Bio351
Group Project 1:

Bitter taste perception

places. Environmental variation arises when external factors influence how much protein is made from particular genes, or how the proteins work. When individuals experience different environments, they make different amounts of proteins or show differences in protein function. Genotype-by-environment interaction is the result of differences among individuals that are encoded into their DNA and that make them differ in their sensitivity to environmental influences. Different individuals thus react differently to a changing environment.

We will illustrate these generalizations with examples.

Genetic Variation

Humans show considerable variation in their perception of taste. One way to demonstrate this variation is to offer people small quantities of the chemical phenylthiocarbamide (PTC). Some individuals find it intensely bitter and unpleasant; others can scarcely taste it at all (Wooding 2006).

An experience of taste begins at a taste receptor protein in the surface membrane of a taste receptor cell in a taste bud on the tongue (Yarmolinsky et al. 2009). Taste receptor proteins have diverse shapes and chemical properties. Each kind of taste receptor protein binds with a subset of the chemicals in food, corresponding to sweet, sour, salty, umami (savory), and bitter flavors **(Figure 5.6a)**. When the right chemical binds to the receptors on its membrane, a taste receptor cell sends nerve impulses to the brain. The brain integrates messages from taste receptors across the tongue and generates a conscious sensation of flavor.

The receptor proteins responsible for bitter flavors are the type 2 taste receptors (TAS2Rs). The one that binds PTC is TAS2R38, encoded by a gene on chromosome 7 (see Figure 5.5). The coding region of the gene specifies a sequence of 333 amino acids. Un-kyung Kim and colleagues (2003) examined the TAS2R38 genes of a number of individuals. They found three places where different versions of the gene encode different amino acids. The 49th codon in the gene may specify either proline or alanine, the 262nd codon may specify either alanine or valine, and the 296th codon either valine or isoleucine. Different versions of a gene are called **alleles**. The most common TAS2R38 alleles, named for the amino acids they specify at the variable sites, are *AVI* and *PAV*.

Everyone has two chromosome 7s: one inherited from their mother, the other from their father. The two chromosomes may carry the same allele of the TAS2R38 gene, or they may carry different alleles. The combination of alleles an individual carries is called his or her **genotype**. Considering just alleles *AVI* and *PAV*, the three possible genotypes are *AVI/AVI*, *AVI/PAV*, and *PAV/PAV*.

The suite of traits an individual exhibits is called his or her **phenotype**. The aspect of phenotype we are interested in here is sense of taste. To show that TAS2R38 genotype influences sensory phenotype, Richard Newcomb and colleagues (2012) asked people with different genotypes to taste a standard PTC solution and rate the intensity of the flavor. Figure 5.6b presents the results. There is variation among the subjects plotted on each graph, showing that factors other than TAS2R38 genotype influence an individual's sensitivity to PTC. But TAS2R38 genotype clearly matters (see also Bufe et al. 2005). Individuals with genotype *PAV/PAV* are most sensitive, those with *AVI/AVI* are least sensitive, and those with *AVI/PAV* fall in between. Switching just 3 of the 333 amino acids in TAS2R38 changes the protein's shape and/or chemical properties enough to alter either the protein's ability to bind PTC (Figure 5.6c), its ability to trigger a nerve impulse in response to binding, or both (see Biarnés et al. 2010).

