CHAPTER

4

Physiological Development and Epigenetics

The Australian sea lion (*Neophoca cinerea*) is an endangered species of diving mammal endemic to the southwestern seacoasts of Australia. Although the adults do not engage in particularly long dives compared with many other diving mammals (see Chapter 26), they are highly accomplished divers in their own way. They feed on fish, squids, octopuses, lobsters, and other prey that they capture by diving to the bottom, or near the bottom, of modestly deep (e.g., 70–100 m) offshore waters. They spend approximately half their time at sea. When they are at sea, they forage incessantly by undertaking about 10 to 11 dives every hour, thereby spending almost 60% of their time at depths in excess of 6 m. With all these considerations in mind, the intensity of foraging effort by Australian sea lions ranks as exceptionally high. Because the sea lions forage underwater, their unusually intense foraging places high demands on their physiological capacities for functioning without breathing.

Infant Australian sea lions cannot dive at all (they depend on their mother to feed them). Thus, a dramatic change in diving capacity occurs in the life of each individual as it grows and matures. Each sea lion starts life with essentially no diving capacity but must develop a sophisticated diving capacity by the time it is a mature adult.

Oxygen stores play a crucial role. During many dives—although not all (see Chapter 26)—the tissues of a diving mammal maintain their metabolism by using O_2 that the animal stored in its body while it was at the surface, breathing air. Oxygen is stored in three major ways. First, during diving, an animal's lung air contains O_2, which can potentially be transported from the lungs to other tissues. Second, O_2 is combined with hemoglobin in the blood; as a dive progresses, this O_2 is released from the blood hemoglobin and taken up by tissues. Third, within the cells of the heart and skeletal muscles, O_2 is combined with myoglobin, a red, iron-containing protein that gives red muscles their reddish color. Myoglobin is a specialized form of hemoglobin. The O_2 combined with myoglobin is released for use in muscle metabolism during a dive.

Biologists studying the individual development of Australian sea lions have discovered that the capacity of a young animal to store O_2 undergoes a major developmental change as the animal matures to adulthood. This can be seen in **Figure 4.1**. Note that the amount of O_2 that can be stored at the start of a dive—plotted on the *y* axis—is expressed per unit of body weight. By use of this form of expression, body size is removed as an immediate consideration. As you can see, by the time an individual has grown to be an adult, it is able to store almost three times as much O_2 as it could store when it was 6 months old. This is not because the animal is bigger. Instead, what the data show is that the amount of O_2 that can be stored *per unit of tissue* triples during development.

Australian sea lions (*Neophoca cinerea*) searching for prey near the bottom of the coastal ocean As adults, these sea lions dive 10–11 times per hour in search of fish, squids, octopuses, lobsters, and other prey. Young Australian sea lions are more limited than adults in their physiological capacities for diving, and they only gradually acquire adult competence as they mature.

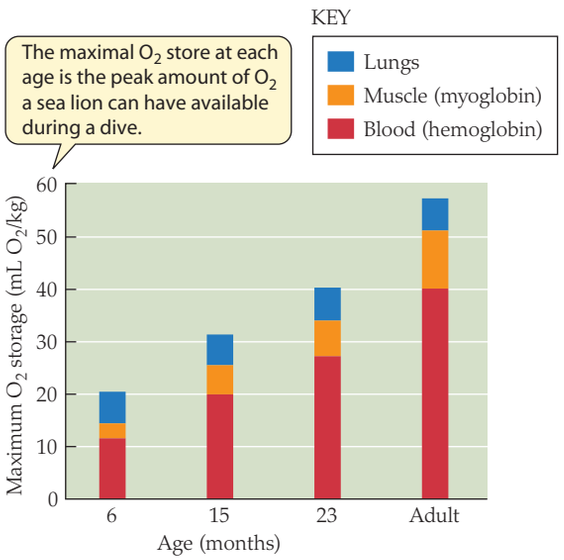


FIGURE 4.1 The maximum O_2 stores of Australian sea lions at four different ages The total O_2 available per kilogram of body weight at each age is estimated by adding (from bottom to top) the O_2 in blood (mostly bound to hemoglobin), the O_2 bound to myoglobin in muscles, and the O_2 in lung air. (After Fowler et al. 2007.)

Some of the reasons for this change are known. A young sea lion increases the concentration of hemoglobin in its blood by about 1.4 times as it matures from 6 months of age to adulthood. The greater concentration of hemoglobin enables each volume of blood to combine with more O_2. In addition, the volume of blood increases. Blood volume per unit of body weight is more than twice as great in an adult as in a 6-month-old. These two developmental changes—the increase in amount of hemoglobin per unit volume of blood and the increase in blood volume—mean that an animal has more and more blood hemoglobin per unit of body weight as it grows older. The capacity for O_2 storage by myoglobin also increases. In fact, myoglobin accounts for the most dramatic of all the changes in O_2 storage capacity. Muscles become more than three times more concentrated with myoglobin (and become strikingly redder) as a sea lion develops from 6 months of age to adulthood.

Each individual sea lion must pass successfully through all the stages of maturation, from infancy to adulthood, if it is ever to reproduce. Each individual must therefore function with the physiological capabilities of a 6-month-old, then the physiological capabilities of a 15-month-old, and so forth.

Recognizing that the O_2 storage capacities of immature sea lions are limited (see Figure 4.1), we might well expect that when a sea lion is young, its diving capabilities are also limited, compared with an adult. This expectation is supported by the available evidence. Young sea lions at 6, 15, and 23 months of age do not dive as long or as deep as adults (Table 4.1). Six-month-olds exhibit very limited diving performance and get most of their food from nursing. Even 23-month-olds dive to depths averaging only 62% as deep as those attained by adults. A youngster does not have as much hemoglobin or myoglobin per unit of body weight as an adult. Consequently, its O_2 store is lower, and its capacity to function without breathing is more limited. Its capacity to gather food in each dive is therefore also more limited. Despite such constraints, the animal must find ways to succeed as a youngster, or it will never be a reproductive adult. In this way, the physiology of all the successive stages of individual development—**developmental physiology**—is a life-and-death matter for every individual.[1]

The Physiology of Immature Animals Always Differs from That of Adults

Universally, the physiology of youngsters differs in important ways from that of the adults of their species, gradually changing in age-specific ways as postnatal development takes place. For one way to see these points, consider the maturation of three categories of tissues in humans, shown in Figure 4.2*a*. The brain matures rapidly. Already by 7 years of age, a person's brain has reached its full adult size, whereas general body tissues are less than half developed at that age and the reproductive organs have hardly started to grow. For comparison, note that this developmental program differs dramatically from that in rats, in which reproductive maturity is attained before full brain growth (Figure 4.2*b*).

The developmental program evolved by humans is unique because of the rate of brain maturation, which is far higher than in any other species of mammal. A consequence of this fast brain development is that young children are in a dramatically different

[1]Another, commonly used term for individual development is *ontogeny*.

TABLE 4.1 Diving durations and depths observed at four different ages in Australian sea lions (*Neophoca cinerea*)[a]

Age	Maximum duration (min)	Mean duration (min)	Maximum depth (m)	Mean depth (m)
6 months	2.7	0.4	29	7
15 months	5.8	3.2	68	40
23 months	5.8	2.8	78	44
Adult	**7.5**	**3.3**	**103**	**71**

Source: Fowler et al. 2006.

[a]Averages are listed. Statistically, all the values for subadult ages are significantly different from the corresponding adult values (bold text), with the exception of the mean duration for 15-month-olds. Numbers of animals studied were 7–9 in each subadult category and 25 in the adult category.

