The Evolution of Developmental Patterns in Unicellular Protists

Every living organism develops. Development can be seen even among the unicellular organisms. Moreover, by studying the development of unicellular protists, we can see the simplest forms of cell differentiation and sexual reproduction.

Control of developmental morphogenesis: The role of the nucleus

A century ago, it had not yet been proved that the nucleus of the cell contained hereditary or developmental information.

Some of the best evidence for this theory came from studies in which unicellular organisms were fragmented into nucleate and anucleate pieces (reviewed in Wilson 1896). When various protists were cut into fragments, nearly all the pieces died. However, the fragments containing nuclei were able to live and to regenerate entire complex cellular structures.

Nuclear control of cell morphogenesis and the interaction of nucleus and cytoplasm are beautifully demonstrated in studies of the protist *Acetabularia*. This enormous single cell (2–4 cm long) consists of three parts: a cap, a stalk, and a rhizoid (Figure 2.5A; Mandoli 1998). The rhizoid is located at the base of the cell and holds it to the substrate. The single nucleus of the cell resides within the rhizoid. The size of *Acetabularia* and the location of its nucleus allow investigators to remove the nucleus from one cell and replace it with a nucleus from another cell. In the 1930s, J. Hämmerling took advantage of these unique features and exchanged nuclei between two morphologically distinct species, *A. mediterranea** and *A. crenulata*. As Figure 2.5A shows, these two species have very different cap structures. Hämmerling found that when he transferred the nucleus from one species into the stalk of another species, the newly formed cap eventually assumed the form associated with the donor nucleus (Figure 2.5B). Thus, the nucleus was seen to control *Acetabularia* development.

The formation of a cap is a complex morphogenic event involving the synthesis of numerous proteins, which must be accumulated in a certain portion of the cell and then assembled into complex, species-specific structures. The transplanted nucleus does indeed direct the synthesis of its species-specific cap, but it takes several weeks to do so. Moreover, if the nucleus is removed from an *Acetabularia* cell early in its development, before it first forms a cap, a normal cap is formed weeks later, even though the organism will eventually die. These studies suggest that (1) the nucleus contains information specifying the type of cap to be produced (i.e., it contains the genetic information that specifies the proteins required for the production of a certain type of cap), and (2) material containing this information enters the cytoplasm long before cap production occurs. This information in the cytoplasm is not used for several weeks.

One current hypothesis proposed to explain these observations is that the nucleus synthesizes a stable mRNA that lies dormant in the cytoplasm until the time of cap formation (see Dumais et al. 2000). This hypothesis is supported by an observation that Hämmerling published in 1934. Hämmerling fractionated young *Acetabularia* into several parts (Figure 2.6). The portion with the nucleus eventually formed a new cap, as expected; so did the apical tip of the stalk. However, the intermediate portion of the stalk did not form a cap. Thus, Hämmerling postulated (nearly 30 years before the existence of mRNA was known) that the instructions for cap formation originated in the nucleus and were somehow stored in a dormant form near the tip of the stalk. Many years later, researchers established that nucleus-derived mRNA does accumulate in the tip of the

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*After a recent formal name change, this species is now called *Acetabularia acetabulum*. For the sake of simplicity, however, we will use Hämmerling's designations here.
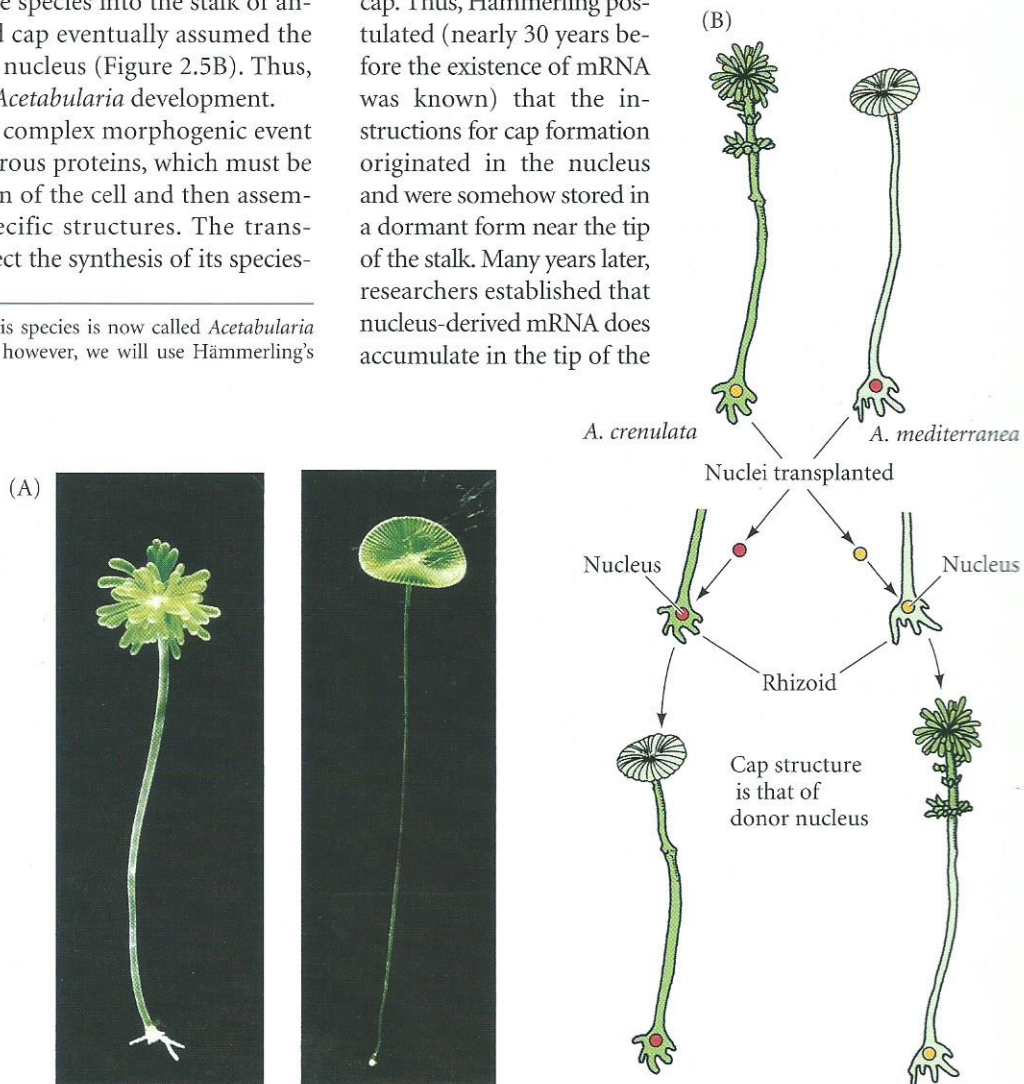