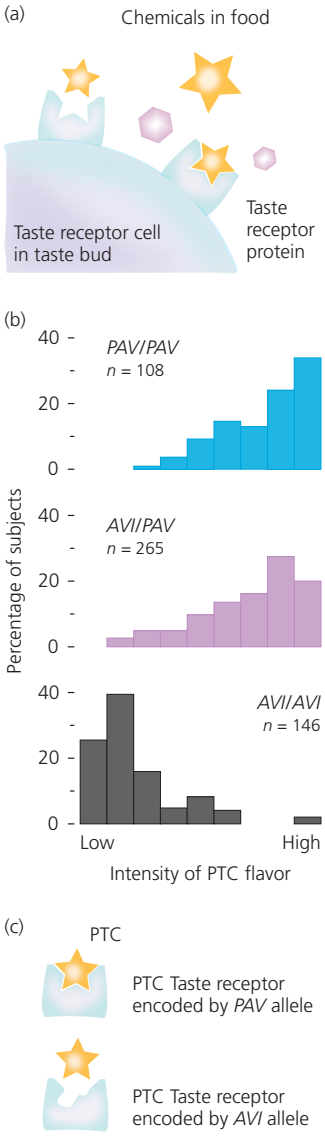


Figure 5.6 Genetic variation for bitter taste perception
(a) Taste receptors bind chemicals in food. (b) PTC tastes different to people with different versions of TAS2R38, perhaps because (c) the version encoded by allele *PAV* binds PTC more strongly. Graphs from Newcomb et al. (2012).

PTC does not naturally occur in food. The ability to taste it might seem unimportant. However, different versions of TAS2R38 also respond differently to other bitter flavors (Sandell and Breslin 2006). Among these flavors are chemicals found in broccoli and its relatives, including mustard greens, turnips, and horseradish. People with genotype *PAV/PAV* rate these vegetables as more bitter than do people with genotype *AVI/AVI*. There is some evidence that *AVI/AVI* individuals eat more vegetables than individuals with other genotypes (Tepper 2008; Duffy et al. 2010; but see Gorovic et al. 2011).

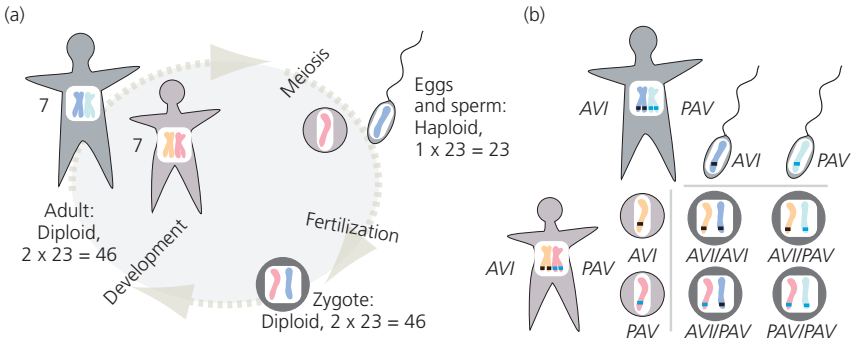


Figure 5.7 The human life cycle (a) We spend most of our lives in a diploid phase, carrying a complete set of chromosomes received via the egg and a complete set received via the sperm. Each gamete we make receives one member of each chromosome pair. (b) We can thus use a Punnett square to calculate the odds that a zygote will have a particular genotype.

To the extent that differences among individuals in the ability to taste bitter flavors are due to differences in genotype, they are transmitted from parents to offspring. Figure 5.7a shows the human life cycle. The figure does the bookkeeping for chromosome counts, highlighting chromosome 7 as an example. For most of our life cycle, our cells carry two chromosome 7s. When we make gametes, each egg or sperm receives a copy, selected at random, of one chromosome 7 or the other. When egg and sperm unite, they yield a zygote that once again has two chromosome 7s. If the gametes were produced by parents who both carried allele *AVI* on one chromosome 7 and allele *PAV* on the other, then all three genotypes are possible among the offspring (Figure 5.7b). In a large sample of offspring, we expect the genotypes to occur in a 1:2:1 ratio.

Genetic variation consists of differences among individuals that are encoded in the genome and transmitted from parents to offspring.