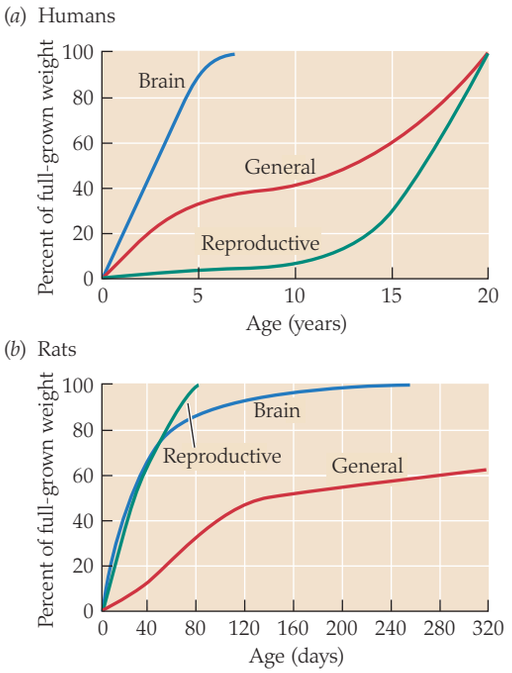


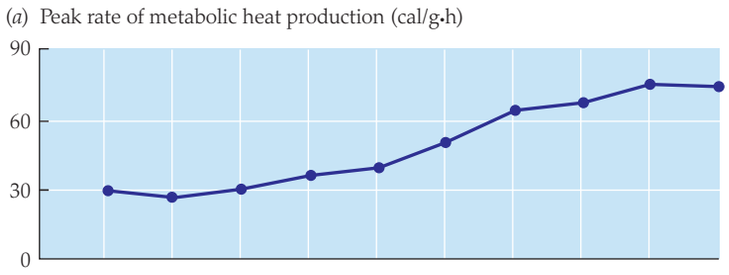
FIGURE 4.2 Growth of the brain, reproductive organs, and other ("general") tissues in humans and laboratory rats Each tissue type is plotted, at each age, as a percentage of its full-grown weight. (After Bogin 1999.)

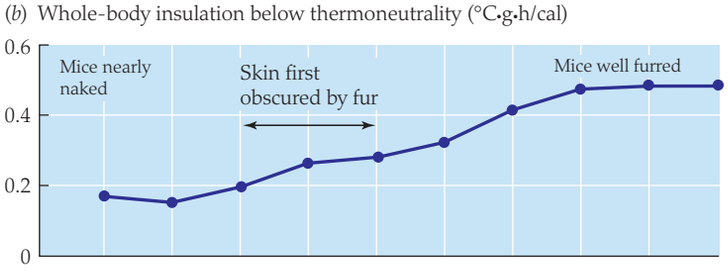
position from adults in the challenge they face to meet their brain's energy needs. Brain tissue in mammals has a particularly high metabolic rate per gram—and therefore a particularly high need for fuel per gram—compared with most tissues; the metabolic rate per gram of brain is more than 10 times that of skeletal muscle, for example. The high per-gram metabolic rate of brain tissue explains why, in human adults, the brain accounts for about 20% of total body metabolism (see p. 181) even though brain weight is only 2% of body weight. In young children, the brain accounts for far more than 20% of total metabolism. Because of the rapid, early development of the brain in humans, a youngster who is 4 to 5 years old has a brain that is almost full size (see Figure 4.2*a*) even though the youngster's body is small. Biologists have calculated that in children of that age, the brain accounts for approximately 50% of total body metabolism! Half of the food metabolized by a 4- or 5-year-old is for his or her brain! One implication is that childhood starvation—a common phenomenon around the world—is a particular threat to the most crucial of all human tissues.

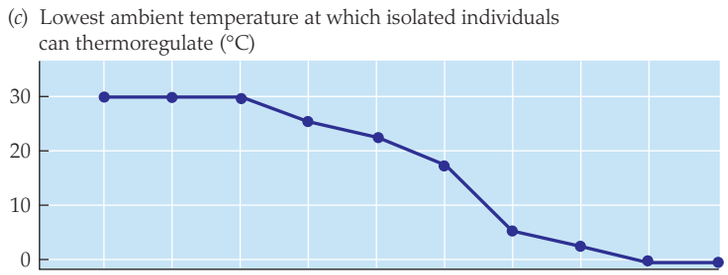
Beyond brain energetics, countless cases are known in which brain performance undergoes maturation during the normal development of young animals, with long-term consequences for each individual. Striking examples come from the study of birds that employ the stars to help guide them during nocturnal migration, such as indigo buntings (*Passerina cyanea*), which breed in North America, and garden warblers (*Sylvia borin*), which breed in Europe. In both of these species, youngsters hatch and undergo their early development in the Northern Hemisphere. As adults, these birds migrate to warmer latitudes each winter. They migrate at night, and during their migration they are able to determine compass directions—such as whether they are flying north or south—by looking at the stars. Obviously, the stars, *in and of themselves*, do not provide compass information. When a person determines north and south from the stars, the person does so by having learned that particular stars serve this purpose. Birds must do much the same thing. Experiments have shown that young indigo buntings and garden warblers, during their first summer of life, observe the rotation of the stars in the sky (**Figure 4.3**) and undergo a maturational change whereby thereafter they possess a brain record of the stars at the center of the rotation. They then associate those stars with north. Without this maturation during their early weeks of life, they cannot determine compass directions from the stars. Normally, of course, they are able to observe the stars during their early development, and consequently, when they head south for the first time in the autumn of their first year of life, they possess a fully functional star compass to guide them. The necessity for star observations during early development was discovered by manipulative experiments in a planetarium, where the stars can be made artificially to rotate around any spot in the sky. If birds undergo their early development in a planetarium where the stars rotate around a spot in the eastern sky, they later treat the eastern stars as if those stars are in the northern sky, so when they travel north based on their internal calculation of direction, they actually travel east.

Thermoregulation is another aspect of physiology that undergoes postnatal development. Adult mammals and birds are noted for physiological regulation of body temperature, termed **homeothermy**. Placental mammals, for example, typically maintain a deep body temperature of about 37°C as adults (see p. 250). Universally, however, newborn mammals are not as effective in maintaining homeothermy as adults of their species. Individuals develop their full capacity for homeothermy as they mature from birth to adulthood.

FIGURE 4.3 The apparent rotation of the stars when looking north in the Northern Hemisphere The stars seem to make circles around the northern end of Earth's axis because of Earth's rotation. This apparent movement of the stars is evident in a long exposure of the northern sky, as in this photograph. During early development, indigo buntings and garden warblers use the apparent rotation of the stars to identify north (the center of the apparent circles), and they undergo a maturational change whereby they thereafter possess a brain record of the stars positioned at the north.







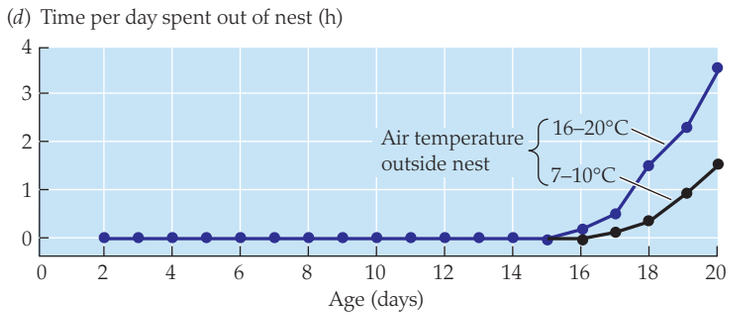


FIGURE 4.4 Development of thermoregulation in white-footed mice (*Peromyscus leucopus*) Four properties are shown as functions of age between birth (age 0) and 3 weeks of age, when weaning occurs. (*a*) Peak thermogenic rate. (*b*) Whole-body insulation (*I*) (see Equation 10.10). (*c*) The lowest air temperature at which an isolated individual can thermoregulate for 2.5–3.0 hours. (*d*) The number of hours per day that nestlings spend out of the nest when the air temperature outside is 7–10°C or 16–20°C. In all parts, the data are means for all individuals studied. (After research by Richard W. Hill; photos courtesy of Robert J. Robbins.)

