Cells and Chromosomes

IN THE NINETEENTH CENTURY, THE MAIN TOPICS OF ZOOLOGY DEALT WITH the discovery, description, and classification of new species. Embryology, the study of the development of animals, reached its heyday at the end of the nineteenth century. Many centers for marine biology, such as those in Naples, Italy or in Woods Hole, Massachusetts, were founded at around this time. The eggs of numerous marine animals were readily available and the embryonic development of the often translucent specimen could be studied in living samples, or with easy methods of fixation and staining. In 1827, German-Estonian biologist Karl Ernst von Baer (1792–1876) described the first human egg cell that was derived from a young woman who had drowned after a wild night. However, as mammalian eggs and embryos were not easy to come by, the study of mammalian development remained on the back burner for a long time. By contrast, egg-laying animals, like sea urchins, frogs, fish, and worms, proved to be excellent objects for the study of the early stages of embryogenesis.

With the help of the microscope it was discovered that organisms consist of cells that divide, and that embryos develop from simple to more complex forms. Even in the mid-nineteenth century people still seriously discussed spontaneous generation, the formation of living beings from dead matter, until the German medical researcher Robert Remak (1815–1865) finally showed that every cell springs from a precursor cell. As the German pathologist Rudolf Virchow in 1855 stated: "omnis cellula e cellula" ... "Wherever a cell develops, another cell must have existed." Improving microscopic methods and staining techniques allowed the structure of both plant and animal cells to be studied. For example, it was discovered that cells of higher organisms contain a cell nucleus, which itself divides prior to the division of the cell. In addition, the nucleus contains structures called chromosomes. At the beginning of the twentieth century, experimental and cytological examinations postulated that it must be the chromosomes that carry the genes because chromosomes and genes are distributed in the same manner from generation to generation.

1. *Cells and Cell Division*

The cell is the smallest unit of an organism. Every cell consists of an outer membrane, a nucleus in its interior, and is filled with cytoplasm. The membrane is built by a double layer of water-repellent lipid molecules together with numerous proteins. The cytoplasm is a viscous liquid containing a high concentration of various proteins, fatty substances, carbohydrates, and salts.

The most important cell components are the proteins, which function as enzymes or as building blocks for membranes, supportive structures, and other structural elements. Not every cell contains every protein, as many of them are produced only when needed and in amounts that differ from cell type to cell type. The cytoplasm includes various structures that assume different biochemical functions in the formation and decay of cellular components. Ribosomes consist of various proteins as well as ribonucleic acid (RNA) that are orderly arranged and play a large role in the synthesis of proteins (see Chapter III). Several organelles create spaces enclosed by membranes where enzymes present in high concentrations can fulfill specific biochemical processes. Interspersed throughout the cell are mitochondria that generate energy for the cell (Figure 2).

The nucleus, encased by its own membrane, is filled with a dense structure named chromatin, so called because it can be stained easily. Before and during the division of the nucleus, chromatin condenses into distinctive, usually threadlike structures named chromosomes. Before cell division, the chromosomes are duplicated lengthwise, and then they are distributed one each to the daughter cells (Figure 3). The centrosome, an organelle in the cell, is particularly important during this process. It is the first structure to divide, with each new centrosome migrating to the opposite poles of the cell. With the centrosomes as the starting point, stellate fibers arranged like a spindle connect to the chromosomes. When these spindle fibers contract, they each pull one of the chromosomes to one of the poles. Each daughter nucleus, therefore, receives one copy of the chromosomes in the original cell. The new nuclei are separated and the two daughter cells finally divide between them. This mechanism of cell division is called mitosis and ensures that the daughter cell receives the same chromosomes as the mother cell.