Given that the *PAV* allele tends to make people who carry it dislike broccoli and related vegetables, and that eating vegetables is good for one's health, we might expect that individuals with allele *PAV* would be less likely to survive and reproduce, and that the allele would disappear. Consider, however, that vegetables contain natural toxins—an adaptive trait that discourages animals from eating them. Consuming a healthy diet thus requires balancing one's intake of nutritious plants against one's ingestion of the toxins they contain, some of which taste bitter. That alleles *PAV* and *AVI* are both common in human populations all over the world suggests that historically the best genotype for survival and reproduction has been *AVI/PAV* (Wooding et al. 2004).

Stephen Wooding (2005, 2006) speculates that although the version of the PTC receptor encoded by allele *AVI* is less sensitive to the toxins in broccoli and its kin, it is perhaps more sensitive to toxins found in other plants. If this is so, then individuals with genotype *AVI/PAV* would be able to detect a wider variety of toxins in their food than individuals with either genotype *AVI/AVI* or *PAV/PAV*. Such individuals might have an advantage in seeking a nutritious diet that avoids an overdose of any particular plant toxin. Note that this is a hypothesis, not an established fact. It will have to be tested with careful experiments.

Bio351
Group Project 2:

Inducible defenses in *Daphnia*

Genetic Variation and Evolution

We have already discussed other examples of genetic variation. We have looked at genetic variation among HIV virions in their susceptibility to the antiretroviral drug AZT as well as genetic variation among humans in the susceptibility to HIV infection. We have considered genetic variation among sticklebacks in the extent of body armor and genetic variation in fruit size in tomatoes. We will see many more examples throughout the book.

We have also discussed the role of genetic variation in evolution. Because genes are passed from parents to offspring, genetic variants associated with higher survival and reproductive success automatically become more common in populations over time, while variants associated with untimely death and reproductive failure disappear. Genetic variation is the raw material for evolution.

But there is more to the story of variation and evolution. That is why we now turn to environmental variation.

Environmental Variation

Our example of environmental variation concerns a prey species, the water flea, and its predator, a larval insect. The fossil record shows that phantom midges have been eating water fleas for 145 million years (Kotov and Taylor 2011).

Water fleas are tiny freshwater crustaceans that inhabit lakes and ponds all over the world (Lampert 2011). Among the traits that make water fleas useful for the study of environmental variation is that when conditions are auspicious they reproduce by cloning, switching to sexual reproduction only when conditions deteriorate. Also useful is that certain environmental cues trigger changes in their morphology, physiology, and behavior. Together these characteristics make it possible for researchers to expose genetically identical water fleas to different cues and get a pure look at how changes in the environment influence phenotype.

The water flea *Daphnia pulex* suffers substantial predation by phantom midge larvae, but only at certain times and places. *Daphnia pulex* is capable of developing a morphology that is well defended against phantom midges **(Figure 5.8a)**. It can nearly double the strength and thickness of its carapace and grow ridges, called neckteeth, on the back of its head (Laforsch et al. 2004). These defenses are costly, however (Hammill et al. 2008). Apparently as a result, *D. pulex* has evolved the capacity to grow anti-midge armor only when it smells midges. The water fleas in Figure 5.8 look different not because they carry different genes, but because they have been exposed to different chemical environments.

The chemical the water flea can detect emanating from phantom midges remains to be identified. Biologists refer to it by the generic term *kairomone*. *Daphnia pulex*'s growth of armor in response to phantom midge kairomone is an example of an **inducible defense**.

Hitoshi Miyakawa and colleagues (2010) suspected that to grow anti-midge armor, a water flea has to boost its production of a variety of proteins involved in development. The researchers exposed *Daphnia pulex* to kairomone from the phantom midge *Chaoborus flavicans* and compared them to genetically identical unexposed individuals. The researchers looked at the production, or **expression**, of dozens of candidate proteins they had reason to think, from earlier research, might play a role in *Daphnia*'s inducible defenses. They measured protein production indirectly, by quantifying the production of messenger RNA, the molecular intermediary that carries genetic information from the DNA in the nucleus to the ribosomes in the cytoplasm where proteins are built.

