CHAPTER 11

Molecular Genetics of Insect Behavior

Chapter Outline

11.1 Overview

The study of insect behavior involves the analysis of any and all activities performed by an insect in relation to its surrounding environment. Behavior genetics is the study of the underlying hereditary basis of behavior. For many years, Mendelian

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genetic analyses were conducted on a few traits determined by one or a few genes, or quantitative genetic methods were used for traits determined by "many" genes. The sequencing of whole genomes and the use of molecular-genetic methods are revolutionizing the genetic analysis of insect behavior. However, one caveat should be recognized, based on a recent discovery about the discrepancy between behavior in the laboratory and the field by the well-studied *Drosophila melanogaster*: the behavior you evaluate in the laboratory should be compared under conditions approaching the natural environment or erroneous conclusions could be reached.

Circadian behaviors, mating behavior, and learning in *Drosophila* have been dissected with the tools of molecular genetics and inter- and intraspecific comparisons can be made of the DNA sequences associated with these behaviors. The molecular genetics of circadian behavior really opened the field. The circadian clock of *Drosophila* involves several genes, including *period*$^+$ (*per*$^+$). Mutants of *period*$^+$ influence activity patterns and other circadian rhythms, as well as affecting song cycles in courting males. The *per*$^+$ locus has been cloned and sequenced in *D. melanogaster* and *D. simulans*. After the *per*$^+$ gene of *D. simulans* was inserted by *P*-element-mediated transformation into a strain of *D. melanogaster* that is arrhythmic, transgenic *D. melanogaster* males produced song cycles like those of *D. simulans*. As few as four amino acids may account for differences in song rhythm of determined by *per*$^+$. The *timeless*$^+$, *doubletime*$^+$, *cycle*$^+$, *cryptochrome*$^+$, and *Clock*$^+$ genes also are involved in the circadian clock. The clock involves transcription of *per*$^+$ and *tim*$^+$ genes, followed by production of PER and TIM proteins and subsequent negative feedback on self-transcription. Degradation of proteins then releases the negative feedback, allowing a new round of transcription, resulting in oscillations of RNA and protein.

Drosophila learning mutants, such as *dunce* and *couch potato*, provide insights into fundamental processes involved in short-term, intermediate, long-term, and anesthetic-resistant learning. Analyses of sleep behaviors in *D. melanogaster* offer clues to sleep in humans. The discovery of "personalities" in insects challenges interpretations of insect behavior. Studies of behavior determined by many genes are being revolutionized by use of molecular-genetic methods and whole-genome sequencing projects. It now is possible to map the number and location of genes or single nucleotide polymorphisms (SNPs) affecting complex traits by correlating their inheritance with a variety of DNA markers.

11.2 Introduction

Insect behavior covers a very wide range of activities, including locomotion, grooming, feeding, communication, reproduction, dispersal, flight, learning, migration, host or prey selection, diapause, and various responses to environmental hazards such as temperature, humidity, parasites, and toxins (Beck, 1980; Alcock, 1984;

Tauber et al., 1986; Gatehouse, 1997; Bazzett, 2008; Ikeno et al., 2011). Understanding the behavior of pest and beneficial insects could improve pest-management programs (Renou and Guerrero, 2000; Bendena, 2010).

One definition of **behavior** is any action that an individual carries out in response to a stimulus or its environment, especially an action that can be observed and described. However, insects also behave spontaneously, in the absence of any obvious stimulus. Thus, behavior includes studies to understand how an insect takes in information from its environment, processes that information, and acts. Processing information in the central nervous system may involve integrating information over time, including stimuli such as hormones coming from within the insect. Thus, the connection between **stimulus** and **response** can be delayed and indirect.

The genetic analysis of behavior rightfully has been perceived to be more complex than analysis of morphological or anatomical traits (Vanin et al., 2012). One complication in genetic analyses of behavior is the difficulty in defining the behavior in a clear manner. Often "a behavior" may consist of multiple components, which can lead to confusion regarding the number of genes involved. Distinguishing between behavior and physiology can be particularly difficult. The same behavior can be examined from at least four different viewpoints: (1) the immediate cause (or control); (2) its development during the individual's lifespan; (3) the function of the behavior; and (4) how the behavior evolved (Wyatt, 1997).

Behavior genetics began to develop as a field of study in the 1960s, but was limited to demonstrating that a behavioral trait was heritable, determining whether its **mode of inheritance** was dominant or recessive, sex-linked or autosomal, and resolving whether the variation was due to single or multiple genes. Genetic analyses of insect behavior require careful control of environmental conditions, because even subtle differences in test conditions can influence results of assays (Vanin et al., 2012). Objective measures of insect behavior are difficult, and considerable efforts have been devoted to devising specific and appropriate assays. The possible influence of learning always must be considered and, to complicate matters further, learning rates vary among populations of the same species and among individuals so both heredity and environment must be considered. Furthermore, recent studies indicate individuals have "personalities"; for example, some may be more adventurous than others. Genetic analyses of insect behavior involve, in many cases, analyses of the physiological or morphological changes associated with the change in behavior. Sometimes, however, behavior is changed in an insect because a morphological trait has been altered through mutation.

The genetic basis of insect behavior initially was analyzed most extensively using *Drosophila melanogaster* and honey bees, grasshoppers, *Nasonia* parasitoids, and crickets. Now, molecular genetic techniques provide powerful methods to analyze olfaction, learning, circadian rhythms, and mating behavior in many species. Having the complete genomes insects simplifies the isolation of specific genes involved in behavior. *P*-element-mediated transformation makes it possible to insert genes from one species of *Drosophila* into the genome of another to determine their effect(s).

Molecular genetic analyses of learning and memory in *Drosophila* provided a means to study one of the most challenging frontiers in neurobiology (Waddell and Quinn, 2001). Molecular genetics may allow us to localize and identify some of the individual genes among the "many" involved in interesting and complex behaviors exhibited by insects (Doerge, 2002). Perhaps the most significant advance in the study of behavior has been the sequencing of genomes of insects other than *Drosophila*. This has allowed novel and detailed studies in a variety of insects and promises to provide exceptional new insights.

Analyses of insect behavior employ techniques from several disciplines including anatomy, biochemistry, ecology, **ethology** (study of animal behavior in the natural environment), genetics, psychology, physiology, and statistics (Matthews and Matthews, 1978; Hay, 1985; Bell, 1990; Via, 1990; Barton Browne, 1993; Heisenberg, 1997; Doerge, 2002). These disciplines are required because an insect perceives the environment through its sensory systems. The external sensory stimuli are transduced into electrical information, which is then processed and decoded, leading to a behavioral response. Behavior can be divided into several sequential steps: **stimulus recognition**, **signal transduction**, **integration**, and **response** or motor output.

11.3 The Insect Nervous System

The insect brain contains around 10^5–10^6 neurons. It consists of three main divisions, the **protocerebrum**, **deutocerebrum**, and **tritocerebrum**. In each division, different **neuropil** regions are located; a neuropil is a dense network of interwoven axons and dendrites of neurons and neuroglial cells in the central nervous system and parts of the peripheral nervous system.

In the protocerebrum, centers are associated with vision and other sensory receptors (the mushroom bodies and central complex). The superior protocerebrum, with the pars intercerebralis, contains sets of neurosecretory cells that supply neurohemal organs in the corpora cardiaca and corpora allata, which are located in the head or prothorax in insects. The optic lobes flanking the protocerebrum contain the most well organized neuropiles in the brain.