Animals differ with respect to the number and the types and shapes of cells they have. The worm *Caenorhabditis elegans*, for example, has exactly 959 body cells composed of about 10 different types such as muscle cells, nerve cells, and skin cells. Most animals, however, are composed of a variable number of cells. Human cells, for example, come in more than 200 different shapes and functions. It is interesting to note that smaller animals actually have fewer cells, and,

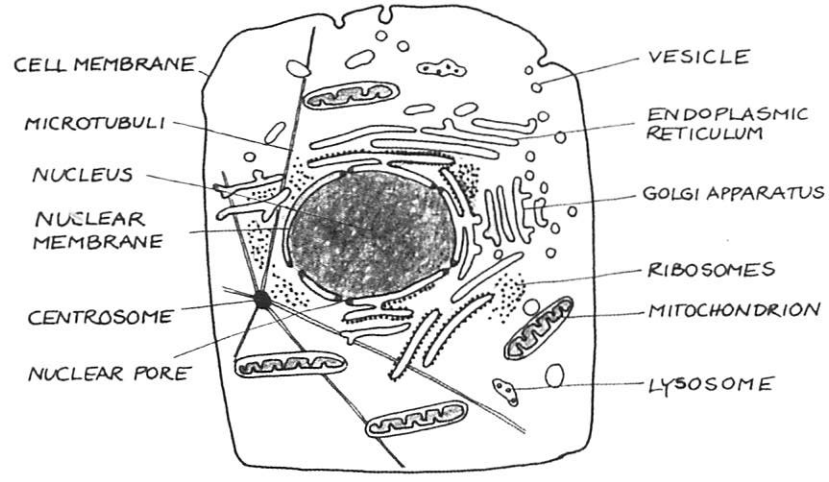

Figure 2. *An Animal Cell.* The cell is surrounded by a membrane, which, via vesicles can exchange material with the outside. A double membrane punctuated by nuclear pores surrounds the nucleus. These pores are highly organized protein complexes which regulate traffic in and out of the nucleus. The endoplasmic reticulum, associated with ribosomes, and the Golgi apparatus are compartments where proteins for excretion are produced. Mitochondria contain membranes with a large surface. In the mitochondria, energy for the cell is generated from nutrients. Microtubules are the building blocks of the cytoskeleton; they originate at the centrosome. Organelles, like the lysosomes, rid the cell of materials that are superfluous or even harmful.

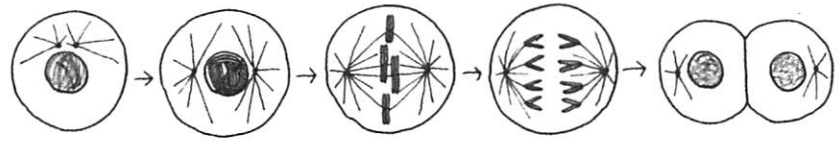

Figure 3. *Cell Division.* For simplicity, only two pairs of chromosomes are shown (red and gray). First the chromosomes are doubled and a spindle forms. Then the nuclear envelope breaks down and the spindle microtubules attach to the chromosomes. Next the chromosomes are pulled into opposing halves of the cell so that they separate into two chromatids per chromosome. Finally, the cell divides and the nuclear envelope reconstitutes itself.

likewise, larger animals do not simply have larger cells but more of them. The mouse has 2×10^9 cells and the human has 3×10^{13} cells. This implies that there is a certain minimum size for cells and that cells cannot be built any smaller. Mammalian cells, for example, have a diameter of about 10 micrometers.

2. *Fertilization*

Animal development begins with the fusion of the egg cell and the sperm, commonly known as fertilization. The resulting zygote receives its cytoplasm exclusively from the egg cell, but the nucleus is composed equally of maternal and paternal chromosomes. As embryonic development begins, the fertilized egg cell divides many times. In the early stages of animal development, known as blastula, the cells look the same. Shortly thereafter, however, the cells start to become visibly different, and the embryo takes shape. This important process is called gastrulation. Cells sort themselves into groups, they ingress or fold into the embryo, shift, continue to divide, and form the primordia of the various tissues and organs. Finally, cells differentiate into organs, which fulfill various functions in the animal (Figure 4).