Environmental variation consists of differences among individuals due to exposure to different environments. One way environments can influence phenotype is by altering gene expression.

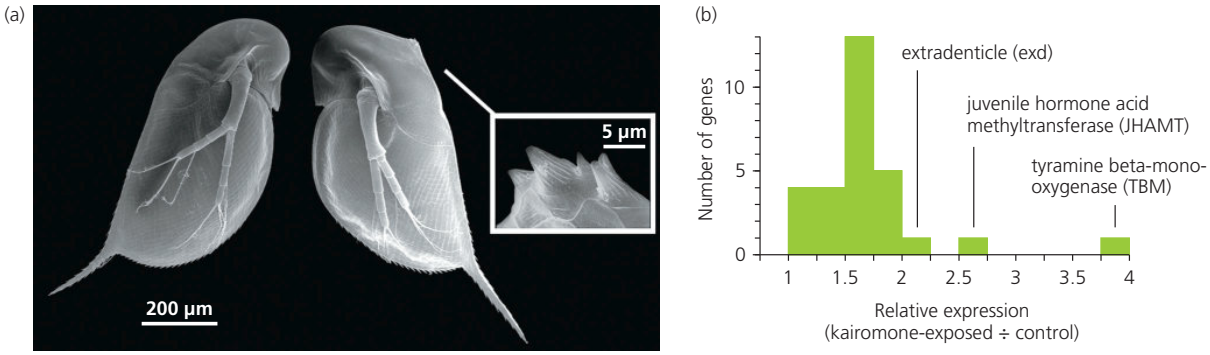


Figure 5.8 Inducible defenses in *Daphnia* (a) Juveniles that smell phantom midge larvae grow neck teeth and other defenses. Photo by Christian Laforsch. (b) This involves boosting production of many proteins. From data in Miyakawa et al. (2010).

The graph in Figure 5.8b shows the expression of 29 candidate genes in kairomone-exposed *D. pulex* relative to their expression in unexposed individuals. In every case, the exposed water fleas made more messenger RNA and thus presumably more protein. The proteins with the largest increase in expression upon exposure to kairomone were extradenticle (exd), juvenile hormone acid methyltransferase (JHAMT), and tyramine beta-monooxygenase (TBM). Exd acts during development to influence the identity of appendages in arthropods. JHAMT is an enzyme required for the synthesis of juvenile hormone, a major regulator of arthropod development. TBM is an enzyme that catalyzes the synthesis of neurotransmitters, chemicals used by nerve cells to send messages to each other.

Many details remain to be discovered, but Miyakawa's results show that the mechanism by which *D. pulex* changes its phenotype when it smells phantom midges involves changes in the production of a variety of proteins.

Environmental Variation and Evolution

Many other organisms alter the identity or quantity of the proteins they make in response to changes in the environment, thereby altering their phenotype. Human athletes living at low altitude, but training at simulated high altitude, produce more vascular endothelial growth factor (VEGF) than athletes living and training at low altitude (Hoppeler and Vogt 2001). The extra VEGF stimulates the growth of capillaries in the muscles. Environmental variation is ubiquitous.

The non-genetic influences on protein expression, and thus phenotype, even include chance. The *Escherichia coli* bacteria in Figure 5.9 are genetically identical. Michael Elowitz and colleagues (2002) inserted into the DNA of their common ancestor two copies of the gene for green fluorescent protein (GFP). The two copies encode distinct variants of GFP that emit different colors of light when they fluoresce. They are controlled by identical promoters—the switches that turn genes on or off. A bacterium making equal amounts of both versions of GFP would be yellow, a cell making more of one version would be green, and a cell making more of the other version would be orange. The explanation for the diversity of colors in the photo is random variation in the interactions between the promoters and the regulatory proteins that activate and deactivate them.

