum of small (0.1–1.5 mm)

segmented animals with eight legs. Some 1150 species have been described that occur in aquatic environments all over the world. Tardigrades mate during moulting and are **oviparous**. Fertilization is usually external. Eggs hatch into juveniles that already have the adult appearance and grow by enlargement of the individual cells.

Most tardigrades are gonochoric, but a number of species are simultaneous hermaphrodites (TYPE 3.2a/b; Rebecchi *et al.* 2000). They typically reproduce sexually, but several species can be partially or completely parthenogenetic with females only (Bertolani 1982, 2001). Diploid or polyploid parthenogenetic cytotypes have been reported for *Macrobiotus pseudohufelandi* (Rebecchi 1991), *Richtersius coronifer* (Rebecchi *et al.* 2003), and *Milnesium tardigradum* (Heinz *et al.* 2005). It is not known whether occasional male production in parthenogenetic strains is due to genetic or environmental factors (Suzuki 2008). Karyotypes have been studied in a number of species, with no heteromorphic sex chromosomes found in sexual populations of *Richtersius coronifer* (Rebecchi *et al.* 2003) and *Macrobiotus richtersi* (Rebecchi *et al.* 2002). Interestingly, Bertolani (1982) found both gonochoric and hermaphroditic strains in *Isohypsibius granulifer* that differed in one chromosome pair. Thus, sex determination in tardigrades is likely genotypic in most species (TYPE 3.1), but epigenetic effects in gonochorists (TYPE 3.2c) cannot be ruled out at this point.

The velvet worms, phylum **Onychophora**, are segmented animals with multiple pairs of legs. There are about 180 species that are largely confined to the southern hemisphere. They prey on small animals, such as insects, by squirting an adhesive slime on to them. They have conspicuous mating behaviour and bear live young. Fertilization takes place internally, but the mode of sperm transmission varies widely from spermatophore transmission into the female's genital opening, placement of spermatophores on the female's skin followed by penetration into the haemocoel and intrusive insemination through spine-like penises. Velvet worms are found in egg-laying (oviparous), egg-live-bearing (ovoviparous) and live-bearing (viviparous) forms.

Almost all species of velvet worm reproduce sexually, are gonochoric, and sexually dimorphic,

but *Epiperipatus imthurni* is parthenogenetic and no males have been observed for this species (Read 1988). Several *Cephalofovea* and *Planipapillus* species appear to have XX-XY sex determination, although in some populations no dimorphic pair of chromosomes could be discerned (Rowell *et al.* 1995; 2002). A heteromorphic pair of XY chromosomes is clearly present in *Cephalofovea tomahmontis* (Reid *et al.* 1995; Rowell *et al.* 1995) and *Ooperipatellus insignis* (Rowell *et al.* 2002). Genetic sex determination (TYPE 3.1a) is therefore most likely for this group, consistent with a 1:1 primary sex ratio observed in the viviparous neotropical *Plicatoperipatus jamaicensis* (Havel *et al.* 1989).

Nematomorpha, commonly known as horsehair worms or Gordian worms is a phylum of parasitic animals that morphologically resemble nematodes. There are 350 described species and an estimated 2000, which greatly range in size from 1 mm to 200 cm. The adult worms are free living in moist habitats, but the larvae are parasitic on insects and crustaceans. After being released from their hosts, males and females pair and mate in large clusters, the so-called Gordian knots.

The majority of nematomorphs reproduce sexually and are gonochoric, although a case of genetically based parthenogenesis was reported in *Paragordius obamai* and may occur more widely (Hanelt *et al.* 2012). Except that primary sex ratios are probably equal (Hanelt *et al.* 2005), no information is available on karyotypes and modes of sex determination in this group.

The nematodes or roundworms (**Nematoda**) form a very diverse phylum of invertebrates containing over 28,000 described and about 500,000 estimated species. More than 50 % of species are parasitic. They occur worldwide in freshwater, marine, and terrestrial environments in nearly every ecosystem. Nematodes are slender worms, typically less than 2.5 mm long, but some species can reach up to 5 cm in length. Some species are parasitic on humans (e.g. *Ascaris*), others can be very detrimental in horticulture, like the root-knot nematode (*Meloidogyne*) and detrimental for livestock and pets, although beneficial species are also known (e.g. entomopathogens). In free-living roundworms, the eggs hatch into larvae, which are essentially identical in appearance to

the adults. Parasitic roundworms have more complicated life cycles.

Nematodes have evolved a large variety of reproductive modes (reviewed in Denver *et al.* 2011). Most species are gonochoric, with males typically smaller than females, but species can also be partially or completely hermaphroditic. Reproduction is usually sexual, but obligate and cyclical parthenogenesis as well as pseudogamous parthenogenesis also occurs widespread, for example, in the plant-parasitic genus *Meloidogyne*, the free-living *Mesorhabditis*, and several species of the Secernentia, including the human parasitic *Strongyloides*. Several species are heterogonic, alternating different reproductive modes across generations, such as gonochorism and hermaphroditism, for example, in the Panagrolaimoidea. *Meloidogyne* species typically reproduce by parthenogenesis but males are produced under stress. In contrast to some of its close relatives that are gonochoric, the genetic model organism *C. elegans* (and *C. briggsae*) exhibits androdioecy, with XO males and XX hermaphrodites, a rare mechanism among animals (Box 3.15). Androdioecy apparently evolved three times independently from dioecy in this genus and a similar polymorphism of both reproductive modes is found in the genus *Oscheius* and *Pristionchus* (Félix 2006; Mayer *et al.* 2007).

The predominant chromosomal mode of nematode sex determination is XX in females and XY or XO in males (Triantaphyllou 1983; Mutafova 1995; Špakulová & Casanova 2004; Hasegawa *et al.* 2006; Eamsobhana *et al.* 2009). Multiple X chromosome systems have been reported from the Ascarididae (Mutafova 1995). As is likely typical for an XX-XO system, sex is determined by the ratio between X chromosomes and sets of autosomes. Interestingly, Chandler (2010) found genetic variation and genotype by environment interactions for sex determination in mutant *C. elegans* laboratory populations. In addition, Chandler *et al.* (2012) showed with laboratory crosses that some of the sex determination genes of *C. elegans* are temperature sensitive. In laboratory cultures of *Rhabditis* sp. SB347 two developmental paths can occur (Félix 2004). Embryos either go through four larval stages and develop into mature males and females (gonochoric), or they have a non-feeding third larval stage and become

reproductively mature hermaphrodites. *Strongyloides* species have a mixture of GSD and ESD. Some species, including *S. ratti* have an XX-XO genetic system but male and female development is also affected by host-specific and external environmental factors (Streit 2008). All these studies point at the existence of a blurred distinction between genetic (TYPE 3.1) and epigenetic (environmental, TYPE 3.2c) sex determination in these species.

Pure ESD (TYPE 3.2c) is known in some nematode groups, including the Mermithidae (Poinar 1979, cited in Blackmore & Charnov 1989); only females are born in lightly infected hosts, and only males in heavily parasitized hosts. At intermediate parasite densities mixed populations are produced (Christie 1929; Harlos *et al.* 1980). In the potato cyst nematodes *Globodera*, sex is also affected by host resistance; larvae are more likely to develop as males when conditions are unfavourable (Schouten 1993). In the mosquito parasite *Octomyomermis muspratti*, several environmental factors matter, including the density of individuals in the host (Petersen 1977). This large variety and multiple shifts in reproductive modes make nematodes particularly promising for studying evolutionary transitions in sex-determination mechanisms (Denver *et al.* 2011).

