Ecological Developmental Biology

The Environmental Regulation of Development, Health, and Evolution

Second Edition

Scott F. Gilbert • David Epel

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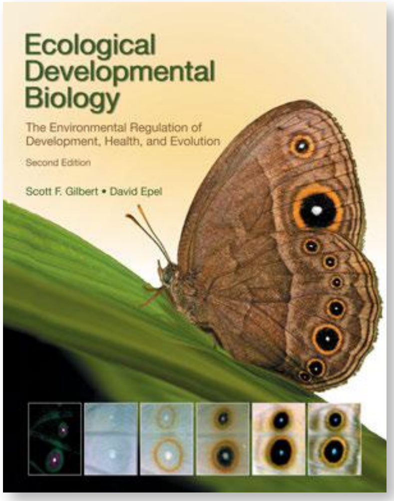

The Cover

Bicyclus anynana, a butterfly native to Malawi, has two forms, each determined by temperature. Warm weather (front cover) induces brightly colored eyespots that provide the flying butterfly with the ability to deflect predators. During cooler weather (back cover), the butterfly remains on the ground. The butterfly does not form such obvious eyespots, and it can be protected by blending into the brown-colored leaf litter. The hormone ecdysone is made during the warm weather, and it stabilizes the transcription of the *Distal-less* gene (left-most panel), which organizes each eyespot. The concentric circles of pigmented scales then develop onto the nascent wing according to the factors organized by *Distal-less*. Photographs courtesy of Patricia Beldade, Sean Carroll, Steve Paddock, Leila T. Shirai, Steven Woodhall, and Oskar Brattström.

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To Anne and Talia
and
To Lois

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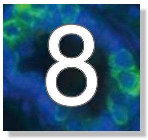

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Preface

A quiet biological revolution, driven by new technologies in molecular, cell, and developmental biology and ecology has made the biology of the twenty-first century a different science than that of the twentieth.

This revolution was not the one expected. Rather than confirm and deepen what we already knew, these new technologies uncovered new layers of inheritance, development, and evolution. They have given us a new humility. There is plenty we don't know, and many of our assumptions about the mechanisms of development, inheritance, physiology, disease, and evolution have to be questioned.

These unexpected challenges have given rise to Ecological Developmental Biology, the science seeking to understand how environments interact with developing organisms to produce new phenotypes, and how these interactions affect disease and evolution. It is a science that may be transforming our thinking about life as profoundly as evolution, the cell theory, or the gene theory.

Some unexpected ideas must be integrated into our new thinking about inheritance, development, evolution, and health. These include:

- **Symbiosis**. Once thought of as the exception to the rules of life, symbiosis is now recognized as a signature of life, including its development and evolution. We function, develop, and evolve as consortia.
- **Developmental plasticity.** Also thought of as an exception to the rules of life, developmental plasticity is also ubiquitous. A single genome can generate numerous phenotypes, depending on environmental conditions.
- **Epialleles, environmentally induced modifications of the genome.** Formerly considered impossible, such environmentally modified chromatin not only exists but can be inherited for many generations.

These new discoveries alter the way we think about the world:

- **Evolutionary biology** must more fully integrate genetics with epialleles, symbiosis, and plasticity to generate a new evolutionary framework for the origins and maintenance of biodiversity.
- **Disease susceptibilities**, especially to diseases such as cancer, diabetes, asthma, autism, and obesity, may be caused by environmental toxins, mismatches in developmental plasticity,

particular combinations of symbionts, or epialleles, Our understanding of disease has to change.

- **Global climate change** and **endocrine disruptors** are affecting how organisms develop and how they behave. These often concern changes in symbionts, the limits of plasticity, and the generation of epialleles.

Ecological Developmental Biology was published almost six years ago, and the above challenges and new ideas form an important part of our revision. The resulting book is organized into four parts. The first part concerns *the ways by which the developing organism interacts with its environment during normal development*. It focuses on the three newly appreciated mechanisms of development that were mentioned above: developmental plasticity, inherited epigenetic modification, and developmental symbioses. Evidence will be given that these three phenomena are crucial to understanding the generation of phenotypes. In each case, the environment is not a merely permissive agent, but one that helps instruct development.

The second part of the book examines *how the environment can cause development to go awry*. First, it looks at the physiological functions and strategies that have evolved to protect the embryo before the developing organism has its adult defense systems. It also details how climate change can circumvent some of those strategies. The next chapters looks at those chemicals—teratogens and endocrine disruptors—that can disrupt normal development, and the final two chapters of this section look at the ways that developmental information coming from the environment can predispose us to develop diseases later in life..

The third part of the book presents *the evidence for a new evolutionary synthesis*. Sometimes called "the expanded synthesis," "eco-evo-devo," or "the developmental synthesis," this synthesis seeks to bring into evolutionary biology the rules by which an organism's genes, environment, and development interact to create the variation and selective pressures needed for evolution. It starts with traditional evolutionary biology, proceeds through evolutionary developmental biology, and then builds on this foundation with ecological evolutionary developmental biology.

A philosophical coda and a series of appendices that go more deeply into the historical, philosophical, and scientific matters discussed in the body of the text follow the final chapter. This book has not hesitated to discuss public policy issues. Indeed, it would be a caricature of science to discuss modern science as if it were done without an eye on funding questions, government regulations, economic considerations, and even ideological issues.

Given that we live in a world characterized by the accelerating reduction of species, the sudden increase in non-infectious diseases, and the breakdown of ecological communities, ecological developmental biology is a critically important science. When we sent the chapters out for review, we found that we were not alone in this feeling. One reviewer, calling eco-devo "the most important field of science at the moment," noted that:

> Ecology isn't prepared to analyze at the molecular level the ills of our present world, genetics doesn't contain the background in tissue

> interactions, and developmental biology has the tools but is only just now turning its attention to an environment outside of cells and the individual organism. Eco-devo is the synthesis that combines all of the above, and we and our students desperately need to have a basic understanding of this new field to become proper stewards of our planet.

Ecological developmental biology integrates molecular biology, ecology, developmental biology, evolutionary biology, physiology, cell biology, and genetics into a syncytial science that is at the core of twenty-first century concerns.

This book is intended both for students and for our scientific colleagues. While it would help students to have had courses in developmental biology, cell biology, ecology, or evolution, a good first-year biology course should be adequate. This book is also for specialists who would like to learn something about how their particular subdiscipline might interact with other biological sciences.

We hope that the examples presented here will reinforce the sense of wonder that biologists find in the world, and at the same time be a jumping-off point for discussions about both the integration of different areas of biology as well as the increasingly critical question of biology's relation to public policy. While we have tried to be integrative, we realize that we are still bound by our past history and training. So we hope that college students, still relatively undifferentiated, will come up with their own connections and syntheses and that they will see patterns that we haven't yet imagined. We are extremely glad that this book has been used in senior and graduate seminars to unite students of different backgrounds. Indeed, we have to thank the students in Jeannette Wyneken's seminar at Florida Atlantic University for giving us a running commentary as they went through the book, augmenting and critiquing the text as they discussed its contents.

Finally, we hope the ideas in this book evoke a way of approaching nature, an approach exemplified in the banner that hangs over the library of the Woods Hole Marine Biology Laboratory, reminding us that we should "Study Nature, Not Books." One is constantly surprised by the wonderful improvisation of development. The photograph, sent by Dr. Bill Bates, a friend of both authors, shows a clutch of toad eggs developing in a small pond in north India. Only, the "pond" is rainwater collected in the footprint of an elephant. Who would have thought that elephants might be necessary for the completion of a toad's life cycle? As Dr. Ian Malcolm says in Jurassic Park, "I'm simply saying that life finds a way."

Toad eggs developing in the footprint of an Indian elephant. (Courtesy of W. Bates.)

Acknowledgments

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Part 1

Environmental Signals and Normal Development

Ecological developmental biology studies the interactions between developing organisms and their environments. Part 1 identifies three major ways through which these interactions occur. **Chapter 1** details the phenomenon of *developmental plasticity*, documenting that the genome is a repertoire for the production of numerous different phenotypes. Various environmental agents—such as temperature and diet—elicit a particular phenotype from the possible range. **Chapter 2** details the mechanisms by which the environment might elicit those particular phenotypes. This chapter thus introduces us to the concept of the *environmentally induced epiallele*, inherited differences in chromatin structure rather than in DNA sequence, which can produce phenotypic differences. **Chapter 3** demonstrates that *symbiotic organisms*—usually commensal bacteria—are important sources of chemical signals that enable normal development. Thus, symbionts, epialleles, and developmental plasticity allow the environment to help construct the phenotypes of organisms.

Developmental Plasticity

The Environment as a Normal Agent in Producing Phenotypes

My soul is wrought to sing of forms transformed to bodies new and strange.

Ovid, 1 CE

A single genotype can produce many phenotypes, depending on many contingencies encountered during development. That is, phenotype is an outcome of a complex series of developmental processes that are influenced by environmental factors as well as genes.

H. F. Nijhout, 1999

Imagine a young aquatic organism developing in a particular pond. This organism has the ability to sense soluble biochemicals in the water—chemicals given off in the saliva or urine of its major predator. In the presence of these chemical signals, the organism's pattern of development changes, resulting in a phenotype that is less likely to be eaten by its predator. For instance, in the presence of the dragonfly larvae that feed on them, tadpoles of the gray tree frogs *Hyla chrysoscelis* and *H. versicolor* develop bright-red tails that deflect the predators' attention, and a set of trunk muscles that enables them to make "ice hockey turns" to escape being eaten (McCollum and Van Buskirk 1996; Relyea 2003a; Figure 1.1A).

Imagine an organism that develops different phenotypes depending on the season. *Nemoria arizonaria* larvae hatching on oak trees in the spring have a form that blends remarkably with young oak flowers ("catkins"). But caterpillars that hatch in the summer would be very conspicuous if they looked like the long-fallen oak flowers; thus the summer caterpillars resemble newly formed twigs (Figure 1.1B). Here, it is the larva's diet that determines its phenotype. Larvae who feed on young oak leaves will look like the catkins, while larvae eating older leaves (which have a different chemical composition) will develop to resemble twigs (Greene 1989).

Figure 1.1 Environmental cues can result in the development of completely different phenotypes in individuals of the same species. (A) Tadpoles of the tree frog *Hyla chrysoscelis* developing in the presence of cues from a predator's larvae (left) develop strong trunk muscles, and a red "warning" coloration. When predator cues are absent (right), the tadpoles grow longer and sleeker. (B) *Nemoria arizonaria* caterpillars that hatch in the spring (left) eat young oak leaves and develop a cuticle that resembles the oak's flowers (catkins). Caterpillars that hatch in the summer (right), after the catkins are gone, eat mature oak leaves and develop a cuticle that resembles young twigs. (C) A single male blue-headed wrasse (*Thalassoma bifasciatum*) swims with a cohort of the less colorful females. Should the male die, one of the females will grow testes, changing phenotype completely to become a male. (A courtesy of T. Johnson/USGS; B courtesy of E. Greene.)

Next, imagine an organism whose sex is determined not by its chromosomes, but by the environment the embryo experiences during a particular time during its development. In many species of fish, turtles, and alligators, sex is determined by the temperature of incubation. The same egg developing at one temperature will be male, but at another temperature it will be female. The blue-headed wrasse (*Thalassoma bifasciatum*), a Caribbean reef fish, is one of several fish species whose sex depends on the other fish it encounters (Figure 1.1C). When an immature wrasse reaches a reef where a single male lives and defends a territory with many females, the newcomer develops into a female. If the same immature wrasse had reached a reef that was undefended by a male, it would have developed into a male (Warner 1984). If the territorial male dies, one of the females (usually the largest) becomes a male; within a day, its ovaries shrink and testes grow (Godwin et al. 2000, 2003).