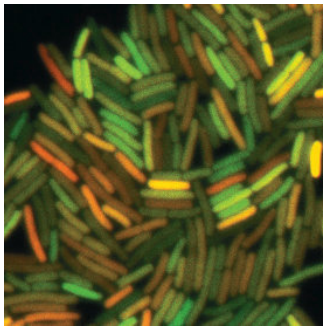
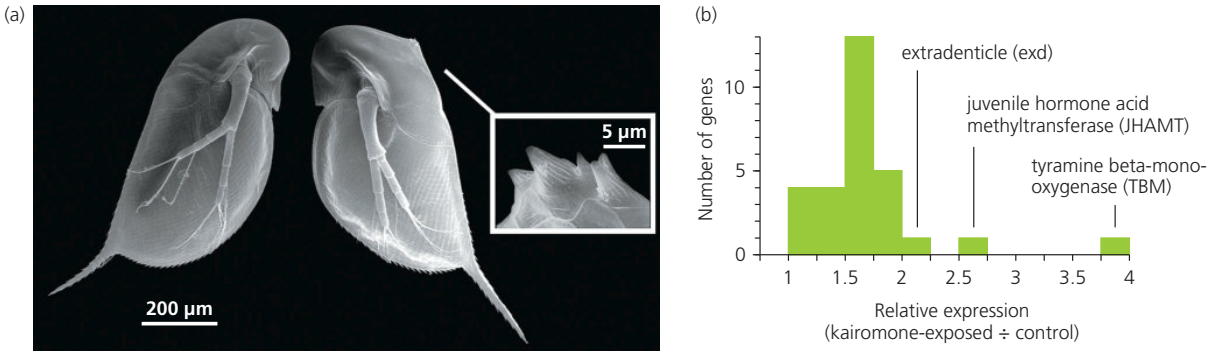


Figure 5.9 Random variation in protein production in genetically identical bacteria These cells are different colors because they are making different amounts of two fluorescent proteins. From Elowitz et al. (2002).

Despite its ubiquity, environmental variation supplies no raw material for evolution. This is because environmentally induced changes in phenotype are not transmitted to future generations. Whether a water flea born by clonal reproduction has neckteeth is determined not by the genes she inherits, but by the

Bio351
Group Project 3:

Random variation in protein production



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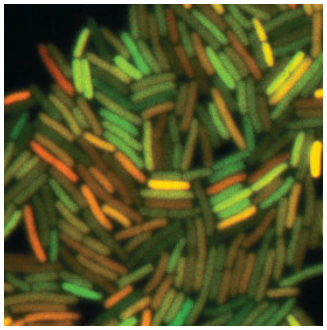

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Bio351
Group Project 4:

A heritable epigenetic variant

COMPUTING CONSEQUENCES 5.1

Epigenetic inheritance and evolution

Under Darwin's theory of evolution by natural selection, populations change from one generation to the next if there is particulate—that is, non-blending—inheritance of variable traits associated with fitness. There are examples of environmental factors that induce phenotypic changes subsequently transmitted to offspring (Cropley et al 2006; Li et al. 2011). Could such environmental variation provide raw material for evolution?

One mechanism of non-genetic inheritance involves the chemical modification—attachment of a methyl group—of cytosine nucleotides in DNA (Richards 2006). These and other modifications, sometimes referred to as **epigenetic marks**, are managed by enzymes encoded in the genome and can influence phenotype by altering gene expression. Epigenetic marks may be transmitted from parent to offspring because they are copied during DNA replication or even because they trigger behavior by parents that provokes their fresh establishment in offspring (Danchin et al. 2011). In some cases, including that of the toadflax variant shown in Figure 5.10, epigenetic marks can be propagated for many generations (Cubas et al. 1999; Johannes et al. 2009). Researchers working with bacteria and plants have found evidence that a few generations of selection on populations in which most of the variation is epigenetic, rather than genetic, can produce lineages with measurably distinct phenotypes (Adam et al. 2008; Hauben et al. 2009). Together these facts suggest that environmentally induced epigenetic differences could, in principle, serve as raw material for evolution.