Mushroom bodies in the brain are associated with olfactory pathways, including olfactory learning (Figure 11.1). Mushroom bodies differ greatly in size and shape,

Figure 11.1 Photograph of brain structures known as mushroom bodies in *D. melanogaster*. The dark areas show mushroom bodies stained with an antibody to the *dunce*-encoded enzyme. *dunce*$^+$ encodes cyclic AMP phosphodiesterase, which destroys cyclic AMP, which is important in learning and memory. (Photograph provided by R. L. Davis.)

with the number of cells ranging from 2500 in *Drosophila* to 50,000 in the cricket *Acheta*, 170,000 in the honey bee and 200,000 in the cockroach *Periplaneta* (Heisenberg, 1998; Strausfeld et al., 1998).

The antennal centers are found in the deutocerebrum; in the tritocerebrum, neurosecretory neurons and neurons associated with the control of feeding and foregut activity are found (Homberg et al., 1989). The brain is connected to the subesophageal ganglion via connectives and to thoracic and abdominal ganglia, or ventral nerve cord (Strausfeld, 1976).

Behavior and development are coordinated in the insect by both nerves and neuropeptides. Both use **neuropeptides** as messengers. Many types of neuropeptides have been identified (Bendena, 2010; Yeoh et al., 2017). Neuropeptides range in size from three amino-acid residues (thyrotropin-releasing hormone) to more than 50 (insulin). They are generated from larger precursor proteins, ranging from 90 to 250 amino acids in length (Bendena, 2010).

Neuropeptides are released as cotransmitters and modulate fast transmission at neuromuscular junctions. A given neuropeptide may occur at several sites, including central nervous system circuits, peripheral synapses, and at the peripheral targets (muscles and glands). Neuropeptides regulate behavior by coordinating temporal and spatial activity of many neuronal circuits. Each circuit controlling behavior employs sets of sensory neurons, interneurons, and motor neurons. Thus, multiple neural networks share neural elements. Molecular-genetic analysis is providing rapid progress in understanding neuropeptide receptors and second-messenger pathways. Research on neuropeptides and their receptors indicates they have roles during development, growth, reproduction, metabolism and behavior. Neuropeptides are being studied as an alternative to chemical pesticides. However, neuropeptides offer a challenge because the insect cuticle contains a lipid component that inhibits penetration of peptides and the gut, hemolymph, and membranes of tissues contain peptidases that rapidly degrade peptides so topical application or ingestion are unlikely to be effective in delivering these proteins (Bendena, 2010).

11.4 Traditional Genetic Analyses of Behavior

Sometimes, as will be demonstrated in examples below, mutations in a single gene or a few major genes alter a behavior and the mode of inheritance can be assessed by "traditional" methods. Traditional behavior-genetic analysis employs two main approaches: **crossing** and **selection**. A third, limited to *D. melanogaster*, involves analysis of **fate maps** in genetic mosaics to locate the anatomical site of abnormalities that affect behavior (Hotta and Benzer, 1972). Many genes may influence an insect's behavior, although a specific behavior sometimes can be altered by the mutation of a single gene (Plomin, 1990). When many genes are involved, analyses of behavior traditionally have required the use of **quantitative genetic** methods and sophisticated statistical analysis.

11.4.1 Crossing Experiments

A crossing experiment involves mating individuals that differ in a particular kind of behavior and then examining the behavior of their F_1 and backcross progeny. (A **backcross** is a cross of F_1 individuals to a parental line, usually the homozygous recessive one.) Ideally, the environment is controlled so that all individuals experience the same conditions. It is easiest to interpret results *if* individuals that are crossed differ *only* with regard to a single behavioral attribute.

The phenotype of F_1 and backcross progeny indicates whether a single gene or multiple genes determine the behavior, and whether there is dominance, sex linkage, or maternal influences. If many genes influence the trait, it is difficult to determine the number of loci, their relationship to each other, or their location on specific chromosomes because many insects lack sufficient genetic markers. New molecular and statistical methods using quantitative trait loci (QTLs) may provide greater power to study and locate multiple and interacting loci (Doerge, 2002).

Honey bee hygienic behavior provides an example of a trait that appears determined by a few genes. Other behaviors, including house-entering behavior in the mosquito *Aedes aegypti*, foraging in *D. melanogaster* and host choice in the parasitoid *Nasonia* also appear determined by one or a few genes.

Susceptibility to foulbrood disease, caused by *Bacillus larvae*, originally was analyzed by crossing two inbred *A. mellifera* strains with differing levels of resistance (Table 11.1). The differences in resistance were attributed to “hygienic behavior” in worker (sterile female) bees (Rothenbuhler, 1964). Resistant workers (= hygienic) consistently remove dead larvae and pupae from the brood nest at a high rate, thus slowing spread of bacteria through the colony. Crosses between “hygienic” queens and susceptible “nonhygienic” haploid males yield F_1 worker progeny that are nonhygienic, indicating genes conferring resistance are recessive.

Progeny produced by backcrosses to the homozygous-recessive hygienic strain yielded approximately 25% hygienic workers, which is consistent with the hypothesis hygienic behavior is determined by two recessive loci (Table 11.1). Under a two-locus model, hygienic worker queens are homozygous for two genes, *uu* and *rr*. The hygienic workers (*uu*, *rr*) both uncap the cells (*uu*) containing dead brood **and** remove them (*rr*). However, uu, r^+r individuals will uncap the cells, but not remove dead brood. The u^+u, rr individuals do not uncap brood, but will remove them if the cells are uncapped for them. Individuals that are u^+u, r^+r are unhygienic, and will neither uncap nor remove brood. Hygienic behavior appears to be a general response to remove pathogens and parasites from the nest (Spivak and Gilliam, 1993).

Table 11.1: Crossing Experiments Explain Differences in Nest-Cleaning Behavior Among Inbred Lines. The phenotypes of the F_1 and Backcross Progeny of the Haplo-Diploid Honeybee *Apis mellifera* Support the Two-Locus, Two-Allele Model.

Parental lines	Hygienic (diploid) queen X Unhygienic haploid male uu, rr u^+, r^+ ↓ Unhygienic F_1 progeny u^+u, r^+r Backcross progeny (workers)			
Cross of u^+u, r^+r queen X hygienic u, r male	1: uu, rr Hygienic	1: uu, r^+r Uncaps, Doesn’t Remove	1: u^+u, rr Removes, Doesn’t Uncap	1: u^+u, r^+r Unhygienic

Data from Rothenbuhler (1964).

Rothenbuhler's research on hygienic behavior became a classic in textbooks because it was one of the first examples demonstrating that behavior is inherited (Rothenbuhler, 1964). More recently, Moritz (1988) proposed a three-locus model for hygienic behavior in bees. Expression of hygienic behavior depends on colony strength and composition of worker types within the colony (Spivak and Gilliam, 1993; Arathi et al., 2000). Electro-antennogram analyses of olfactory and behavioral responses of hygienic and nonhygienic bees to diseased brood indicated hygienic bees have a higher sensitivity to low concentrations of the odor of diseased bee pupae. Thus, nonhygienic bees may be unable to detect diseased brood. Understanding hygienic behavior in *A. mellifera* has resulted in practical recommendations to beekeepers for selecting colonies resistant to chalkbrood (a fungal disease) and the pest bee mite *Varroa*. So far, no negative effects have been found associated with hygienic colonies and such colonies produce as much honey as nonhygienic ones (Moritz, 1994; Spivak and Gilliam, 1998).