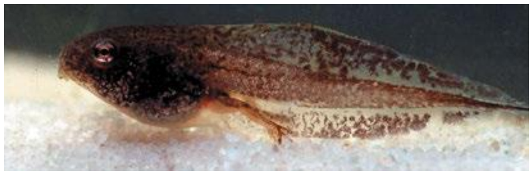
Consider now an organism with a set of cells that can recognize and attack invading viruses and bacterial cells. It has billions of such immune cells, each of which will divide and produce antibodies only when it binds to a particular virus or bacterium. While its genetic repertoire allows this organism to form billions of different types of immune cells, the actual number of different antibody-producing cell types in a given individual is only a fraction of this potential and will depend on which bacteria and viruses infect that individual. This same organism has the ability to regulate its muscular phenotype such that continued physical stress on a particular muscle will cause that muscle to grow. Furthermore, the brain development of this organism can be altered by experience, making learning possible. Moreover, parts of its digestive system develop in response to the many different bacteria residing symbiotically in its gut. This species, in which so much of the phenotype is due to environmental circumstances, is *Homo sapiens*.

Plasticity Is a Normal Part of Development

In each of the above instances, the environment has profound effects on the animal's phenotype. In other words, everything one needs for phenotype production is *not* packaged in the fertilized egg. This ability of a single individual to develop into more than one phenotype has been called **phenotypic plasticity** (Nilsson-Ehle 1914). Phenotypic plasticity was well known to late nineteenth-century embryologists, who showed that different environmental conditions produced different phenotypes during normal

(A) *Hyla chrysoscelis*

Predator present



Predator absent

(B) *Nemoria arizonaria*

Spring morph among catkins

Summer morph on twig

(C) *Thalassoma bifasciatum*

development (Nyhart 1995). Today we define phenotypic plasticity as the ability of an organism to react to an environmental input with a change in form, state, movement, behavior, or rate of activity (West-Eberhard 2003; Duckworth 2009). This plasticity is the property of the trait, not the individual; indeed, most individuals have several plastic traits. When seen in embryonic or larval stages of animals or plants, phenotypic plasticity is often referred to as **developmental plasticity**.

A century of studies

In his 1894 volume *The Biological Problem of Today: Preformation or Epigenesis?*, Oscar Hertwig summarized the studies demonstrating that development involved not only the interactions between embryonic cells, but also important interactions between developing organisms and their environments. He cited numerous cases of developmental plasticity, especially instances in which the sex of an organism was determined by the environment. These included the well-known case of *Bonellia viridis* (see the box), as well as temperature-dependent sex determination in rotifers, nutrition-dependent production of workers and queens in ant colonies (see Figure 1.8B), and temperature-dependent pigmentation patterns of butterfly wings (see Figure 1.5). Hertwig wrote (1894, p. 122), "These seem to me to show how very different final results may grow from identical rudiments, if these, in their early stages of development, be subjected to different external influences."

In 1909, two publications brought the concept of phenotypic plasticity to the awareness of many biologists. The Danish biologist Wilhelm Johannsen's *Elemente der Exakten Erblichkeitslehre* made clear the distinction between the genotype and phenotype (Figure 1.2). Rejecting August Weismann's 1893 proposal that all the causes of an embryo's development were compressed into the nucleus of the egg, Johannsen stated specifically that phenotype (what the organism looks like and how it behaves) is not merely the expression or actualization of the genotype (the set of inherited genes), but rather depends on the interactions of inherited genes with components of the environment. Like Hertwig, Johannsen felt that early development was genetically controlled but that the environment could effect changes in the later developmental stages (Moss 2003; Roll-Hansen 2007). Johannsen

Figure 1.2 One hundred years ago, Wilhelm Johannsen noted that the phenotype is the product of both the genome and environmental circumstances. Here he writes on the board that *Anlaegspraeg* ("genotype") + *Kaar* (Danish for "conditions" or "circumstances") gives *Fremtonnigspraeg* ("phenotype"). (Photograph from a movie of Professor Johannsen at www.wjc.ku.dk/library/video/original.avi.)

also believed that Weismann's refutation of the inheritance of acquired characteristics had not been complete.

Another important paper published in 1909 was from the German biologist Richard Woltereck, who reported that genetically identical lines of *Daphnia* (a water flea that reproduces asexually) could produce different phenotypes during different times of the year (see Figure 1.16). Woltereck argued that what actually was inherited was the *potential* to generate an almost infinite number of small variations in phenotype in response to environmental cues. He called this potential the **Reaktionsnorm** (**reaction norm** or **norm of reaction**).* Moreover, different pure lines (genotypes) responded differently to seasonal cues. This suggested to Woltereck that the reaction norm was heritable and that, as with any other trait, natural populations would harbor genetic variation in this potential.

However, after the 1920s and the rise of genetics, plasticity dropped out of the study of animal development. In the laboratory, we usually study development of only a few organisms and over a very narrow set of conditions. *Drosophila* are bred at 18°C, chick embryos at 37°C. Nutrition is similarly controlled. Indeed, as Bolker (1995, 2014) has pointed out, our "model organisms" for studying development have been selected for traits (early separation of germline and somatic lines; rapid generation) that would suppress the environmental contributions to the study of development. Since most of the contemporary developmental biology has focused on the genetic causation of cell differentiation and morphogenesis, the environmental effects have been seen as "noise." As evolution and developmental biology became sciences of gene frequency and gene expression, respectively, nongenetic sources of variation became marginalized and stopped being taught or discussed. So for most of the past half century, developmental biologists have not studied plasticity.

A contextually integrated view of life

The view expressed in the preceding examples is that the environment is not merely a filter that selects existing variations. Rather, it is a *source of variation*. The environment contains signals that can enable a developing organism to produce a phenotype that will increase its fitness in that particular environment. This isn't the view of life usually presented in today's textbooks or popular presentations of biology.

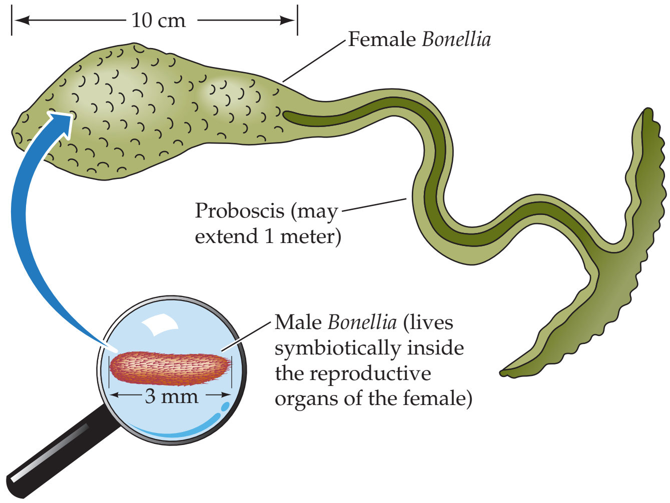
Since World War II, the dominant paradigm for explaining biodiversity has been genetics. Indeed, there has been marked antipathy against the notion of phenotypic plasticity and the inheritance of nonallelic phenotypic variation (Sarkar 2006; see Appendices A and C). The architects of twentieth-century biology (including such remarkable people as Ernst Mayr, Eric Davidson, and Jacques Monod) have emphasized the gene as

*Sarkar (1999) has pointed out that Woltereck actually concluded the *Reaktionsnorm* is what is inherited and that hereditary change consists of an alteration of the *Reaktionsnorm*. Woltereck identified the *Reaktionsnorm* with the genotype: "*Der 'Genotypus' ... eines Quantitativmerkmals ist die vereberte Reaktionsnorm*" (Woltereck 1909, p. 136). Johannsen agreed, saying that Woltereck's *Reaktionsnorm* was "nearly synonymous" with his own conception of genotype (Johannsen 1911, p. 133). As we will see later in this chapter, the ability of enzymes to have different properties at different temperatures ensures that all organisms have plasticity during their development.

Bonellia viridis: When the Environment Determines Sex

Some early studies of the effect of the environment on development included the fascinating case of sex determination in echiuroid worms, specifically *Bonellia viridis* (the green spoonworm). Females of this marine species have a deep-green, round body that burrows into rock crevasses and gravel on the seafloor, and from which is extended a projection (proboscis) that can grow up to a meter long. Males of *B. viridis* have an amazingly different phenotype; indeed, the males are rarely seen, being colorless, only about 3 mm long, and living parasitically inside the female's genital sac, where their sole function is to produce sperm to fertilize the female's eggs.

It has been known since the nineteenth century that the sex of a *B. viridis* individual depends solely on the environment in which the larva develops (Baltzer 1914; Leutert 1974; Jaccarini et al. 1983). Fertilized *B. viridis* eggs are expelled into the seawater. Larvae that settle and develop on the seafloor become female, maturing over several years as the proboscis extends. The female's cells, especially those of its proboscis, generate a powerful attractant to *B. viridis* larvae. Larvae passing within range of these signals will land on the proboscis of the sessile female and then crawl up into her mantle and/or be sucked into her gut, where they develop into the miniscule males.



Sexual dimorphism in *Bonellia viridis*. While the body of the adult female remains buried in rocks or ocean sediments, her proboscis extends widely across the seafloor, where it is used for feeding. The proboscis also produces chemical signals that attract other *B. viridis* larvae, which, upon landing on the female, develop into males.

This mode of environmental sex determination enables the optimum use of space by females and the prevention of further competition for the limited burrowing regions (Beree et al. 2005).

being the core of animal identities and the "master molecule" of life. James Watson (1989) claimed, "We used to think our fate was in the stars. Now we know in large measure, our fate is in our genes." In his popular book *The Selfish Gene*, Richard Dawkins (1976) wrote of the genome as "the book of life" and proposed that our bodies are merely transient vehicles for the survival and propagation of our immortal DNA. In 1995, Nelkin and Lindee reviewed the popular accounts of DNA and concluded that DNA is being perceived as the secular equivalent of the soul. It is thought of as the essence of our being and that which determines our behaviors.* Richard Lewontin (1993) has also documented the dominance acquired by genetic determinism as an explanation of behavioral phenotypes.

But as we have seen (and will see much more of), genes are not the only explanation for animal diversity. During the past decade, interest in

*This is still a popular theme in the anti-Choice websites, where one is told that at fertilization, we receive our new DNA that pre-determines our physical and psychological traits for the rest of our lives (Gilbert 2008).

the environmental mechanisms of variation proposed early in the twentieth century has been renewed, fueled by new findings in conservation biology, developmental biology, public health, and evolutionary biology. The breakthroughs in molecular biology that have led to our exponentially expanded genomic knowledge also led to our current understanding of molecular signaling, casting a brilliant new light on the work of a century earlier. Just as in the 1600s the microscope revolutionized our view of life by revealing a previously invisible world, so in the twenty-first century technologies such as PCR (the polymerase chain reaction) and high-throughput RNA analysis have allowed us glimpses of a hitherto unsuspected world of interactions and interrelationships between genes and the environment. The result is a new perspective on life, its origins, and its interconnections.

"Eco-Devo": Embryology Meets Developmental Plasticity

Ecological developmental biology, casually known as **eco-devo**, is an approach to embryonic development that studies the interactions between a developing organism and its environment (Gilbert 2001; Sultan 2007). It focuses on how animals have evolved to integrate signals from the environment into their normal developmental trajectories. As we detail in this section, ecological developmental biology has three major sources: developmental plasticity (this chapter), environmentally induced gene configurations (Chapter 2), and developmental symbiosis (Chapter 3). Each of these phenomena is a means of producing phenotypic variation.

In many ways, ecological developmental biology is the extension of embryology to levels above that of the individual. In standard embryology, the focus has always been on the internal dynamics through which the genes of an individual's cell nuclei produce the phenotype of the organism. Within the past century, we have discovered that cell-cell communication is key to this phenomenon. By itself, the genetic information in a cell's nucleus cannot directly produce the many differentiated cell types in a multicellular organism; cells must interact, reciprocally instructing each other as they differentiate. Molecular signals called **paracrine factors** are released by one set of cells and induce gene expression changes in the cells adjacent to them. These neighboring cells, with their newly acquired characteristics, then produce their own paracrine factors that can change the gene expression of *their* neighbors—sometimes including the cell that originally induced them! By such cooperative signaling between cells, organs are formed.

But as Paul Weiss (1970) and others hypothesized, such molecular signals are not limited to the internally generated paracrine factors, but can also come from sources outside the organism. Oscar Hertwig (1894), Curt Herbst (1901), and others catalogued these environmental agents and discussed them as normal components in determining the phenotype of the embryo. Thus the same genotype can generate different phenotypes depending on what cues are present in the environment, allowing the embryo to change its developmental trajectory in response to environmental input.