This suggestion is tempered by consideration of the crucial functions epigenetic marks serve in organisms. There are at least three (Feng et al. 2010; Shea et al. 2011). Epigenetic marks silence transposons, integrated viruses, and other genomic parasites. They allow individuals to communicate to their offspring, and sometimes their grand-offspring, useful information about the state of the environment they are likely to encounter (Whittle et al. 2009; Scoville et al. 2011). And in multicellular organisms, they facilitate and maintain cell differentiation. Nicholas Shea and colleagues (2011) point out that the latter two functions require that epigenetic marks be periodically reset. This may explain why many epigenetic marks induced by environmental factors appear to be reprogrammed at some point in the life cycle or to decay over several generations (Feng et al. 2010; Paszkowski and Grossniklaus 2011; Shea et al. 2011). The impermanence of most epigenetic marks precludes a substantial contribution by epigenetic variation to long-term evolution (Slatkin 2009).

(a) Typical toadflax (b) Radially symmetrical variant

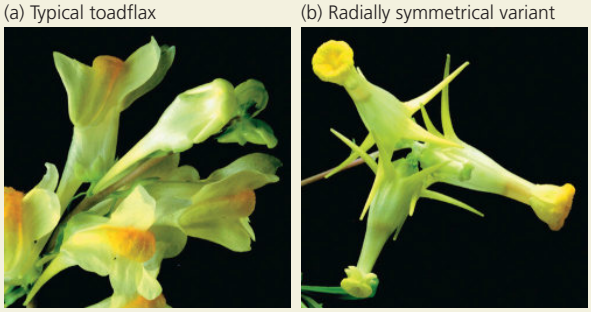

Figure 5.10 A heritable epigenetic variant (a) Typical flowers of common toadflax (*Linaria vulgaris*). (b) Radially symmetrical flowers from a plant of the same species. This variant was described by Linnaeus over 250 years ago and has been inherited since (Paun et al. 2010). It is caused by epigenetic marks that prevent expression of a gene called *Lcyc* (Cubas et al. 1999). From Grant-Downton and Dickinson (2006).

presence or absence of kairomones. (For exceptions to this general rule, and consideration of their implications, see **Computing Consequences 5.1**).

This is not to say that how the relationship between genotype and phenotype is altered by the environment is irrelevant to descent with modification. Indeed, as Theodosius Dobzhansky pointed out in 1937, "Selection deals not with the genotype as such, but with its dynamic properties, its reaction norm, which is the sole criterion of fitness in the struggle for existence." To understand this claim, we turn to genotype-by-environment interaction.

Bio351
Group Project 5:

Sex determination in leopard geckos

Genotype-by-Environment Interaction

We will start with another example of environmental variation, this time in the leopard gecko (Figure 5.11a). In leopard geckos, as in many other reptiles (see Bull 1980), an individual's sex is determined largely by the temperature at which it incubates while developing in the egg. Leopard geckos that develop at cool or hot temperatures become female, whereas those that grow at intermediate temperatures tend to be male (Figure 5.11b). Development along the female versus the male pathway involves changes in the production of a variety of proteins (Shoemaker and Crews 2009). For example, expression of the gene *Sox9* in the gonad ceases earlier in leopard geckos developing as females than as males (Valleley et al. 2001). *Sox9* encodes a transcription factor that directs the expression of other genes and thus commits the gonad to being a testis versus an ovary.

Turk Rhen and colleagues (2011) wanted to know whether leopard geckos harbor genetic variation in the threshold temperatures for developing as female versus male. Analyzing data from 13 generations of geckos maintained in a breeding colony, the researchers compared the sex ratios among offspring that shared a father and hatched from eggs that had incubated at different temperatures.