(A)

(B)

Figure 2.5
(A) *Acetabularia crenulata* (left) and *A. mediterranea* (right). Each individual is a single cell. The rhizoid contains the nucleus. (B) Effect of exchanging nuclei between two species of *Acetabularia*. Nuclei were transplanted into enucleated rhizoid fragments. *A. crenulata* structures are darker, *A. mediterranea* structures lighter green. (Photographs courtesy of S. Berger.)
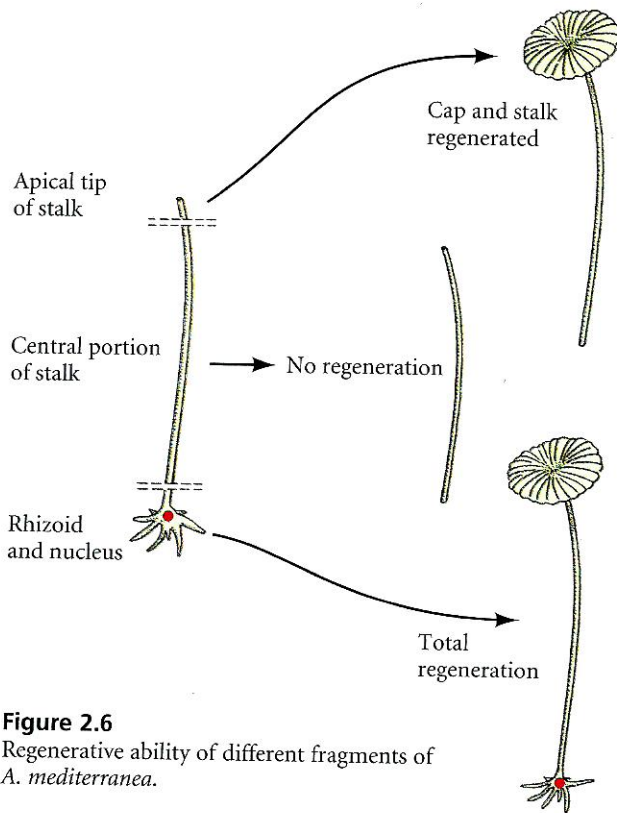
A. crenulata   *A. mediterranea*
Nuclei transplanted

Nucleus   Nucleus

Rhizoid

Cap structure is that of donor nucleus

Figure 2.6
Regenerative ability of different fragments of
A. mediterranea.

stalk, and that the destruction of this mRNA or the inhibition
of protein synthesis in this region prevents cap formation
(Kloppstech and Schweiger 1975; Garcia and Dazy 1986;
Serikawa et al. 2001).

It is clear from the preceding discussion that nuclear
transcription plays an important role in the formation of the
Acetabularia cap. But note that the cytoplasm also plays an es-

sential role in cap formation. The mRNAs are not translated
for weeks, even though they are present in the cytoplasm.
Something in the cytoplasm controls when the message is uti-
lized. Hence, the expression of the cap is controlled not only
by nuclear transcription, but also by the translation of the cy-
toplasmic RNA. In this unicellular organism, "development" is
controlled at both the transcriptional and translational levels.

WEBSITE 2.3 **Protist differentiation.** Three of the most
remarkable areas of protist development concern the con-
trol of sex type in fission yeast, the transformation of
Naegleria amoebae into streamlined, flagellated cells, and
the cortical inheritance of the cell surface in paramecia.

Unicellular protists and the origins of sexual reproduction

Sexual reproduction is another invention of the protists that
has had a profound effect on more complex organisms. It
should be noted that sex and reproduction are two distinct
and separable processes. **Reproduction** involves the creation
of new individuals. **Sex** involves the combining of genes from
two different individuals into new arrangements. Reproduc-
tion in the absence of sex is characteristic of organisms that
reproduce by fission (i.e., splitting into two); there is no sort-
ing of genes when an amoeba divides or when a hydra buds
off cells to form a new colony.

Sex without reproduction is also common among unicel-
lular organisms. Bacteria are able to transmit genes from one
individual to another by means of sex pili. This transmission is
separate from reproduction. Protists are also able to reassort
genes without reproduction. Paramecia, for instance, repro-
duce by fission, but sex is accomplished by **conjugation**. When
two paramecia join together, they link their oral apparatuses
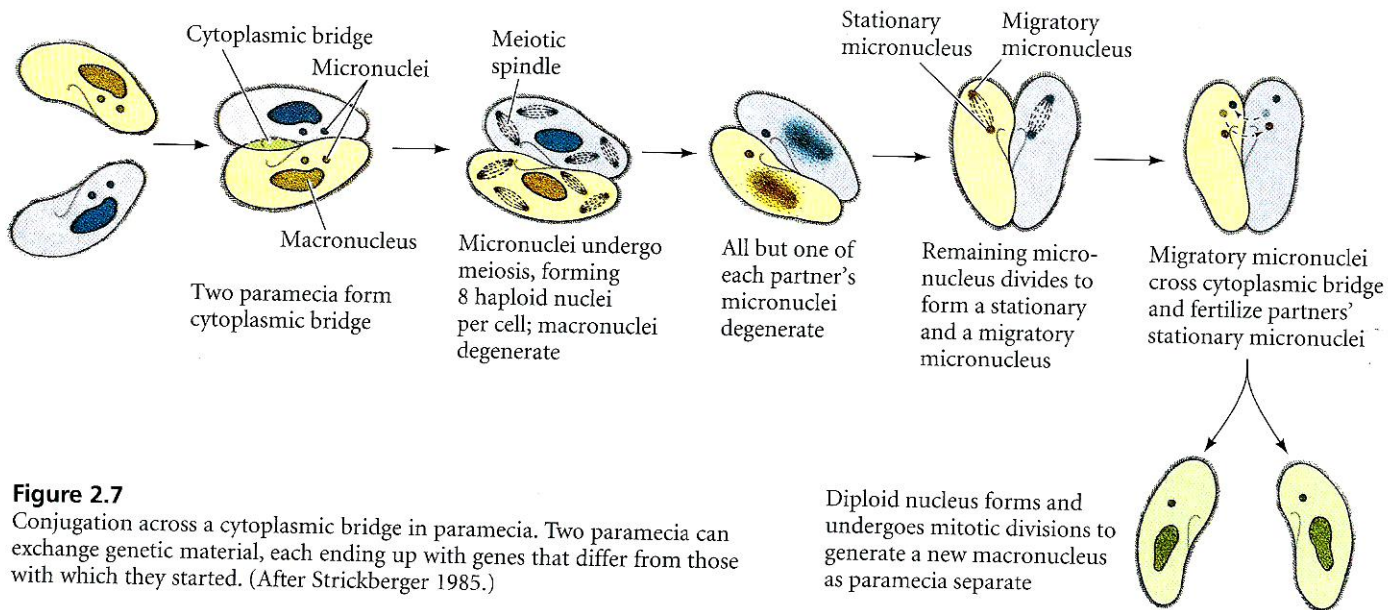and form a cytoplasmic connection through which they can