Sonia Sultan (2007, p. 575) summarized the modern status of ecological developmental biology:

> Ecological developmental biology ("eco-devo") examines how organisms develop in "real-life" environments... [and] aims to provide an integrated framework for investigating development in its ecological context…. Eco-devo is not simply a repackaging of plasticity studies under a new name…. Whereas plasticity studies draw on quantitative genetic and phenotypic selection analyses to examine developmental outcomes and their evolution as adaptive traits, eco-devo adds an explicit focus on the molecular and cellular mechanisms of environmental perception and gene regulation underlying these responses, and how these signaling pathways operate in genetically and/or ecologically distinct individuals, populations, communities, and taxa.

Developmental plasticity is usually adaptive—that is, it makes the organism more fit for its environment. This idea that the developing organism has evolved mechanisms to receive and to respond to environmental cues to produce particular phenotypes has important evolutionary implications, which will be discussed more fully in Chapter 11. We will see that for evolutionary change to occur, the phenotypically plastic change may come first, followed by the genetic change. Moreover, as we will see in later chapters, there are times when plasticity is maladaptive—either when environmental cues alter development in a pathological manner, or when there is a mismatch between the phenotype induced by the embryonic environment and the environment experienced by the organism later in life. In both instances, developmental plasticity can give rise to disease, as will be discussed in the second section of the book, Chapters 4–8.

In most developmental interactions, the genome provides specific instructions, while the environment is permissive. That is to say, the genes determine what structures get made, and the only requirement of the environment is that it support and not disturb the developmental processes. Dogs will generate dogs and cats will beget cats, even if the animals live in the same house. However, in most species, there are instances in development when the *environment* plays the instructive role and the genome is merely permissive. In these instances, the environment determines what type of structure is made—but the genetic repertoire has to be capable of building that structure. The genetic ability to respond to environmental factors has to be inherited, of course, but in these cases it is the environment that directs the formation of the specific phenotype (Sarkar 1998; Gilbert 2001; Jablonka and Lamb 2005).

Reaction norms and polyphenisms

Two main types of phenotypic plasticity are currently recognized: reaction norms and polyphenisms (Woltereck 1909; Schmalhausen 1949; Stearns et al. 1991; West-Eberhard 2003). As mentioned earlier, in a reaction norm, the genome encodes a *continuous range* of potential phenotypes, and the environment the individual encounters determines the phenotype. One obvious example is muscle hypertrophy in humans. The size of our muscle depends on environmental conditions—how much load it experiences.

(A) (B)

Figure 1.3 Density-induced polyphenism in the desert (or "plague") locust *Schistocerca gregaria*. (A) The low-density morph has green pigmentation and miniature wings. (B) Triggered by crowding, the high-density morph develops with deep pigmentation and wing and leg development suitable for migration. (From Tawfik et al. 1999, courtesy of S. Tanaka.)

Those people who exercise have larger muscles—but only within hereditarily defined limits. Reaction norms allow developing organisms to "titrate" their responses to the strength of a signal. Tadpoles of the wood frog *Rana sylvatica*, for instance, respond to the presence of predators by developing deeper tails and shorter bodies. Moreover, the larger the predation risk (as measured by the chemical secreted by the predators), the deeper the tail and the shorter the body (Relyea 2004).

The second type of phenotypic plasticity, **polyphenism**, refers to *discontinuous* (either/or) phenotypes elicited by the environment. One obvious example is sex determination in turtles, where one range of temperatures induces female development in the embryo, while a different range of temperatures elicits male development. Between these two ranges is a small band of temperatures that will produce different proportions of males and females—but these intermediate temperatures do not induce intersexual animals.

An important example of polyphenism is seen in the migratory locust *Schistocerca gregaria*. These plant-eating grasshoppers exist in two mutually exclusive forms (Figure 1.3): they are either short winged, uniformly green, and solitary or they are long winged, brightly colored, and gregarious (Pener 1991; Rogers et al. 2003, 2004). The phenotypic differences between these two morphs are so striking that only in 1921 did the Russian biologist Boris Usarov finally realize they were the same species. Cues in the environment determine which morphology a young locust will develop. The major stimulus appears to be population density, as measured by the rubbing of legs. When locust nymphs get crowded enough that a certain neuron in the hind femur is stimulated by other nymphs, their developmental pattern changes, and the next time they molt, they emerge with long, brightly colored wings, as well as with gregarious (flocking) and migratory behaviors.

The different phenotypes induced by the environment are sometimes called **morphs** or **ecomorphs**. Genetically identical animals can have different morphs depending on the season, their larval diet, or other signals present in the environment. Confusingly, the phenotypes produced by different genetic alleles are called either "mutants" (if rare) or "polymorphisms" (if common—arbitrarily defined as found in more than 5% of the population). Polymorphism is therefore a condition where variation is the product of genetic differences, while polyphenism is a condition where variation (different morphs) is the product of environmental signals (Mayr 1963).

Epigenetics

In 1968, Waddington coined the term "epigenetics" to describe a way of integrating the series of ordered interactions in development (epigenesis) with genetics (see Van Speybroeck 2002). Since then, epigenetics has been redefined as the set of mechanisms involved in regulating gene activity during development. For instance, the methylation of certain regions of genes suppresses their expression. This is very important in preventing genes from being expressed in the wrong types of cells or at the wrong times. By these epigenetic mechanisms, hemoglobin is made in red blood cell precursors and in no other type of cell, and insulin is made only in the beta cells of the pancreas. This is discussed more fully in Appendix B. **Epigenetics** is defined in this book as those genetic mechanisms that create phenotypic variation without altering the base-pair nucleotide sequence of the genes. Specifically, we use this term to refer to those mechanisms that cause variation by altering the *expression* of genes rather than their sequence.

As we will see in the next chapter, epigenetic mechanisms can integrate genomic and environmental inputs to generate the instructions for producing a particular phenotype. Epigenetic investigations have become focused on the mechanisms of phenotypic plasticity and on how changes in gene expression patterns mediated by the environment can cause diseases (such as cancers and hypertension).

The term **epigenetic inheritance** has been used to denote heritable phenotypes that are not encoded in the genome (Jablonka and Lamb 2005; Jablonka and Raz 2008). Epigenetic inheritance includes:

- Variations inherited over *cell* generations, such as changes in chromatin that stabilize a particular cell type during normal development. For instance, during the course of their differentiation, mammalian liver precursor cells obtain a chromatin configuration that instructs their liver-specific genes to function, and henceforth all liver cells "remember" this chromatin configuration and maintain it over progressive cell divisions.
- Variations inherited from one *organismal* generation to the next. For instance, certain drugs can induce changes in the chromatin structure in the nuclei of mouse cells, including the mouse's germ cells. The progeny of such a mouse can inherit the drug-induced chromatin change from its parents, even if the drug is no longer present (see Chapter 6). There are also some variations that can be inherited from one generation to the next by modifying maternal nursing behavior (see Chapter 2).

- The inheritance of symbionts from one generation to another. Chapters 3 and 11 will document that these microorganism help construct the animal body and can be a source of heritable variation.

Among humans (and possibly among other animals; see Avital and Jablonka 2000), variations in cultural inheritance represent another inheritance pattern that is not mediated by changes in DNA.

Agents of developmental plasticity

Possibly, every organism has environmentally determined components in its phenotype. When asked, "Where does polyphenism occur among the insects?" Simpson and colleagues (2011) reply, "'Everywhere' is the brief answer." Therefore, a complete list of organisms with phenotypic plasticity would resemble a survey of all the planet's eukaryotes. Examples of how the environment acts in normal development are given in Table 1.1,

TABLE 1.1 Some environmental contributors to phenotype development

Context-dependent normal development (Chs. 1, 2)

A. Morphological polyphenisms
  1. Nutrition-dependent (*Nemoria*, hymenoptera castes)
  2. Temperature-dependent (*Arachnia*, *Bicyclus*)
  3. Density-dependent (locusts)
  4. Stress-dependent (*Scaphiopus*)

B. Sex determination polyphenisms
  1. Location-dependent (*Bonellia*)
  2. Temperature-dependent (*Menidia*, turtles)
  3. Social-dependent (wrasses, gobys)

C. Predator-induced polyphenisms
  1. Adaptive predator-avoidance morphologies (*Daphnia*, *Hyla*)
  2. Adaptive immunological responses (mammals)
  3. Adaptive reproductive allocations (ant colonies)

D. Stress-induced bone formation
  1. Prenatal (fibular crest in birds)
  2. Postnatal (patella in mammals; lower jaw in humans?)

Context-dependent life cycle progression (Chs. 2, 3)

A. Larval settlement
  1. Substrate-induced metamorphosis (bivalves, gastropods)
  2. Prey-induced metamorphosis (gastropods, chitons)
  3. Temperature/photoperiod-dependent metamorphosis

B. Diapause
  1. Overwintering in insects
  2. Delayed implantation in mammals

C. Sexual/asexual progression
  1. Temperature/photoperiod-induced (aphids, *Megoura*)
  2. Temperature/colony-induced (*Volvox*)

D. Symbioses/parasitism
  1. Blood meals (*Rhodnius*, *Aedes*)
  2. Commensalism (*Euprymna*/*Vibrio*, eggs/algae, mammalian gut microbiota)
  3. Parasites (*Wolbachia*)

E. Developmental plant-insect interactions

Adaptations of embryos and larvae to environments (Ch. 4)

A. Egg protection
  1. Sunscreens against radiation (*Rana*, sea urchins)
  2. Plant-derived protection (*Utetheisa*)

B. Larval protection
  1. Plant-derived protection (*Danaus*, tortoise beetles)

Source: Gilbert 2001.

Note: This list should not be thought to be inclusive. For example, the list is limited to animals; plant developmental plasticity and many plant-animal interactions have not been included here.

which shows some of the numerous environmental agents that contribute to producing normal phenotypes, including:

- Temperature
- Nutrition
- Pressure and gravity
- Light
- The presence of dangerous conditions (predators or stress)
- The presence or absence of conspecifics (other members of the same species)

The remainder of this chapter describes how environmental cues affect the course of normal development in a variety of species. In subsequent chapters, more specific details about the mechanisms of developmental plasticity will be discussed.

Temperature-Dependent Phenotypes

Temperature is the causal factor in a number of phenotypes: when temperature differences cause amino acid chains to fold differently, traits (determined by enzymes) are turned on or off. Often, temperature will cause a suite of morphological and behavioral changes. Indeed, in some species, different temperatures cause embryos to develop as either male or female, each with its own set of behavioral characteristics. Thus, the organism is born with the possibility for both sets of organs and behaviors, and the temperature will select both the organ and the behavior that goes with it.