Priapulida, or penis worms, form a phylum of marine worms consisting of 16 extant species. They are named for their extensible proboscis, which has a human penis like shape in some species. They are gonochoric, but virtually nothing is known about their life cycles and no information is available about sex determination.

Loricifera is a phylum of very small (up to 1 mm) animals with 22 described species. They inhabit marine sediments where they attach themselves firmly to the substrate. Development occurs through so called Higgins larvae, which differ from adults in several respects. Reproduction is generally sexual, but parthenogenesis and paedogenesis also occurs. Adults occur as separate sexes, but no information is available on the mechanisms of sex determination.

Kinorhyncha is a phylum of small (less than 1 mm) marine invertebrates that live in mud or sand and are also called mud dragons. The larvae

are free-living, but little else is known of their reproduction. They are gonochoric with both sexes looking alike, but nothing is known about their sex determination.

Arthropoda are invertebrates with segmented bodies and jointed limbs. The phylum covers an estimated 1,170,000 described species accounting for over 80% of all known living animal species. The number of extant species may well be 5–10 million. Arthropods are found in virtually every ecosystem worldwide. The vast majority of species are sexual and gonochoric, but parthenogenesis and hermaphroditism also occurs. The four subphyla (Chelicerata, Myriapoda, Crustacea, and Hexapoda) will be treated separately below.

The subphylum **Chelicerata** includes the horseshoe crabs, scorpions, spiders, and mites. Horseshoe crabs (Merostomata) are marine with external fertilization, whereas all other chelicerates have internal fertilization. Sperm transfer can be in the form of spermatophores or by direct insemination.

Reproduction in chelicerates is typically sexual, but clusters of parthenogenetic species occur widespread in the phylogeny of the group. Pseudoscorpions (class Arachnida) have male XO sex determination (TYPE 3.1a) which is likely ancestral for this group (Štáhlavský *et al.* 2006). Spiders (Araneae) also have male heterogametic XO sex determination, but frequently with multiple X chromosomes, up to four or five in number, which probably arose by duplication of an ancestral X chromosome (Rodríguez *et al.* 2002; Král *et al.* 2011). No heteromorphic sex chromosomes were found in the primitive palpigrades (Král *et al.* 2008). Female heterogamety and epigenetic sex determination have not been reported from the Chelicerata.

The Acari (mites and ticks) exhibit a large variety of genetic sex-determination systems. Diploidy and male heterogamety (TYPE 3.1a) without distinct sex chromosomes appears to be ancestral in both major orders, the Parasitiformes and Acariformes (Norton *et al.* 1993). The ticks (Ixodida) are diploid with male heterogamety of the type XY or XO. Other groups in the Parasitiformes have retained the ancestral system with undifferentiated sex chromosomes, but many derived groups have haplodiploid sex

determination. The Acariformes also contain groups with haplodiploid and diploid reproduction, the latter with male heterogamety of the XY or XO type, or without heteromorphic sex chromosomes. Haplodiploidy occurs widely in mites and can be of two types: arrhenotoky with haploid males developing from unfertilized eggs (TYPE 3.1c), or paternal genome loss (also called parahaploidy or **pseudoarrhenotoky**) in which males develop from fertilized eggs from which the paternal genome is eliminated (Sabelis *et al.* 1993). In addition, many arrhenotokous species have shifted to reproduce by **thelytoky** in which diploid females are parthenogenetically produced and males are absent. Weeks *et al.* (2001) found that the false spider mite *Brevipalpus phoenicis* consists of haploid female parthenogens only, this form of thelytoky being controlled by endosymbiotic *Cardinium* bacteria.

The **Myriapoda** are a subphylum of arthropods containing the millipedes, centipedes, and others. There are about 13,000 described species, all of which are terrestrial. Most myriapods are sexual and gonochoric, but some reproduce by parthenogenesis. The mode of sex determination in the order Diplopoda (millipedes) is male heterogamety (TYPE 3.1a) with XY or XO systems (White 1979, Fontanetti *et al.* 2002, Warchałowska-Śliwa *et al.* 2004; de Godoy *et al.* 2008), although the differentiation between the X and Y chromosome is often very slight. Woznicki *et al.* (2003) found no heteromorphic sex chromosomes in the centipede *Lithobius forficatus* (order Chilopoda). In a karyotype study of the order Symphyla, Fischer (1987) found a case of male XO in *Sympylella isabellae*, but no heteromorphic chromosomes in males and females in a number of other species. The order Pauropoda contains species with male heterogamety of the XY and XO type and others with no heteromorphic sex chromosomes (Fratello & Sabatini 1990). Thus, all myriapods studied to date appear to have genetic sex determination of TYPE 3.1a although the sex chromosomes cannot be distinguished morphologically in many species. There are no indications for epigenetic components.

Crustacea (crustaceans) form a very large subphylum of arthropods, which includes over 67,000

described species as diverse as crabs, krill, water fleas, and barnacles. Most crustaceans are free-living and aquatic, but some are sessile (e.g. barnacles), terrestrial (e.g. woodlice), or parasitic (e.g. fish lice, tongue worms, *Sacculina*). In many groups, the fertilized eggs are simply released into the water column, while others have developed a number of mechanisms for holding on to the eggs until they are ready to hatch, such as attachment to their limbs or a brood pouch. Eggs hatch into several types of larvae that develop, often after several instars, into the adult phenotype. Most crustaceans are gonochoric. A small number are simultaneous hermaphrodites, mostly in groups that are sessile (barnacles) or live at low densities (Remipedia, Cephalocarida). Some are sequential hermaphrodites (see below). Parthenogenesis is also widespread, such as in many branchiopods, and some ostracods, isopods, and crayfish. Water fleas (*Daphnia*) are known for their cyclical parthenogenesis, the alternation of a number of asexual cycles with a sexual generation (Box 3.16).

Sex determination in gonochoric crustaceans may be both genetic (TYPE 3.1a and b) and environmental (TYPE 3.2c), often with a combination of factors (Legrand *et al.* 1987). Male heterogamety appears to be more common (Legrand *et al.* 1987). Both female (ZW or ZO) and male (XY or XO) heterogamety have been reported for isopods (Rigaud *et al.* 1997; Tomaszkiewicz *et al.* 2010) and copepods (Lécher *et al.* 1995; reviewed in Legrand & Juchault 2006). Decapoda and Ostracoda appear to only have male heterogamety (XY and XO), sometimes with multiple X and Y chromosomes (Lécher *et al.* 1995). Multiple W chromosomes have been reported from the isopod *Jaera albifrons* (Staiger & Bocquet 1954). No heteromorphic sex chromosomes could be identified in many species, such as the branchiopod *Triops* (Ombretta *et al.* 2005), the decapod *Cherax quadricarinatus* (Tan *et al.* 2004), the isopod *Hemilepistus elongatus* (Roder *et al.* 1996), and the krill *Meganyctiphanes norvegica* (Thiriot-Quiévreux & Cuzin-Roudy 1995), making it impossible to distinguish between heterogamety or polygenic sex determination. Most cases likely correspond to early stages of sex chromosome differentiation, a common phenomenon in this group, which is further supported by the viability and fertility of YY and WW individuals in

some groups (Legrand *et al.* 1987) and the presence of male as well as female heterogamety in many groups, sometimes at the family level, for example, the Porcellionidae and Armadillidiidae (Juchault & Rigaud 1995). Another good illustration of young sex chromosomes is the common isopod *Asellus aquaticus*, which usually has $2n=16$ chromosomes without any morphological difference between each pair, but in some populations a heteromorphic pair of chromosomes (XY) is present (Rocchi *et al.* 1984). Polygenic sex determination has been proposed for the marine isopod *Idotea balthica* (Legrand *et al.* 1987). A role of B chromosomes in sex determination has been implied in the paternal inheritance of male-biased sex ratios in the fairy shrimp *Branchipus schaefferi* (Beladjal *et al.* 2002). Natural populations of the clam shrimp *Eulimnadia texana* consist of self-compatible hermaphrodites and males, a rare case of animal androdioecy (Sassaman & Weeks 1993). Gender is determined by incipient sex chromosomes; males are ZZ, while hermaphrodites are either ZW or WW (Weeks *et al.* 2010). The former are termed amphigenic (selfing produces both males and hermaphrodites), while the latter are monogenic (selfing only produces hermaphrodites). The system might be thought of as a mixture of TYPES 3.1b and 3.2a.