An example is provided by the white-footed mouse (*Peromyscus leucopus*), one of the most common native rodents in North America (see Chapter 11 for additional examples). Individual adult white-footed mice can maintain a deep body temperature near 37°C when the air temperature is below freezing. Individual newborns, however, quickly cool to the environmental temperature; in a near-freezing environment, their tissues cool to near-freezing temperatures (which they tolerate). As young white-footed mice grow and mature during their 3-week-long nestling period, the peak rate per gram at which they can metabolically generate heat increases dramatically (**Figure 4.4*a***). In addition, they develop fur, and their resistance to heat loss (their whole-body insulation) increases (**Figure 4.4*b***). The mice therefore become increasingly able to thermoregulate as isolated individuals, away from their nest and siblings (**Figure 4.4*c***). By 18 days of age, a lone youngster can thermoregulate for several hours even in freezing-cold air, and it capitalizes on this newfound ability by making its first excursions outside its nest (**Figure 4.4*d***).

Physiological development occurs at all scales. The scale of individual proteins is particularly fundamental, because proteins determine the structural and metabolic properties of a tissue.

One class of proteins of great importance is the enzymes. A common pattern during development is for enzymes in a tissue to be upregulated—increasing their amounts and activities—in an almost stepwise fashion, under genetic control, as the tissue matures. For example, **Figure 4.5** shows such stepwise upregulation of three enzymes in a single tissue, the liver, of developing rats. As new enzymes appear in cells (and old ones disappear), their

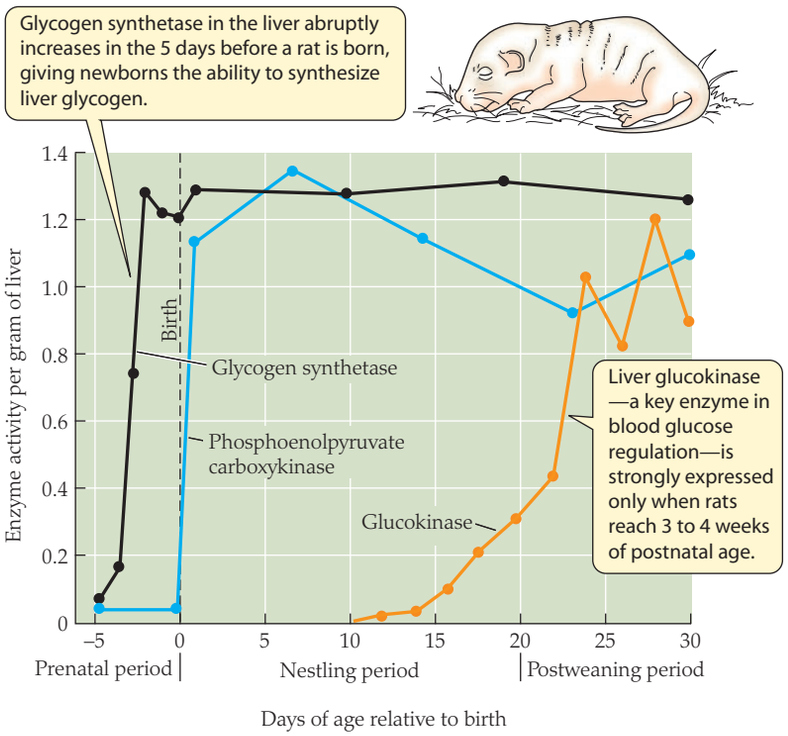


FIGURE 4.5 Sequential upregulation of enzymes during development in laboratory rats Activities of three enzymes in the developing liver are shown. Day 0 on the *x* axis is the day of birth; negative times are days before birth. (After Walker 1983.)

collective catalytic and regulatory properties alter cell metabolism. For instance, although early fetal rats cannot synthesize liver glycogen because they lack glycogen synthetase, newborn rats *can* because the required enzyme is suddenly expressed during the 5 days before birth.

The tissues in which a protein is highly expressed may also undergo developmental change. To illustrate this phenomenon, we will use a fish example. In many species of fish, the importance of the physiology of young animals is particularly evident because over 99% of individuals die before they complete their embryonic and larval life. In such cases, if you want to know the physiological characteristics that help to determine whether *most* individuals live or die, you need to look at the physiology of embryos and larvae.

Many species of fish complete a substantial portion of their early development before their gills form, raising the question of how and where gill functions are met before the gills develop. For fish living in seawater, Cl^- ions need to be transported out of the body to prevent the body fluids from becoming overly salty. In adults, this transport is carried out by the gills, but where does this transport occur in embryos and larvae? Often, eggs are spawned into the ambient water, where fertilization occurs. The embryos and larvae thus develop in seawater and have a need to transport Cl^- out of the body, like adults do, despite having no gills or only partly developed gills.

The cells that transport Cl^- ions out of the body, known as **chloride cells** (see Box 28.1), can be identified because they express the transporter protein Na^+–K^+-ATPase (see page 110) in abundance (the protein is crucial for their ion-transport function). Because these cells profusely express this specific protein, they can be located using immunocytochemistry. In this technique, an antibody is made against a protein of interest—Na^+–K^+-ATPase in this case—and when tissues are exposed to this antibody, the antibody binds wherever its target protein occurs. By visualizing the binding sites, one can learn the locations where the target protein is expressed (and how abundant it is). This method has revealed the fascinating developmental patterns depicted in **Figure 4.6**. In the species shown, a killifish, young that are 8 days old or younger do not have gills.[2] They do, however, have chloride cells, which—at these early ages—are found in the yolk sac membrane and scattered over the surface of the skin (see Figure 4.6*a*). By 15 days of age, the young have emerged from the egg membrane (typically at 11 days), and the yolk sac has been reabsorbed. Still, chloride cells are found widely on the skin surface (see Figure 4.6*b*). In addition the gills have started to develop in 15-day-olds and are populated to some extent with chloride cells (see Figure 4.6*c*). Later, as killifish progress to adulthood, chloride cells cease to be found on the general skin surface and become mostly localized in the gills (and inner opercular membranes). In all, a major shift occurs in the location of outward Cl^- transport as a killifish develops. At first, the yolk sac and skin are the principal sites of transport. Later, the skin and gills become the principal sites, and still later, transport becomes localized principally to the gills.

Lately, investigators have become able to study gene transcription itself during the course of development by using DNA microarrays and other transcriptomic methods (see Chapter 3). For an example, we return to the developing lab rat, one of the most intensely studied model systems. **Figure 4.7** shows the temporal pattern of expression of spinal cord mRNAs. As you can see, different sets of mRNAs are upregulated sequentially during development, confirming that different sets of genes are transcribed at different times in a developmental program. Some of the mRNAs in the studied sets code for enzymes. Programmed transcription of the sort observed in Figure 4.7 can thereby give rise to sequences of enzyme upregulation such as those seen in Figure 4.5.

[2]Ages are counted from conception.