House-entering behavior by the mosquito *A. aegypti* from East Africa was analyzed by crossing different populations with different behaviors (Trpis and Hausermann, 1978). One population commonly entered houses (domesticated or D), while others rarely did (either peridomestic, P, or feral, F). House-entering behavior is important in determining whether a population transmits yellow fever to humans. Three populations of *A. aegypti* collected either inside houses (D), near a village (P), or from tree holes in a forest (F) were bred in insectaries and crossed to produce hybrid (DP, PD, DF, FD, PF, FP) populations (Trpis and Hausermann, 1978). The original and hybrid populations were then marked with different colored fluorescent powders and released near houses. Of the mosquitos entering houses, 45% were from the D population, 13.9% from hybrids (DP and PD), 9.8% from the P population, and 5.7% were hybrids (DF and FD). Only 1.5 and 0.6% of the PF and FP hybrids were collected in the house, and the feral population entered the house with a frequency of only 0.6%. The recapture rates in the village were in the reverse order. The data indicate a few genes with additive effects determine this behavior.

"Domesticity" in *A. aegypti* is a complex phenomenon that includes a preference for ovipositing in man-made containers, the ability of larvae to develop in drinking water stored in clay pots with a low nutritional content, and preferences for feeding on man (rather than birds) inside houses, as well as resting and mating indoors. No doubt *A. aegypti* speciated long before man began to build houses, but *A. aegypti* has adapted rapidly to human habitats, and the domestic form of *A. aegypti* is the only one known entirely dependent on man (Trpis and Hausermann, 1978).

D. melanogaster larvae feed on yeast growing on decaying fruit. Naturally occurring populations contain larvae that vary in the distance they travel while foraging, a difference attributed to a single *foraging* gene (Osborne et al., 1997; Sokolowski et al., 1997). Natural populations comprise approximately 70% "rovers" (who forage long distances) and 30% "sitters" (short-distance foragers), with the rover phenotype dominant to sitter, indicating a single-gene mode of inheritance (Sokolowski, 2001). Sitter larvae grow at a normal rate and are of normal size. Both sitters and rovers are maintained in the field by natural selection; density-dependent selection shifts allele frequencies so rovers are selected for in crowded larval environments and sitters in less-crowded ones. The *foraging* gene encodes a cyclic guanosine monophosphate (cGMP)-dependent protein kinase, and rovers have higher kinase activity levels than sitters (Shaver et al., 1998). Sleep deprivation and starvation studies with *D. melanogaster* individuals with natural variants of *foraging* suggest one reason the *foraging* polymorphisms persist in wild populations is due to fitness tradeoffs (Donlea et al., 2012). *foraging* influences sleep and learning and memory, as well as feeding. Flies with the "rover" genotype have better short-term memory than "sitters," but sitter flies have better long-term memory. Rovers do not lose their short-term memory if well fed, but do so if starved overnight. Sitter flies have reduced short-term memory after sleep deprivation, but are able to learn after 12 h of starvation. Thus, *foraging* alleles provide benefits in some environments, but not others. Donlea et al. (2012) conclude that "…understanding how these tradeoffs confer resilience or vulnerability to specific environmental challenges may provide additional clues as to why an evolutionary alternative to sleep has not emerged," although it is not clear how the ability to survive sleep loss can confer a fitness advantage. Another gene, *Chaser*, affects larval foraging by increasing foraging path length (Pereira et al., 1995). Anreiter et al. (2017) found an epigenetic regulator interacts with the *for* gene to regulate adult foraging behavior through histone methylation of a *fo*r promoter.

In the Amazonian ant *Allomerus octoarticulatus*, which defends the plant *Cordia nodosa* against herbivores, variability in protectiveness occurs among different colonies. Male et al. (2017) found *foraging* gene expression in ant workers correlated with whether an ant colony discovered an herbivore and how much damage the pest caused.

Crossing experiments indicate that one or a few genes influence a specific behavior in: the flour moth *Ephestia kuhniella* (silk-mat spinning by larvae prior to pupation), the mosquito *Aedes atropalpus* (egg maturation without an exogenous source of protein such as blood), and the parasitoid wasp *Habrobracon juglandis* (flightlessness) (Ehrman and Parsons, 1981). In *Bombyx mori*, females with the *piled egg* gene deposit eggs in a peculiar manner; *B. mori* larvae with the *Nonpreference* gene are unable to discriminate mulberry leaves from others (Tazima et al., 1975), and Huettel and Bush (1972) found when two monophagous tephritid flies

(*Procecidochares*) were crossed, the host-preference behavior segregated in a manner consistent with control by a single locus. Desjardins et al. (2010) found crosses in the laboratory between *Nasonia vitripennis* and *N. giraulti* resulted in a change in host-preference behavior, which was dominant and localized to 16 Mb of sequence on chromosome four.

A variety of behavioral mutants determined by single-major genes were identified in *D. melanogaster* (Grossfield, 1975; Hall, 1985; Pavlidis et al., 1994), including a group of sex-linked, incompletely dominant mutants (*Shaker*, *Hyperkinetic*, and *eag*) that are expressed when flies are anesthetized with ether. The sex-linked temperature-sensitive recessive mutant $para^{ts}$ causes *D. melanogaster* to become immobile above 29°C. The *couch potato* (*cpo*) locus causes flies to be hypoactive and exhibit abnormal geotaxis (response to gravity), phototaxis (response to light), and flight behavior. This gene is unusually complex, spanning more than 100 kb and encoding three different messages (Bellen et al., 1992).

Many "single-gene" mutants affect morphology of *D. melanogaster* and thus affect behavior because flies are unable react to a stimulus due to altered effector structures. Other mutants exhibit altered behavior because perception of cues is impaired. For example, flies with *white* eyes exhibit abnormal courtship behaviors (Grossfield, 1975).

Pheromone communication in the European corn borer *Ostrinia nubialis* is genetically determined (Klun and Huettel, 1988; Lofstedt, 1990). Females of the E- and Z-strains of *O. nubialis* produce different **enantiomeric** ratios of sex pheromone. Hybrids between these two strains produce an intermediate pheromone blend. Analysis of the F_2 and backcross progeny indicates pheromone type is controlled by two alleles at a single autosomal locus, although one or more modifier genes control the ratio of the isomers in heterozygous females. Males of the two *O. nubialis* strains are attracted to the appropriate pheromone blends in the field, and hybrid males respond preferentially to the pheromone produced by hybrid females rather than to the two parental female types. A single sex-linked gene with two alleles determines the response of males to the pheromone. The olfactory sensillae of the two males are different, which is controlled by an autosomal locus with two alleles. Yasukochi et al. (2011) found sex-linked pheromone receptor genes of *O. nubialis* were present in tandem array on the Z chromosome. In addition, an autosomal locus responsible for male response to the sex pheromone contains at least four odorant-receptor genes, suggesting additional copies of odorant-receptor genes can increase the potential for male moths to acquire altered specificity for pheromone components (Yasukochi et al., 2011).

Sexual isolation of the moths *Heliothis subflexa* and *H. virescens* was determined to be due to a single quantitative trait locus (QTL) that consists of at least four odorant receptor genes (Gould et al., 2010).

11.4.2 Selection Experiments

Selection experiments provide another traditional method to determine the degree to which a given behavior is determined genetically. In a selection experiment, individuals with a specific behavioral attribute are allowed to reproduce and this process is repeated over succeeding generations. Eventually, behavior of the selected population is altered *if* genetic variation for the attribute is present in the initial colony *and* the selection procedures were appropriate. The response of the population to selection can be analyzed to estimate the heritability of the trait.