Enzyme activity as a function of temperature

Nearly all enzyme activity is temperature-dependent. This concept is often expressed as the enzyme's Q_{10}, or the ratio of its activities at two temperatures, one 10°C higher than the other. Temperature can cause changes in the way a protein folds and thereby determine the shape of an enzyme's active site and the sites of interaction with other proteins. One example of such a protein is the tyrosinase enzyme variant found in Siamese cats and Himalayan rabbits (Figure 1.4). Tyrosinase is critical for making melanin, the dark pigment of vertebrate skin. (Indeed, mutations that block melanin production result in albinism, the lack of dark pigment throughout the body.) The mutation that creates the phenotype of Siamese cats and Himalayan rabbits transforms tyrosinase from an enzyme that is not temperature-dependent (in the physiological ranges expected in an organism) into a temperature-dependent enzyme. In these animals, tyrosinase folds properly at relatively cold temperatures but does not fold properly—and thus does not work—at warmer temperatures. Cooler temperatures are normally found at the extremities (the tips of the ears, the paws and tail, and part of the snout), with warmer temperatures throughout the major parts of the body (Schmalhausen 1949). Thus, tyrosinase functions (and melanin pigment is made) only in the extremities of Siamese cats and Himalayan

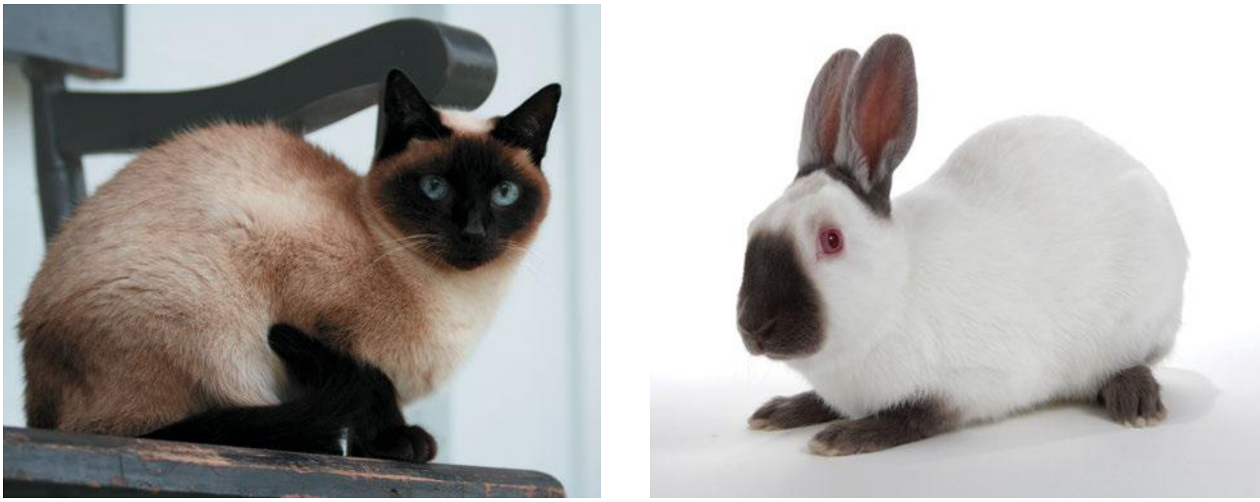


Figure 1.4 The dark pigment melanin is synthesized only in the colder areas of the vertebrate skin in Siamese cats and Himalayan rabbits. This is due to a mutation in the gene for tyrosinase—the rate-limiting enzyme of melanin synthesis—that renders the protein heat-sensitive. In the colder extremities of the body, tyrosinase folds properly and melanin (dark pigment) is produced. In warmer regions of the body, however, the enzyme folds improperly and cannot function, thus limiting the production of melanin.

rabbits, demonstrating that enzymes are affected by temperature and that their subsequent responses can have large impacts on phenotype.*

There are analogous conditions in humans in which only the hair at the extremities is pigmented (Berson et al. 2000). These conditions result from a single G $\rightarrow$ A mutation that replaces the positively charged amino acid arginine at position 402 of tyrosinase with the uncharged glutamine.

The induction of melanin pigment by the environment is part of our plastic response to the environment and is the basis for suntanning† (D'Orazio et al. 2006; April and Barsh 2007). By increasing epidermal melanin content, tanning is the skin's major response against acute and ultraviolet light-induced damage (Chen et al. 2014). Plants also have an inducible system for melanin production. When certain fruits are cut, melanin is induced, creating a dark, protective meshwork that prevents bacterial and fungal penetration. This is why apples, potatoes, and bananas turn brown

*Both Siamese and Burmese cats possess mutations of the tyrosinase gene, but they occur at slightly different sites within the gene. This apparently produces different thresholds for gene activity, allowing the Burmese breeds to have darker body color (Schmidt-Küntzel et al. 2005).

†There is an unexpected side-effect to this induction. UV light induces the epidermal cells to produce the pro-hormone proopiomelanocortin. This protein is processed into several other smaller proteins. One of these is melanocyte-stimulating hormone, which induces the production of melanin that is characteristic of tanning. Another product, however, is β-endorphin, which gives pleasurable sensations. Fell and colleagues (2014) have evidence that this β-endorphin can cause addiction to tanning and a subsequent increase in melanoma tumors.

when sliced. (See Szent-Györgyi 1966 and Bachem et al. 2004 for discussion of the importance of this reaction.)

Seasonal polyphenism

Since enzymes (and presumably other proteins, such as transcription factors) can be influenced by temperature, it is not surprising that animals have evolved such that thermal cues can cause different phenotypes at different seasons. Ecologists have long known that in North America, the pigmentation of many butterfly species follows a seasonal pattern. Throughout much of the Northern Hemisphere, one can see such a polyphenism in butterflies of the family Pieridae (the cabbage whites), with phenotypes that differ between individuals that eclose from their pupa during the long days of summer and those that eclose at the beginning of the season, in the shorter, cooler days of spring. The hindwing pigments of the spring forms are darker than those of the summer butterflies (Figure 1.5). Pigmentation has a functional advantage during the cooler months: darker pigments absorb sunlight more efficiently than lighter ones, raising the body temperature more rapidly (Shapiro 1968; Watt 1968; see also Nijhout 1991). As we will see later, temperature may effect these changes in color by affecting the production of hormones needed for growth and differentiation.

Seasonal changes in fur color are typical for many mammals that thrive in winter snow. Their summer pelage is brown or gray, blending into the trees and grasses, but when the average amount of daylight (photoperiod) gets progressively less, hormones in their bodies activate those genes producing the white fur that camouflages them in the snow. The photoperiod,

Figure 1.5 Polyphenic variation in *Pontia* (Pieridae) butterflies. The top row shows summer morphs: *P. protodice* female (left) and male (center), *P. occidentalis* male (right). The bottom row shows spring morphs, which have a more highly pigmented ventral hindwing: *P. protodice* female (left) and male (center), *P. occidentalis* male (right). (Photograph courtesy of T. Valente.)

not temperature, is the cue, and it has been shown to be a very accurate one (Grange 1932; Flux 1970). Global climate change, however, can cause mismatch between the coat color and background. As snow is coming much later to areas of the northern United States, snowshoe hares are turning white long before the first snowfall (Mills et al. 2013; Zimova et al. 2014). Conservation biologists are concerned that this might lead to the elimination of this species in many areas of its range.

Temperature and sex

Aristotle—a noteworthy naturalist and history's first embryologist—made few major errors in his embryological descriptions. One of these, however, was to attribute human sex determination to temperature (Aristotle 355 BCE). He felt that maleness was generated through the heat of the semen, and he encouraged elderly men to mate in the summertime if they desired male heirs.

Although Aristotle was wrong about temperature having a role in human sex determination, in many species, temperature *does* control whether an embryo develops testes or ovaries. Indeed, among certain reptile groups (turtles and crocodilians), there are many species in which the temperature at which an embryo develops determines whether an individual is male or female (Figure 1.6). This type of environmental sex determination, which also is found in certain fishes, has advantages and disadvantages.

One probable advantage is that it gives the species the benefits of sexual reproduction without tying the species to a 1:1 sex ratio. In crocodiles, in which temperature extremes produce females while moderate temperatures produce males, the sex ratio may be as great as 10 females to each male (Woodward and Murray 1993). In instances where the population size is limited by the number of females, such a ratio is more advantageous than the 1:1 ratio usually resulting from genotypic sex determination.

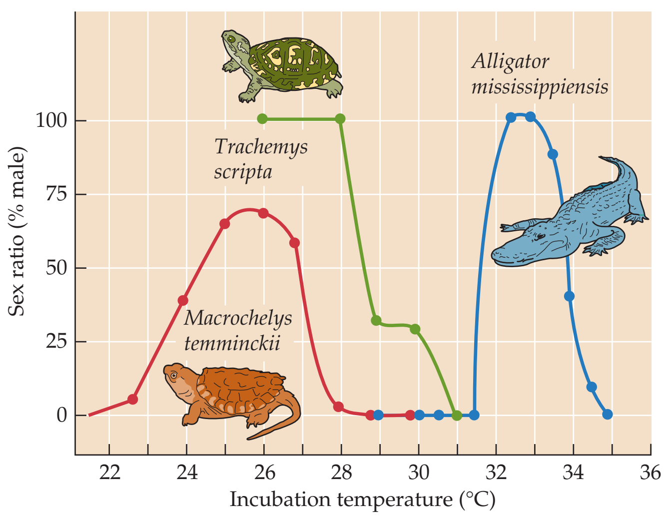


Figure 1.6 Temperature-dependent sex determination in three different reptilian species: the American alligator (*Alligator mississippiensis*), the red-eared slider turtle (*Trachemys scripta*), and the alligator snapping turtle (*Macrochelys temminckii*). (After Crain and Guillette 1998.)

The major disadvantage of temperature-dependent sex determination may involve its narrowing of the temperature range within which a species can persist. Thus, thermal pollution (either local or due to global warming) could conceivably eliminate a species from a given area (Janzen and Paukstis 1991). Researchers have speculated that dinosaurs may have had temperature-dependent sex determination, and that their sudden demise may have been the result of a slight change in temperature that created conditions wherein only males or only females hatched (Ferguson and Joanen 1982; Miller et al. 2004). Unlike turtles, which have long reproductive lives, can hibernate for years, and whose females can store sperm, dinosaurs may have had a relatively narrow window of time in which to reproduce and lacked the ability to hibernate through long stretches of bad times.

Charnov and Bull (1977) argued that environmental sex determination would be adaptive in those habitats characterized by patchiness—that is, habitats having some regions where it is more advantageous to be male and other regions where it is more advantageous to be female. Conover and Heins (1987) provided evidence for this hypothesis. In certain fish species, females benefit from being larger, since larger size translates into higher fecundity. If you are a female Atlantic silverside (*Menidia menidia*), it is advantageous to be born early in the breeding season, because you have a longer feeding season and thus can grow larger. (The size of males in this species doesn't influence mating success or outcomes.) In the southern range of *M. menidia*, females are indeed born early in the breeding season, and temperature appears to play a major role in this pattern. However, in the northern reaches of its range, the species shows no environmental sex determination. Rather, a 1:1 sex ratio is generated at all temperatures (Figure 1.7). Conover and Heins speculated that the more northern populations have such a short feeding season, there is no reproductive advantage for females in being born earlier. Thus, this fish has environmental sex determination in those regions where it is adaptive, and genotypic sex determination in those regions where it is not.

In mammals, primary sex determination is controlled by chromosomes and not by hormones. This is important because we develop inside the hormonal milieu of our mothers. If the determination of mammalian gonads were accomplished through hormones, there would

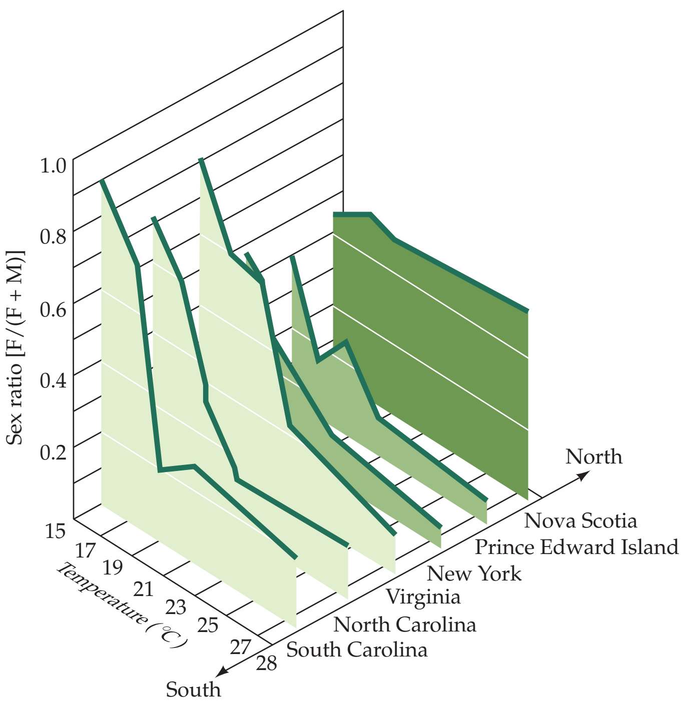


Figure 1.7 Relationship between temperature during the period of sex determination and sex ratio [F/(F + M)] in *Menidia menidia*. In fish collected from the northernmost portion of its range (Nova Scotia), temperature had little effect on sex determination. However, among fish collected at more southerly locations (especially from Virginia through South Carolina), temperature had a large effect. (After Conover and Heins 1987.)

be no males. In mammals, two stages of sex determination have evolved. Primary sex determination is controlled by the X and Y chromosomes, which determine whether the gonads differentiate as ovaries or as testes. Secondary sex determination is accomplished by the hormones (testosterone, estrogen, and others) made by the gonads. This second stage is responsible for the male- and female-specific external genitalia, as well as for the differentiation of the uterus and oviducts in females and the development of the spermatic ducts in males.