ESD (TYPE 3.2c) occurs in several groups, with documented effects of temperature, nutrition, population density, parasitism, acidity, and photoperiod (e.g. *Gammarus duebeni*; Naylor *et al.* 1988), often in interaction with genetic factors. In the copepod *Cyclops viridis*, temperature affects the differential elimination of one of the sex chromosomes (Legrand *et al.* 1987). A well-studied example is that of water fleas (*Daphnia*) (Hobæk & Larsson 1990; Yampolsky 1992, Box. 3.16). In *Daphnia pulex*, cyclical parthenogens reproduce clonally during most of the year, but at the onset of harsh conditions females start the parthenogenetic production of male individuals and switch to sexual reproduction. Mated females produce resting eggs able to overcome the adverse conditions. The capacity to epigenetically produce males is controlled genetically, and is polymorphic within populations: some lines of females are unable to produce males. A similar situation has been reported for *Daphnia magna* (Galimov *et al.* 2011).

Hermaphroditism is well illustrated by fish-cleaning shrimps of the genus *Lysmata* (Bauer 2000; Baldwin & Bauer 2003). A number of species are protandric sequential hermaphrodites in which males turn to female with increasing size. Other species are protandric simultaneous hermaphrodites, with males turning into hermaphrodites that retain their male function and outcross with other hermaphrodites (TYPE 3.2b). Protandrous hermaphroditism has also been documented in copepods (Gusmão & McKinnon 2009) and isopods, in particular *Porcellionides pruinosus* where it apparently stems from the temperature sensitivity of feminizing *Wolbachia* bacteria (Rigaud *et al.* 1997). Sex can also be reverted experimentally by implanting or surgically removing the androgenic gland. Intersexuality and gynandromorphism is commonly observed (e.g. in crayfish, Rudolph 1999; Parnes *et al.* 2003; anostracans, Sassaman & Fugate 1997; the clam shrimp *Eulimnadia*, Weeks *et al.* 2006), and may be due to exposure to unusual environments (e.g. high temperature in *D. magna*; Mitchell 2001), incomplete feminization by microorganisms (Sassaman & Fugate 1997) or artificial compounds and environmental pollution (Ford 2012).

Another major characteristic of sex determination in some crustacean groups is the existence of 'cytoplasmic sex factors'. They are in most cases vertically transmitted microorganisms that manipulate the sex of an individual or the sex ratio of its progeny (Bouchon *et al.* 1998). They have been well studied, for example, in the amphipods *Gammarus* (Bulnheim 1978; Terry *et al.* 2004; Haine *et al.* 2007), *Orchestia* (Ginsburger-Vogel & Charniaux-Cotton 1982) and *Corophium volutator* (Mautner *et al.* 2007), as well as in shrimps (Baldwin & Bauer 2003). The best-studied example is that of the terrestrial isopod (woodlice) *Armadillidium vulgare* by Legrand, Juchault, Rigaud and colleagues (Rigaud 1997; Rigaud *et al.* 1997; Bouchon *et al.* 2008). This species has ZZ-ZW sex determination, but some females are infected with *Wolbachia* bacteria that convert ZZ genetic males into females by preventing androgenic gland differentiation during sexual development. Feminized ZZ individuals, upon mating with ZZ males produce all-female progenies. Yet, other strains have been found to carry feminizing elements and resistance genes preventing

feminization, making the *Armadillidium* a highly interesting system for studying transitions in sex determination (Cordaux *et al.* 2011; Box 7.1).

The subphylum **Hexapoda**, known since the Palaeozoic, is the largest group of arthropods in terms of number of species. It includes the paraphyletic Entognatha, with approximately 9000 species of Protura, Diplura, and Collembola (springtails), and the Insecta. Insects are among the most diverse groups of animals with a described number of 1 million species, but an estimated number of 2–30 million species, representing more than half of all known organisms. They occur all over the world but are mostly confined to terrestrial and freshwater habitats. Most insects reproduce through an egg stage and can undergo complete metamorphosis with a pupal stage (holometabolic), or incomplete metamorphosis with a number of nymphal stages (hemimetabolic). Insects are very important for human welfare: some species are agricultural pests or transmit diseases and can cause enormous damage, whereas others are beneficial, such as pollinators and honey bees, essential for human food production, or silkworms for silk production.

The vast majority of insects are sexual and gonochoristic, sometimes with strong sexual dimorphism (such as in horned beetles and lepidopterans). Hermaphroditism is very rare, being found only found in some scale insects (Hemiptera). Parthenogenesis (thelytoky) occurs widely and has evolved multiple times independently in most groups. Cyclical parthenogenesis (where a sexual generation alternates with several rounds of asexual reproduction) occurs in aphids (Hemiptera). In other groups, such as *Ips* beetles and *Bacillus* stick insects, parthenogenesis is sperm-dependent (Beukeboom & Vrijenhoek 1998), whereas in yet others (such as many hymenopterans), thelytokous reproduction is controlled by endosymbiotic bacteria like *Wolbachia* and *Cardinium* (Werren *et al.* 2008; Duron *et al.* 2008; Bourtzis & O'Neill 1998). Other developmental variations include oviparity, ovoviparity, vivipary, paedogenesis (juvenile reproduction), and androgenesis.

Sex determination in insects is largely genetic, with either heterogamety (often involving heteromorphic sex chromosomes) or haplodiploidy (Blackman 1995, Table 2.1c). Male heterogamety (XY or XO; TYPE 3.1a) is more common, occur-

ring, for example, in almost all hemimetabolous insects and in most beetles (Coleoptera) and flies (Diptera). Female heterogamety (ZW; TYPE 3.1b) occurs in all butterflies and moths (Lepidoptera), all caddisflies (Trichoptera, with a ZO system), and in some flies. Multiple sex chromosomes (e.g. XXY, XYY or more complex) are generally rare. Table 2.1c gives an overview of the phylogenetic distribution of the main sex determination types in the Hexapoda.

Haplodiploidy (TYPE 3.1c) occurs in all Hymenoptera (ants, bees, wasps, and sawflies) and thrips (Thysanoptera) as well as in some beetles (Coleoptera) and true bugs (Hemiptera). Unlike the situation found in monogonont rotifers, inseminated females can produce both fertilized eggs (developing into daughters) and unfertilized eggs (developing into sons); accordingly, this mode of sex determination may also qualify as epigenetic, insofar as offspring sex ratio is under maternal control. Research on parasitoid wasps has been instrumental for testing predictions of adaptive sex-allocation theories (West 2009). Parental imprinting has been shown to be involved in some modes of haplodiploid sex determination (Verhulst *et al.* 2010; Box 3.21).