Migratory behavior of the large milkweed bug, *O. fasciatus*, is under genetic control (Palmer and Dingle, 1989). Bidirectional selection on wing length (for increased and decreased wing length) was performed for 13 generations. Individuals also were selected for flight time, and those whose flight times totaled 30 min were considered "fliers," while those with a shorter flight time were labeled "nonfliers." Response to selection on wing length was rapid, and flight tests of long- and short-winged insects indicated a positive correlation between wing length and flight duration. Selection after two generations for flight or nonflight likewise resulted in divergent responses, indicating a large genetic component to flight behavior.

To estimate the degree of genetic influence on a specific behavior, two measures are used, the selection differential and the estimate of heritability. The **response to selection** (**R**) is the difference in mean phenotypic value between offspring of the selected parents and mean phenotypic value of the entire parental generation before selection (Falconer, 1989).

$$R = h^2 S$$

R is the improvement or response to selection, h^2 is the heritability of the characteristic under selection in the population, and S is the selection differential. The **selection differential** (**S**) is the average superiority of the selected parents expressed as a deviation from the population mean (Falconer, 1989). The selection differential measures the difference between the average value of a quantitative character in the whole population and the average value of those selected to be parents of the next generation. It is measured in standard deviation units.

Heritability *in the broad sense* is the degree to which a trait is genetically determined. Because both genes and environment influence behavioral traits, heritability is expressed as the ratio of the total genetic variance to the phenotypic variance (V_G/V_P). Heritability *in the narrow sense* is the degree to which a trait is transmitted from parents to offspring, and is expressed as the ratio of the additive genetic variance to the total phenotypic variance (V_A/V_P) (Falconer, 1989).

Heritability could be estimated to be "zero" if the specific population being selected had no variability for the behavioral attribute under study because it was inbred. Heritability could be estimated to be "one" if the trait was completely determined by genes, and environment had little effect, although this would be unusual.

Heritability estimates provide no information about the mode of inheritance because they represent the cumulative effect of all loci affecting the trait. The number of loci involved generally can be determined only with elaborate and specially designed experiments. A number of assumptions are made when estimating heritability: (1) all loci act *independently* of one another and, (2) the loci are *unlinked* (located on different chromosomes). Another assumption (3) is environment affects all genotypes in a similar fashion. These three assumptions are not always justified. Thus, heritability estimates are difficult to interpret, although useful for *predicting response to selection under specific environmental conditions.*

Heritability estimates are usually made by regression-correlation analyses of close relatives (parent-offspring, full sibs, half sibs), experiments involving response to selection, or analysis-of-variance components. Traits with high heritability respond readily to selection with an appropriate selection method. The magnitude of response to selection, the differences in mean values between parent and progeny generations, provides an estimate of heritability in the narrow sense (h_n^2). This estimate is valid **only** for the population being examined, under the test conditions employed, for the behavior observed, and for the method of measurement employed.

Heritability of most insect behaviors is relatively high, because many arthropod behaviors appear stereotyped (Ehrman and Parsons, 1981). For example, heritability of locomotor activity of *D. melanogaster* is estimated to be 0.51, and heritability of mating speed of male *D. melanogaster* is estimated to be 0.33. Heritability for honey production from honey bees ranged from 0.23 to 0.75, depending upon experimental conditions and colonies tested (Rinderer and Collins, 1986). Italian honey bees are less able to remove parasitic *Varroa* than Africanized bees, and heritability was estimated to be 0.71 (Moretto et al., 1993). Heritability of the length of the prereproductive period in *Helicoverpa armigera*, which is when migratory flight occurs, ranged from 0.54 to 0.16 (Colvin and Gatehouse, 1993). Heritability of host selection behavior by *Asobara tabida*, a parasitoid of *Drosophila subobscura*, ranged from 0.03 to 1.0 depending upon test method (Mollema, 1991), illustrating that test conditions are important.

11.4.3 Some Polygenically Determined Behaviors

Behavior often is a continuous variable, controlled by multiple genes with small additive effects. The task of teasing apart the respective roles of genes and environment requires statistical analysis (Doerge, 2002). *Drosophila* behaviors determined by multiple genes include locomotor activity, chemotaxis, duration of copulation, geotaxis, host-plant preference, mating speed, phototaxis, preening, and the level of sexual isolation within and between species. Multiple genes influence host-plant adaptation and host preference in insects, and learning also may affect host preference (Papaj and Prokopy, 1989; Via, 1990). Host-plant choice is usually a hierarchy of several components. For example, attraction to a site from a distance and oviposition-site preference (egg laying at the site) are genetically distinct in *Drosophila tripunctata* (Jaenike, 1986).

The genetic basis of host-plant specialization in *Drosophila sechellia* and *D. simulans* is determined by a minimum of three or four loci that affect egg production, survival, and host preference (R'Kha et al., 1991). *D. sechellia* breeds in a single plant, *Morinda citrifolia*, which is toxic to other *Drosophila* species. Its sympatric relative, *D. simulans*, breeds on a variety of plants. The two species can be crossed, and F_1 hybrid embryos produced by *D. simulans* females are susceptible to *Morinda* fruit because susceptibility is maternally inherited and fully dominant. Females of *D. sechellia* are stimulated by *Morinda* to produce eggs, but this plant inhibits oviposition in *D. simulans*. In hybrid progeny, the inhibition observed in *D. simulans* is dominant. F_1 hybrids and backcross progeny exhibit intermediate, approximately additive, behavior. These differences result in isolation of the two species in nature, although their ranges overlap geographically. Thus, their ecological niches are determined by tolerance to toxic products in the ripe *Morinda* fruit, with *D. sechellia* exhibiting a strong preference for *Morinda*, an ability to detect fragrant volatiles from *Morinda* over a long distance, and a stimulation of egg production. By contrast, *Morinda* inhibits egg production in *D. simulans*.

Other specific behavioral attributes inherited in a complex manner include: *Musca domestica* (number of attempts to mate by males); *Phormia regina* (high and low ability to learn to extend the proboscis to a stimulus applied to the forelegs); hybrid crickets (call rhythm of males; female response to calling songs); *Anopheles albimanus* (ability to avoid pesticides); *Apis mellifera* (high and low collection of alfalfa pollen, and stinging behavior) (Ehrman and Parsons, 1981; Hall, 1985; Rinderer, 1986). Gould (1986) found the propensity for cannibalism by larvae of *Heliothis virescens* is polygenically determined.

11.5 Molecular-Genetic Analyses of Insect Behavior

Molecular-genetic analyses are providing significant advances in our knowledge of behavior. The ability to identify, clone, and sequence specific genes makes it easier to understand several behaviors, including the periodicity of biological rhythms, mating behavior, locomotion, and learning. It is possible to clone a gene from one *Drosophila* species, insert it into a *P*-element vector, and introduce the exogenous gene into mutant strains of *D. melanogaster* to confirm the putative gene does, in fact, code for the behavior of interest. Cloned genes from *Drosophila* can, in some cases, be used as probes to identify genes from other arthropods, which then can be sequenced and compared. The availability of complete genomes of insects allows analyses of behavior that could not be conducted previously.

11.5.1 The Photoperiodic Clock

The potential that molecular genetics offers was first exemplified by analyses conducted using the *period*$^+$, and other clock genes, of *D. melanogaster* (Table 11.2). Most insects, and other organisms, exhibit particular behaviors at specific times of the day due to a **circadian clock** that allows the insect to measure

Table 11.2: Some Genes Involved in the Circadian Clock of *Drosophila melanogaster*.