Figure 2.7
Conjugation across a cytoplasmic bridge in paramecia. Two paramecia can
exchange genetic material, each ending up with genes that differ from those
with which they started. (After Strickberger 1985.)

exchange genetic material (Figure 2.7). The macronucleus of each individual (which controls the metabolism of the organism) degenerates, while each micronucleus undergoes meiosis to produce eight haploid micronuclei, of which all but one degenerate. The remaining micronucleus divides once more to form a stationary micronucleus and a migratory micronucleus. Each migratory micronucleus crosses the cytoplasmic bridge and fuses with ("fertilizes") the partner's stationary micronucleus, thereby creating a new diploid nucleus in each cell. This diploid nucleus then divides mitotically to give rise to a new micronucleus and a new macronucleus as the two partners disengage. Therefore, no reproduction has occurred, only sex.

The union of these two distinct processes, sex and reproduction, into **sexual reproduction** is seen in other unicellular eukaryotes. Figure 2.8 shows the life cycle of *Chlamydomonas*. This organism is usually haploid, having just one copy of each chromosome (like a mammalian gamete). The individuals of each species, however, are divided into two **mating types**: plus
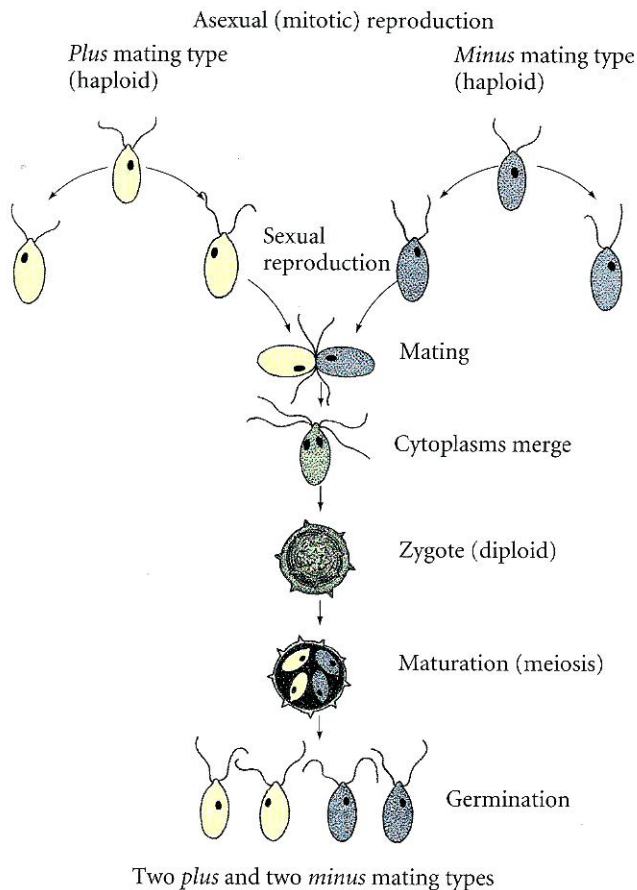
and minus. When a plus and a minus meet, they join their cytoplasms, and their nuclei fuse to form a diploid zygote. This zygote is the only diploid cell in the life cycle, and it eventually undergoes meiosis to form four new *Chlamydomonas* cells. This is true sexual reproduction, for chromosomes are reassorted during the meiotic divisions and more individuals are formed. Note that in this protist type of sexual reproduction, the gametes are morphologically identical; the distinction between sperm and egg has not yet been made.

In evolving sexual reproduction, two important advances had to be achieved. The first was the mechanism of meiosis (Figure 2.9), whereby the diploid complement of chromosomes is reduced to the haploid state (discussed in detail in Chapter 19). The second was a mechanism whereby the two different mating types could recognize each other. In *Chlamydomonas*, recognition occurs first on the flagellar membranes (Figure 2.10; Bergman et al. 1975; Wilson et al. 1997; Pan and Snell 2000). The flagella of two individuals twist around each other, enabling specific regions of the cell membranes to come together. These specialized regions contain mating type-specific components that enable the cytoplasms to fuse. Following flagellar agglutination, the plus individual initiates fusion by extending a fertilization tube. This tube contacts and fuses with a specific site on the minus individual. Interestingly, the mechanism used to extend this tube—the polymerization of the protein actin to form microfilaments—is also used to extend the processes of sea urchin eggs and sperm. In Chapter 7, we will see that the recognition and fusion of sperm and egg occur in an amazingly similar manner.

Unicellular eukaryotes appear to possess the basic elements of the developmental processes that characterize more complex organisms: protein synthesis is controlled such that certain proteins are made only at certain times and in certain places; the structures of individual genes and chromosomes are as they will be throughout eukaryotic evolution; mitosis and meiosis have been perfected; and sexual reproduction exists, involving cooperation between individual cells. Such intercellular cooperation becomes even more important with the evolution of multicellular organisms.

Figure 2.8
Sexual reproduction in *Chlamydomonas*. Two mating types, both haploid, can reproduce asexually when separate. Under certain conditions, the two mating types can unite to produce a diploid cell that can undergo meiosis to form four new haploid organisms. (After Strickberger 1985.)