It became clear that heredity is tied to the process of reproduction. Often, plants, and even certain animals such as polyps or sponges, reproduce asexually by budding. It is easy to see that the buds, or layers, develop into the image of their parents. However, all plants and animals of higher order have two sexes. Both sexes produce reproductive cells that are called germ cells, or

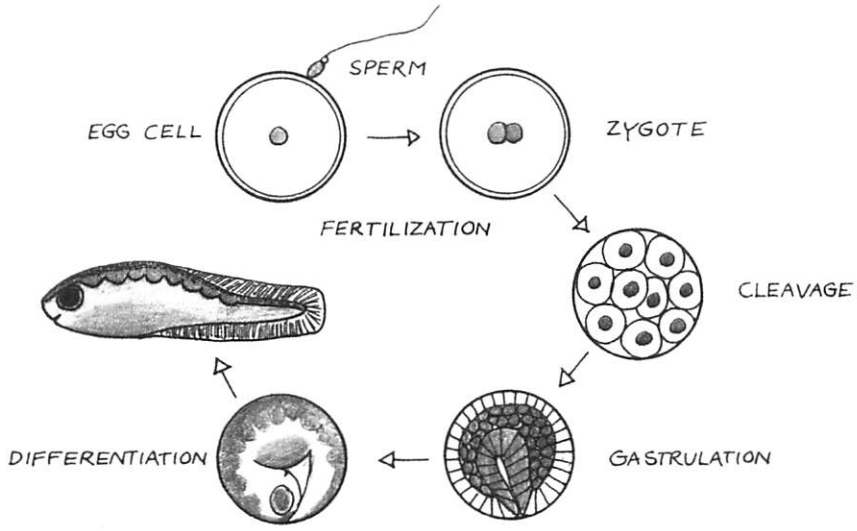

Figure 4. *Development.* A simplified and generalized view of embryonic development, beginning with fertilization. Cleavage follows, dividing the fertilized egg into many small cells. During the process of gastrulation, the overall organization of the body plan is laid down involving cell rearrangements. Then organs start to form and begin to differentiate into various tissues.

gametes. The female egg cells that eventually become the embryo are large and immobile while the smaller male sperm cells are produced in often incredibly large numbers.

The development of a new individual begins with fertilization. The female and male gametes, the egg cells and the sperm cells, each have a distinct shape. The egg cell is rich in cytoplasm and often contains a large amount of yolk that provides nutrients to the embryo up to the moment of hatching. The sperm cell is tiny by comparison, and contains only the nucleus and the centrosome, an organelle not present in the egg cell. Often, the sperm cell also has an organelle for locomotion known as the flagellum, which allows the sperm to swim toward the egg cell.

In marine animals, the fusion of egg cell and sperm cell takes place in the water outside of the body. With the rise of land animals, fertilization adapted to the fluids contained within the body. As soon as a sperm cell penetrates the egg cell, the flagellum of the sperm is shed, and the nucleus and centrosome enter the egg. At the same time, a membrane quickly encloses the egg cell ensuring that not more than one sperm enters. The nucleus of the sperm then approaches the nucleus of the egg and they fuse.

Embryonic development begins with the initial cell divisions. The cytoplasm of the egg cell is distributed to the daughter cells, which become smaller in size as their numbers increase through a process known as cleavage. Later in development, at the point when the organism is no longer confined to the size of the original egg cell, the division of the nucleus is preceded by a growth of the cytoplasm so that each daughter cell may become as big as the mother cell.

Certain aspects of fertilization deal specifically with the role of the chromosomes, the centrosome, and the cytoplasm. Usually, the egg cell can only develop if fused with a sperm cell. One interesting finding was that the nucleus of the sperm cell is not necessary for the early divisions at the very beginning of development. The centrosome, which is also part of the sperm, however, is essential for the initial divisions. But there are some cases in which a centrosome can actually be created within the egg. This process is called parthenogenesis, or virgin fertilization, as development begins without fertilization. Male bees, for example, develop through parthenogenesis.