Paternal genome loss, a special form of haplodiploidy in which the paternally inherited genome is eliminated from the zygote after fertilization, occurs in several modes among scale insects (Hemiptera). It seems also vulnerable to maternal control: in the mealybug *Planococcus citri*, offspring sex ratio is affected by the rearing conditions of mothers (temperature, age of mating, crowding, and starvation; Ross *et al.* 2010). There is also some evidence to suggest paternal imprinting effects in mealybug sex determination (Buglia & Ferraro 2004).

Sex determination and offspring sex ratios are also under epigenetic control in cyclical parthenogenesis. In the pea aphid *Acyrthosiphon pisum*, viviparous XX females asexually produce all-daughter generations during the summer and a mixture of XO males and XX females at the onset of winter, sex being determined by the random loss, in the apomictic egg, of one of the two maternal X chromosomes (Jaquiéry *et al.* 2012).

Primary sex ratios may also be affected by microbial endosymbionts. In the moth *Ostrinia scapulalis*, *Wolbachia* feminizes genotypic males via alternative splicing of the *doublesex* gene (Sugimoto *et al.* 2010;

Table 2.1c Phylogenetic overview of sex-determination systems among hexapoda

Taxon		Approx. number described (estimated) species	Sex determination TYPE			GSD mechanism				
			3.2a Epigenetic hermaphrodite	3.2b Epigenetic gonochorist	3.1 Genetic gonochorist	No heteromorphic sex chromosomes	Male heterogamety (XX/XY)	Male heterogamety (XX/XO)	Female heterogamety (ZW/ZZ)	Female heterogamety (ZO/ZZ)
Hexapoda	Entognatha (Springtails, Diplura, Protura)	9,300			●	●	●	●		
	Insecta (Insects)	950,000	•		●	●	●	●	●	●

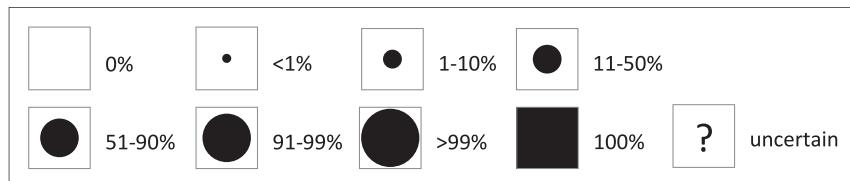


Table 2.1c *continued*

Insecta : hemimetabola	Archaeognatha (Jumping bristletails)	350			?						
	Zygentoma (=Thysanura) (Silverfishes, firebrats)	300			?						
	Ephemeroptera (Mayflies)	2,100					•	●			
	Odonata (Dragonflies, damselflies)	5,000					•	●			
	Plecoptera (Stoneflies)	3,000					•	●			
	Embioptera (Webspinners)	360						■			
	Zoraptera (Angel insects)	34					■				
	Dermaptera (Earwigs)	2,000					●		•		
	Orthoptera (Grasshoppers, crickets)	25,000					•	●			
	Phasmatodea (Stick insects)	3,000					•	●			
	Grylloblattodea (=Notoptera) (Gladiators, ice crawlers)	30					■				
	Blattodea (Cockroaches, termites)	8,500					●	●			
	Mantodea (Mantids)	1,900					•	●			

Table 2.1c *continued*

Insecta : Hemimetabola	Psocoptera (Book lice, bark lice)	3,000								
	Thysanoptera (Thrips)	5,100								
	Phthiraptera (Lice)	12,000								
	Hemiptera (True bugs)	112,100	•		●	●	●	●	●	●
Insecta : Holometabola	Hymenoptera (Ants, bees, wasps, sawflies)	150,000								
	Strepsiptera (Twisted-winged parasites)	600				●				
	Coleoptera (Beetles)	350,000			●	●	●			
	Megaloptera (Alderflies a.o.)	200								
	Rhaphidioptera (Snake flies)	210								
	Neuroptera (Net-winged insects)	6,010				●				
	Trichoptera (Caddisflies)	12,000						●		
	Lepidoptera (Butterflies, moths)	120,000					●	●	●	
	Diptera (True flies)	125,000			●	●	●	●		●
	Siphonaptera (Fleas)	1,740								
	Mecoptera (Scorpion flies, hanging flies)	550			●	●	●			

Sugimoto & Ishikawa 2012). Temperature may interfere with the process by affecting the survival of endosymbionts. Although direct environmental effects on the expression of sex determination genes have been rarely documented in insects, they may be more prevalent than previously thought. The distribution of different sex-determination systems in houseflies can be explained by temperature and seasonality (Feldmeyer *et al.* 2008), which suggests that sex determination genes in this species are somehow temperature sensitive. Thus, although genetic sex determination is the rule in insects, there are many examples to suggest an interaction with environmental components.

Some groups show a surprising diversity of systems, pointing to frequent transitions. Although being male heterogametic in their vast majority, Coleoptera show a variety of XY, XO, neo-XY, and multiple XY systems (Smith & Virkki 1978). Dipterans are also predominantly male heterogametic, but show in addition other systems such as female heterogamety, monogeny (e.g. *Chrysomya*), and X-chromosome elimination (e.g. *Sciara*). The housefly (*Musca domestica*) carries multiple different sex-determination mechanisms, including populations with male and female heterogamety, that are clinally distributed (Feldmeyer *et al.* 2008). True bugs (Hemiptera) also display a remarkable lability of sex-determination systems, containing all the variation that is present at the level of the insect class (Table 2.1c).

2.2.9f Deuterostomia

Table 2.1d gives an overview of the phylogenetic distribution of the main sex determination types in the Deuterostomia. The ~7,000 living species of **Echinodermata** form a large and ecologically diversified group of marine invertebrates, comprising phenotypes as different as Echinoidea (sea urchins), Asteroidea (sea stars), Ophiuridea (brittle stars), Holothuridea (sea cucumbers), and Crinoidea (sea lilies). The vast majority of echinoderms are gonochoric with exclusively sexual reproduction. Differentiated sex chromosomes have been documented in a few species (e.g. the male heterogametic sea urchin *Paracentrotus lividus*; Lipani *et al.* 1996). A few species of brittle stars and sea stars (e.g. *Asterina phylactica*) are simultaneous hermaphrodites, and may show high rate of selfing.

Among **Hemichordata**, the Enteropneustes (~70 species of acorn worms) are gonochoric with almost exclusively sexual reproduction, but no differentiated sex chromosomes. Pterobranchia (10–20 species known) form colonies by asexual reproduction (budding). Colonies are usually dioecious, but hermaphroditic individuals have also been documented (e.g. *Cephalodiscus nigrescens*).

The phylum **Chordata**, already known from the Cambrian explosion (530 Mya), comprises more than 60,000 species, grouped in three sub-phyla. The **urochordates** (Tunicata), which evolved in early Cambrian period, contain ~3000 species of filter feeders, either sessile (Asciidae; sea squirts) or actively swimming (Appendicularia and Thaliacea). Appendicularia (or Larvacea) are solitary, reproduce only sexually, and are mostly hermaphroditic (exceptions include *Oikopleura dioica*, also known for possessing one of the smallest genomes among Metazoa). Asciidae and Thaliacea, which also are mostly hermaphroditic, often live in clonal colonies that reproduce asexually (budding). Salps (Thaliacea) show an obligate alternation between sexual and asexual generations. The solitary form produces asexually a chain of tens to hundreds of individuals, which are released from the parent at a small size. Individuals from the chain are sequential hermaphrodites, first maturing as females (and fertilized by male gametes from older chains). The growing embryo remains attached to the parent body wall before being released and developing into the solitary phase. Most ascidians are simultaneous hermaphrodites, except for a few sequential hermaphrodites (e.g. *Botryllus schlosseri*). Several species evolved SI systems to prevent selfing (TYPE 3.2b), similar to angiosperms (e.g. *Ciona intestinalis*; Harada *et al.* 2008; Section 3.4.1b).