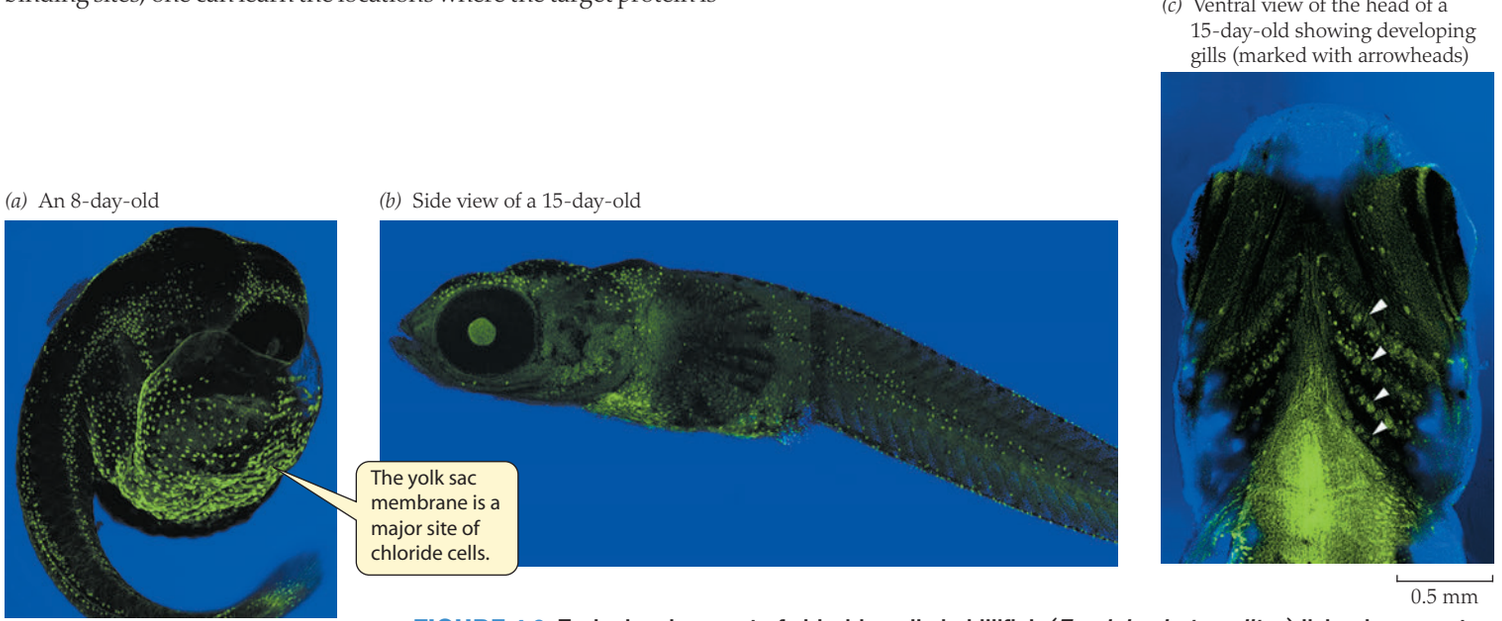


FIGURE 4.6 Early development of chloride cells in killifish (*Fundulus heteroclitus*) living in seawater The chloride cells are responsible for transporting Cl^- ions out of the body so the tissue fluids do not become overly salty. In these images, the chloride cells have been labeled with an antibody that glows green in the light used to produce the images. Ages are measured from conception. (*a*) The cells occur in the yolk sac membrane (which is highly vascularized) and skin at 8 days of age, when the gills have yet to develop. At 15 days of age, chloride cells (*b*) still occur widely on the skin and (*c*) occur in the gills, which have started to develop. (After Katoh et al. 2000; images courtesy of Toyoji Kaneko.)

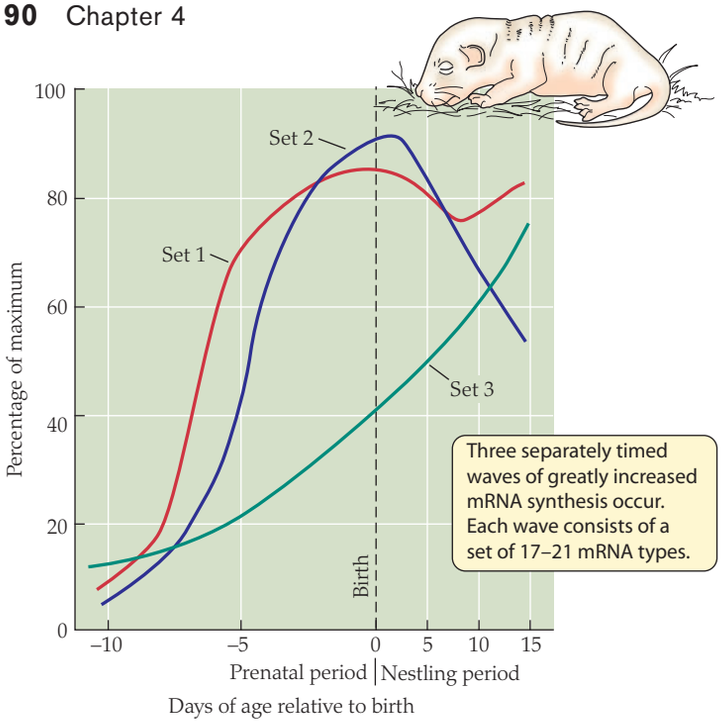


FIGURE 4.7 Developmental programming of gene transcription in fetal and newborn rats The graph shows quantities of three sets of mRNAs in the developing spinal cord. Almost 120 mRNAs were monitored in this study. Some (not shown here) were steady or declined. Note that time is scaled differently before and after birth. Day 0 on the *x* axis is the day of birth; negative times are days before birth. (After Wen et al. 1998.)

Phenotypic Plasticity during Development

Phenotypic plasticity, as discussed in Chapter 1 (see pages 15–16), is the ability of a single animal—with a fixed genotype—to express two or more genetically controlled phenotypes. Phenotypic plasticity is frequently observed during development. An animal's adult phenotype, for instance, often differs if the animal matured in one type of environment rather than another. Two human examples will provide a good starting point for discussing this highly significant phenomenon.

The first example concerns the age at which **menarche**—first menstruation—occurs in girls. Many European countries have historical records on menarche dating back to at least the nineteenth century. Analyses of these records show that, on average, girls did not attain menarche until 16 to 17 years of age in the mid-nineteenth century, whereas they attained menarche at about 13 years of age in the second half of the twentieth century. Most scholars have concluded that this dramatic shift toward earlier menarche between the mid-nineteenth and late twentieth centuries was a consequence of improved nutrition, public health, and medical care. That is, population genotypes did not change in ways that caused the recorded shifts in menarche. Instead, changes in the environment were responsible. Girls in the late twentieth century reached reproductive maturity when they were several years younger than girls in the mid-nineteenth century because they enjoyed a more benign childhood environment.

The second human example concerns the restriction of growth that is often observed in people who live in a relative state of nature. Based on recent, detailed studies, 4- to 12-year-old children in populations of Maya people in Guatemala are, on average, 5.5 cm (2 inches) shorter at each age than children in populations of the *same* ethnic group who recently settled in the United States. From every perspective—public health, sufficiency of the food supply, access to medical care, and political security—Maya populations in Guatemala face greater environmental challenges than do those in the United States. Evidently, the environmental stresses in Guatemala impair growth, and when those stresses are relaxed, children attain greater stature at each age.

Several historical comparisons—although not as well documented as that of the Maya—also point to a strong environmental effect on human growth. Medieval armor on display in museums, for example, is often striking for how small it is. Clearly the medieval people who wore the armor were smaller than the modern people who today look at it. **Figure 4.8** compares the stature of youngsters in two early populations in the British Isles with the stature of recent British youngsters. The factory children studied in 1833 were far shorter than modern children at each age —16–20 cm (6–8 inches) shorter in their teenage years. A 14-year-old factory child in 1833 was about as tall as a 10- or 11-year-old today! The factory children were, in fact, dramatically shorter than aristocratic children living at the same time they lived; Francis Galton (1822–1911) commented that working-class children and aristocratic children of that time could be told apart by their heights, and the fact that the elite grew faster helped reinforce their social status. As seen in Figure 4.8, youngsters in a medieval English rural population were even a bit shorter than the nineteenth-century factory children. The rude conditions of life that existed for medieval people and nineteenth-century factory children are believed to have been responsible for their short statures.

Examples such as these emphasize that the developmental environment can exert strong effects on an individual's phenotype. The developmental program does not unfold as a strictly genetic process, and the phenotype is not simply a fixed property set by genes (as some popularizations of genetic determinism argue). The phenotype, instead, is a product of interaction between genes and environment—with the developmental environment often being particularly important.