Multicellularity: The Evolution of Differentiation

One of evolution's most important experiments was the creation of multicellular organisms. There appear to be several paths by which single cells evolved multicellular arrangements; we will discuss only two of them here (see Chapter 22 for a fuller discussion). The first path involves the orderly division of the reproductive cell and the subsequent differentiation of its progeny into different cell types. This path to multicellularity can be seen in a remarkable series of multicellular organisms collectively referred to as the family Volvocaceae, or the volvocaceans (Kirk 1999, 2000).
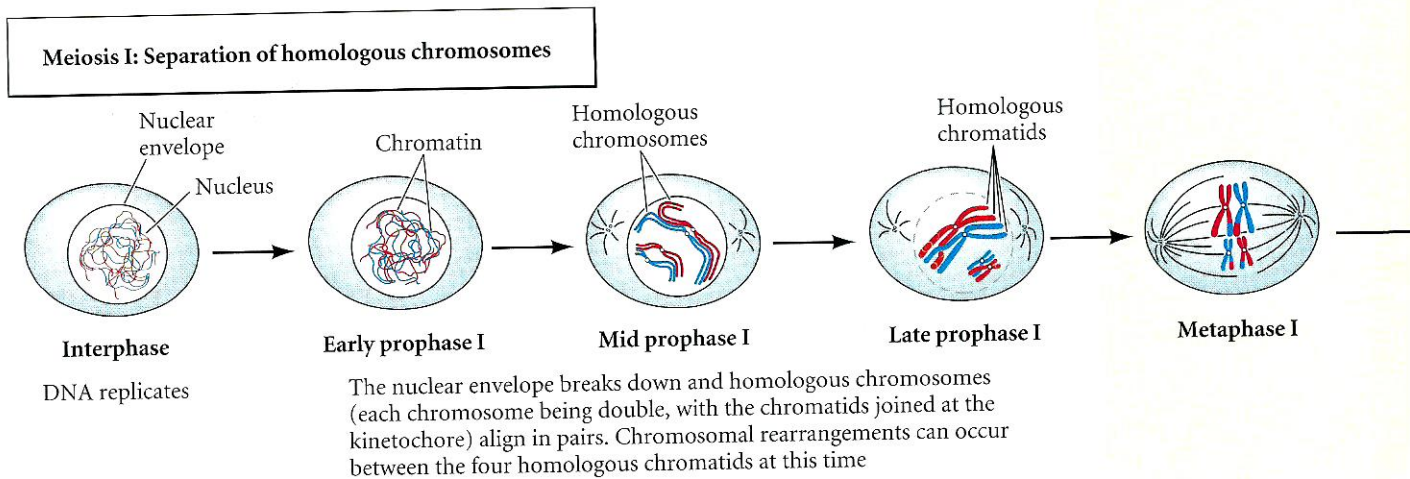
Meiosis I: Separation of homologous chromosomes

| Interphase | Early prophase I | Mid prophase I | Late prophase I | Metaphase I |

DNA replicates

The nuclear envelope breaks down and homologous chromosomes (each chromosome being double, with the chromatids joined at the kinetochore) align in pairs. Chromosomal rearrangements can occur between the four homologous chromatids at this time

Figure 2.9
Summary of meiosis. The DNA and its associated proteins replicate during interphase. During prophase, the nuclear envelope breaks down and homologous chromosomes (each chromosome is double, with the chromatids joined at the kinetochore) align in pairs. Chromosomal rearrangements between the four homologous chromatids can occur at this time. After the first metaphase, the two original homologous chromosomes are segregated into different cells. During the second meiotic division, the kinetochore splits and the sister chromatids separate, leaving each new cell with one copy of each chromosome.

Figure 2.10
Two-step recognition in mating *Chlamydomonas*. (A) Scanning electron micrograph (7000×) of mating pair. The interacting flagella twist around each other, adhering at the tips (arrows). (B) Transmission electron micrograph (20,000×) of a cytoplasmic bridge connecting the two organisms. The actin microfilaments extend from the donor (lower) cell to the recipient (upper) cell. (From Goodenough and Weiss 1975 and Bergman et al. 1975; photographs courtesy of U. Goodenough.)

The Volvocaceans

The simpler organisms among the volvocaceans are ordered assemblies of numerous cells, each resembling the unicellular protist *Chlamydomonas*, to which they are related (Figure 2.11A). A single organism of the volvocacean genus *Gonium* (Figure 2.11B), for example, consists of a flat plate of 4 to 16 cells, each with its own flagellum. In a related genus, *Pandorina*, the 16 cells form a sphere (Figure 2.11C); and in *Eudorina*, the sphere contains 32 or 64 cells arranged in a regular pattern (Figure 2.11D). In these organisms, then, a very important developmental principle has been worked out: the ordered division of one cell to generate a number of cells that are organized in a predictable fashion. Like cleavage in most animal embryos, the cell divisions by which a single volvocacean cell produces an organism of 4 to 64 cells occur in very rapid sequence and in the absence of cell growth.