But for some time one question remained. Within this process of fertilization, where were the carriers of hereditary factors located—in the cytoplasm or in the nucleus? Clever experiments helped find the answer. It had been common knowledge that when crossbreeding two different species, the hybrid would show both maternal and paternal characteristics. An exchange of parents did not result in major differences even though the cytoplasm was

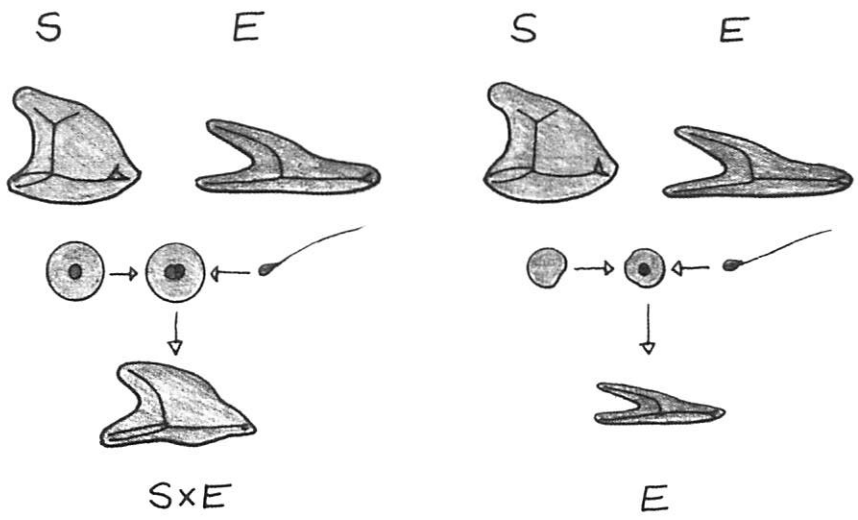

Figure 5. *The Importance of the Nucleus.* When two different species of sea urchins (S and E) are crossed (left panel), the resulting hybrid larva shows a mixture of maternal and paternal qualities. By heavily shaking the eggs, Theodor Boveri could produce eggs without a nucleus. If such eggs are fertilized with the sperm of another species (right panel), larvae develop that only resemble the father even though the cytoplasm was entirely maternal.

exclusively maternal, in other words, solely from the egg. The German zoologist Theodor Boveri (1862–1915) observed that when clumps of cytoplasm without any nucleus were fertilized with the sperm of a different species, the resulting mini larva looked like the father and not like the mother. This proved that it is the nucleus rather than the cytoplasm that carries all genetic information (Figure 5).

3. *Chromosomes and Genes*

The American biologist Walter Sutton (1877–1916) examined the development of germ cells. He showed that a certain species of grasshopper had 11 chromosomes that were visibly distinct from each other. Furthermore, he showed that within the body cells the chromosomes appear in duplicate, termed the "diploid" state. During the cell division that precedes the development of egg cells and sperm cells, the corresponding homologous chromosomes arrange in pairs and are distributed one each into one of the daughter cells by the dividing spindle. Which copy of the two homologous

chromosomes is distributed into which daughter cell is left to chance. Such a division results in daughter cells that contain only one copy of each chromosome, and are thus called haploid.

This form of cell division is called reduction division or meiosis in contrast to the normal division called mitosis. In the course of fertilization, the haploid cell nucleus of the maternal egg cell fuses with that of the paternal sperm cell. The result is named a diploid zygote. Mitotic divisions result in the multiplication of diploid cells, whereas meiotic divisions reduce the chromosome number back to the haploid state during the formation of egg and sperm cells.

The distribution of chromosomes from generation to generation corresponds exactly to the distribution of hereditary factors according to Mendel's laws (Figure 7). Walter Sutton's theory predicted that there should only be as many independent hereditary characteristics as there are pairs of chromosomes. In 1902, he published the possible combinations in a table. The table is relatively easy to read with just two chromosome pairs. For example, there are 2×2 totaling 4 different combinations in the haploid nucleus, and 4×4 totaling 16 different combinations in the zygote. If there are 10 pairs of chromosomes there are more than a thousand possible combinations in the egg or sperm cell, and 1 million in the zygote. Humans have 23 pairs of chromosomes.