Gene abbreviation	Mutant phenotype(s)	Function(s)
period$^+$ *per*$^+$	Short-period, long-period and arrhythmic flies	Negative transcription element; *per*$^+$ mRNA levels rise late in the day
	Affects locomotion, eclosion, courtship rhythms	Activated by the heterodimer of CLOCK and CYCLE proteins
		PER proteins feedback negatively on their own transcription
		PER contains a protein dimerization domain called PAS
timeless$^+$ *tim*$^+$	Short-period, long-period, and arrhythmic flies	Negative element; *tim*$^+$ mRNA levels rise late in the day TIM protein destabilized by light
	Affects locomotion, eclosion, sleep	TIM proteins feedback negatively on their own transcription, interacts with PER
Clock$^+$ *Clk*$^+$	Arrhythmic	CLK, in combination with CYC, activates transcription of *per*$^+$ and *tim*$^+$
	Affects locomotion, eclosion, Rhythm	CLK negatively regulates itself Mutants blind for “lights-on” response
cycle$^+$ *cyc*$^+$	Arrhythmic	CYC, in combination with CLK, activates transcriptions of *per*$^+$ and *tim*$^+$

Table 11.2: (Continued)

Gene abbreviation	Mutant phenotype(s)	Function(s)
	Affects locomotion and Eclosion	CYC negatively regulates itself Mutants respond poorly to light-dark cycles
cryptochrome^{+} *cry*$^{+}$	Photoreceptor Resets rhythms	Sequence homologous to a photolyase; binds TIM in a light-dependent manner Altered light response in mutants
doubletime^{+} *d*$^{+}$	Lengthens cycle in constant darkness Affects locomotion, sleep	Casein kinase I involved in phosphorylating PER, rendering it unstable in absence of TIM

Adapted from Zerr et al. (1990), Takahashi (1992), Kyriacou (1993), Young (1998), and Sokolowski (2001).

time (Kyriacou, 1993; Takahashi, 1992; Young, 1998). Such circadian rhythms have a number of characteristics:

(1) The clocks are usually "free running" in constant environments and are not simple responses to changes in light or temperature.
(2) Although the rhythms are free running, an initial environmental signal is required to start the clock. Among the cues that "set" the clock are alternating light and dark cycles, high and low temperature cycles, or short pulses of light.
(3) The circadian rhythm is relatively insensitive to changes in temperature (temperature compensated).
(4) Altering the cues that entrain the clock can reset the clock.

D. melanogaster adults reared in constant darkness exhibit circadian locomotor activity rhythms. However, rhythms of individual flies are not synchronized with one another (Sehgal et al., 1992). Rhythms can be synchronized if dark-reared flies are exposed to light as first-instar larvae. Light treatments occurring prior to hatching of first-instar larvae fail to synchronize adult locomotor activity, indicating the clock functions continuously from larval hatch until adulthood. The rhythm can be advanced, delayed, or unchanged, depending on the phase of the cycle at which the cue is given.

The circadian rhythm has an approximate periodicity of 24 h. Molecular-genetic analyses of *Drosophila* mutants provided fundamental understanding of the mechanisms of the circadian clock (Hogenesch and Ueda, 2011). Circadian rhythms are found in all organisms and probably evolved early. Common genetic elements are present in *Drosophila*, *Neurospora*, mammals, and cyanobacteria. In *Drosophila*, the genes *period*$^+$, *timeless*$^+$, *Clock*$^+$, *cycle*$^+$, *doubletime*$^+$, and *cryptochrome*$^+$ are important (Lakin-Thomas, 2000, Table 11.2).

Numerous reviews compared the molecular, genetic, and neurological components of biological rhythms, reflecting the great excitement of the scientific community in learning the molecular basis of this complex behavior. Hall (1998) questioned how it is possible "…to review an over-reviewed subject–one whose reviews have even been reviewed." As an indication of the importance of clock research, Jeffrey Hall, Michael Rosbash and Michael Young received the Nobel Prize in Physiology or Medicine in 2017 for their work on circadian rhythms in *Drosophila,* which "…gave us our first working model of how the molecular clock might tick" (Offord, 2017).

The *Drosophila per*$^+$ locus is on the X chromosome and mutations of it influence eclosion, locomotor activity, and the length of the interpulse interval of the courtship song (Table 11.2). Eclosion of wild-type flies (emergence of adults from the pupal case) typically occurs around dawn, when dew and high relative humidity increases survival (Figure 11.2A). Locomotor activity decreases during midday and is followed by increased activity in the evening. Three classes of alleles exist; they shorten (*per*^{S} mutants have 19-h eclosion rhythms), lengthen (*per*^{L} mutants have 29-h eclosion rhythms), or completely abolish circadian eclosion and locomotor activity rhythms (*per*^{0} mutants). Flies with the *per*^{0} mutation eclose arrhythmically, but periodicity in eclosion can be restored by *P*-element-mediated transformation of arrhythmic flies using the wild-type *per*$^+$ allele (Bargiello et al., 1984, Figure 11.2B and C).

The *per*$^+$ gene is approximately 7 kb long, encodes a 4.5-kb transcript with eight exons, the first of which is noncoding (Figure 11.3). One of the most striking features of the protein is a series of threonine-glycine (Thr-Gly) repeats in the gene middle (Costa et al., 1992). The region encoding the Thr-Gly repeats is polymorphic in length within and between *Drosophila* species and plays a role in thermal stability of the circadian phenotype. For example, 17, 20, or 23 repeats are found in *melanogaster* populations, and a clinal pattern occurs along a north-south axis in Europe and North Africa, with the shorter sequences in southern Europe (Costa et al., 1992). Costa et al. (1992) suggested that the length polymorphism cline is maintained by natural selection under different temperature conditions.

A large number of tissues express the *per*$^+$ product, including embryonic, pupal, and adult nervous systems, as well as esophagus, gut, and ovaries. Hardin et al.