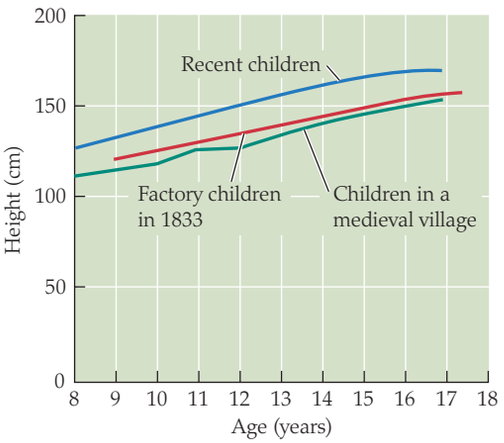


FIGURE 4.8 Height as a function of age in three groups of children in England The "Recent children" and "Factory children in 1833" were living subjects when measured. The "Children in the medieval village" (Wharram Percy) had died in childhood, and their skeletons were later excavated; height was calculated from bone lengths, and age was estimated from dental features. (After Mays 1999.)

Phenotypic plasticity is known today to occur in such a wide range of contexts that the question arises of whether a single term—"phenotypic plasticity"—is appropriate. Possibly several distinct types of phenomena are being lumped under that one rubric. Here, nonetheless, we use the global term "phenotypic plasticity" because a consensus has not yet emerged on an alternative terminology.

Environmental effects during development may arise from programmed responses to the environment or may be forced by chemical or physical necessity

Now we need to focus on a significant question that arises in all studies of phenotypic plasticity—the question of whether environmental effects develop as programmed responses or are forced on the animal. Two further examples—deliberately chosen to illustrate extremes—will set the stage.

Recent research has shown that when marine periwinkle snails *Littorina obtusata* or blue mussels (*Mytilus edulis*) grow in the presence of crabs over a period of weeks, they upregulate shell deposition and develop thicker shells than members of the same species that grow without predators being present. No contact is required between the crabs and molluscs. For example, when snails share the same water with crabs, the snails develop shells that are more than 10% thicker, even when the snails and crabs are kept separate; the snails detect that crabs are nearby by use of chemical cues in the water. To eat the flesh of a snail or mussel, a crab must first crack the mollusc's shell with its claws. A thicker shell therefore protects against crab predation.

A different effect of the environment on development is seen when studies are directed at brain function following malnutrition. Particularly rigorous studies can now be conducted on *spatial learning*—the ability to learn and remember locations—in rats and mice, by using two devices, the *Morris water maze* and *radial-arm maze* (see page 497). In the Morris water maze, a small platform is positioned beneath the water surface in a pool in which a test animal swims (rats and mice swim readily). An opaque dye is added to the water so that the animal cannot see the platform. When an animal is first introduced into the water maze, it swims at random until it finds the platform and can crawl out of the water. Later, as the procedure is repeated over and over, the animal will exhibit learning with each repetition. The rat or mouse will arrive at the platform sooner and travel less distance to find it. Many studies have shown that when recently born rats or mice are subjected to a low-protein diet during their postnatal development, they exhibit impaired performance in the water maze, even long after being returned to a fully adequate diet. Thus, deficiencies in an animal's nutritional environment during development cause an easily demonstrated, long-term deficit in capacity to learn.

A persistent question in studies of phenotypic plasticity is whether adaptive evolution in the past is responsible for the pattern of phenotypic plasticity observed. In the case of snails having their development altered by the presence or absence of predators, the pattern of phenotypic plasticity is summarized as follows: When predators are absent, a snail grows an ordinary shell, but when predators are present, a snail grows a thickened shell.[3] This pattern of plasticity could readily be advantageous in all respects, in that a snail does not expend extra energy to synthesize and carry a thickened shell when such a shell is unneeded, but it develops an augmented shell in the presence of danger. Accordingly, natural selection could well have favored the evolution of this pattern of plasticity. That is, the plasticity might well be genetically programmed because it has become inbuilt over evolutionary time by positive selection.

In contrast, in the case of rats having their brain development altered by availability of dietary protein, the pattern of plasticity is summarized as follows: When dietary protein is adequate, a rat develops a normal capacity for learning, but when dietary protein is inadequate, a rat grows up to be deficient in its ability to learn. Probably, this pattern of plasticity, instead of being a product of adaptive evolution, simply represents pathology. In a protein-poor environment, the relevant part of a rat's brain (the hippocampus) may simply be unable to develop normally, and in that case the observed learning deficiency would be *forced* on the rat by chemical or physical necessity.

In many cases of developmental phenotypic plasticity, no means presently exist to resolve the question of causation. Accordingly, different biologists sometimes take opposite stances. Recall, for example, the low growth rates and delay of menarche in human populations living under stressful environmental conditions. Most biologists tend to think that these effects are simply pathologies; according to this reasoning, poor public health and uncertain nutrition prevent normal growth and maturation. Some biologists, however, argue that when environmental conditions are harsh, growth and maturation are programmed to be slowed because there are advantages to being small-bodied when food is scarce, and there are advantages to delaying reproduction when daily life is difficult.

Insect polyphenic development underlies some of the most dramatic cases of phenotypic plasticity

Many species of insects exhibit **polyphenic development**, a phenomenon in which *genetically identical* individuals can assume two or more distinct body forms, induced by differences in the environment. The body forms of a polyphenic species can be so dramatically different that anyone but a specialist would think they represent different species. In **Figure 4.9**, for example, both butterflies shown

FIGURE 4.9 Polyphenic development In the western white butterfly (*Pontia* [*Pieris*] *occidentalis*), any one individual can express either of the phenotypes shown; the difference between phenotypes represents phenotypic plasticity and is not caused by differences in genotype. This species goes through more than one generation each year, and the phenotype changes with the season in which an individual develops—a case of seasonal polyphenism. (Courtesy of Tom Valente.)

[3] In technical terms, this sentence is a statement of the norm of reaction discussed in Box 1.2.

belong to one species, and indeed either of the individuals could have developed either of the wing patterns. Polyphenic development is a particularly striking form of phenotypic plasticity.

The species shown in Figure 4.9 exemplifies a particular type of polyphenic development. Many species, including the one shown in the figure, go through two or more generations each year, and individuals that develop in one season differ in body form from those that develop in another season—a phenomenon termed **seasonal polyphenism**. Day length and temperature during development often serve as cues that help determine which body form an individual will assume. Two hormones, juvenile hormone and ecdysone (see Table 16.5)—and the timing of their secretion relative to endogenously timed sensitive periods—are frequently implicated in the control of seasonal polyphenism.

Extensive studies have established that in the western white butterfly (*Pontia* [*Pieris*] *occidentalis*) shown in Figure 4.9—and in several other butterflies —seasonal polyphenism aids thermoregulation. In these butterflies, an individual requires a high thoracic temperature to fly; if the thorax is too cool, the flight muscles cannot develop sufficient power. The required high body temperature is achieved by complex sun-basking behaviors. Owing to seasonal polyphenism, adults have different amounts and patterns of dark pigment (melanin) in their wings during different seasons as exemplified by Figure 4.9. Careful studies have shown that seasonal timing of the different phenotypes aids thermoregulation in that (1) during cool seasons, the prevailing wing phenotypes aid absorption of solar radiation for warming (as in the spring butterfly in Figure 4.9, which has dark wings), whereas (2) during hot seasons, the prevailing wing phenotypes offer more options for avoiding solar overheating.

Probably the most stunning examples of phenotypic plasticity in the animal kingdom are provided by migratory locusts—notorious since ancient times for their capacity to descend from the sky in incomprehensible numbers. Swarms are large and fast-moving—capable of decimating vegetation over large swaths of land (**Figure 4.10**). In some cases, a swarm may comprise tens of millions of locusts per square kilometer, and it may extend at any single time over a total land area of 100 km^2 or more.

