

Volvox: When Heat Brings Out Sex

One of evolution's most important products was the multicellular organism. There appear to be several paths by which single cells evolved multicellular arrangements; we will discuss two of them here. One 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 among a group of multicellular organisms collectively referred to as the family Volvocaceae, or the volvocaceans (Kirk 1999, 2000).

The volvocaceans

The simpler organisms among the Volvocaceae are ordered assemblies of numerous cells, each resembling the unicellular protist *Chlamydomonas*, to which they are related. A single organism of the volvocacean genus *Gonium* 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; and in the genus *Eudorina*, the sphere contains 32 or 64 cells arranged in a regular pattern. 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, which are then 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*, the cells in the anterior region are restricted to somatic (nonreproductive) functions, and only those cells on the posterior side can reproduce. In this species, 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.

In *Volvox*, almost all the cells are somatic; only a very few are able to produce new individuals. In some *Volvox* species, reproductive cells are similar to those of *Pleodorina*, in that they are derived from cells that 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 as it forms a new individual. These reproductive cells never develop functional flagella and never contribute to motility or other somatic functions; 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 whose two distinct and interdependent cell types (somatic and reproductive) are both required for perpetuation of the species. In *V. carteri*, three genes play critical roles in the distinction between somatic cells and germ cells.

First, the *gonidialless (gls)* gene is needed for the asymmetric divisions after the fifth symmetrical division. The product of *gls* appears to bind to the mitotic spindle and displace it to one side of the cell (Miller and Kirk 1999; Cheng et al. 2003), thus establishing the big and small cells. Once this is

done, two other sets of genes are employed. The *Late gonidia* (*Lag*) genes are active in the large cells and are “turned off” in the small cells. The *Lag* gene products repress those genes that would help form the smaller somatic cells. Conversely, the *somatic regulator A* (*regA*) gene is active in the small cells, where it plays a central role in regulating cell death and prevents the expression of the gonidial genes (Kirk 1988, 2001a). (It seems that the *regA* product suppresses cell growth by inhibiting chloroplast functioning.)

So the differentiation switch in *Volvox carteri* is controlled by negative regulators: the products of the *Lag* genes repress somatic cell genes, while the *regA* product represses the gonidial genes (Schmitt 2003; Kirk and Kirk 2004). In laboratory strains possessing mutations of the *regA* gene, somatic cells express *regA* and redifferentiate into gonidia, thus gaining the ability to reproduce asexually and become potentially immortal. 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.

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.

Sex and Individuality in *Volvox*

Simple as it is, *Volvox* shares many of the features that characterize the life cycles and developmental histories of much more complex organisms, including ourselves. *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, and 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. 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. Each cell is haploid (like the sex cells of most animals). When mature, each gonidium divides rapidly 11 or 12 times. The first five divisions are symmetrical, resulting in a 32-cell embryo. At the sixth division, 16 of the cells divide asymmetrically producing 16 pairs of big and small sister cells. Each of these large cells will eventually produce a gonidium. At the end of cleavage, all the cells that will be present in an adult have been produced from the original gonidium.

As the cells of *V. carteri* divide, they produce an embryo that is “inside out.” This embryo is 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. Clusters of bottle-shaped cells open a hole at one end of the embryo by producing tension on the interconnected cell sheet. At the same time, the ATPase protein kinesin accumulates near the tips of

the bottle-shaped cells, providing the energy (by splitting ATP) needed for the inversion to occur (Nishii et al. 2003). 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* once its young “leave home”? Having produced offspring and being incapable of further reproduction, these somatic cells die. Actually, they “commit suicide,” synthesizing proteins that cause their own death and dissolution (Pommerville and Kochert 1982). In death, the somatic cells release for the use of others (including their own offspring) all the nutrients they stored during life. “Thus emerges,” notes David Kirk, “one of the great themes of life on planet Earth: ‘Some die that others may live.’”

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 die in minutes once the pond dries up. *V. carteri* is able to survive by turning sexual shortly before the pond disappears, producing sperm and eggs that unite to form dormant diploid zygotes that survive the heat and drought of late summer and the cold of winter. When rain fills the pond the following spring, the zygotes break their dormancy, undergo meiosis, and hatch out a new generation of haploid individuals (of both mating types) that reproduce asexually until the pond is about to dry up once more. Thus, fertilization in Volvocaceans is a survival tactic that allows the production of a dormant diploid zygote capable of surviving harsh environmental conditions.

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. carteri* is known to be a 30-kDa sexual inducer protein. This protein is so powerful that concentrations as low as $6 \times 10^{-17} M$ 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. 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). 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.

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 heat-induced chemical changes in the somatic cells of the asexual volvoxes produced the sexual inducer protein (Nedelcu et al. 2003, 2004). 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.

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