The next two genera of the volvocacean series exhibit another important principle of development: the differentiation of cell types within an individual organism. In these organisms, the reproductive cells become differentiated from the somatic cells. In all the genera mentioned earlier, every cell can, and normally does, produce a complete new organism by mitosis. In the genera *Pleodorina* and *Volvox*, however, relatively few cells can reproduce. In *Pleodorina californica* (Figure 2.11E), the cells in the anterior region are restricted to a somatic function; only those cells on the posterior side can reproduce. In *P. californica*, a colony usually has 128 or 64 cells, and the ratio of the number of somatic cells to the number of reproductive cells is usually 3:5. Thus, a 128-cell colony typically has 48 somatic cells, and a 64-cell colony has 24.
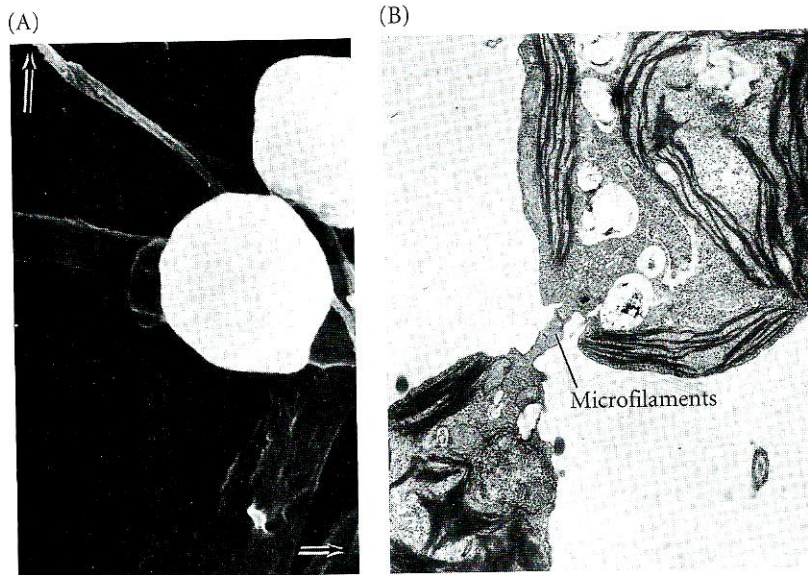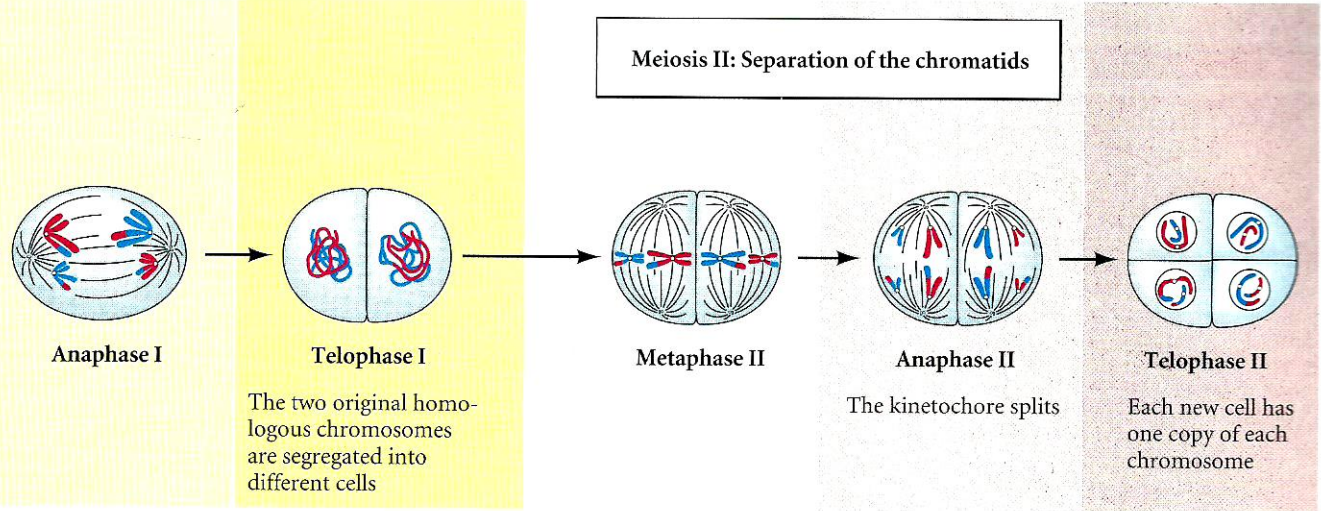
Meiosis II: Separation of the chromatids

Anaphase I

Telophase I

The two original homo-logous chromosomes are segregated into different cells

Metaphase II

Anaphase II

The kinetochore splits

Telophase II

Each new cell has one copy of each chromosome

In *Volvox*, almost all the cells are somatic, and very few of the cells are able to produce new individuals. In some species of *Volvox*, reproductive cells, as in *Pleodorina*, are derived from cells that originally look and function like somatic cells before they enlarge and divide to form new progeny. However, in other members of the genus, such as *V. carteri*, there is a complete division of labor: the reproductive cells that will create the next generation are set aside during the division of the original cell that is forming a new individual. The reproductive cells never develop functional flagella and never contribute to motility or other somatic functions of the individual; they are entirely specialized for reproduction.

Thus, although the simpler volvocaceans may be thought of as colonial organisms (because each cell is capable of independent existence and of perpetuating the species), in *V. carteri* we have a truly multicellular organism with two distinct and interdependent cell types (somatic and reproductive), both of which are required for perpetuation of the species (Figure 2.11F). Although not all animals set aside the reproductive cells from the somatic cells (and plants hardly ever do), this separation of germ cells from somatic cells early in development is characteristic of many animal phyla and will be discussed in more detail in Chapter 19.

WEBSITE 2.4 *Volvox* **cell differentiation.** The pathways leading to germ cells or somatic cells are controlled by genes that cause cells to follow one or the other fate. Mutations can prevent the formation of one of these lineages.
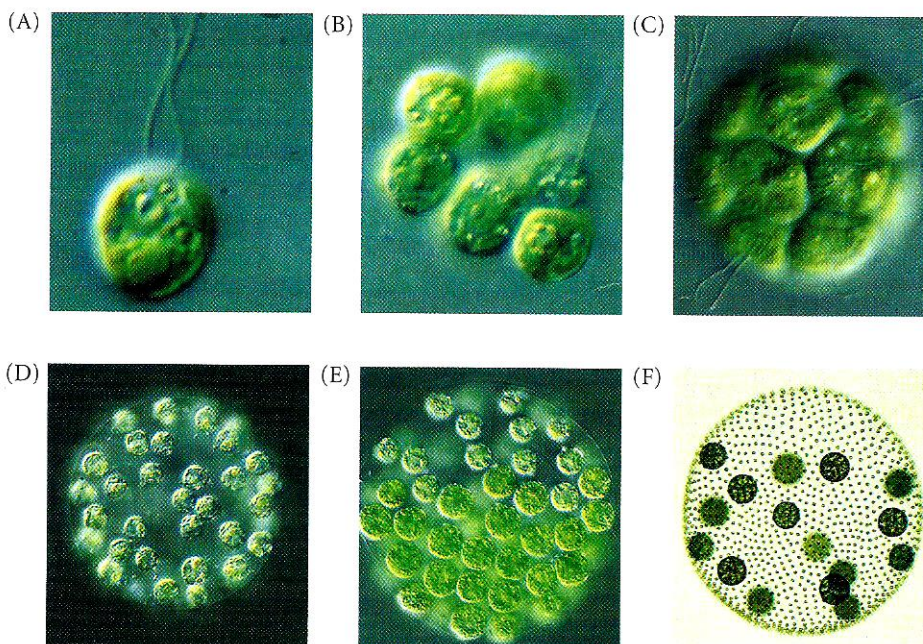
(A)   (B)   (C)

(D)   (E)   (F)

Figure 2.11
Representatives of the order Volvocales. All but *Chlamydomonas* are members of the family Volvocaceae. (A) The unicellular protist *Chlamydomonas reinhardtii*. (B) *Gonium pectorale*, with 8 *Chlamydomonas*-like cells in a convex disc. (C) *Pandorina morum*. (D) *Eudorina elegans*. (E) *Pleodorina californica*. Here, all 64 cells are originally similar, but the posterior ones dedifferentiate and redifferentiate as asexual reproductive cells called gonidia, while the anterior cells remain small and biflagellate, like *Chlamydomonas*. (F) *Volvox carteri*. Here, cells destined to become gonidia are set aside early in development and never have somatic characteristics. The smaller somatic cells resemble *Chlamydomonas*. Complexity increases from the single-celled *Chlamydomonas* to the multicellular *Volvox*. (Photographs courtesy of D. Kirk.)