Cephalochordates comprise 25 species of lancelets (*Branchiostoma*). All seem gonochoric with equal sex ratio, suggesting GSD (TYPE 3.1). Differentiated XY chromosomes have been described in a population of *Branchiostoma belcherii* (Nogusa 1957), but with no confirmation from other populations (Saotome & Ojima 2001).

The **Vertebrates** include the overwhelming majority of living chordates, with currently about 64,000

Table 2.1d Phylogenetic overview of sex-determination systems among Deuterostomia

Taxon		Approx. number described (estimated) species	Sex determination TYPE				GSD mechanism		
			3.2a Epigenetic herma-phrodite - simultaneous	3.2a Epigenetic herma-phrodite - sequential	3.2b Epigenetic gonochorist	3.1 Genetic gonochorist	No heteromorphic sex chromosomes	Male heterogamety (XX/XY)	Female heterogamety (ZW/ZZ)
Deuterostomia	Echinodermata (Echinoderms)	7,000 (14,000)	●	●		●	●	●	
	Hemichordata (Hemichordates)	120	●	●		●	●		
	Chordata (Chordates)	67,000	●	●	●	●	●	●	●
	Tunicata (Tunicates)	3,000	■						
	Cephalochordata (Cephalochordates)	25				?	?	●	
	Vertebrata (Vertebrates)	64,000	•	●	●	●	●	●	●

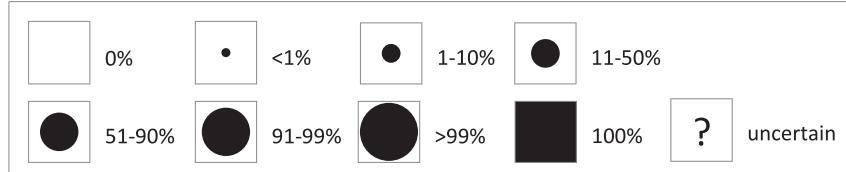


Table 2.1d *continued*

Vertebrata	Agnatha (Jawless fishes)	110		●	●						
	Chondrichtyes (Cartilaginous fishes)	1,000				●					
	Osteichtyes (Bony fishes)	30,000 (40,000)	●	●	●	●	●	●	●	●	●
	Amphibia (Amphibians)	7,000 (15,000)	●	●	●	●	●	●	●	●	●
	Squamata (Lizards, snakes)	9,000		●	●			●	●		
Reptilia (Reptiles)	Chelonia (Turtles)	270		●	●			●	●		
	Crocodylia (Crocodylians)	25			●						
	Aves (Birds)	10,000				●					
	Mammalia (Monotremes, therians)	5,500					●				

species described. **Agnatha** (jawless fishes), which comprise lampreys (~ 30 species), and hagfish (~ 80 species), are mostly gonochoric, but hermaphroditic individuals may coexist with males and females in hagfish (Powell *et al.* 2005). Sex ratios are often biased, and several lampreys show environmental effects on sex ratios (including temperature, pH, and density; Docker & Beamish 1994; Beamish 1993). Hence, sex determination appears mostly epigenetic (TYPE 3.2c). **Chondrichtya** are also gonochoric, but genetic effects seem predominant, with differentiated sex chromosomes found both in Rajiformes and Carcharhiniformes.

More is known from **Osteichtya**, a group paraphyletic to the tetrapods and comprising the Actinopterygii and the non-tetrapod Sarcopterygii (dipneusts and Crossopterygii). The Class Actinopterygii contribute 99% of modern fish diversity, mostly within the Teleostei, an infraclass that diverged some ~235 Mya and contains ~30,000 species described (i.e. about half of known vertebrates). Some lineages are parthenogenetic (Vrijenhoek 1994), often recently derived from sexual species (Ryskov 2008) and normally requiring sperm to initiate embryonic development (Lampert 2008). Most bony fishes display meiotic sex, with a large diversity of sex-determination mechanisms. The majority of species are gonochoric, with sex determined by genes, environment, or both (TYPES 3.1 and 3.2). GSD may involve either one single bi- or multi-allelic locus, different loci spread over several chromosomes, or differentiated sex chromosomes.

Male heterogamety is about twice as common as female heterogamety, but whether it constitutes the ancestral state is difficult to assess, given the extreme lability of sex-determination systems in teleosts (Mank *et al.* 2006). Sex chromosome losses (X0 or Z0) and autosomal fusions (XY₁Y₂, YX₁X₂) are not rare. Sex chromosome differentiation has only been documented in about 10% of species investigated, and is often slight. The non-recombining segment may be very small (e.g. in catfish, *Ictalurus punctatus*: Waldbieser *et al.* 2001; three-spined sticklebacks, *Gasterosteus aculeatus*: Peichel *et al.* 2004) or even absent, as in the tiger pufferfish *Takifugu rubripes*, where sex is determined by one single nucleotide polymorphism in the *Amhr2* gene (Kamiya *et al.* 2012). Ancient ZW systems seem conserved

for at least 60 My in some Aulopidae and Synodontidae, but most systems are much more recent. The identity of sex chromosomes and the patterns of heterogamety often differ among species from the same family or the same genus. In Salmonidae, for instance, all species investigated so far are XY (sometimes with differentiated Y chromosomes), but sex is associated with different linkage groups depending on species (Thorgaard 1977; Phillips *et al.* 2001; Woram *et al.* 2003, 2004). Similarly, most sticklebacks (Gasterosteidae) show GSD with male heterogamety, but sex chromosomes differ between species (e.g. sex-determination genes are on LG19 in *G. aculeatus*, but LG12 in *P. pungitius*), and are often not differentiated. An X₁X₂Y system is found in *G. wheatlandi* (the sister species to *G. aculeatus*), derived from a Y-autosome fusion (Ross *et al.* 2009). The more distant *Apeltes quadratus* is female heterogametic with heteromorphic sex chromosomes. *Culaea inconstans* (sister to *P. pungitius*) shows no evidence for GSD or sex-linked markers (Ross *et al.* 2009). In Atherinopsidae, the XY system of medaka fish *Oryzias latipes* (with sex determined by a duplication of *Dmrt1* on the Y chromosome; *DmY*) is also found in the closely related *O. curvinotus*, but was already lost from its sister species *O. luzonensis* (Myosho *et al.* 2012). The more distant *O. javanicus* and *O. hubbsi* are female heterogametic (Tanaka *et al.* 2007; Takehana *et al.* 2008). Among Cichlidae, sex determination is associated with at least four different LGs: in Tilapiinae, a male-heterogametic system (XY) on LG1 coexists with a female-heterogametic system (ZW) on LG3, while in Haplochrominae, a male-heterogametic system (XY) on LG7 coexists with a female-heterogametic system (ZW) on LG5 (Ser *et al.* 2010).