In other vertebrates (including fishes, amphibians, and birds), the hormones estrogen and testosterone appear to be responsible for making ovaries or testes, respectively. The enzyme responsible for controlling the ratio of these hormones is **aromatase**, which converts testosterone into estrogen. Aromatase has been found to be temperature-regulated in several vertebrate species (Kroon et al. 2005). As we will see in later chapters, the enzyme is a target for environmental mutagens that can seriously alter the sexual development of a number of vertebrate species.*

Nutritional Polyphenism: What You Eat Becomes You

The food an organism eats may contain powerful chemical signals that induce phenotypic changes. We saw at the start of the chapter that the larval phenotype of the moth *Nemoria arizonaria* depends on its diet (see Figure 1.1B). Such effects are not uncommon among insects.

Royal jelly and egg-laying queens

In hymenopteran insects (bees, wasps, and ants), the determination of queen and worker castes can be effected by several factors, including genes, nutrition, temperature, and even volatile chemicals secreted by other members of the hive. In the honeybee, new queens are generated within 2 weeks after the death of the preceding queen (or in anticipation of the colony's splitting and a second queen being needed); they are almost never produced otherwise. Queen formation is dependent almost entirely on diet. A larva fed "royal jelly" (a protein-rich food that contains secretions from the workers' salivary glands) for most of its larval life will be a queen (with functional ovaries), while a larva fed a poorer diet (and given royal jelly for only a brief time late in larval development) will become a sterile worker (Figure 1.8A). In addition to nutritive proteins, the royal jelly contains a relatively small protein dubbed "royalactin" that increases the juvenile hormone titer, increases body size, and speeds up development (Kamakura 2011).

*This sex-altering property of aromatase was useful in an experiment that demonstrated the adaptive value of temperature-dependent sex determination. In the jacky dragon lizard (*Amphibolurus muricatus*), males are produced at intermediate temperatures (around 27°C), whereas both higher and lower temperatures produce females. By using aromatase inhibitors to block the conversion of testosterone to estrogen, Warner and Shine (2008) were able to produce males throughout the temperature range 23°C–33°C. There were no morphological differences among the males produced at any of these temperatures, but males produced at the intermediate temperatures had significantly better fitness (i.e., they sired more progeny) than the males produced at the extreme (normally female-producing) temperatures. The reason for this increased fitness as yet remains unknown.

(A) (B)

Figure 1.8 Reproductive queens in hymenopteran colonies. (A) The queen of the honeybee (*Apis mellifera*) with her sister workers. (B) Gyne (reproductive queen) and worker of the ant *Pheidologeton*. This picture shows the remarkable dimorphism between the large queen and the small worker (seen near the queen's antennae). The difference between these two sisters involves larval feeding and juvenile hormone synthesis.

The increase in juvenile hormone is very important for the development of fertile queens. Larvae can become queens only if they reach a certain size before metamorphosis. A larva continually fed royal jelly from a relatively early stage retains the activity of a structure called the corpora allatum throughout its larval stages. The corpora allatum secretes juvenile hormone (JH), which delays metamorphosis, allowing the larva to grow larger and to have functional ovaries (Brian 1974, 1980; Plowright and Pendrel 1977). The rate of JH synthesis in the "queen larvae" is 25 times greater than the rate of synthesis in larvae not fed royal jelly. Applying large amounts of JH to worker larvae late in life can transform them into queens (Wirtz 1973; Rachinsky and Hartfelder 1990). Thus, the queen does not achieve her large and fertile status due to a genetic predisposition, but from nutritional supplementation.

Similarly, ant colonies are predominantly female, and the females can be very different in size and function. The much larger reproductive females ("gynes" or "queens") have fully functional ovaries and wings; the workers do not (Figure 1.8B). These striking differences in anatomy and physiology are also regulated through juvenile hormone (Wheeler 1991). The influence of the environment on hormone levels and gene expression in ants was analyzed by Abouheif and Wray (2002), who found that nutrition-induced JH levels regulated wing formation. In the queen, both the forewing and the hindwing disc undergo normal development, expressing the same genes as *Drosophila* wing discs. However, in the wing imaginal discs of workers, some of these genes remain unexpressed, and the wings fail to form.

Horn length in the male dung beetle

The structural and behavioral male phenotypes of some male dung beetles depend on the quality and quantity of the nutrition—in the form of maternally provided dung—that they have access to during development (Emlen 1997; Moczek and Emlen 2000). In dung beetle species such as *Onthophagus taurus* and *O. acuminatus*, males have the ability to grow horns while females do not. The hornless female beetle gathers manure, digs tunnels, and places balls of dung in brood chambers that she constructs at the ends of the tunnels. She then lays a single egg on each cluster of dung, and when the larvae hatch, they eat the dung. Metamorphosis occurs when the dung cluster is consumed.

The amount of food affects the titer of juvenile hormone present during the developing beetle's last larval molt. In the males, the last organs to form are the horns. The size of the larva at metamorphosis determines the titer of JH, and the titer of JH affects the growth of the ectodermal regions that make the horns (Emlen and Nijhout 1999; Moczek 2005; Figure 1.9A). If juvenile hormone is added to an *Onthophagus* male larva during the sensitive period of his last molt, the cuticle in its head expands to produce a horn. The male horn does not grow unless the beetle larva reaches a certain size. Above this threshold body size, horn size is proportional to body size. Thus, although body size has a normal distribution, there is a bimodal distribution of horn sizes. About half the males (the small-bodied ones) have no horns, while the other half have horns of considerable length (Figure 1.9B).

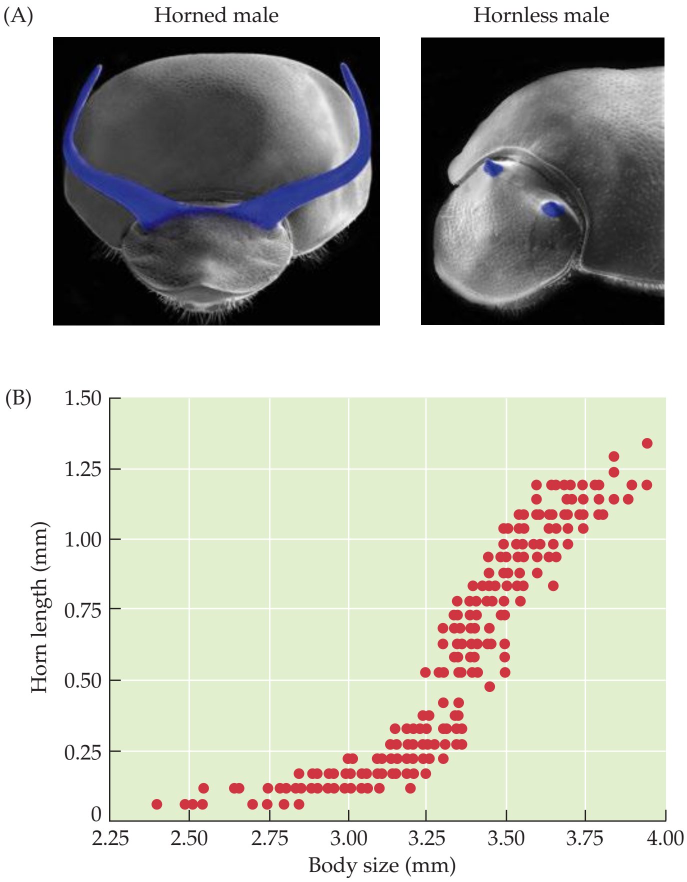


Figure 1.9 Diet and *Onthophagus* horn size. (A) Horned and hornless males of the dung beetle *Onthophagus acuminatus* (horns have been artificially colored). (B) Whether a male of this species is horned or hornless is determined by the titer of juvenile hormone at the last molt. This hormone titer depends in turn on the size of the larva. There is a sharp threshold of body size, before which horns fail to form and after which horn growth is linearly correlated with the size of the beetle. This threshold effect produces populations with no horns and with large horns, but very few with horns of intermediate size. (After Emlen 2000, photographs courtesy of D. Emlen.)

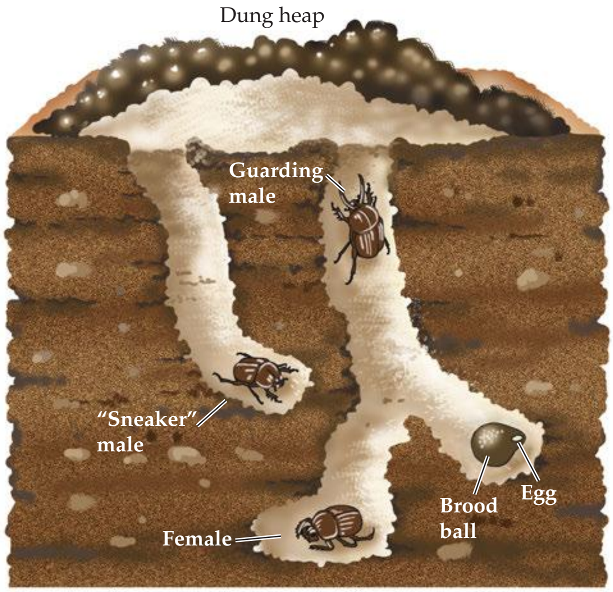


Figure 1.10 The presence or absence of horns determines the male reproductive strategy in some dung beetle species. Females dig tunnels in the soil beneath a pile of dung and bring dung fragments into the tunnels. These will be the food supply of the larvae. Horned males guard the entrances to the tunnels and mate repeatedly with the females. They fight to prevent other males from entering the tunnels, and those males with long horns usually win such contests. Smaller, hornless males do not guard tunnels. Rather, they dig their own tunnels to connect with those of females, mate, and exit. (After Emlen 2000.)

The behaviors of horned and hornless males also differ. Horned males guard the females' tunnels and use their horns to prevent other males from mating with the females; the male with the biggest horns wins such contests. But what about the males with no horns? Hornless males do not fight with the horned males for mates. Since they, like the females, lack horns, they are able to dig their own tunnels without the horns getting in the way. These "sneaker" males dig tunnels that intersect with those of the females and mate, while the horned males stand guard at the tunnel entrances (Figure 1.10; Emlen 2000; Moczek and Emlen 2000). Both strategies appear to be highly successful.

The heritability of horn length is zero; it is a phenotype that is environmentally determined by the response of the endocrine system to food intake. However, the size threshold a male larva must reach in order to produce a horn is a property of the genome that can be selected. Different species of beetle are expected to differ in the direction and amount of plasticity they are able to express (Gotthard and Nylin 1995; Via et al. 1995). Interestingly, large horns also appear to correlate with reduced penis and testis size (Simmons and Emlen 2006; Parzer and Moczek 2008). This **trade-off** is probably due to altered allocation of resources during development, since experimentally ablating the male genital disc (from which the penis originates) results in a male with larger horns (Moczek and Nijhout 2004).