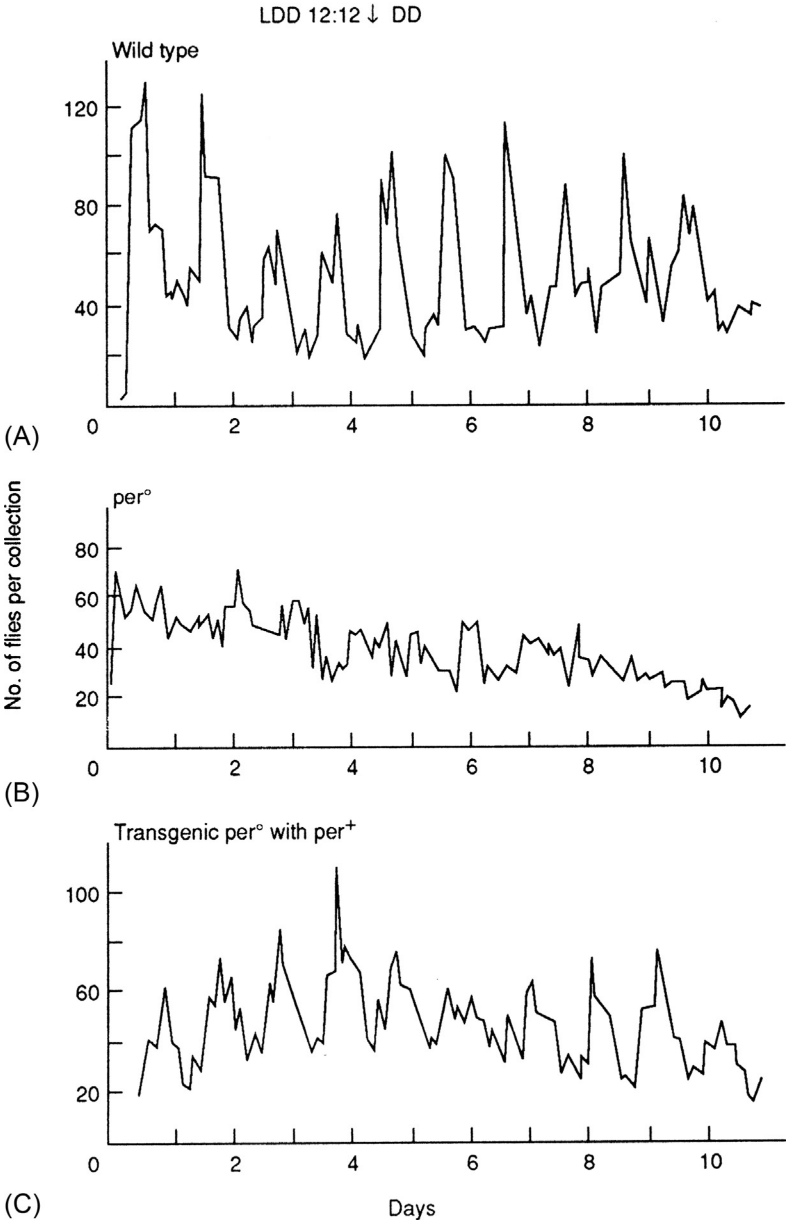

Figure 11.2 Profiles of eclosing adults (emergence from pupal cases) for populations of: (A) *D. melanogaster* wild-type females, (B) *per*^{0} males and females, (C) and transgenic *per*^{0} individuals that have received a wild-type *per*$^+$ gene by *P* element-mediated transformation. LDD 12:12 indicates there is a 12-h light-dark cycle. (Modified from Bargiello et al. (1984).)

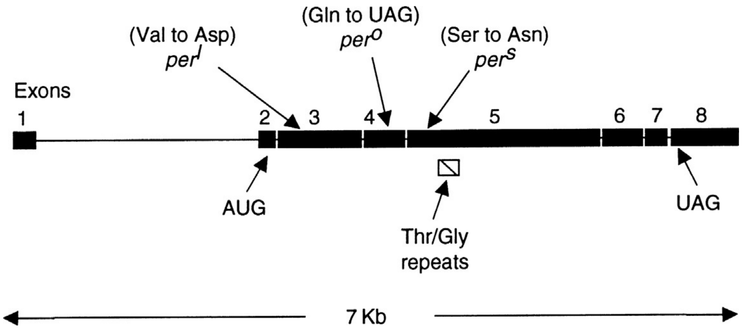

Figure 11.3 The exon/intron structure of the *D. melanogaster per* gene. The gene is approximately 7 kb long, with 7 exons. The location of the per^{L}, per^{0}, and per^{S} mutations are indicated, as is the region coding for the variable number (17, 20, or 23) of Thry/Gly repeats.

(1992) showed per^{+} mRNA levels undergo daily fluctuations, which constitutes a **feedback loop** in which PER affects the oscillations of its own mRNA. The fluctuations in per^{+} mRNA are due to fluctuations in gene transcription because per^{+} mRNA has a relatively short half-life (Zerr et al., 1990), consistent with the hypothesis PER acts as a transcription factor (Table 11.2).

The per^{+} genes from *D. simulans*, *D. virilis*, *D. pseudoobscura*, and *D. yakuba* have parts of the gene conserved and parts highly diverged, suggesting conserved regions may encode basic functions common to all (clock-type functions), while species-specific differences, such as love songs, locomotor activity and eclosion profiles, may be encoded within variable regions (Kyriacou, 1990).

$Clock^{+}$, $timeless^{+}$, $cycle^{+}$, and $doubletime^{+}$ are components of the circadian clock (Kyriacou, 1993, Table 11.2). Flies with mutations of $phase\text{-}angle^{+}$ emerge in predawn instead of just after dawn, while flies with mutations of $gate^{+}$ fail to eclose during this narrow time window.

The $cryptochrome^{+}$ (cry^{+}) gene is important because it encodes a critical circadian photoreceptor in *Drosophila* (Egan et al., 1999). The gene product, CRY, belongs to a family of blue-light-sensitive proteins. Flies overexpressing CRY are hypersensitive to light. The CRY protein is probably the only dedicated circadian photoreceptor in *Drosophila*.

Courtship song is produced when Drosophila males vibrate their wings. The song consists of two components: (1) hums, and (2) a series of pulses with interpulse intervals that can fluctuate between 15 and 85 ms (Kyriacou and Hall, 1989). The variation in interpulse intervals ranges from 56 s in *D. melanogaster* and 35–40 s in *D. simulans*. The males of *D. melanogaster* with the per^{S} mutation sing with 40-s periods, per^{L} males sing with 76-s periods, and per^{0} males are arrhythmic.

The genetic basis of species-specific song instructions was confirmed by transfer of the *per*$^+$ gene from *D. simulans* into *D. melanogaster* via *P*-element-mediated transformation (Wheeler et al., 1991). The *D. simulans per*$^+$ gene restored a rhythm in *D. melanogaster* and the males produced song cycles characteristic of *D. simulans* males. Wheeler et al. (1991) concluded substitutions in four or fewer amino acids in the *per*$^+$ locus are responsible for the species-specific behavior.

per alleles affect locomotion, cellular rhythms, and development time. Flies with *per*^{S} develop faster than wild-type flies, and *per*^{L} flies develop more slowly (Kyriacou, 1993). It has been hypothesized that circadian oscillations provided the clock for photoperiodically induced **diapause** in insects (Takeda and Skopik, 1997). Diapause is a genetically determined state of arrested development induced prior to the onset of detrimental conditions. Hibernal diapause, which allows insects to survive over winter, can be induced when insects develop during a period of cooling temperatures under shortening daylengths, which means they measure light and dark cycles. However, *per*$^+$ appears to have no influence on diapause in *D. melanogaster* (Saunders, 1990).

However, Ikeno et al. (2011) found the circadian clock genes *period*$^+$ and *cycle*$^+$ regulate reproductive diapause in males of the bean bug *Riptortus pedestris*. RNAi analyses showed disruption of *per*$^+$ and *cycle*$^+$ disrupted the rhythm of cuticle deposition. RNAi of *per*$^+$ also induced development of male reproductive organs even under diapause-inducing short-day conditions. RNAi of *cycle*$^+$ suppressed development of reproductive organs under diapause-preventing long-day conditions. Larval diapause in *Chymomyza costata* also is affected by mutations affecting the photoperiod clock (Kostal and Shimada, 2001).

Many behaviors, including learning, involve temporally patterned events. The interval between presentation of the conditioned stimulus and reinforcement is important in associative learning. It was thought the *per*$^+$ gene could be involved in learning, based on the observation *Drosophila* males with the *per*^{L} allele in one experiment did not exhibit normal courtship behavior. However, males with the wild type or *per*^{S} and *per*^{0} alleles could be conditioned normally (Gailey et al., 1991).