Multiple alleles (X, Y, and W) may segregate at the same locus, such as found in the poeciliid *Xiphophorus maculatus* (Kallman 1968). Allele Y is dominant over X, and W over Y, so that YY and XY individuals develop into males, whereas XX, and WY individuals develop into females. Hence, family sex ratios vary from 0:1 (in XX × YY pairs) to 3:1 (in WX × XY pairs) (Volff & Schartl 2001). This system differs from other species in the genus, which may show strict ZW or XY systems (*X. alvarezi* and *X. cortezii* respectively), or XY systems with autosomal influence (*X. nezahualcoyotl*; *X. milleri*).

Polymorphisms in sex determination may also occur among or within populations from the same species. In rainbow trout *Oncorhynchus mykiss*, sex chromosomes are heteromorphic in some populations, not in others. A Y chromosome to autosome fusion is found in some populations of *Blennius tentacularis* but not others. In the rainbow wrasse *Coris julis* (Labridae), sex chromosomes seem heteromorphic in some females and secondary males, but homomorphic in primary males (Duchac *et al.* 1982). Both *Oreochromis aureus* and *O. mossambicus* (Tilapiinae) simultaneously possess an XY system on LG1 and a ZW system on LG3 (Lee *et al.* 2004; Cnaani *et al.* 2008). In *Metriacalma pyronotus* (Haplochrominae) similarly, an XY system on LG7 coexists with a ZW system on LG5. In these cases, W is dominant over Y, so that ZWXY individuals develop into females (Ser *et al.* 2010).

Polygenic sex determination often involves minor autosomal factors, which may coexist or not with a main pair of sex chromosomes. Sex is then determined by a balance between masculinizing and feminizing factors, distributed across the genome. The polygenic nature of sex determination is often documented by 'sex reversed' XY females or ZW males, as well as by biased and heritable sex ratios at the family level. Sex-ratio heritability was estimated to $h^2 = 0.26$ in the Nile tilapia *Oreochromis niloticus* (Lester *et al.* 1989) and up to 0.62 in the European sea bass *Dicentrarchus labrax* (Vandepitte *et al.* 2007). In the zebra fish *Danio rerio*, family sex ratios vary from 4.8 to 97.3%, with a repeatability of about 90% (Liew *et al.* 2012), which sets the upper limit for broad sense heritability. Autosomal factors may increase in frequency under selection (Aida 1936; Liew *et al.* 2012), and override primary sex chromosomes. In laboratory strains of the common carp, selection for a recessive masculinizing autosomal mutation (*mas*) allowed to replace the ancestral male heterogamety by a female-heterogametic system, with XX *mas*/+ females and XX *mas*/*mas* males (Komen *et al.* 1992 a, b, c).

In several gonochoric species, sex ratio is affected by environmental factors likely to show relevant fluctuations in natural environments (Baroiller *et al.* 2009). The pH value sometimes plays a role (Rubin 1985; Baroiller *et al.* 2009), but incubation temperature is by far the most important factor, with effects

found in over 60 species belonging to very divergent orders (Godwin *et al.* 2003; Baroiller *et al.* 2009). The thermosensitive period takes place before the onset of histological differentiation of the gonads. In most cases, the proportion of males increases with temperature (FM pattern), but the reverse may also occur (MF pattern; e.g. in the catfish *Ictalurus punctatus* and the sockeye salmon *Oncorhynchus nerka*). In the Japanese flounder *Paralichthys olivaceus* and the Southern flounder *P. lethostigma*, both low and high temperatures give male-biased sex ratios, with a balanced sex ratio in between (MFM; Yamamoto 1999; Luckenbach *et al.* 2009).

Temperature effects often occur in association with major (XY/ZW) or minor genetic factors. High temperatures (32°C) induce XX male sex reversal in medaka fish (Sato *et al.* 2005; Hattori *et al.* 2007). Similarly, the European sea bass *D. labrax*, with polygenic sex determination, shows an excess of males at high temperature (20–24°C) and an excess of females at cold temperatures (13–15°C) (Vandepitte *et al.* 2007); temperature-induced sex reversal also seems to occur in nature (Saillant *et al.* 2002, 2003a,b,c). Nile tilapias have sex mostly determined by major (XY) and minor genetic effects, but in addition XX individuals develop as males at high temperature (>32°C; Baroiller *et al.* 1995). *O. niloticus* is a strict maternal mouth brooder from 0 to ~9 days post fertilization (dpf). The thermosensitive period is about 10 dpf; during this period, wild fry shoals visit shallow water where they can encounter masculinizing temperatures (32–35°C). Accordingly, sex-reversed XX males (as well as XY females) have been found in nature (Scott *et al.* 1989; Bezault *et al.* 2007).

One particularly well-studied case is the Atlantic silverside *Menidia menidia* (Atherinopsidae), which lives along the Atlantic coast of North America (from Florida to Northern Quebec), attaining maturity in its first year. Southern populations exhibit pure temperature sex determination (TSD) (with no genotypic effect documented). Offspring produced early in the season (at low temperature) develop into females, while those produced later in the season (in warmer conditions) develop into males (Conover & Heins 1987). Females, which benefit more than males from a large body size, have more time to grow before the onset of reproduction. Interestingly, temperature sensitivity decreases with

increasing latitude: northern populations of Nova Scotia have pure GSD with stable 1:1 sex ratio. Short breeding seasons presumably erase the potential benefits from temperature effects. The related South American pejerrey *Odontesthes bonariensis* (Strüssmann *et al.* 1996) show a similar dependence, producing 0% males at 19°C, 100% males at 29°C, and a balanced sex ratio at intermediate temperatures (25°C). Populations from Argentina living in lagoons can encounter masculinizing temperature during hot summers in their natural habitats (Strüssmann & Patiño 1999; Strüssmann *et al.* 2008).

Many environmental effects might actually be mediated by their effect on growth (Kraak & de Looze 1993). Growth and body size are known to have sex-specific effects on fitness. Females often benefit more than males from better growth conditions (as in *Menidia*). Accordingly, high density, low feeding rates, or poor growth conditions tend to produce males (e.g. Roncarati *et al.* 1997; Davey & Jellyman 2005; Lawrence *et al.* 2008; Vandepitte *et al.* 2007; Chapter 4). The reverse may also occur, however, depending on mating systems. In the monogamous Midas cichlid, large males are better in defending the breeding territory against competitors (and are preferred by females). Hence, sex is determined by the relative (and not absolute) size as a juvenile: Individuals that are relatively larger than average members of their group tend to develop as males (Francis & Barlow 1993). Social effects are actually important determinant of sex in many fish species (Godwin *et al.* 2003), particularly widespread in reef-dwelling species. In the presence of a conspecific adult of a given sex, juveniles *Gobiodon erythrosipilus* rapidly mature into the opposite sex (Hobbs *et al.* 2004).

Sequential hermaphroditism, in which adults may change sex depending on social context (or other environmental variables), is also widespread (TYPE 3.2). Several hundred species, from at least 25 families and 9 orders (many of them from Perciformes) are known to be hermaphroditic (Baroiller *et al.* 1999; Devlin & Nagahama 2002; Avise & Mank 2009), a trait often associated with reef-dwelling habits, patchy habitats, and/or low population densities (while diadromous species tend to be gonochoric with chromosomal sex determination; Mank *et al.* 2006). Hermaphroditic Aulopiformes

were shown to derive from gonochoristic ancestors with heteromorphic sex chromosomes (Ota *et al.* 2000), so that transitions occur in both ways. Most hermaphroditic species are either protandrous or protogynous, with sex change triggered by temperature, age, or social factors. In the protandrous anemone fish (*Amphiprion*) females are the largest and dominant members of a social group; when a position becomes vacant, the largest male in the group differentiates into a female (Fricke & Fricke 1977; Fricke 1979; Hattori 1991). Conversely, when the dominant male disappears from a polygynous group of the cleaner wrasse *Labroides dimidiatus* (Labridae), the largest female may change sex within a few weeks and take control over the harem (Robertson 1972). Protogynous species are termed monandrous when males necessarily derive from initially female individuals (secondary males), and diandrous when primary males may also derive from immature individuals. A few species from the family Gobiidae have been found to be able to change sex repeatedly in both directions. This includes coral-dwelling species of the genus *Gobiodon* and *Paragobiodon* (e.g. Kuwamura *et al.* 1994; Nakashima *et al.* 1996; Munday *et al.* 1998).