Both *Locusta migratoria* and *Schistocerca gregaria*, the species of migratory locusts that have been best studied, exhibit polyphenic development. They have two behavioral phenotypes—*solitary* and *gregarious*. These two phenotypes, besides differing in behavior, also differ strikingly in their morphology (e.g., coloration). No genetic difference exists between individuals that show the solitary and gregarious phenotypes. Instead, any individual can exhibit either phenotype. When an individual exhibits the solitary phenotype, it avoids other individuals. Solitary individuals are inconspicuous and difficult to find in their natural setting. However, when circumstances bring individuals displaying the solitary phenotype into contact with each other for a few hours, they transform to the gregarious phenotype and begin to associate avidly with other locusts. That transformation is the starting point for swarm formation. The adaptive advantage is postulated to be that the fast-moving, voracious behavior of swarms enables locusts in a swarm to collect large quantities of food. When locusts in the solitary phenotype become crowded together—signaling that numbers in their population may be outstripping their food supply—the transformation to swarming poises them to collect food at an enhanced rate.

FIGURE 4.10 **Migratory locusts in their gregarious phenotype move across the landscape in voracious, fast-moving swarms**

Recent research has pinpointed two biogenic amines (see Table 13.2) as key agents that physiologically mediate the transformation from solitary to gregarious. In *Schistocerca gregaria*, serotonin is both necessary and sufficient to induce transformation to the gregarious phenotype. In *Locusta migratoria*, dopamine is the principal agent of phenotype change, although serotonin also plays a role.

SUMMARY Phenotypic Plasticity during Development

- Phenotypic plasticity is the capacity for an individual of fixed genotype to exhibit two or more genetically controlled phenotypes. Because the phenotype expressed is often dependent on the prevailing environment, phenotypic plasticity is a process by which genotype and environment interact to determine the phenotypic characteristics of an individual.
- Phenotypic plasticity is often programmed by a genetically coded, physiological control system that determines which specific phenotypes are expressed under which specific environmental conditions. Such control systems are subject to natural selection over evolutionary time and may, therefore, in themselves represent adaptations. At the opposite extreme, changes of phenotype in different environments may simply be forced by chemical or physical necessity. A major challenge for biologists is to develop empirical ways to determine whether instances of phenotypic plasticity represent adaptations or products of chemical or physical forcing.

- Polyphenic development in insects is perhaps the most striking form of phenotypic plasticity. In a species with polyphenic development, an individual with a fixed genotype can express two or more highly distinct phenotypes. Solitary and gregarious forms of migratory locusts provide an example.

Epigenetics

Epigenetics refers to modifications of gene expression—*with no change in DNA sequence*—that are transmitted when genes replicate.[4] Although we usually think of *genes* being transmitted during gene replication, *gene expression* differences are transmitted when epigenetic effects are at work. Some epigenetic modifications of gene expression are environmentally induced. Some, having been environmentally induced in embryonic cells during the early development of an animal, are passed to all the cells in the adult animal that arise, during development, from the cell lineages initially affected. Some are passed, many researchers argue, from parents to offspring—meaning that epigenetics provides a mechanism whereby environmental effects can be transmitted from one generation to the next. The existence of this stunning phenomenon discredits the old dogma that, in the transmission of hereditary material, the genes are isolated from environmental influences. Interest in epigenetics has soared in the past 15 years, as evidenced by a 10-fold increase in the number of pertinent scientific papers published each year. Because most of the flurry of interest is focused on human disease processes and effects of toxic chemicals,[5] the degree to which epigenetics is a factor in normal animal physiology is only starting to be clarified. A great deal of attention from physiologists can be expected in the near future.

Epigenetics provides an entirely new dimension in which genes and environment interact. To see this, let's first reflect again on programmed phenotypic plasticity, which—as we have just seen—is another process that mediates interaction between genes and environment in the determination of phenotype. In cases of programmed phenotypic plasticity, although genes control the program, the genes themselves are not modified in any way. For example, consider the case of snails and crabs discussed earlier. Snails develop an ordinary shell when crabs are not present in their environment, but they develop a thickened shell when crabs are present in their environment. A genetic program orchestrates the control sequence by which snails use sensory information from their environment to modulate shell formation. However, the genes responsible for this program—speaking loosely—*use* the environmental information *without being modified* by it.

By contrast, in some forms of epigenetic modification, the expression of genes is semi-permanently altered by interaction with the environment. An affected gene is said to be **marked** in such a way that its expression is modified, and when the gene replicates, its marking also replicates so that the resulting gene copies also have their expression modified in the same way. The environmental modification of gene expression is thereby passed along as gene replication takes place. All the while, the DNA sequence of the gene remains unaltered. Phenotype is modified by the sort of gene–environment interaction seen in epigenetics, but it is modified in a different way than in programmed phenotypic plasticity. In the case of epigenetics, phenotype is altered because genes are directly modified in a transmissible way by marking, and the marking causes their expression to be different that it was before the environmental effect.

Phenotypes arising from epigenetic modification (similarly to ones arising from phenotypic plasticity) may, in principle, be adaptive for the organism or not. If a particular epigenetic modification has been subject to natural selection over evolutionary time, it may prove to be a product of positive selection and advantageous. However, when environmental agents chemically force epigenetic marking (as is known to occur), the epigenetic modifications may have negative effects. Adaptive effects cannot be assumed.

Two major mechanisms of epigenetic marking are DNA methylation and covalent modification of histones

One of the most widely observed and best-understood mechanisms of epigenetic marking is DNA methylation: the attachment of methyl ($-CH_3$) groups by covalent bonds to cytosine residues in the structure of DNA. In the most common version of this process, methylation is at a promoter region of DNA, and when it occurs there, the gene regulated by the promoter is repressed or silenced. When a marked cell replicates, an enzyme (usually the DNA methyltransferase DNMT1) acts to *perpetuate* the mark by methylating the same cytosine residues in each daughter cell (Figure 4.11), meaning that

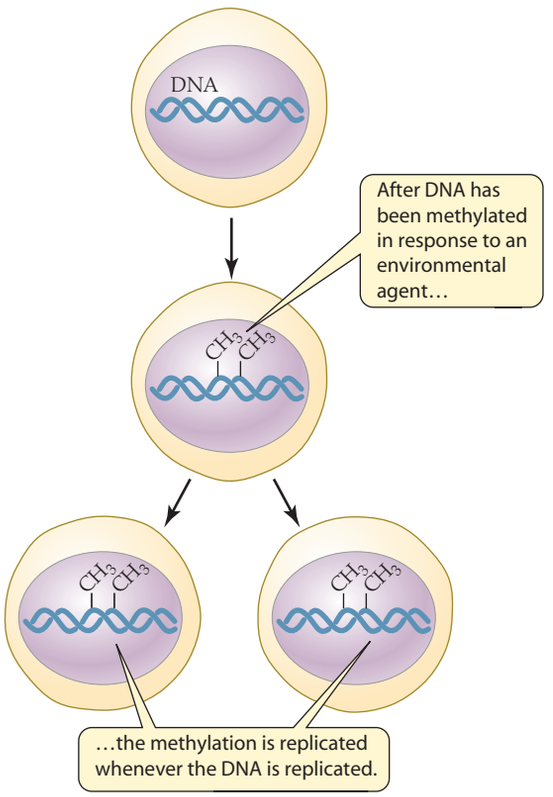


FIGURE 4.11 Methylation of DNA, a type of epigenetic mark, is perpetuated during DNA replication In this way, the gene expression modification caused by methylation is transmitted to daughter cells.

[4] In the research literature, other definitions can be found, but this is the definition around which the greatest consensus now exists.

[5] Strong evidence for transmission of epigenetic effects across generations, from parents to offspring, comes mostly from studies of the effects of toxic agents—putting these effects outside the scope of this text.

the affected gene is repressed or silenced in the daughter cells. The end result is that a modification of gene expression in the initial cell is transmitted to the daughter cells during cell replication, while the DNA sequence remains unchanged.

Another important mechanism of epigenetic marking is covalent modification of the histones (basic proteins) around which DNA is wrapped in the nucleosomes of the chromatin of a cell. Modification can be by methylation, acetylation, phosphorylation, or other binding of modulators, and it usually occurs on the NH_2-terminal tails of the histone molecules. Distinctive histone variants, resulting from covalent modification, act as epigenetic marks.

Additional mechanisms of epigenetic marking also exist. Small RNA molecules play roles, for example.