Although the above-described analyses of *Drosophila* circadian behavior informed us about the genetics of circadian rhythms, Vanin et al. (2012) discovered the behavior of flies under natural conditions is different from that observed in laboratory conditions with constant temperatures and light-dark cycles without dawn- or dusk-lighting regimes. Vanin et al. (2012) recommended that future studies of circadian rhythms be conducted using more-natural temperature and light regimes in the laboratory. Under artificial laboratory conditions, where lights were either on or off, flies anticipated lights turning on by increasing locomotor activity about 2 h prior to that event. However, under field conditions, the flies did not behave in this manner. In fact, their sleep-wake cycle was quite different when temperature, sunlight, moonlight, and humidity varied. Instead of sleeping during the middle of the day, as they did in the laboratory, they became active. Instead of anticipating dawn, they reacted to changing light during twilight, but did not respond to dawn. In addition, moonlight had no effect on behavior under field conditions, although it does under artificial laboratory conditions.

Sandrelli et al. (2008) note the mammalian genes *Clock* and *Period* share a common evolutionary origin with insects, although the mammalian-clock components consist of multiple gene copies, increasing complexity and redundancy. They also reviewed what has been learned about circadian clock genes in insects (Diptera, Lepidoptera and Orthoptera) other than *Drosophila*. Sandrelli et al. (2008) note peripheral clocks are common in insects and can be entrained by light in extra-retinal photoreceptors, but these have not yet been studied.

11.5.2 Learning in Drosophila

It is difficult to produce a single definition of learning. **Learning** can be defined as a change in behavior with experience, but this would not exclude responses such as growth and maturation, or other processes triggered by events such as mating or feeding. Another definition is a reversible change in behavior with experience, but excludes phenomena in which the modification caused by some experience is fixed and resistant to further change. Another definition is learning is a more-or-less permanent change in behavior that occurs as a result of practice, but this definition is ambiguous.

Papaj and Prokopy (1989) suggested that the following properties are characteristic of learning in insects: (1) An individual's behavior changes in a repeatable way as a consequence of experience. (2) Behavior changes gradually with continued experience, often following a "learning curve" to an asymptote. (3) The change in behavior accompanying experience declines in the absence of continued experience of the same type or as a consequence of a novel experience or trauma.

Insect populations and species vary in their ability to learn (Hoedjes et al., 2011). Genetic variability within strains has been used to analyze learning in *Drosophila*, *Phormia* flies, and the honey bee (McGuire and Hirsch, 1977; McGuire, 1984; McGuire and Tully, 1987; Tully, 1996; Menzel, 1999). *D. melanogaster* can be sensitized and habituated, learn associations with positive or negative reinforcement, and be classically conditioned (Davis and Dauwalder, 1991). *D. melanogaster* can learn to run away from odors they previously experienced with an electric shock and hungry flies can learn to run toward odors previously associated with a sugar reward. Flies can learn visual, tactile, spatial, and proprioceptive cues (Waddell and Quinn, 2001). Analyses of memory mutants in *Drosophila*, including *dunce*, *rutabaga*, *amnesiac*, *radish*, *zucchini*, *cabbage*, *tetanic*, *turnip*, *linotte*, and *latheo*, indicate that memory consists of distinct phases: short-term, intermediate, long-term, and anesthesia-resistant memory (Table 11.3, Davis, 1996, Sokolowski, 2001).

Table 11.3: Some Single Genes Involved in Learning and Memory of *Drosophila melanogaster*.

Gene	Mutant phenotype(s)	Function(s)
dunce^{+}	Short-term memory defective	cAMP-specific phosphodiesterase involved inolfactory learning and memory Affects locomotor rhythms, ethanol tolerance, learning
rutabaga^{+}	Short-term memory defective	Adenylate cyclase decreases expression of cAMP, affects courtship, learning, ethanol tolerance, grooming
amnesiac^{+}	Middle-term memory defective	Neuropeptide, stimulates cAMP synthesis Affects ethanol tolerance, sleep regulation
radish^{+}	Anesthesia-resistant long-term memory	Affects only one type of long-term memory
cabbage^{+}	Long- and short-term memory (?)	Involved in olfactory learning
turnip^{+}	Long- and short-term memory	Involved in the protein kinase C pathway Affects olfactory discrimination, larval, visual and reward learning
latheo^{+}	Acquisition of initial memory defective?	Involved in short-term memory; affects DNS replication and synaptic plasticity?
linotte^{+}	Retarded learning Mutants have structural brain defects (mushroom bodies and central complex)	Encodes receptor tyrosine kinase

(*Continued*)

Table 11.3: (Continued)

Gene	Mutant phenotype(s)	Function(s)
Volado$^+$	Short-term memory Expressed in mushroom	Cell surface receptor altered, involved in synaptic remodeling underlying learning and memory; two variants of ∀-integrin coded for bodies
leonardo$^+$	Short-term and olfactory learning	Affects protein 14-3-3-, which is involved in intracellular signaling that activates and represses protein kinase-C activity, activates tyrosine hydroxylase and tryptophan hydroxylase (enzymes involved in catecholamine and serotonin synthesis)

Adapted from Sokolowski (2001), Waddell and Quinn (2001), and FlyBase.

Genetic analyses of learning in *D. melanogaster* began in the mid-1970s in Seymour Benzer's laboratory when *D. melanogaster* was trained to avoid an odor associated with a shock (Benzer, 1973; Vosshall, 2007). The learned avoidance lasted only a few hours, but the odor-avoidance test was used to screen mutagenized flies for strains with normal olfaction and aversion to shock, but an abnormally low ability to associate odors with shocks. The mutant flies obtained were poor learners, but each had different phenotypes (Table 11.3). Flies with the mutant gene *amnesiac* had nearly normal learning ability, but forgot rapidly. Flies with *dunce* genes had shortened memory for several conditioned behaviors (Davis and Dauwalder, 1991) due to a defective gene for cAMP-specific phosphodiesterase, an enzyme that regulates levels of cyclic AMP (cAMP). The *dunce* flies have elevated cAMP levels (Zhong and Wu, 1991). cAMP is part of a "second-messenger" signaling pathway in nerve cells that help form associative memories.

The *dunce*$^+$ gene is one of the largest and most complex identified in *Drosophila*, extending over 140 kb. *dunce*$^+$ is expressed in mushroom bodies in the brain of *D. melanogaster* (Figure 11.1). It produces, by the use of multiple transcription start sites and alternative splicing of exons and differential processing of $3'$ sequences, at least 8–10 RNAs ranging in size from 4.2 to 9.5 kb. One unusually large intron, 79 kb in length, contains at least two genes (*Sgs-4* and *Pig-1*) within it (Chen et al., 1987; Qiu et al., 1991). This "genes-within-an-intron" arrangement is uncommon.

One contained gene, *Sgs-4*$^+$, is expressed in larval salivary glands and provides glue used by larvae to attach them to the surface for pupation. The second gene, *pre-intermolt*$^+$, is expressed in larval salivary glands. Genes homologous to *dunce*$^+$ have been identified in mice, rats, and humans, and the mammalian counterpart of *dunce*$^+$ functions in regulating mood.

Fly brains were screened and some 50 learning mutants identified, including several alleles of *rutabaga*$^+$. Mutations of *rutabaga*$^+$ cause decreased expression of cAMP and rutabaga protein was identified as an adenylate cyclase (Han et al., 1992, Table 11.3).

In *D. melanogaster* mushroom bodies are paired and consist of about 2500 neurons (Heisenberg, 1998, Figure 11.1). Mushroom bodies receive olfactory information from antennal lobes. Mushroom bodies house part of the short-term memory for odors, are required for courtship conditioning memory, and are necessary for context generalization in visual learning, as well as regulating the transition from walking to rest (Zars, 2000).