Gravity and Pressure

Embryologists have long appreciated the critical role that gravity plays in frog and chick body axis formation. For instance, if a frog egg is rotated during the first cell division cycle, the dense yolk will fall to the bottom

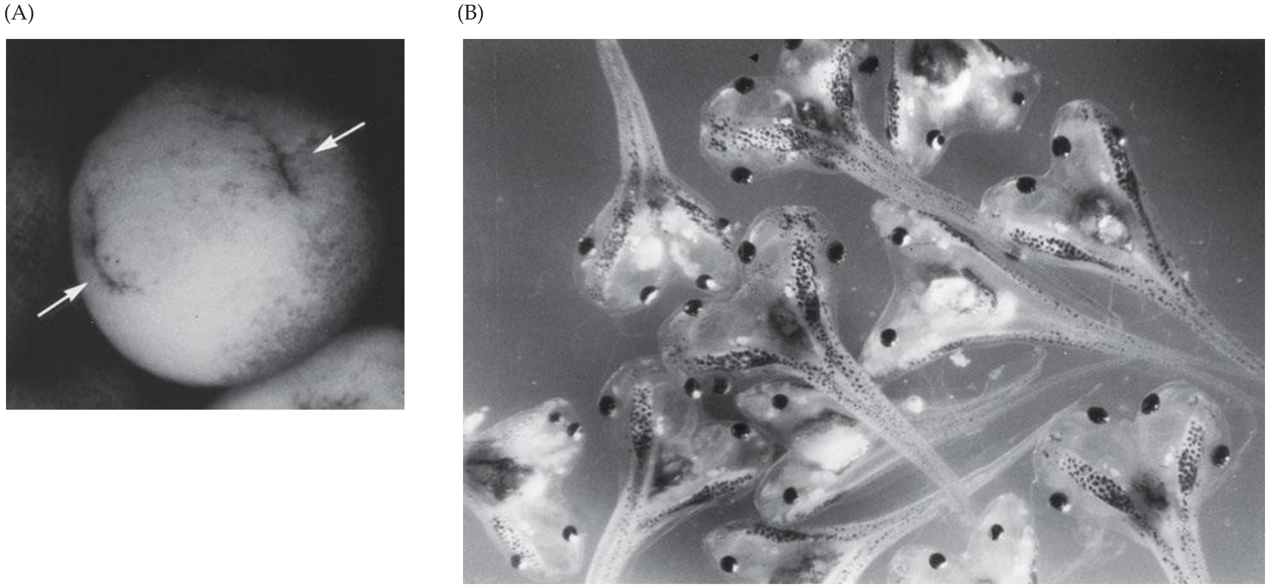


Figure 1.11 Expectation of a 1-G gravitational field in frog development. The dense yolk of a frog egg instructs a single axis to form, defined by the gravitational force on the yolk. If a frog egg is rotated during first cleavage, the yolk travels into a new area of the egg, displacing its contents. As a result, gastrulation (the beginning of organ formation) is initiated at two sites rather than one site (A) and two axes form, each with a fully developed head (B). (Photographs courtesy of J. Gerhart.)

of the rotated egg and displace the proteins and mRNA molecules there. This can result in the formation of two heads, one defined by the old axis, and one defined by the new axis (Kirschner et al. 1980; Figure 1.11). The axes will not form correctly, or several axes will form—in which case the embryo will have more than one head. Bird eggs use the force of gravity to form their anterior-posterior (head-to-tail) axis (see Gilbert 2013).

More recent experiments have shown that the human body requires a 1-G gravitational field for the proper development and maintenance of bones and muscles. Astronauts experiencing weightless conditions undergo severe muscle atrophy. As Figure 1.12 shows, weightlessness results in dramatic structural changes in the muscles, leading to tears and loss of strength and coordination. Spending 11 days in microgravity (without exercising) can cause a 30% shrinkage in the mass of certain muscles (NASA 2003). Several genes necessary for muscle differentiation and maintenance—including those genes encoding the transcription factors MyoD and myogenin—are not expressed in microgravity conditions (Inobe et al. 2002). Moreover, in mice and rats, genes encoding proteins that support mitochondria and muscle growth also fail to function without normal gravity (Nikawa et al. 2004; Allen et al. 2009).

In addition to muscle, the formation of several vertebrate bones is dependent on gravity (or on pressure from the environment). Such stresses are known to be responsible for the formation of the human patella (kneecap)

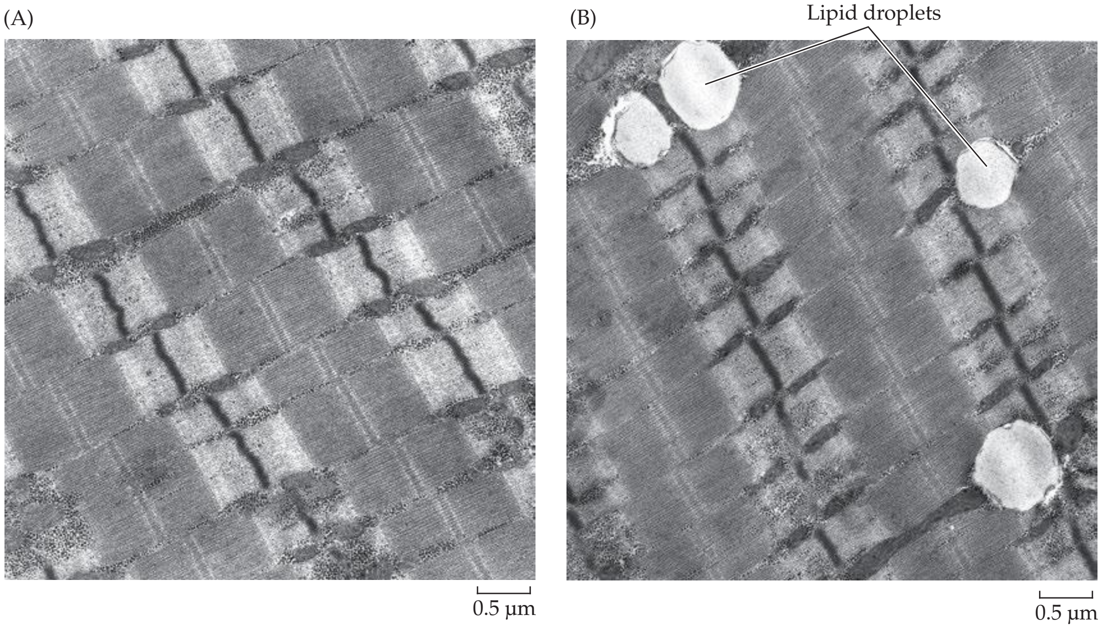


Figure 1.12 Human soleus muscle tissues, showing the effects of exercise in weightless conditions. (A) Exercised tissue, where gravitational load stimulates the production of proteins that keep muscle fibers strong. (B) After 17 days in microgravity, muscle protein synthesis has slowed down. The muscle cells have grown more irregular and show signs of atrophy. The prevalence of lipid droplets indicates that in microgravity, the muscle cells store fat instead of using it for energy. (From Widrick et al. 1999.)

after birth and have also been found to be critical for jaw growth in humans and fish. The jaws of cichlid fish differ enormously, depending on the food they eat (Figure 1.13; Meyer 1987). Similarly, normal human jaw development may be predicated on expected tension due to grinding food: mechanical tension appears to stimulate the expression of the *indian hedgehog* gene in mammalian mandibular cartilage, and this paracrine factor stimulates cartilage growth (Tang et al. 2004). If an infant monkey is given

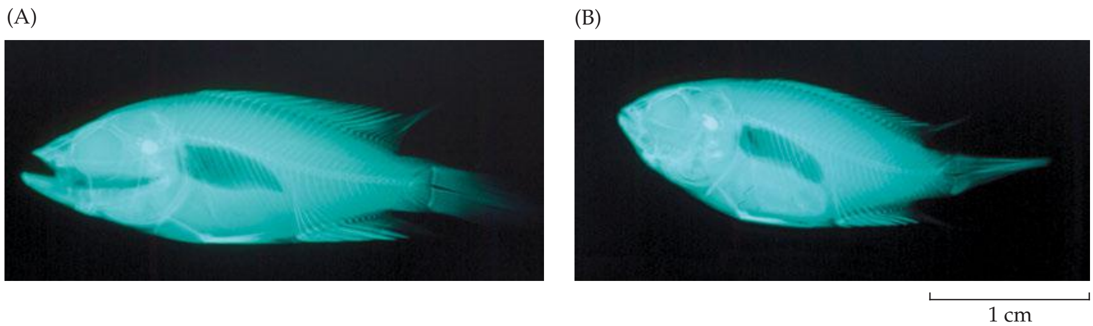


Figure 1.13 X-rays of cichlid fish fed different diets for 8 months. (A) Fish fed shrimp larvae developed a narrow-angled jaw. (B) Fish fed commercial flaked food and nematodes had a wider-angled jaw. (From Meyer 1987, photographs courtesy of Axel Meyer.)

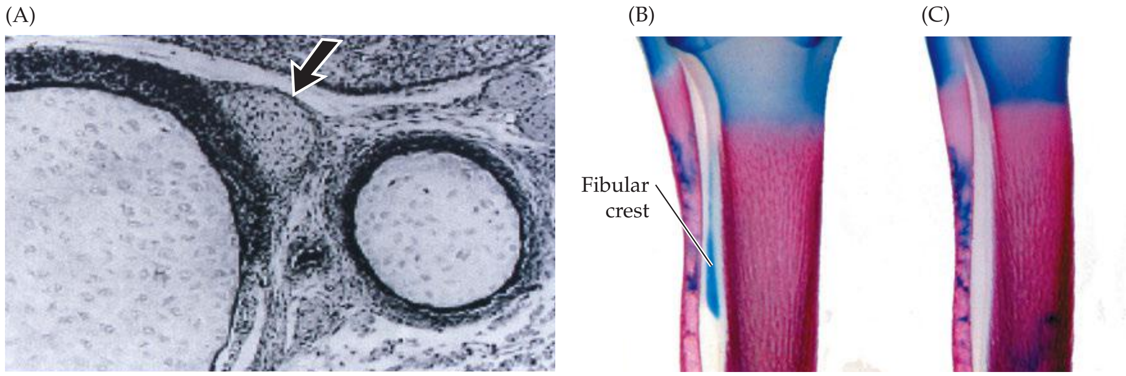


Figure 1.14 Activity-induced formation of the fibular crest. The fibular crest (syndesmosis tibiofibularis) is formed when the movement of the embryo in the egg puts stress on the tibia. (A) Transverse section through the 10-day embryonic chick limb, showing the condensation (arrow) that will become the fibular crest. (B) A 13-day chick embryo, showing fibular crest forming between the tibia and fibula. (C) Absence of fibular crest in the connective tissue of a 13-day embryo whose movement was inhibited. The blue dye stains cartilage; the red dye stains the bone elements. (From Müller 2003, photographs courtesy of G. Müller.)

soft food, its lower jaw is smaller than normal. Corruccini and Beecher (1982, 1984) and Varela (1992) have shown that people in cultures where infants are fed hard food have jaws that "fit" better, and they speculate that soft infant food explains why so many children in Western societies need braces on their teeth. Indeed, the notion that mechanical tension can change jaw size and shape is the basis of the functional hypothesis of modern orthodontics (Moss 1962, 1997).

In the chick, several bones do not form if the embryo's movement inside the egg is suppressed. One of these bones is the fibular crest, which connects the tibia directly to the fibula. This direct connection is believed to be important in the evolution of birds, and the fibular crest is a universal feature of the bird hindlimb (Müller and Steicher 1989). When the chick is prevented from moving within its egg, the fibular crest fails to develop (Figure 1.14; Wu et al. 2001; Müller 2003).

Predator-Induced Polyphenisms

At the beginning of this chapter, we asked you to imagine an animal that is frequently confronted by a particular predator, that could recognize soluble molecules secreted by that predator, and that could use those molecules to activate the development of structures that would make this individual less likely to be eaten. This ability to modulate development in the presence of predators is called predator-induced defense, or **predator-induced polyphenism**.