Each of the dozen lines in Figure 5.11c represents the offspring of a particular father who had anywhere from 7 to 27 offspring reared at each temperature. Some offspring were full siblings and others were half sibs (with different mothers). The ends of each line show the sex ratio among the offspring reared at each temperature. Analyzing offspring with the same father but a variety of mothers allowed the researchers to statistically factor out variation due to non-genetic influences (called maternal effects) mothers might have had on their offspring via hormones, proteins, or messenger RNAs they might have placed in the eggs.

Note the variation among paternal families in the effect of temperature on sex ratio. For some families, such as the one highlighted in green, incubation at 30°C versus 32.5°C had little effect on sex ratio. For other families, such as the one in orange, incubation at different temperatures had a dramatic effect. The pattern of phenotypes an individual may develop upon exposure to different environments is called its **reaction norm**. The reaction norms in Figure 5.11c, and data on the

Genotype-by-environment interactions consists of differences among individuals, encoded in the genome, in the way the environment influences phenotype.

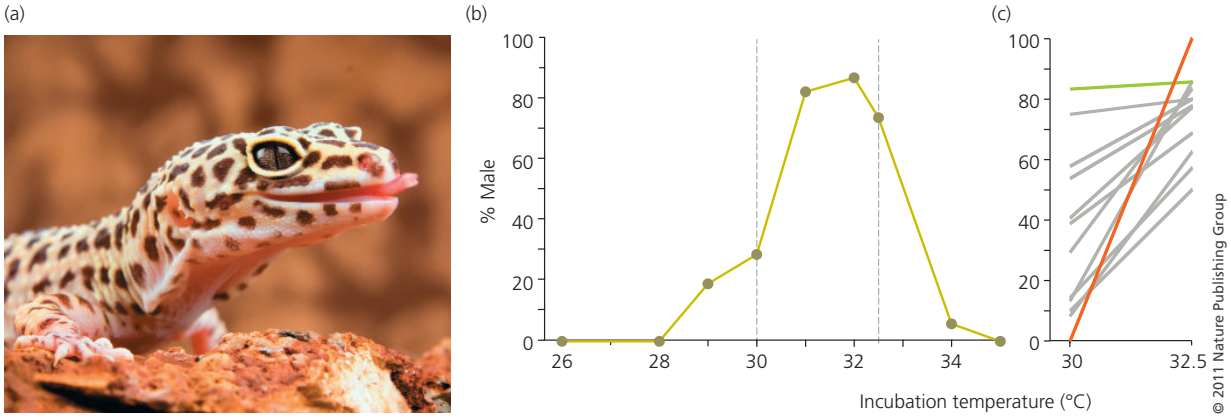

Figure 5.11 Both genotype and temperature influence sex in leopard geckos (a) A leopard gecko (*Eublepharis macularius*). (b) Individuals incubated at intermediate temperature are more likely to be male. This graph, drawn from data in Viets et al. (1993), shows the average relationship for the species. (c) There is variation among fathers in the effect of temperature on the sex ratio of their offspring. Redrawn from Rhen et al. (2011).

offspring of many more sires in the breeding colony, reveal significant variation in temperature sensitivity due to the genes bequeathed by different fathers. This is a striking example of genotype-by-environment interaction.

The identities of the gene or genes responsible for variation in temperature sensitivity, and the proteins they encode, remain to be discovered.

Genotype-by-Environment Interaction and Evolution

Like genetic variation and environmental variation, genotype-by-environment interaction is common. Many instances have been documented in humans. Figure 5.12 shows an example. Among people with genotype *ll* for the serotonin transporter gene, maltreatment in childhood has little effect on the probability of major depression in early adulthood, whereas among people with genotype *ss* maltreatment increases the probability of depression substantially (Caspi et al. 2003; Brown and Harris 2008; Caspi et al. 2010). The serotonin transporter is a cell-surface protein that mops up the neurotransmitter serotonin after nerve cells in the brain have used it to send messages to each other. The two alleles of the gene encode identical versions of the protein, but the *l* allele specifies production of the transporter in higher quantities. Individuals with genotype *ss* make less serotonin transporter, and are more sensitive to trauma in childhood.