Sex and Individuality in Volvox

Simple as it is, *Volvox* shares many features that characterize the life cycles and developmental histories of much more complex organisms, including ourselves. As already mentioned, *Volvox* is among the simplest organisms to exhibit a division of labor between two completely different cell types. As a consequence, it is among the simplest organisms to include death as a regular, genetically regulated part of its life history.

Death and Differentiation

Unicellular organisms that reproduce by simple cell division, such as amoebae, are potentially immortal. The amoeba you see today under the microscope has no dead ancestors. When an amoeba divides, neither of the two resulting cells can be considered either ancestor or offspring; they are siblings. Death comes to an amoeba only if it is eaten or meets with a fatal accident, and when it does, the dead cell leaves no offspring.

Death becomes an essential part of life, however, for any multicellular organism that establishes a division of labor between somatic (body) cells and germ (reproductive) cells. Consider the life history of *Volvox carteri* when it is reproducing asexually (Figure 2.12). Each asexual adult is a spheroid containing some 2000 small, biflagellated somatic cells along its periphery and about 16 large, asexual reproductive cells, called **gonidia**, toward one end of the interior. When mature, each gonidium divides rapidly 11 or 12 times. Certain of these divisions are asymmetrical and produce the 16 large cells that will become a new set of gonidia in the next generation. At the end of cleavage, all the cells that will be present in an adult have been produced from the gonidium. But the resulting embryo is "inside out": it is now a hollow sphere with its gonidia on the outside and the flagella of its somatic cells pointing toward the interior. This predicament is corrected by a process called **inversion**, in which the embryo turns itself right side out by a set of cell movements that resemble the gastrulation movements of animal embryos (Figure 2.13A–H). Clusters of bottle-shaped cells open a hole at one end of the embryo by producing tension on the interconnected cell sheet (Figure 2.13I). The embryo everts through this hole and then closes it up. About a day after this is done, the juvenile *Volvox* are enzymatically released from the parent and swim away.

What happens to the somatic cells of the "parent" *Volvox* now that its young have "left home"? Having produced offspring and being incapable of further reproduction, these somatic cells die. Actually, these cells commit suicide, synthesizing a set of proteins that cause the death and dissolution of the cells that make them (Pommerville and Kochert 1982). Moreover, in death, the cells release for the use of others, including their own offspring, all the nutrients that they had stored during life. "Thus emerges," notes David Kirk, "one of the great themes of life on planet Earth: 'Some die that others may live.'"

In *V. carteri*, a specific gene, *somatic regulator A*, or *regA*, plays a central role in regulating cell death (Kirk 1988, 2001a). This gene is expressed only in somatic cells,

Figure 2.12
Asexual reproduction in *V. carteri*. (A) When reproductive cells (gonidia) are mature, they enter a cleavage-like stage of embryonic development to produce juveniles within the adult. Through a series of cell movements resembling gastrulation, the embryonic *Volvox* invert and are eventually released from the parent. The somatic cells of the parent, lacking the gonidia, undergo senescence and undergo programmed cell death, while the juvenile *Volvox* mature. The entire asexual cycle takes 2 days. (B) Micrograph showing young adult spheres of *Volvox carteri* being released from parent to become free-swimming individuals. (A after Kirk 1988; B from Kirk 2001b.)
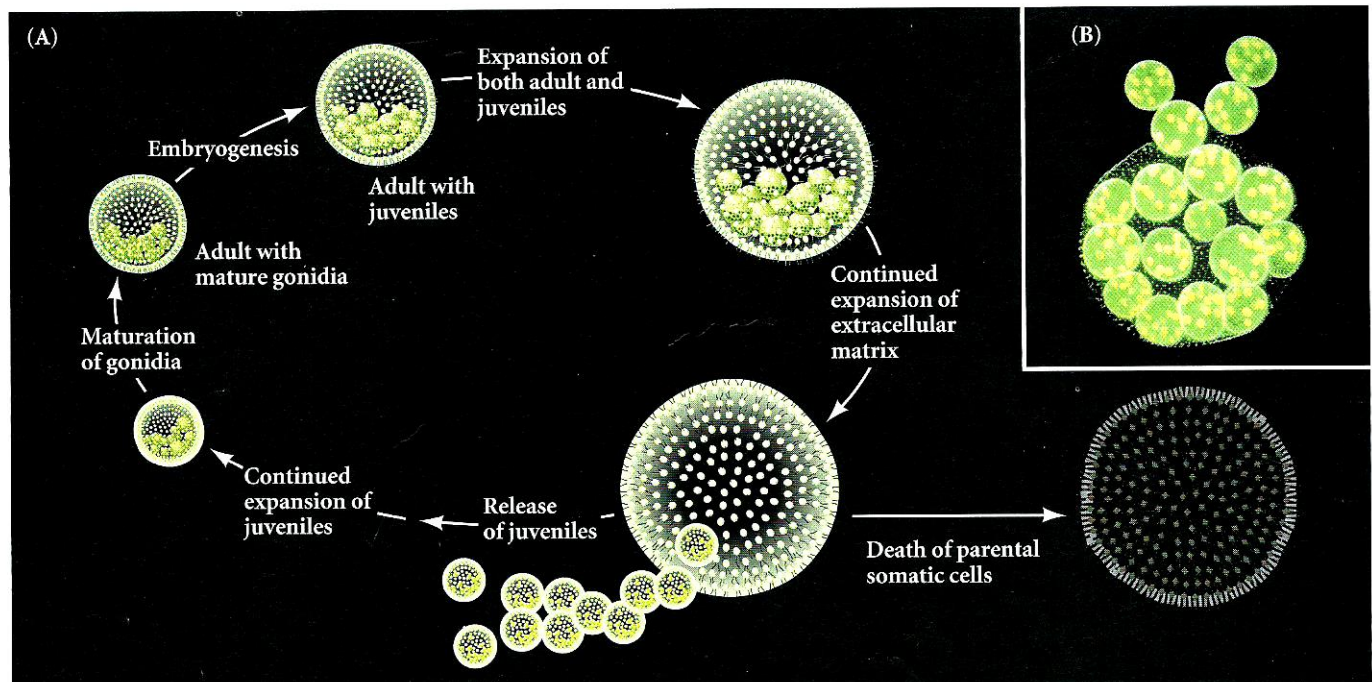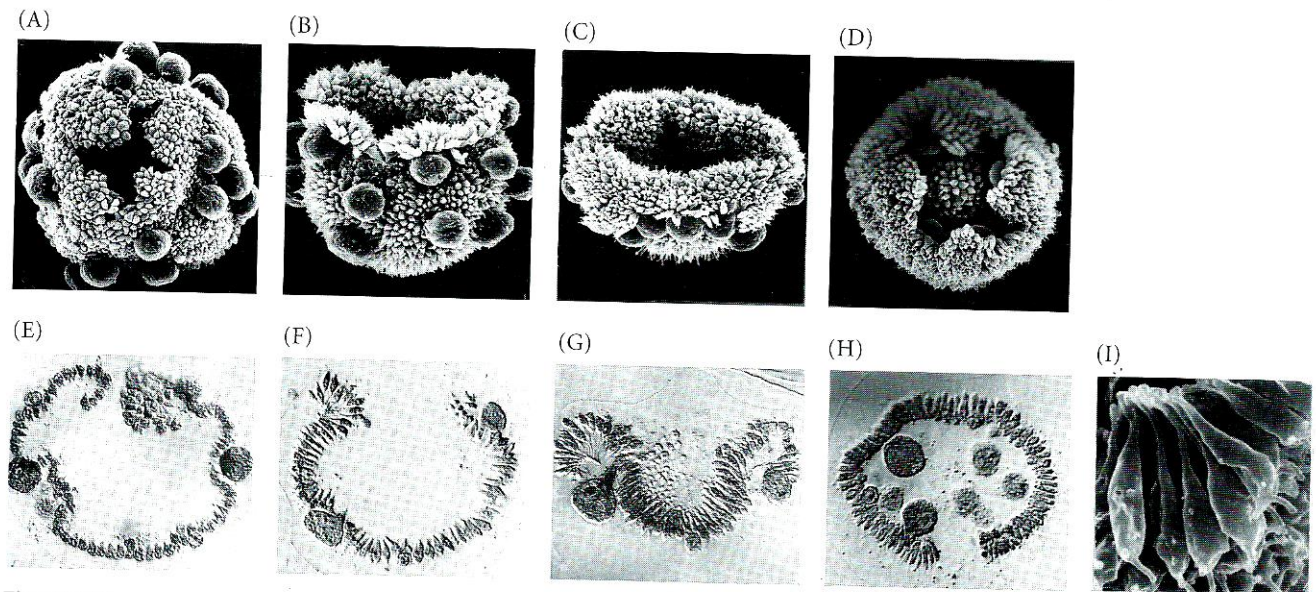
Figure 2.13
Inversion of embryos of *V. carteri*. A–D are scanning electron micrographs of whole embryos. E–H are sagittal sections through the center of the embryo, visualized by differential interference microscopy. Before inversion, the embryo is a hollow sphere of connected cells with the new gonidia on the outside. When the "bottle cells" change their shape, a hole (the phialopore) opens at the apex of the embryo (A, B, E, F). Cells then curl around and rejoin at the bottom (C, D, G, H). The new gonidia are now inside. (I)"Bottle cells" near the opening of the phialopore in a *V. carteri* embryo. These cells remain tightly interconnected through cytoplasmic bridges near their elongated apices, thereby creating the tension that causes the curvature of the interconnected cell sheet. (From Kirk et al. 1982; photographs courtesy of D. Kirk.)