Simultaneous hermaphroditism is rare and confined to the tips of the phylogeny (hence, of recent origin; Mank *et al.* 2006). This rarity might be due to high fixed costs for alternative sexual function and inherent antagonisms between male and female physiological systems (Heath 1977; Bull & Charnov 1985). With few exceptions, simultaneous hermaphrodites are obligate outcrossers (TYPE 3.2b): in the chalk bass *Serranus tortugarum*, individuals alternate sexual roles in close succession, spawning serially during an encounter as a male and as a female. In the barred serrano *Serranus fasciatus*, individuals mature as simultaneous hermaphrodites, but larger individuals later may lose female function and become functional males (Petersen 1990). The mangrove rivulus *Kryptolebias marmoratus* (cyprinodont) is the only simultaneous hermaphrodite reported to self-fertilize routinely (TYPE 3.2a; Harrington 1961; Avise 2008). Populations of this species also harbour rare males, and can therefore be classified as androdioecious, as does the goby *Lythrypnus dalli* (Drilling & Grober 2005).

In sharp contrast with teleosts, the **tetrapods** (which evolved from the Sarcopterygia some 410 Mya, during the Devonian period) are strictly gonochoric. The basal clade **amphibians** comprise three extant orders (Gymnophiona, Caudata, and Anura) and about 7000 living species described. Although older than teleosts, this class exhibits much less variation in sex-determination mechanisms. Most species investigated so far display a simple GSD with either male or female heterogamety. However, sex chromosomes are differentiated in only ~4% of species, and usually only slightly so. The phylogenetic analysis of Hillis & Green (1990) suggested a ZW system to be ancestral, with several independent evolutions towards XY. Eight heterogametic transitions only were identified in this study, which certainly is widely underestimated, given the cryptic nature of sex chromosomes in amphibians. In the genus *Rana*, most species are male heterogametic, but at least five different pairs of chromosomes (out of 13) may display sex linkage, depending on species or populations. Polymorphism may also occur within populations, and transitions are obviously frequent. In the Japanese wrinkled frog *R. rugosa*, a single pair has been identified as sex chromosomes, but presents either an XY or a ZW system, depending on populations. Alternative GSD systems are rare: multiple sex chromosomes have been identified in a few species (e.g. *Eleutherodactylus maussi*), and a B chromosome bears a dominant feminizing allele in the endemic New Zealand frog *Leiopelma hochstetteri*, leading to a OW/OO system. The only gene with a known sex-determination role in amphibians is the dominant W-specific *DmW*, which resulted from a partial duplication of *Dmrt1* in the common ancestor of *Xenopus laevis* and *X. clivii* (Section 3.3.2a).

Despite absence of pure ESD, environmental effects (mostly temperature) have been identified in a few species: XX juvenile crested newts (*Triturus cristatus*), for instance, may develop as males at high temperature, and XY juveniles as females at low temperature (Wallace & Wallace 2000). Effects may differ between species: high temperatures make ZW juvenile *Pleurodeles waltl* develop into males, but ZZ juvenile *P. poireti* into females (Dournon *et al.* 1990). Sex reversal is easily triggered in the laboratory by temperature or steroid

hormones, as well as by surgical treatment (the Bidder's organ of castrated *Bufo* males develops into a fully functional ovary; Ponse 1942). YY males and WW females are often viable, testifying to the lack of sex-chromosomes decay. Field occurrence of sex reversal has been documented for several anuran species (e.g. *Rana temporaria*, *Hyla japonica*). As very few species have been properly examined for their response to temperature and possible sex reversal, temperature effects might actually be more influential than conventionally thought.

The **amniotes**, which diverged from amphibians some 360 Mya, comprise two main lineages, the Synapsida, now represented by mammals, and the Sauropsida, now represented by the Lepidosauria (tuataras and squamates), Chelonia (turtles), and Archosauria (including crocodiles and birds). TSD, which was first described in an agamid lizard (Charnier 1966), seems ancestral in **Lepidosauria**, being the only system found in the two living species of the basally diverged Rhynchocephalia (*Sphenodon*). It coexists with GSD among many families of lizards, including the Gekkonidae, Scincidae, Lacertidae, and Agamidae / Chamaeleonidae, pointing to several independent transitions to and from GSD (Janzen & Krenz 2004; Pokorna & Kratochvil 2009). Sex determination seems particularly labile in Gekkonidae, where GSD can be either male- or female heterogametic, and co-occur with TSD in different species from the same genus (e.g. *Phelsuma*), or different populations from same species (e.g. *Gekko japonicus*). Genotypes and temperature may interact to determine sex: the dragon lizard *Pogona vitticeps* (Agamidae) is female heterogametic with differentiated Z and W chromosomes, but ZZ juveniles develop as females at incubation temperatures exceeding 32°C (Quinn *et al.* 2007). Similarly, *Bassania duperreyi* (Scincidae) presents heteromorphic X and Y chromosomes, but XX juveniles develop as males at low incubation temperatures (Radder *et al.* 2008). More generally, all species considered as TSD show standing genetic variance for sensitivity to temperature, as investigated in detail in the leopard gecko *Eublepharis macularius* (Rhen *et al.* 2011). Other groups have more strict GSD, with female heterogamety in Serpentes, Varanidae, and Amphisbaenidae, and male heterogamety

in Iguanidae, Teidae, and Gymnophtalmidae. All snakes share the same pair of sex chromosomes, but the extent of Z-W differentiation varies greatly across families, being absent in Boidae (no differential segment was found in *Python molurus*; Matsubara *et al.* 2006), moderate in Colubridae, and strong in Viperidae and Crotalidae (the short arm of the W chromosome is extensively degenerated in *Trimeresurus flavoviridis*, and almost no homology between the Z and W chromosomes remains except for the telomeric regions; Matsubara *et al.* 2006). Sex chromosome evolution in lizards is reviewed in Ezaz *et al.* (2010).

Chelonia form an ancient group represented in the fossil record as far back as 200 Mya, and comprise approximately 270 living species. TSD is widespread and very likely ancestral in turtles. It is still the only sex-determination system found in a majority of families, but was independently lost in the male-heterogametic Chelidae and in the female-heterogametic Trionychidae. It coexists with GSD within a few families (Emydidae, Geoemydidae, and Kinosternidae), sometimes within the same genus (e.g. *Clemmys guttata* has TSD, while *C. sculpta* has GSD). In the European pond turtle *Emys orbicularis*, eggs produce all-female broods when incubated above 30°C, all-male broods below 25°C, and even sex ratios at 28.5°C (the pivotal temperature; Pieau *et al.* 1994). This MF pattern (with males produced at lower temperatures than females) is widespread among TSD turtles. The change from 100% males to 100% females sometimes occurs within 1–2°C. In a few species, females are produced at either warm or cool temperatures, and males in between (FMF; Ewert & Nelson 1991).