To demonstrate predator-induced polyphenism, one has to show that the phenotypic modification is caused by the presence of the predator. In addition, many investigators say, the modification should increase the

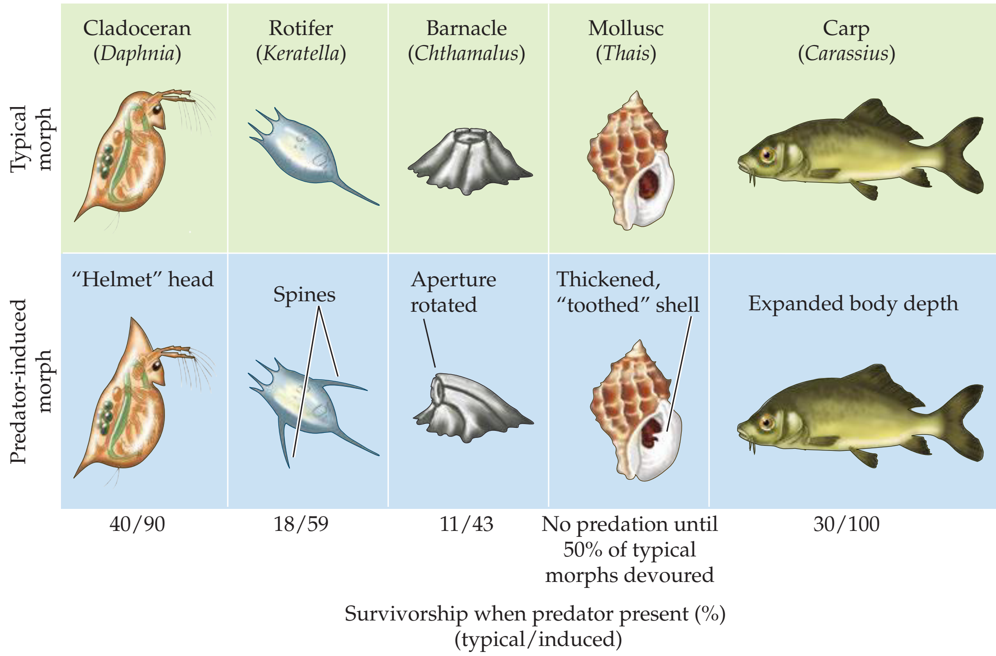


Figure 1.15 Predator-induced defenses. Typical (upper row) and predator-induced (lower row) morphs of various organisms are shown. The numbers beneath each column represent the percentages of organisms surviving predation when both induced and uninduced individuals were presented with predators (in various assays). (Data from Adler and Harvell 1990 and references cited therein.)

fitness of its bearers when the predator is present (see Adler and Harvell 1990; Tollrian and Harvell 1999). Figure 1.15 shows both the typical and predator-induced morphs for several species. In each case, the induced morph is more successful at surviving the predator, and soluble filtrate from water surrounding the predator is able to induce the changes. The chemicals that are released by a predator and that induce defenses in its prey are called **kairomones**.

One important concept to remember is that, as with the larger horns of male dung beetles mentioned earlier, there is usually a trade-off. That is, the energy and material used to produce the adaptation to the predator often come at the expense of making other organs or cells. Thus, what is adaptive in one environment is less adaptive in another. *Daphnia*, for instance, make a spiked "helmet" in the presence of kairomones (Figure 1.16). However, helmeted *Daphnia* individuals produce fewer eggs than their smaller counterparts. Similarly, tadpoles that develop quickly in order to escape predators are usually less robust than those that take the full time developing.

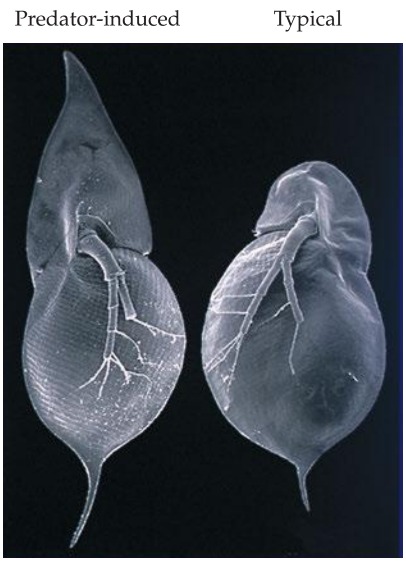


Figure 1.16 Scanning electron micrographs showing predator-induced and typical morphs of genetically identical individuals of the water flea genus *Daphnia*. (Photographs by C. Laforsch and R. Tollrian, courtesy of A. A. Agrawal.)

Predator-induced polyphenism in invertebrates

Several rotifer species will alter their morphology when they develop in pond water in which their predators were cultured (Dodson 1989; Adler and Harvell 1990). The predatory rotifer *Asplanchna* releases a soluble compound that induces the eggs of a prey rotifer species, *Keratella slacki*, to develop into individuals with slightly larger bodies and anterior spines 130% longer than they otherwise would be (see Figure 1.15), making the prey more difficult to eat. Also shown in Figure 1.15, the snail *Thais lamellosa* develops a thickened shell and a "tooth" in its aperture when exposed to water that once contained the crab species that preys on it. In a mixed snail population, crabs will not attack the thicker snails until more than half of the typical-morph snails are devoured (Palmer 1985).

When parthenogenetic water fleas (*Daphnia cucullata*) encounter the predatory larvae of the fly *Chaoborus*, the heads of the *Daphnia* grow to twice the normal size, becoming long and helmet shaped (see Figure 1.16). This increase in size lessens the chances that *Daphnia* will be eaten by the fly larvae. This same helmet induction occurs if the *Daphnia* are exposed to extracts of water in which the fly larvae have been swimming. Further, the predator-induced polyphenism of the *Daphnia* is beneficial not only to itself, but also to its offspring. Agrawal and colleagues (1999) have shown that the offspring of such induced *Daphnia* are born with this same altered head morphology. It is possible that the *Chaoborus* kairomone regulates gene expression both in the adult and in the developing embryo.

Predator-induced polyphenism in vertebrates

Predator-induced polyphenism is not limited to invertebrates. Indeed, predator-induced polyphenisms are abundant among amphibians. Tadpoles found in ponds or reared in the presence of other species may differ significantly from tadpoles reared by themselves in aquaria. For instance, newly hatched wood frog tadpoles (*Rana sylvatica*) reared in tanks containing the predatory larval dragonfly *Anax* (confined in mesh cages so that they cannot eat the tadpoles) grow smaller than those reared in similar tanks without predators. Moreover, as with the *Hyla* species shown in Figure 1.1A, the wood frog tadpoles' tail musculature deepens, allowing faster turning and swimming speeds (Van Buskirk and Relyea 1998). The addition of more predators to the tank causes a continuously deeper tail fin and tail musculature, and in fact what initially appeared to be a polyphenism may be a reaction norm that responds to the number (and type) of predators. In some species, phenotypic plasticity is even reversible, and removing the predators can restore the original phenotype (Relyea 2003a).

Predator-induced defensive reactions in some other frogs involve responding to specific vibrational cues produced by predators. The embryos of the Costa Rican red-eyed tree frog (*Agalychnis callidryas*) use vibrations transmitted through the egg mass to escape egg-eating snakes. These egg masses are laid on leaves that overhang ponds. The embryos usually develop into tadpoles within 7 days, at which time the tadpoles wiggle out of the eggs and fall into the pond. However, when snakes attempt to feed on the frog eggs (Figure 1.17A), the vibrations from the snakes' movements cue any embryos remaining inside the eggs to begin the twitching

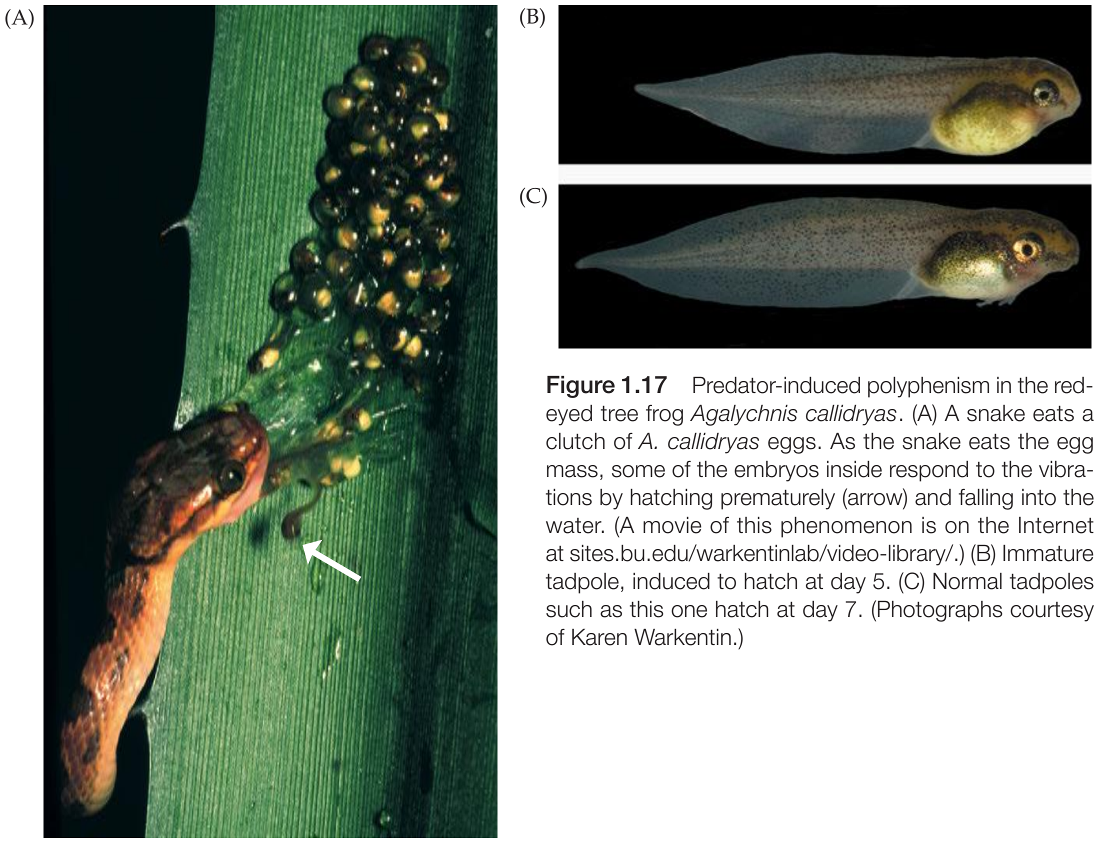


Figure 1.17 Predator-induced polyphenism in the red-eyed tree frog *Agalychnis callidryas*. (A) A snake eats a clutch of *A. callidryas* eggs. As the snake eats the egg mass, some of the embryos inside respond to the vibrations by hatching prematurely (arrow) and falling into the water. (A movie of this phenomenon is on the Internet at sites.bu.edu/warkentinlab/video-library/.) (B) Immature tadpole, induced to hatch at day 5. (C) Normal tadpoles such as this one hatch at day 7. (Photographs courtesy of Karen Warkentin.)

movements that initiate their hatching (within seconds!), and they drop into the pond, escaping the snakes. Embryos are competent to begin these hatching movements as early as day 5 (Figure 1.17B). Interestingly, the frog embryos respond this way only to vibrations given at a certain frequency and interval, and research has shown that these vibrations alone (and not sight or smell) cue the hatching movements (Warkentin 2005; Warkentin et al. 2006). Up to 80% of the remaining embryos can escape snake predation in this way. However, though these embryos escape their snake predators, they are at greater risk from water-borne predators than are "full-term" embryos, since the musculature of the early hatchers has not fully developed (Figure 1.17C).

The Presence of Conspecifics: It's Who You Know

Cues to change phenotype can come not only from predators but also from conspecifics (organisms of the same species); an individual in a large population can have a markedly different phenotype from that of an individual of the same species that is solitary. As mentioned above, the presence of predators induces wood frog (*Rana sylvatica*) tadpoles to develop thicker trunk muscles and shorter bodies. In contrast, when the tadpoles are raised together in high population density (in the absence of predators), development is slowed down, resulting in shallower tails and longer bodies relative to those raised in isolation. Thus, predation results in the development of short, muscular tadpoles, while competition from conspecifics results in long, sleek tadpoles (Relyea 2004). A developing tadpole apparently integrates competing signals from predators and conspecifics to produce a body shape that will optimize its performance.

A swarm of locusts: Polyphenism through touch

Crowding among conspecifics can produce remarkably different phenotypes; this phenomenon is especially obvious in migratory desert locusts, *Schistocerca gregaria*. In this species, mechanoreceptors are responsible for the induction of the crowding phenotype. Locusts in the low-density "solitary" phase are usually green, have short wings and large abdomens, and avoid each other. However, when forced to crowd together (as in small areas of patchy food), they actively aggregate to form a high-density "gregarious" or "migratory" phase. Individuals change their behavior (from avoidance to attraction), change their color (from green to brown, black, and orange), and molt into adults with longer wings and more slender abdomens (see Figure 1.3). These profound changes in color and behavior can be accomplished by subjecting solitary locust nymphs to buffeting with small paper mache balls (Roessingh et al. 1998).

The behavioral phase of the phenotypic change is mediated by direct physical contact among locusts, and the major sites of this mechanosensory input are the femurs of the hind legs. Repeatedly touching a minute region of the outer surface area of a hind femur with a fine paintbrush produces full behavioral gregarization within 4 hours (Simpson et al. 2001; Rogers et al. 2003). The colorization of the gregarious phenotype, however, may come from different cues. The smell of other locusts appears able to induce dark coloration in solitary nymphs by inducing the secretion of the neuropeptide hormone corazonin (Lester et al. 2005).