An organism that develops different phenotypes in different environments is said to exhibit **phenotypic plasticity**. When populations harbor genetic variation for environmental sensitivity, populations can evolve greater or lesser plasticity. Documentation of this claim comes from an elegant study by Yuichiro Suzuki and Frederik Nijhout on tobacco hornworms (*Manduca sexta*).

Ordinary tobacco hornworm caterpillars are green (Figure 5.13a). Suzuki and Nijhout (2006) worked with a laboratory strain in which the caterpillars are black (Figure 5.13b). They are black, that is, unless they are exposed to high temperature shortly before molting. After a heat shock, they may emerge from molting with green coloration. Figure 5.13c shows the variation in color among individuals in Suzuki and Nijhout's laboratory population following exposure to 42°C. Note that some individuals are highly sensitive to heat shock. These are the ones with a color score of 3. They turned nearly as green as ordinary caterpillars, which have a color score of 4. Other individuals are insensitive to heat shock. They remained black, earning a color score of 0. This variation in sensitivity is an example of genotype-by-environment interaction.

Suzuki and Nijhout took the most sensitive caterpillars and used them as founders of a high-plasticity line. They took the least sensitive and used them as founders of a low-plasticity line. And they picked caterpillars at random and used them as founders of an unselected line. The researchers maintained the three lines for 13 generations. Each generation they gave the caterpillars a heat shock, then selected breeders according to the same criteria they used for the founders.

Suzuki and Nijhout's artificial selection program yielded dramatic evolution in both selected lines. Figure 5.14a documents change over time in the heat-induced color of caterpillars in each line. The low-plasticity line lost all sensitivity to heat shock. Its caterpillars remained completely black regardless. The high-plasticity line became extremely sensitive. Many of its caterpillars turned as green as ordinary tobacco hornworms and the rest nearly so. The unselected line, as expected, retained roughly the same sensitivity over time.

Figure 5.14b compares the reaction norms of the three lines in the 13th generation. To prepare this graph, Suzuki and Nijhout reared caterpillars at a variety

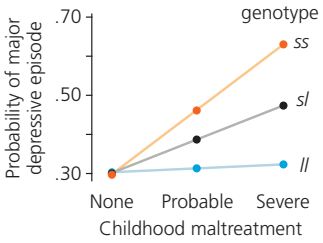

Figure 5.12 Human genotype-by-environment interaction People with different genotypes differ in sensitivity to maltreatment during childhood. Redrawn from Caspi et al. (2003).

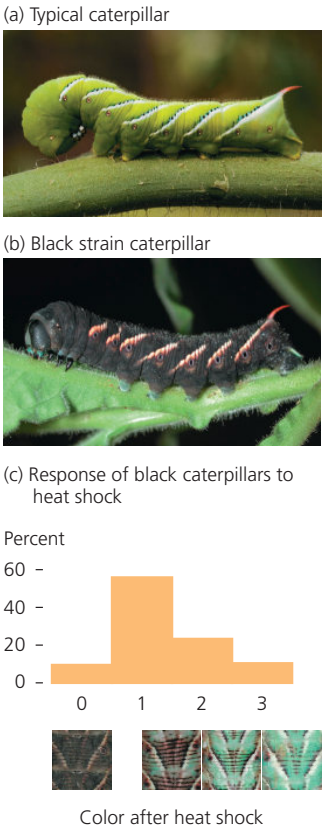

Figure 5.13 Insect genotype-by-environment interaction (a) A normal tobacco hornworm. (b) A black mutant. From Pennisi (2006). (c) Some black mutants turn green after heat shock. Redrawn from Suzuki and Nijhout (2006).