Is it only the number of chromosomes that matters or are chromosomes distinct from each other? If so, what are their functions? Theodor Boveri discovered that at least one copy of every chromosome is necessary for the normal development of the animal. If one or more chromosomes were missing completely, characteristic developmental problems resulted.

The experiment that led him to this conclusion was an examination of sea urchin embryos developing from eggs that had been fertilized by two sperm, something that happens only very rarely (Figure 6). The egg cell then receives four spindles that during the first division form four daughter cells simultaneously. But since every chromosome is present in three copies—one from the egg cell and one each from the two sperm cells—the developing cells would vary in the numbers of chromosomes they receive.

Instead of receiving two copies of each chromosome, Boveri showed that many cells have only one, others three, and others miss that particular chromosome altogether. These descendant cells could not develop normally and showed a characteristic set of defects. This means that chromosomes are individually different from each other and that every one of them carries specific genes for specific functions in the development of the animal. Therefore, genes from different chromosomes cannot substitute for each other.

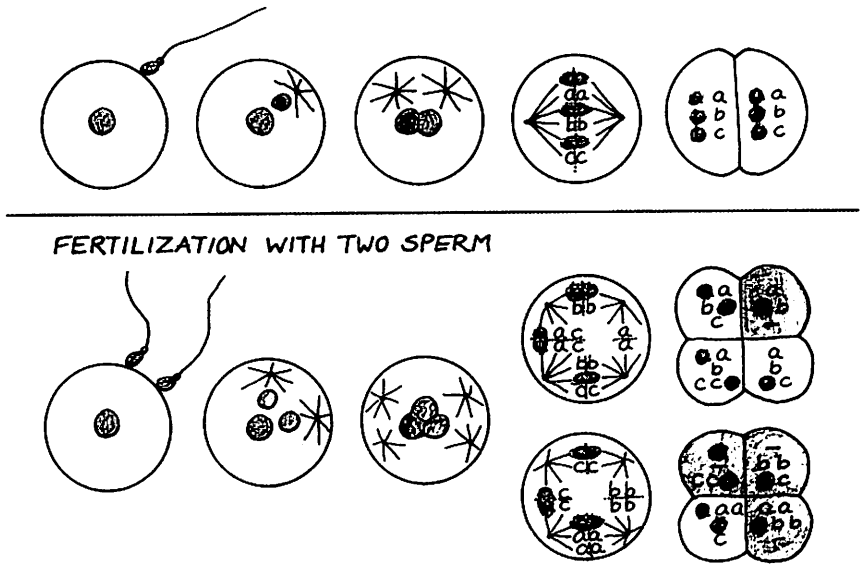

Figure 6. *Double Fertilization.* During normal sea urchin fertilization (upper panel), the spindle that develops between the centrosomes distributes the chromosomes of the maternal (red) and paternal (gray) nucleus to the daughter cells such that each cell receives one paternal and one maternal copy of each chromosome. If two sperm fertilize the cell at the exact same time, four spindles will develop (lower panel). In this case, the three sets of chromosomes (one from the mother and two from the fathers) are distributed among these four spindles. Four cells are created at the same time containing varying numbers and combinations of chromosomes. Many cells do not develop normally. From the frequency of defective cells, Theodor Boveri concluded that these cells (gray) were missing one chromosome altogether and, thus, that the chromosomes must be different.

Sutton and Boveri published their results more than 100 years ago, in 1903. Their results can be summarized as follows: the chromosomes carry the genes that are present in duplicate within the body cells, yet are singular within the gametes. Only one of the pair of homologous chromosomes is passed on to the offspring; which one of the two is left to chance. All body cells contain two copies of all genes, one from the mother and one from the father (Figure 7).