The green coloration of the solitary stage blends in with the background, making the grasshopper harder for predators to see. In contrast, the black-and-orange pattern on the gregarious locusts functions as a warning, telling potential predators that these nymphs have been feeding on toxicity-conferring plants. Moreover, gregariousness is thought to enhance the efficiency of this protective coloration. Thus the cryptic (hiding) coloration of the solitary morph and the aposematic (warning) coloration of the gregarious morph both serve as predator-avoidance strategies (Sword et al. 2000).

As in so many polyphenisms, a suite of behavioral practices are intimately connected with the morphological changes. In this locust, the changes associated with predator avoidance can be observed prior to the morphological changes. Within a few hours of crowding (and before molting), the solitary nymphs that are to change their phenotype start eating plants that had been distasteful previously. The plant-derived chemicals are responsible for make the migrating locusts unpalatable to their predators (Roessingh et al. 1993; Simões et al. 2013).

Sexual polyphenism induced by the community environment

Fish of many species change their sex based on social interactions; the blue-headed wrasse described at the start of the chapter is one good example (see Figure 1.1C). Marine goby fish are among the few fishes that can change their sex more than once—and in either direction. A female goby can become male if the male of the group dies. However, if a larger male enters the group, such males revert to being female (Black et al. 2005). Grober and Sunobe (1996) induced females to become males, males to become females, and females to become males and then females again, merely by changing their companions. A goby can change its sex in about 4 days.

In both the goby and the blue-headed wrasse, the shift of sex is mediated by hormonal changes caused by the environmental conditions. Interestingly, the sex changes in behaviors take place within hours, whereas the female-to-male color changes and gamete production take about a week to complete. The male behavioral changes may arise so quickly from the inactivation of the aromatase gene in certain hypothalamic neurons in the brain. This would enable testosterone to accumulate in these neurons and would prevent the synthesis of new estrogens. Indeed, the addition of estrogen implants into the brains of these fish prevents the female-to-male transition (and raises the levels of aromatase). These neurons are thought to mediate the sex-specific competitive and mating behaviors (Godwin et al. 2003; Perry and Grober 2003; Kroon et al. 2005; Marsh-Hunkin et al. 2013). The gonadal and color changes involved in such sex reversals also seem to be mediated by estrogens, wherein the gonadal aromatases are inhibited and elevated serum estrogen levels are seen. At this point, the ovaries start forming testes and sperm (and estrogen implants into the ovaries can also block these changes) (Horiguchi et al. 2013).

Cannibalism: An extreme phenotype for extreme times

One of the most impressive of these stress-induced phenotypes is cannibalism in some spadefoot toads of the genus *Spea*. These amphibians have a remarkable strategy for coping with a very harsh environment. The toads are called out of hibernation by the thunder that accompanies the first spring storms in Arizona's Sonoran Desert. (Unfortunately, motorcycles produce much the same sound, causing the toads to come out of hibernation only to die in the scorching sun.) The toads breed in temporary ponds formed by the rain, and the embryos develop quickly into larvae. After the larvae metamorphose into toads, the young toads burrow into the sand until the next year's storms bring them out.

Desert ponds are ephemeral pools that can either dry up quickly or persist, depending on the initial depth and the frequency of the rainfall. One might envision two alternative scenarios confronting a tadpole in such a pond: (1) the pond persists until tadpoles have time to metamorphose, and they live; or (2) the pond dries up before the tadpoles' metamorphosis is complete, and they die. *Spea*, however, has evolved a third alternative. The timing of its metamorphosis is controlled by the pond. If the pond persists at a viable level, development continues at its normal rate, and the algae-eating tadpoles develop into juvenile toads. However, if the pond is

Polyphenisms and Conservation Biology

Phenotypic plasticity means that animals in the wild may develop differently than those in the laboratory. Embryos and larvae in the wild develop in the presence of particular plants, predators, and conspecifics, and they experience variations of temperature and day length. In contrast, animals developing in a laboratory are usually grown in a monoculture of conspecific organisms, under a single particular temperature regime. This has important consequences when we apply knowledge gained in the laboratory to a field science such as conservation biology.

For instance, the metabolism of predator-induced morphs may differ significantly from that of the uninduced morphs, and this phenomenon has important consequences. Relyea (2003b, 2004) has found that in the presence of the chemical cues emitted by predators, pesticides such as carbaryl (Sevin®) can become up to 46 times more lethal than they are without the predator cues. Bullfrog and green frog tadpoles were especially sensitive to carbaryl when they were exposed simultaneously to predator chemicals. Relyea (2003b) has related these findings to the global decline of amphibian populations, saying that governments should test the toxicity of the chemicals under natural conditions, including that of predator stress. He concluded that “ignoring the relevant ecology can cause incorrect estimates of a pesticide’s lethality in nature, yet it is the lethality of pesticides under natural conditions that is of utmost interest.”

Temperature-dependent polyphenisms are also important for conservation biology and are likely to become more so with global warming. The significance of thermal polyphenisms was highlighted by Morreale and colleagues (1982) in a paper documenting temperature-dependent sex determination in a range of sea turtle species. Prior to that time, conservation biologists interested in restoring sea turtle populations had been growing the eggs in laboratory incubators set at a certain temperature, or culturing them in a single area of a beach. But these practices result in turtles of only one sex. Thus, Morreale and colleagues concluded that “current practices threaten conservation of sea turtles” rather than enhance them. They suggested protecting existing nests from predators, thereby maintaining the normal sex ratio.

Developmental plasticity also allows invasive species to alter their development to enable them to consume new prey (Bernays 1986; Kishida et al. 2006). The ability of such predators to display such plasticity may be a critically important factor in their success or failure to expand their ranges (Baldridge and Smith 2008).

One of the most interesting types of polyphenisms involves larval cues for metamorphosis. Environmental cues are critical to metamorphosis in many species, and some of the best-studied examples are the settlement cues used by marine larvae. A free-swimming marine larva often needs to settle near a source of food or on a firm substrate on which it can metamorphose. If prey, conspecifics, or substrates give off soluble molecules, these molecules can be used by the larvae as cues to settle and begin metamorphosis. Among the mollusks, there are often very specific cues for settlement (Hadfield 1977). In some cases, the prey supply the cues, while in other cases the substrate itself gives off molecules used by the larvae to initiate settlement. These cues may not be constant, but they need to be part of the environment if normal development is to occur (Pechenik et al. 1998).

The importance of substrates for larval settlement and metamorphosis was demonstrated in 1880 when William Keith Brooks, an embryologist at Johns Hopkins University, was asked to help the ailing oyster industry of Chesapeake Bay. For decades, oysters had been dredged from the bay, and there had always been a new crop to take their place. But suddenly, each year brought fewer oysters. What was responsible for the decline? Experimenting with larval oysters, Brooks discovered that the American oyster (*Crassostrea virginica*) requires a hard substrate on which to metamorphose. For years, oystermen had simply thrown the mollusks’ shells back into the water, but with the advent of suburban sidewalks, they began selling the shells to cement factories. Brooks’s solution: go back to returning the oyster shells to the bay. The oyster population responded, and Baltimore wharves still sell their descendants.

Knowledge of phenotypic plasticity is critical in conservation biology and is necessary for making informed decisions that will benefit the environment. We will revisit this theme several times in this book. For more information on plasticity and conservation biology, see the video “Race for Survival” (Baressi et al. 2013).

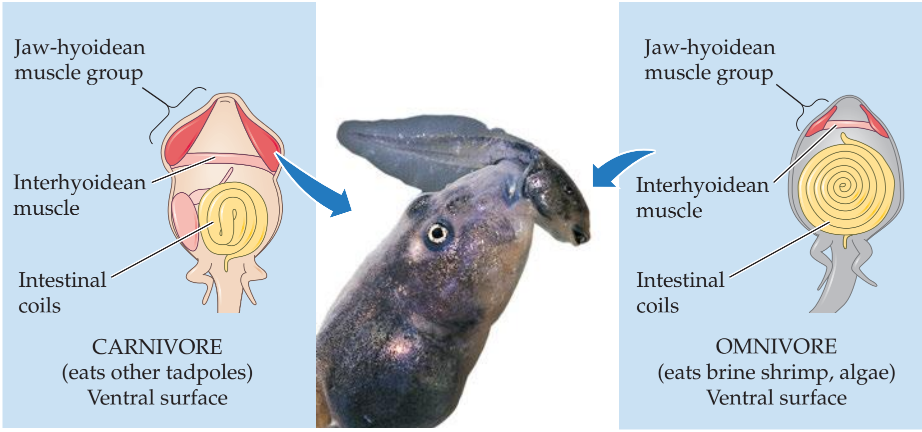


Figure 1.18 Polyphenism in tadpoles of a spadefoot toad (*Spea*). The typical morph (right) is an omnivore, feeding on insects and algae. When ponds are drying out quickly, however, a carnivorous (cannibalistic) morph forms. It develops a wider mouth, larger jaw muscles, and an intestine modified for a carnivorous diet. The center photograph shows a cannibalistic tadpole eating a smaller pondmate. (Photograph © Thomas Wiewandt; drawings courtesy of R. Ruibel.)

drying out and getting smaller, some of the tadpoles embark on an alternative developmental pathway. They develop a wider mouth and powerful jaw muscles, which enable them to eat (among other things) other *Spea* tadpoles (Figure 1.18). These carnivorous tadpoles metamorphose quickly, albeit into a smaller version of the juvenile spadefoot toad. But they survive while other *Spea* tadpoles perish from desiccation (Newman 1989, 1992; Denver 1997).

Convergence on Favorable Phenotypes

One principle of environmentally induced polyphenisms worth stressing is the fact that *different* environmental cues can produce the same favorable phenotype. For instance, the helmet-and-spiked-tailed *Daphnia* morph can be induced by different predators, and the chemical signals eliciting this phenotype are probably different. The water conditioned by dragonfly larvae can induce this phenotype, but so can chemicals released by dead *Daphnia* individuals being digested inside a fish's gut (Stabell et al. 2003). Similarly, the hatch-early-into-the-pond behavior of the red-eyed tree frog can be induced not only by snake vibrations, but also by wasp predation and by fungal infection (Warkentin 2000; Warkentin et al. 2001). The gregarious phase change of desert locusts can be induced by mechanostimulation of the hindlimb neurons (as described on p. 29), but it can also take place in the presence of a combination of visual and olfactory stimuli. In these developing locusts, either cause will induce a rise in the levels of serotonin in the thoracic ganglia of solitary individuals and the subsequent components of the phase change. Drugs that block the action or synthesis of serotonin will prevent the phase change in both cases (Simpson and Sword 2009; Anstey et al. 2009).

Looking Ahead

Plasticity had been considered as an exception to the rule, something seen in odd critters such as *Daphnia*. Now, plasticity is seen as the norm, something common to all animals. Environment is therefore considered to play a role in the generation of phenotypes, in addition to its well-established role in the natural selection of which phenotypes will survive and reproduce. This opens up numerous questions concerning both health and evolution, and we will be dealing with these throughout the book.

But one of the major questions concerns the mechanisms by which several plastic traits in an organism are regulated. This is especially important when looking at morphs differing in both *anatomical* and *behavioral* traits. For instance, the polyphenisms in the male dung beetle involve not only the presence or absence of horns, but also the type of behavior males can express—fighter or sneaker. It seems that the different components of the dung beetle response to diet is controlled by different sets of genes (Snell-Rood et al. 2011). Similarly, wood frog tadpoles not only develop tails of have different shapes and colors, depending on the sensing of predators; their behavior also changes. The first component of the sex change in fish and the gregarious phenotype in locusts involves their new behaviors. Indeed, behavioral plasticity may be more common than we thought. According to John Dupre (personal comm.), plants exhibit their plasticity through morphological change, while animals may manifest their plasticity primarily through behaviors and the morphological structures that enable them. The ability of organisms to be plastic in both their behaviors and their anatomies opens up important areas of study that will be critically important for studying evolution and psychological diseases. Much of the remainder of the book will concern the ramifications of plasticity for disease and evolution.

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