and it prevents their expressing gonidial genes. In laboratory strains possessing regulatory mutations of this gene, somatic cells begin expressing *regA*, abandon their suicidal ways, gain the ability to reproduce asexually, and become potentially immortal (Figure 2.14). The fact that such mutants have never been found in nature indicates that cell death most likely plays an important role in the survival of *V. carteri* under natural conditions.

Enter Sex

Although *V. carteri* reproduces asexually much of the time, in nature it reproduces sexually once each year. When it does, one generation of individuals passes away and a new and genetically different generation is produced. The naturalist Joseph Wood Krutch (1956, pp. 28–29) put it more poetically:

The amoeba and the paramecium are potentially immortal. … But for Volvox, death seems to be as inevitable as it is in a mouse or in a man. Volvox must die as Leeuwenhoek saw it die because it had children and is no longer needed. When its time comes it drops quietly to the bottom and joins its ancestors. As Hegner, the Johns Hopkins zoologist, once wrote, 'This is the first advent of inevitable natural death in

the animal kingdom and all for the sake of sex.' And he asked: 'Is it worth it?'

For *Volvox carteri*, it most assuredly is worth it. *V. carteri* lives in shallow temporary ponds that fill with spring rains but dry out in the heat of late summer. Between

those times, *V. carteri* swims about, reproducing asexually. These asexual volvoxes will die in minutes once the pond dries up. *V. carteri* is able to survive by turning sexual shortly before the pond disappears, producing dormant zygotes that survive the heat and drought of late summer and the cold of winter. When rain fills the pond in spring, the zygotes break their dormancy and hatch out a new generation of individuals that reproduce asexually until the pond is about to dry up once more.

How do these simple organisms predict the coming of adverse conditions so accurately that they can produce a sexual generation in the nick of time, year after year? The stimulus for switching from the asexual to the sexual mode of reproduction in *V.*
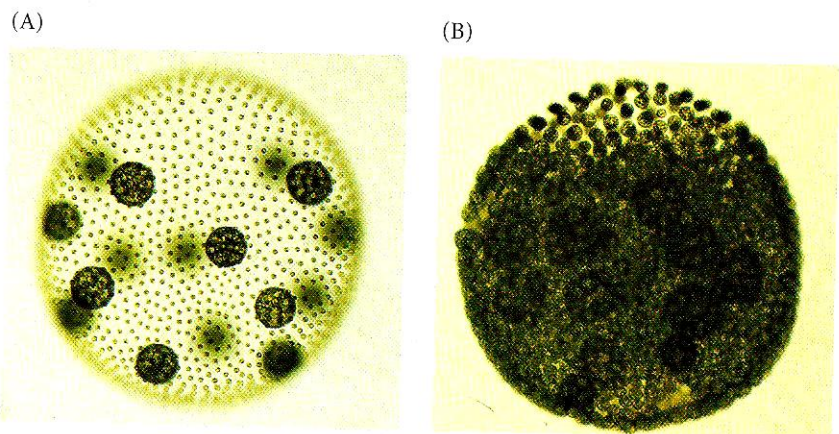

Figure 2.14
Mutation of a single gene (*somatic regenerator A*) abolishes programmed cell death in *V. carteri*. (A) A newly hatched *Volvox* carrying this mutation is indistinguishable from the wild-type spheroid. (B) Shortly before the time when the somatic cells of wild-type spheroids begin to die, the somatic cells of this mutant redifferentiate as gonidia (B). Eventually, every cell of the mutant will divide to regenerate a new spheroid that will repeat this potentially immortal developmental cycle. (Photographs courtesy of D. Kirk.)

carteri is known to be a 30-kDa sexual inducer protein. This protein is so powerful that concentrations as low as 6×10^{-17} *M* cause gonidia to undergo a modified pattern of embryonic development that results in the production of eggs or sperm, depending on the genetic sex of the individual (Sumper et al. 1993). The sperm are released and swim to a female, where they fertilize eggs to produce dormant zygotes (Figure 2.15). The sexual inducer protein is able to work at such remarkably low concentrations by causing slight modifications of the extracellular matrix. These modifications appear to signal the transcription of a whole battery of genes that form the gametes (Sumper et al. 1993; Hallmann et al. 2001).