4. Germ Line and Clones

The discovery that all cells within an organism contain all chromosomes, and thus all genes, was of crucial importance to the study of how cells become

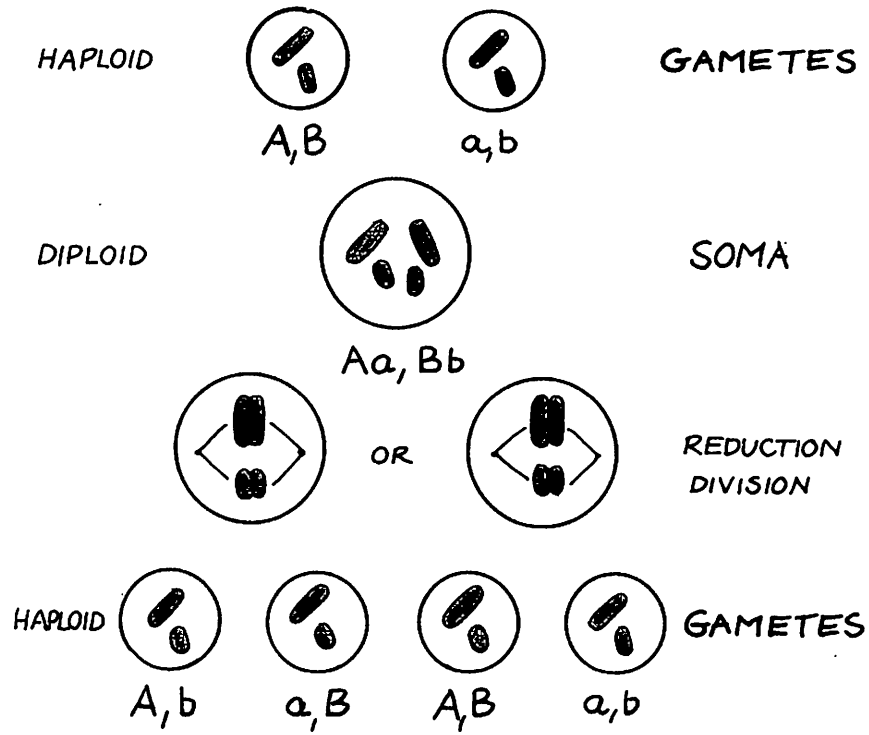

Figure 7. *The Chromosome Theory of Heredity.* Gametes are haploid, they have only one copy of each chromosome, while the diploid body cells, called soma, have two copies, one from the father, one from the mother. For simplicity only two chromosome pairs are illustrated here. When gametes develop, the homologous chromosomes arrange in pairs and are distributed into the gametes independently from each other. This explains Mendel's Laws. This figure does not take into account the phenomenon of recombination (see Chapters III and VI) that was not discovered until later.

different from one another. Previously, the famous yet incorrect thesis of the German embryologist August Weismann (1834–1914) had postulated that the differentiation of cells was caused by an unequal distribution of genes to the daughter cells. In contrast, we now know that an organism is composed of a large number of genetically identical cells. Thus, it is a clone that originates from one founder cell, the zygote.

The observations of Boveri and Sutton may well have sprung from isolated cases, but in the 1960s, the British researcher John Gurdon (1933–) tested and confirmed their theses. He transplanted cell nuclei from differentiated cells using the gut of a tadpole into egg cells from which the nucleus had been

removed. In rare cases these composites developed into a normal tadpole (Figure 8). With this experiment John Gurdon proved that the differentiated gut cells still contain all genes required for the development of a tadpole. The resulting tadpole is identical to the tadpole that donated the gut cells—it is indeed its clone. This way of cloning animals by nuclear transplantation has also been shown to work with other animals, such as sheep. However, the very low success rate of this method raises the question: why is cloning so difficult?