Epigenetic marking during an animal's early development affects the animal's lifelong phenotype

Epigenetic effects are often instrumental in ordinary tissue differentiation. During early development, the developmental program places epigenetic marks on genes in various cell lineages, this being one of the mechanisms whereby different patterns of gene expression are established in different tissue types. The divergent patterns of gene expression help give cells their divergent tissue-specific phenotypes. When a marked cell divides, its epigenetic marks are passed to each daughter cell (see Figure 4.11), serving (along with other mechanisms) to perpetuate the cell's tissue-specific phenotype.

A fascinating recent study has illustrated how an *environmental* epigenetic effect can be added to the sort of ordinary epigenetic developmental effects just discussed. During the winter of 1944–1945, a food embargo was imposed on part of The Netherlands as one of the final spasms of World War II. The citizens were exposed to severe famine in what has come to be called the Dutch Hunger Winter. Studies of pregnant laboratory rats subjected to malnutrition throughout conception and pregnancy have shown that certain genes in their offspring have fewer methylation marks than in ordinary rats. To see if this same phenomenon occurs in humans, researchers tracked down 60 people, all now over 60 years old, who were conceived during the Dutch famine and compared them with same-sex control siblings who were not conceived during the famine. DNA was extracted from the whole blood of each subject and analyzed for methylation marks associated with cytosine residues in a gene of particular relevance. As **Figure 4.12** shows, there is a statistically strong tendency for the individuals who were conceived during the famine to exhibit a lower percentage of marked DNA sites than their sibling controls. As noted already, methylation marks are generally associated with reduced gene expression. Accordingly, reason exists to expect that the reductions of methylation marks induced by famine are allowing greater expression of some genes.

A stunning consideration to recognize in this study of Dutch people conceived in famine is the timescale. The original environmental effect was exerted on their cells when they were embryos. That effect is now evident more than 60 years later in their current blood cells, probably having been transmitted through a great many cell divisions in the cells that multiply to produce blood cells.

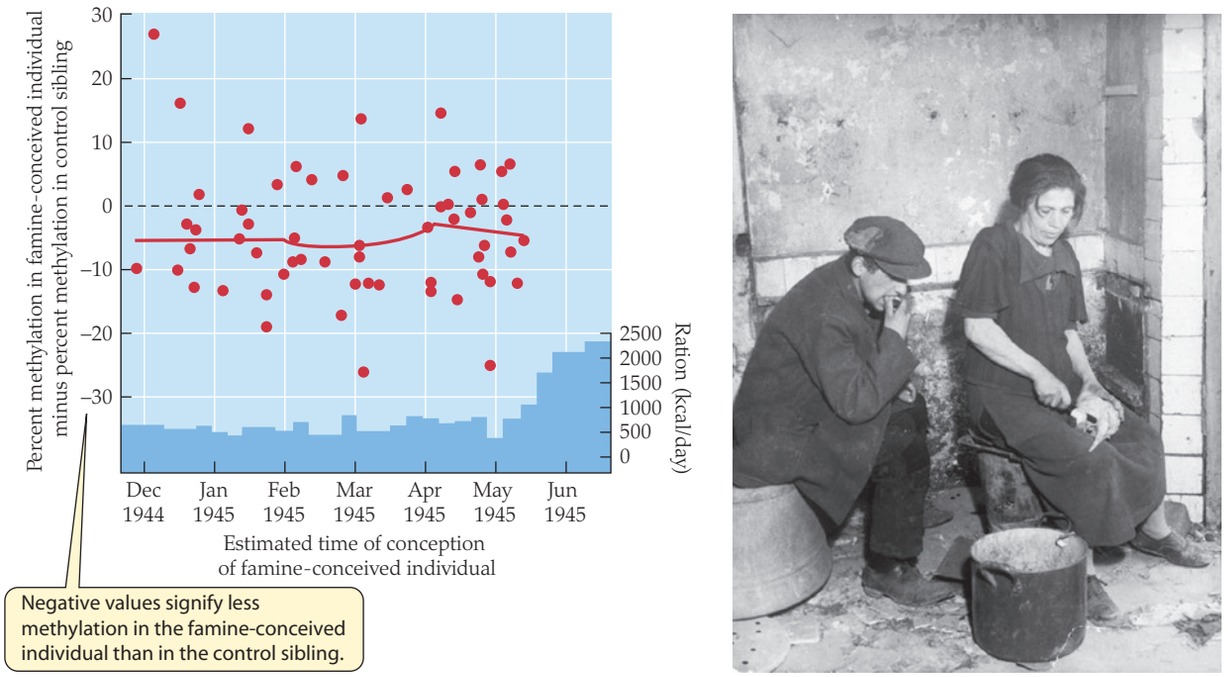


FIGURE 4.12 Conception during famine exerts an epigenetic effect Each of the 60 red symbols on the plot represents a person conceived in famine during the Dutch Hunger Winter of 1944–1945. Along the *x* axis, the symbol is plotted at the time of conception. The scale on the *y* axis shows the difference in percent methylation of sites in a focal gene between the famine-conceived individual and a same-sex sibling not conceived in famine. The red line, depicting the average response, is below zero (0) to a statistically significant extent ($P < 0.00002$), indicating that famine-conceived individuals tend to exhibit a reduced extent of DNA methylation. The daily ration of food per person was tightly regulated and recorded, and is shown at the bottom; note that during the famine (December 1944 to May 1945), the daily ration per person was far below 2000–2500 kcal, the range of values considered ordinary. The two people in the photograph were victims of the food embargo in the winter of 1944–1945. (After Heijmans et al. 2008.)

An additional important consideration is the association that researchers have discovered between (1) early famine or malnutrition and (2) later cardiovascular and metabolic disease. This association is one of the best-documented examples of epigenetic control of individual phenotype. The association has been extensively investigated by means of epidemiological studies of humans and experimental studies of other mammals, most notably lab rats. Famine or malnutrition during pregnancy—especially early pregnancy—predisposes offspring to a variety of disease states that often develop relatively late in life, including cardiovascular diseases and metabolic diseases (e.g., diabetes and obesity). Although the mechanism of this association is not fully understood, strong evidence indicates that epigenetic marks play a role. Possibly, therefore, the alteration of marks observed in the 60-plus-year-old Dutch people is not merely of abstract, academic interest. The research on those people may have directly revealed epigenetic changes—wrought when they were embryos—that now, late in their lives, affect their odds of developing coronary artery disease, hypertension, and other ailments. In fact, separate studies have shown that these people show an unusually high prevalence of obesity and coronary artery disease.

Effects of maternal care represent another of the best-documented examples of epigenetic control of individual phenotype. After genetically homogeneous female lab rats have given birth, they exhibit two patterns of maternal care during their offsprings' first week of life. One pattern—termed *low-LG*—is characterized by low amounts of licking and grooming of the young; the other—*high-LG*—is characterized by high amounts of licking and grooming. Although the young of these two types of mothers are homogeneous in their DNA sequences, they develop into adults that display multiple differences. In adulthood, the high-LG offspring are less behaviorally fearful and show lesser endocrine responses to stress than the low-LG offspring. Direct chemical measurements on genes in hippocampal tissue from the brain show that the high-LG offspring differ significantly in epigenetic marking from the low-LG offspring. The results of extensive experiments show that the differences in maternal behavior between the two types of mothers epigenetically program their offspring to differ in fearfulness and endocrine responsiveness to stress in adulthood!

One of the most striking of all epigenetic effects in the lives of individual animals, an effect just discovered, is the control of caste in honeybees. Worker honeybees and queen honeybees develop from the same genotype. They have radically different phenotypes, however—a case of polyphenic development (discussed earlier). Workers, for example, do not produce offspring, whereas queens produce thousands. A remarkable recent experiment has shown that if DNA methylation—a type of epigenetic marking—is blocked, an individual originally destined to develop into a worker develops, to a great extent, into a queen. This evidence—bolstered by additional observations—points to gene-expression differences, arising from differences in epigenetic marking, as being the cause of caste differentiation. More than 20% of genes in the brains of developing worker and queen bees are expressed differently, depending on epigenetic marks. The differences in epigenetic marking (DNA methylation pattern) between the two castes arise, during early development, based on whether or not an individual is fed extensively with *royal jelly*. The royal jelly, which is fed principally to individuals that are developing into queens, is a complex chemical substance made by glands in the mouths of young nurse bees.