What is the source of this sexual inducer protein? Kirk and Kirk (1986) discovered that the sexual cycle could be initiated by heating dishes of *V. carteri* to temperatures that might be expected in a shallow pond in late summer. When this was done, the somatic cells of the asexual volvoxes produced the sexual inducer protein. Since the amount of sexual inducer protein secreted by one individual is sufficient to initiate sexual development in over 500 million asexual volvoxes, a single inducing volvox can convert an entire pond to sexuality. This discovery explained an observation made over 90 years ago that "in the full blaze of Nebraska sunlight, *Volvox* is able to appear, multiply, and riot in sexual reproduction in pools of rainwater of scarcely a fortnight's duration" (Powers 1908). Thus, in temporary ponds formed by spring rains and dried up by summer's heat, *Volvox* has found a means of survival: it uses that heat to induce the formation of sexual individuals whose mating produces zygotes capable of surviving conditions that kill the adult organism. We see, too, that development is critically linked to the ecosystem in which the organism has adapted to survive.

Figure 2.15
Sexual reproduction in *V. carteri*. Males and females are indistinguishable in their asexual phase. When the sexual inducer protein is present, the gonidia of both mating types undergo a modified embryogenesis that leads to the formation of eggs in the females and sperm in the males. When the gametes are mature, sperm packets (containing 64 or 128 sperm each) are released and swim to the females. Upon reaching a female, the sperm packet breaks up into individual sperm, which can fertilize the eggs. The resulting dormant zygote has tough cell walls that can resist drying, heat, and cold. When spring rains cause the zygote to germinate, it undergoes meiosis to produce haploid males and females that reproduce asexually until heat induces the sexual cycle again.
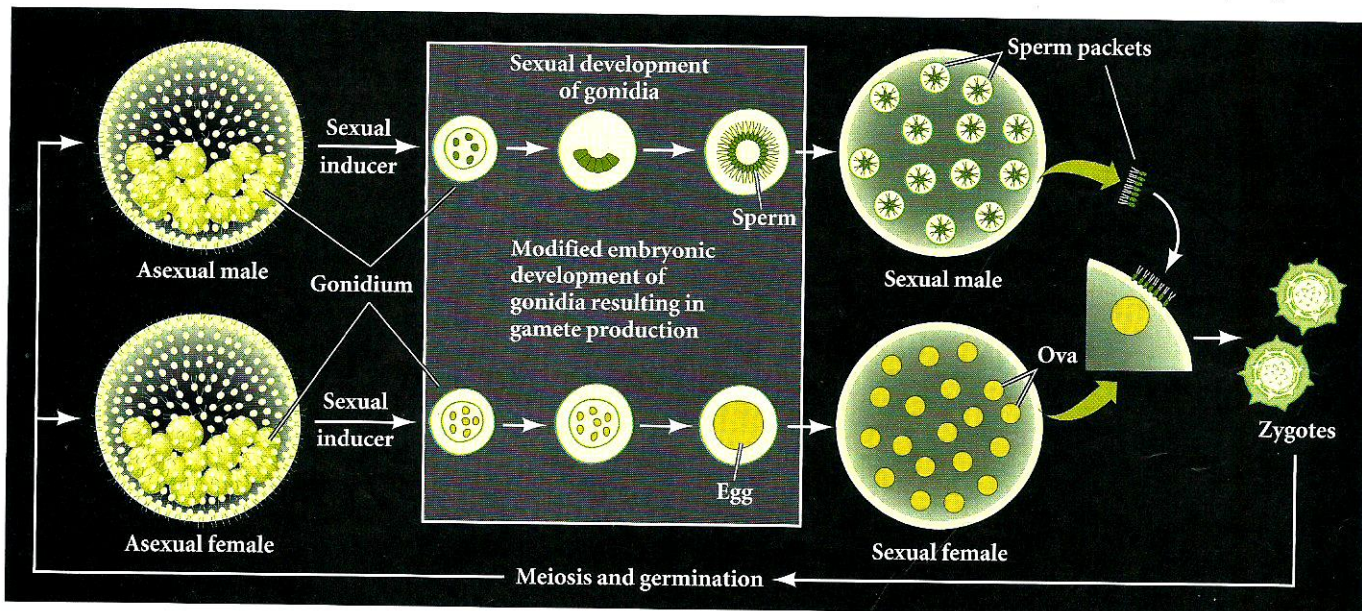
Although all the volvocaceans, like their unicellular relative *Chlamydomonas*, reproduce predominantly by asexual means, they are also capable of sexual reproduction, which involves the production and fusion of haploid gametes. In many species of *Chlamydomonas*, including the one illustrated in Figure 2.10, sexual reproduction is **isogamous** ("the same gametes"), since the haploid gametes that meet are similar in size, structure, and motility. However, in other species of *Chlamydomonas*—as well as many species of colonial volvocaceans—swimming gametes of very different sizes are produced by the different mating types. This pattern is called **heterogamy** ("different gametes"). But the larger volvocaceans have evolved a specialized form of heterogamy called **oogamy**, which involves the production of large, relatively immotile eggs by one mating type and small, motile sperm by the other (see Sidelights & Speculations). Here we see one type of gamete specialized for the retention of nutritional and developmental resources and the other type of gamete specialized for the transport of nuclei. Thus, the volvocaceans include the simplest organisms that have distinguishable male and female members of the species and that have distinct developmental pathways for the production of eggs or sperm.

In all volvocaceans, the fertilization reaction resembles that of *Chlamydomonas* in that it results in the production of a dormant diploid zygote that is capable of surviving harsh environmental conditions. When conditions allow the zygote