In higher animals, specific cells are responsible for the production of gametes. These "primordial germ cells" contain distinctive components that are present only in this cell type. The germ cells separate from the other body cells very early on during development and migrate to the sexual organs, the gonads, where later they form egg or sperm cells. In flies, for example, the first cells that develop are named the pole cells, and they are the ones that will develop into the germ cells (Figure 20). The gametes and their predecessors are called the germ line, as opposed to the body cells, which are called the soma. The germ-line cells go through a special developmental process, including most likely a program protecting the genes from mutations and thus ensuring that the cells responsible for the next generation remain as unharmed as

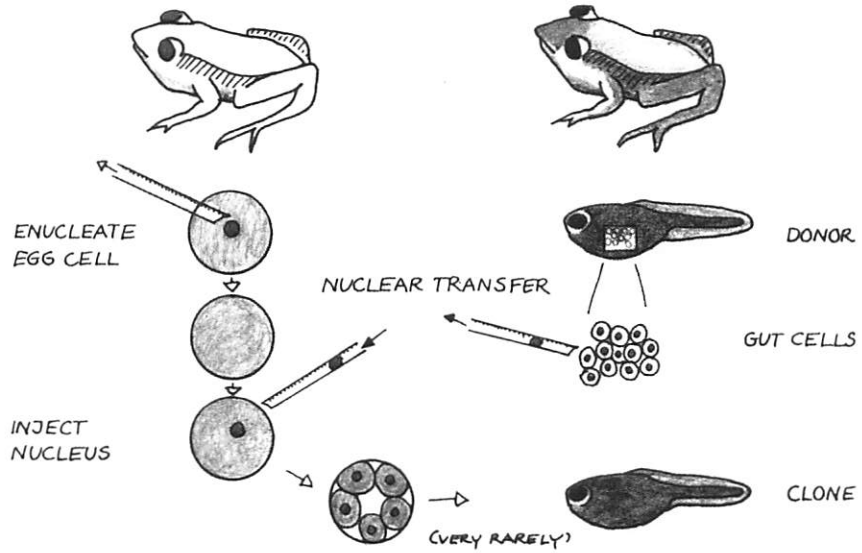

Figure 8. *Cloning.* The nucleus of the egg of an albino frog is removed. It is replaced by the somatic cell of a differentiated tadpole from a normally pigmented frog. The tadpole developing in rare cases from this experiment is normally pigmented and thus has the genotype of the animal from which the nucleus was derived. It is its clone.

possible. It is not yet clear what this program entails exactly, but it is clear that the germ line is necessary as it contains characteristics that the soma does not have. Because any offspring will develop solely from the gametes, changes to somatic cells have no influence on the offspring's hereditary information. In addition, gene mutations will only be inherited if they take place in the germline cells. This is why acquired properties are not inherited.

Asexual reproduction is common among plants, but rare among animals. The freshwater polyp *Hydra* reproduces by budding, and some insects, like aphids, show life phases of quick multiplication through diploid eggs that form large, genetically identical clones. But in difficult times, even these animals reproduce sexually. Sexual reproduction leads to more variations among the descendants, of which some may have a greater chance to survive.

5. *The Influences of the Cytoplasm and the Environment*

If all cells have all genes, the origin of differences arising during development of an organism must reside in the cytoplasm. Factors in the cytoplasm decide the fate of the cells by determining which genes are active and which are not. The two daughter cells that originate from the first cleavage division of a frog can produce a viable, albeit smaller, frog. But just a few divisions later, this is no longer possible as the individual cells do not have enough cytoplasm and the cytoplasm that they do have differs between the individual cells (Figure 9). Theodor Boveri observed that sea urchin eggs have an intrinsic polarity and that an artificial splitting of the eggs at right angles does not produce complete embryos. With regard to their ability to produce an entire embryo, the right and left sides of the egg are identical, but the top and bottom are different. He concluded that cytoplasmic factors are at play and that they gradually increase or decrease in concentration from top to bottom of the egg in determining the developmental fate of the cells. In other words, there are differences within the cytoplasm that determine the fate of the daughter cells.

The secret of embryonic development is the control of gene activity in time and space. Together with the factors in the cytoplasm, the genes constitute a building plan for the developing organism, a blueprint that is realized step by step. As explained earlier, the cytoplasm originates from the egg. But the cytoplasm also receives signals and information from the environment, including the neighboring cells. This information is transmitted to the genes in the nucleus. In this manner, the fate of a cell is dependent on both the cytoplasm and external influences.