Despite being characterized as TSD, turtles obviously show genetic variance (within- and among populations) in their sensitivity to temperature as well as in pivotal temperatures. Sex-ratio heritability has been investigated in several TSD species, including the map turtle *Graptemys* (Bull *et al.* 1990), the snapping turtle *Chelydra* (Janzen 1992), and the painted turtle *Chrysemys picta* (McGaugh *et al.* 2011). The ways in which genotypes interact with temperature in sex determination (in turtles and other vertebrates) are presented in more detail in Chapter 4.

Archosauria, of early Triassic origin, flourished mostly during Mesozoic times. Only two clades survived the KT boundary, namely the crocodilians (25 extant species) and the saurischian class Aves (around 10,000 species of birds). All crocodilians exhibit TSD. A FM pattern was initially described from several species, with females produced at low temperature and males at high temperature. In *Alligator mississippiensis*, Ferguson & Joosten (1982) found all-females broods to develop at 30°C, and all-males broods at 34°C. Similar values were found for *Crocodylus niloticus*, with some among-clutch variance in pivotal temperature (Hutton 1987). More extensive data (Lang & Andrews 1994) then showed that the FMF pattern more commonly applies to crocodilians (including the two above species) and confirmed the large inter-clutch variance in sensitivity to temperature: in *Alligator mississippiensis*, for instance, the sex ratios produced by different clutches incubated at a constant 32°C vary from 0 to 100% males.

Birds, by contrast, display strict GSD with female heterogamety (ZW). The same homologous pair of chromosomes (differing from that in snakes) stably determines sex in birds since approximately 130 Mya. As in snakes, however, Z-W differentiation co-varies with phylogeny. Among paleognaths, sex chromosomes are largely homomorphic in ratites (ostriches and relatives), with cytologically detectable crossovers in female meiosis throughout most of the chromosomes, but tinamous show variable patterns: depending on species, ZW heteromorphism is either only slightly stronger than in ratites (with crossovers restricted to a smaller region) or more similar to the neognath situation, which present a highly diverged and degenerated W chromosome (Pigozzi 2011). Neo-sex chromosomes have been found in Sylvioidea, formed some 40 Mya by a fusion with the first half of autosome 4a (10 Mb, including the androgen-receptor gene; Pala *et al.* 2012).

Sex in birds is likely determined by the dosage of *Dmrt1*, which only occurs on the Z chromosome, and thus presents two copies in males and a single one in females (Smith *et al.* 2009; see Chapter 3). Interestingly, *Dmrt1* is also absent from the W chromosome in ratites, despite extensive ZW homomorphy (Shetty *et al.* 2002). Maternal control

over sex ratios has been documented in a few species such as the European kestrel *Falco tinnunculus* (Dijkstra *et al.* 1990) and the Seychelles warbler *Acrocephalus sechellensis* (Komdeur 1996). The control over sex apparently occurs before ovulation (Komdeur *et al.* 2002), but the exact mechanism is still unknown (Section 2.1.6e).

Synapsida diverged from Sauropsida at the end of the Carboniferous times (310 Mya) and flourished during the Permian, but few species survived the Permian-Triassic extinction (250 Mya). Among these were the ancestors of **mammals**. The prototherians (monotremes), which diverged from therians during Triassic times (~210 Mya), are now limited to one species of platypus (*Ornithorhynchus anatinus*) and a few species of Echidna (*Tachyglossus* and *Zaglossus*), endemic to Australia and New Guinea. All show GSD, with a complex system of multiple sex chromosomes resulting from a series of translocations. Platypus males have five X and five Y chromosomes, forming a chain during meiosis (Veyrunes *et al.* 2008), while Echidna males have nine sex chromosomes (four Y and five X chromosomes), two of which differ from platypus (Rens *et al.* 2007). Which gene constitutes the initial sex-determination trigger is unknown: monotremes lack *Sry* (*Sox3* is autosomal). *Dmrt1* is sex linked, but localized on an X chromosome (X5 in platypus, and its homologue, X4 in Echidna), and is unlikely to play a key role in sex determination.

Most therians possess the same pair of sex chromosomes, with the same sex-determination gene: *Sry* (for Sex-determination Region on the Y), from the *Sox* family, resulted from a mutation of *Sox3* (its X homologue) that occurred shortly before eutherians and marsupials divergence, in Jurassic times (~170 Mya). An autosomal fusion then occurred in the eutherian lineage, linking the X and Y added regions (XAR/YAR) to the X and Y conserved region (XCR/YCR) that is shared by all therians (Graves 1995). Autosomal fusions and neo-sex chromosomes have since occurred independently in several eutherian lineages (Yoshida & Kitano 2012).

The X chromosome is gene rich and highly conserved across eutherians (except for murid rodents which show a scrambled gene order). In

contrast, the Y chromosome is much degenerated, with several evolutionary strata testifying to successive expansions of the non-recombining segment (Lahn & Page 1999). It is also much diverged between lineages, due to independent gene losses (i.e. different lineages did not always retain the same genes). The human Y chromosome only retained a few dozen genes (as contrasted with more than 1000 for the X chromosome). The *Sry* gene is also much diverged between eutherian lineages, except for its central DNA-binding HMG region which is better conserved (Box 3.13).

Weird sex-determination systems have been found in a few rodent species (Fredga 1983, 1988, 1994). In some cases, individuals develop into females despite possessing a Y chromosome and a functional *Sry*. Three sex chromosomes segregate in populations of the wood lemming *Myopus schisticolor*, namely X, Y, and X*. The latter bears a dominant feminizing allele, such that X*Y individuals develop into females. Due to a strong meiotic drive, these females only transmit their X* (and thus only produce daughters), making sex ratios strongly female biased at the population level. Similar systems are found in the collared lemming *Dicrostonyx torquatus* and a few species of South American field mice (*Akodon* ssp.). The X* of African pygmy mice *Mus minutoides*, which results from a chromosomal rearrangement of the normal X chromosome, interferes with the sex-determination cascade downstream of *Sry*, making X*Y individuals develop into functional females. Sex ratios are also female biased in natural populations (Veyrunes *et al.* 2010). One *M. minutoides* population seems composed of only X*Y females and XY males, suggesting that the paternal X is only transmitted with the maternal Y chromosome (to produce sons), while the paternal Y is only transmitted with the maternal X* chromosome (to produce daughters). In other words, sex is not determined anymore by the Y chromosome, but by the presence of X* or X chromosomes (Veyrunes *et al.* 2010). XY females also occur in *Microtus cabrerae* (Burgos *et al.* 1988), with several copies of SRY on both the X and the Y chromosomes (Fernandez *et al.* 2002). In *Microtus oregoni*, males and females are gonosomal mosaic (the females are XO in the soma and XX in the germ

cells, while the males are XY in the soma and OY in the germ cells).

Sex determination may also occur in complete absence of the Y chromosome and *Sry* gene, implying that maleness is determined by an alternative (and still unknown) pathway. Both sexes are XX in the mole vole *Ellobius tancrei* and *E. talpinus*, and XO in *E. lutescens* (XX and OO embryos fail to develop). Similar patterns are found in the Japanese

spiny rats *Tokudaia osimensis* and *T. tokunoshimensis* (Fredga 1988) and the African mouse *Mus triton* (Jotterand-Bellomo 1988).

Maternal control over sex ratios (Section 2.1.6e) has been documented in a few cases such as the red deer *Cervus elaphus* (Clutton-Brock *et al.* 1984) or the Barbary macaque *Macaca sylvanus* (Paul *et al.* 1993), but the underlying mechanisms are not known.