There is a dramatic common denominator to all the effects discussed in this section. Namely, the origin of adult differences is in early development—mediated by epigenetic gene-expression modification.

Epigenetic marks on paternal and maternal copies of genes set the stage in mammals and insects for the two copies to exert nonequivalent effects

An embryo receives one copy of each gene from its father and a second from its mother. Incidental, exceptional examples have long been known in which the two copies—alleles—are unequally expressed. Recent research, however, has brought nonequivalent expression to the fore by demonstrating that a phenomenon termed **genomic imprinting** occurs in some types of organisms. In genomic imprinting, the allele inherited from the father may be expressed exclusively (or predominantly), or the allele from the mother may be expressed exclusively (or predominantly)—because of epigenetic marks. At present, genomic imprinting is known to occur only in mammals, insects, and flowering plants. In mammals, the number of genes subject to imprinting is not particularly great, being roughly in the range of 100 to 1000 (different species are not entirely identical in which genes these are). Although genomic imprinting thus has a restricted distribution, it is nonetheless emerging as a phenomenon likely to be of great importance in certain contexts. Reason exists to believe that genomic imprinting evolved in mammals in association with the evolution of the placenta, although placental functions for imprinting are not yet well known.

An essential feature of genomic imprinting is that *the transcription mechanism recognizes epigenetic imprinting marks during the transcription process*. Suppose, for example, that in the cells of an animal, the allele of a particular gene inherited from the animal's father is marked. The transcription mechanism recognizes this. Accordingly, transcription of the paternal allele may be regulated differently than that of the maternal allele. For example, only the paternal allele might be transcribed, or only the maternal allele might be. In general, marking is by DNA methylation. The usual assumption is that marks are added in the germ line (cells that give rise to sperm and eggs) in the parental generation. Then, after zygote formation—and during growth of an offspring—the marks are maintained and are present in the cells of the offspring, permitting the transcription mechanism to recognize which allele is paternal and which is maternal.

Although most research on genomic imprinting in animals has focused on human diseases, recent papers have suggested that imprinting may play a major role in normal brain development and function. By studying embryonic and adult lab mice using cutting-edge transcriptomic methods, researchers have established that expression of imprinted genes is far greater in some parts of the brain than in others. Moreover, a bias exists for preferential expression of *maternal* alleles during embryonic development, whereas in adults, a bias exists for expression of *paternal* alleles. And there is an effect of offspring gender: Females exhibit three times more expression of imprinting effects from their parents in certain critical brain regions than males. Surveying all these known effects, we can see that imprinting—differential expression of the alleles from mother and father—is implicated in region-

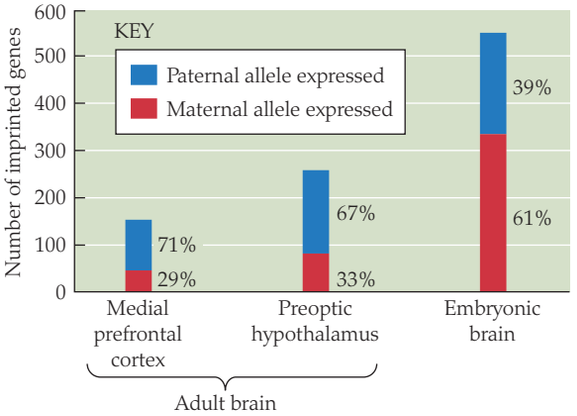


FIGURE 4.13 In the brain of the laboratory mouse, genomic imprinting exhibits differences from region to region and from one stage of development to another In the adult brain, the number of genes showing imprinting differs to a statistically highly significant extent from one region of the brain to another (e.g., medial prefrontal cortex versus preoptic hypothalamus). Comparing the adult and embryonic brain, in genes where genomic imprinting is evident, the paternal allele is expressed in well over half the cases in the adult brain (67–71%), whereas the maternal allele is expressed in over half the cases in the embryonic brain (61%). This difference is also highly significant statistically. (After Gregg 2010.)

specific, time-of-development-specific, and gender-specific brain function (Figure 4.13). Imprinting therefore has the potential to be richly involved in the processes by which the brain develops and functions, although only further research will resolve how much this potential is actually realized.

SUMMARY Epigenetics

- Epigenetics refers to changes in gene expression that are transmitted during gene replication despite the lack of any alteration in the DNA sequence. The mechanism of epigenetic control is that genes are marked in ways (e.g., DNA methylation or histone modification) that modify their expression and the marks are replicated when the genes are replicated.
- Epigenetic marking is often initiated by environmental conditions because of the action of programmed control systems or of chemical or physical forcing. Accordingly, epigenetic control is a specific mechanism by which genotype and environment interact to determine phenotype.
- Because epigenetic marks are replicated from cell to cell as cells divide in an individual, epigenetic marks induced by the environment early in development can be perpetuated throughout life and affect the adult phenotype.
- Genomic imprinting, presently known only in mammals and insects among animals, is a particular case of epigenetic control in which maternal or paternal alleles in an individual are marked and therefore can be differentially transcribed, resulting in nonequivalent expression in determining the individual's phenotype.

Study Questions

1. You are asked to give a 20-minute talk to high-school students on why nutrition matters in a child's first years of life. Your assignment is to present a biologist's perspective. Write your talk.
2. Genotype and environment interact to produce the phenotype of an animal. Explain comprehensively the types of mechanisms by which this interaction takes place.
3. Rigorously define phenotypic plasticity, polyphenic development, and epigenetics. Outline how they relate to each other.
4. Explain why the physiological properties of individual animals *at each stage of their development* are crucially relevant for their ecological and evolutionary success.
5. As seen in Figure 24.20, freshwater water fleas (*Daphnia*) develop without hemoglobin (Hb) and are whitish when living in well-aerated water but produce abundant Hb and are red in O_2-depleted water. Explain how each of these phenotypes could be advantageous (relative to the other) in the environment where it is expressed.
6. Regarding the water fleas in Question 5, describe experiments you could carry out to test empirically whether (1) the low-Hb phenotype is more advantageous than the high-Hb one in well-aerated water and (2) the high-Hb phenotype is superior to the low-Hb one in O_2-poor water.
7. After studying Box 1.2, state in your own words the meaning of norm of reaction. Give an example.
8. Write a short explanatory essay on the following statement: "In cases of genetically programmed phenotypic plasticity, natural selection acts on the norm of reaction. Thus the norm of reaction as a whole—rather than any one phenotype—determines if selection is positive or negative."
9. In northern latitudes, a variety of birds and mammals create storage depots of food, termed *caches*, in the autumn and later can find the caches in winter to obtain food. What experiments could be done to determine if the ability to find caches is fully innate at birth or must undergo postnatal development?
10. Some molecules found in foods are known to be capable of driving DNA methylation or DNA demethylation. For example, folic acid (a vitamin), vitamin B_{12}, and choline are thought to increase methylation. Based on this consideration, why would it be important to avoid eating *excessive* quantities of such molecules (while being certain to eat sufficient quantities for health)?
11. In principle, what consequences might be expected to arise from an environmentally induced shift in the age of reproductive maturation? Think as broadly as possible. Answer the question for the human populations, discussed in this chapter, in which menarche shifted to occur more than 3 years earlier between the mid-nineteenth and late twentieth centuries. Also answer for a nonhuman mammal such as a species of squirrel or antelope.

Go to **sites.sinauer.com/animalphys3e** for box extensions, quizzes, flashcards, and other resources.

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See also **Additional References** *and Figure and Table Citations.*