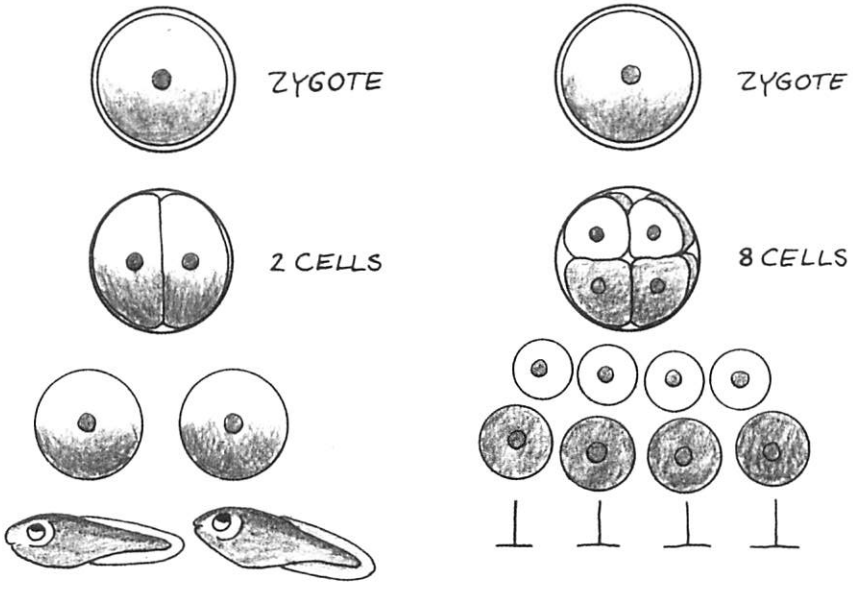

Figure 9. *Multiples.* The separation of cleavage cells at the two-cell stage can produce two normal offspring. With sea urchins, this is even possible at the four-cell stage. At later stages (right panel), this is no longer possible because the cells do not contain all necessary cytoplasmic factors any longer and are too small.

Researchers like Theodor Boveri clearly realized that in their time it would not be possible to find out what genes are, nor what factors influence development. It took nearly a century before modern molecular genetics was capable of identifying and isolating these factors.

From an historic standpoint, it is interesting that at the beginning of the twentieth century, embryology and genetic research began to develop as separate disciplines. One important reason may have been that the organisms most suited to one field of study were not particularly useful to the other. Amphibians, such as frogs and newts were excellent for embryological research, as they lay huge eggs 2–4 millimeters in diameter that could easily be surgically manipulated. For example, experiments in which specific regions of the embryo were isolated and recombined led to the discovery of a special region in the newt egg that came to be called the organizer.

The organizer has a long-range influence on its neighboring cells and can even induce the formation of an additional body axis. In the famous 1923 experiment by the German zoologist Hans Spemann (1869–1941), the organizer cells from one embryo were transplanted into another embryo of the

same age, in the region that develops normally into the stomach. But after the transplantation, a second head and trunk region developed instead of stomach. Since the two embryos were from two different species and easily distinguishable by way of their pigmentation, it was possible to determine that the new axis did not originate from the donor tissue but rather that the donor tissue had influenced the cells in its new environment to form an additional body axis. This crucial experiment prompted attempts to isolate and biochemically characterize the factor operating as the organizer. But despite great efforts, it took 70 years before the gene responsible was isolated and identified.

Aside from some frustrations and false interpretations along the way, several quintessential discoveries had been made at the dawn of the twentieth century. One was the realization that genes are the discrete units of heredity that guide development. Another was that the cytoplasm exerts influence by determining which genes are active and which are not. And a third was that the nucleus contains, with the genes, the entire hereditable information of a developmental program. With these discoveries, the nature of genes and their regulation became the main concern of biology.