Learning requires the antennal lobes, the central complex, and the lateral protocerebrum in insects (Hansson and Anton, 2000). During metamorphosis, the nervous system of holometabolous insects such as *Drosophila* changes significantly. A controversy has existed as to whether flies retain learned behavior after metamorphosis from larvae to adults. There is no evidence larval conditioning induces a change in adult olfactory responses (Barron and Corbet, 1999). This is not surprising, because larval sense organs undergo histolysis during the pupal stage and adult sense organs are formed *de novo* from imaginal discs. The mushroom bodies are extensively rewired during metamorphosis.

Drosophila with a mutant version of the *turnip*$^+$ gene have difficulty in olfactory discrimination, conditioning of leg position, larval, visual and reward learning (Table 11.3, Choi et al., 1991). Additional mutated genes, including *radish*, *amnesiac*, *cabbage*, *latheo*, and *linotte*, are involved in abnormal learning or memory (Table 11.3). For example, flies with the X-linked *radish* mutation initially learn in olfactory tests, but their memory decays rapidly at both early and late times after learning. *radish* flies show normal locomotor activity and sensitivity to odor cues and electric-shock reinforcements used in the learning tests, but anesthesia-resistant memory, or consolidated memory, is strongly reduced with the *radish* phenotype (Folkers et al., 1993).

The *rutabaga*$^+$ gene codes for an adenyl cyclase and is expressed in *Drosophila* mushroom bodies. This gene is involved in olfactory short-term memory (Zars et al., 2000). Likewise, *Volado*$^+$ is expressed in mushroom body cells and mediates short-term memory in olfactory learning (Grotewiel et al., 1998).

How does long-term memory develop and persist? Majumdar et al. (2012) found the *Drosophila* Orb2 protein forms amyloid-like oligomers upon neuronal stimulation and these are enriched in the synaptic membrane, which may be critical for persistence of long-term memory. Mutants of the Orb2 protein had impaired long-term memories. The enlightenment obtained from *Drosophila* learning mutants is providing an understanding of learning in higher organisms (Majumdar et al., 2012).

How might learning and instincts in insects be related? Robinson and Barro (2017) proposed that "instincts" evolve from learning. They note behavioral plasticity allows animals to respond to their environment. If this adaptive response increases fitness, then selection will favor animals that manifest that behavior earlier in development or with less experience. Selection "…acting to adjust the timing and extent of plasticity can thus produce an instinct." They note that "…it is possible that some instincts evolved via the more traditional "mutation first" model of evolution. In this case, mutations cause changes in the timing of the development of neural circuitry, for example, from postnatal to prenatal." They cite research from bees and flies that show both innate and learned olfactory responses are determined by the same neural circuits and suggest the molecular mechanism(s) that change behavioral plasticity to instinct is **epigenetics**, involving DNA methylation, histone modifications and noncoding RNAs. Robinson et al. (2015) showed the migratory locust (*Locusta migratoria*), which has solitary and migratory behaviors, have different levels of DNA methylation gene expression, suggesting epigenetic mechanisms could produce alternative locust behaviors.

11.5.3 Functional Genomics of Odor Behavior in Drosophila

The ability to respond to odors is essential for survival and reproduction, allowing insects to select mates, find and choose food, and locate appropriate oviposition sites. Insects detect odors with antennae and maxillary palps, upon which sensory hairs (sensilla) are present. Each sensillum houses dendrites of a few olfactory-receptor neurons. Insect antennae can contain more than 100,000 sensilla but *D. melanogaster* antennas contain about 400. Sexual dimorphism in antennal structure is common, and immature insects typically contain fewer sensilla than adults, perhaps because eggs are typically deposited in or near the appropriate food.

Studies of the genetic basis of odor behavior in insects first used *D. melanogaster* as a model (Field et al., 2000; Vosshall, 2000; Anholt et al., 2001). Efforts also are being made to evaluate olfaction, learning, and memory in the honey bee. Robertson and Wanner (2006) identified 170 odorant-receptor genes in the honey bee, a dramatic increase compared to the 62 and 79 genes found in *D. melanogaster* and *Anopheles gambiae* genomes.

Odors are received by olfactory receptors located on antennae and maxillary palps, which send their axons to the antennal lobes in insect brains. Each third antennal segment in *D. melanogaster* contains about 1300 olfactory receptor cells and each maxillary palp carries 120 chemosensory neurons (Anholt et al., 2001). These neurons project to 43 glomeruli in the antennal lobe of the brain. From there, processed olfactory information is relayed to higher-order brain centers (the mushroom body and the lateral horn of the protocerebrum). Insect odorant receptors are seven-transmembrane-domain proteins, but are not related to G protein-coupled receptors (Carey and Carlson, 2011).

It is thought there are fewer than 100 *types* of odor receptors in insects, perhaps as few as 50 or 60 (Vosshall et al., 2000; Carey and Carlson, 2011). Insect receptors consist of large multigene families (Clyne et al., 2000). Once an odor or pheromone has activated the olfactory receptors, it needs to be deactivated. Several enzymes appear to degrade odor stimulants, including esterases, oxidases, and glutathione transferases (Field et al., 2000).

D. melanogaster recognizes and discriminates between a large number of odors (Vosshall, 2001; Rutzler and Zwiebel, 2005). Because there are as few as 50 or 60 *types* of receptors in insects, each olfactory sensory neuron responds to several odorants, but responds maximally to one (Dryer, 2000). While the average olfactory-receptor gene is expressed in 20 olfactory neurons, some receptor genes are expressed in only two to three neurons. Seven olfactory receptor genes are expressed solely in the maxillary palp (Vosshall et al., 2000).

In the Chagas disease vector *Rhodnius prolixus*, an odorant co-receptor gene was silenced using RNAi, reducing expression by 73% and persisting for more than 100 days (Franco et al., 2016). RNAi silencing reduced the ability of *R. prolixus* to find a vertebrate host in a timely manner, decreased ingested blood volume, delayed and decreased molt rate, increased mortality, and decreased egg deposition, suggesting this gene could be a target for controlling pest populations.

11.5.4 Behavior of Apis mellifera

Mushroom bodies in the Hymenoptera are much larger than in *Drosophila*, which may reflect the importance of mushroom bodies for social behavior, learning, and memory in social insects (Rinderer, 1986; Rybak and Menzel, 1993; Meller and Davis, 1996).

Social Hymenoptera (ants, bees, wasps) have complex behaviors, including caring for their brood. Social species such as *A. mellifera* feed, protect and nurse larvae, store food, and respond to adverse environmental factors. They search for nectar and pollen at unpredictable sites, learn celestial and terrestrial cues to guide their foraging trips over long distances and allow them to find their nest sites once again. They learn to respond to the changing position of the sun, to a pattern of polarized light during the day, and to landmarks. Associative learning is an essential component to foraging behavior and dance communication. Hive mates attending a dance performance learn the odor the dancing bee carries and seek out that same odor when they forage.

The complexity of bee behavior makes it an ideal organism to better understand learning, as well as to analyze social behavior (Bloch and Grozinger, 2011). Associative olfactory learning in honey bees has several features similar to higher forms of learning in vertebrates (Grunbaum and Muller, 1998).

11.5.5 Pheromones in Insects

Many insects use chemical cues to find mates and molecular genetic methods allow study of various aspects of pheromone-response behavior. For example, genes have been identified that code for proteins involved in **pheromone synthesis** (a substance released by the body that causes a predictable reaction by another individual of the same species), the perception of **semiochemicals** (chemicals that influence insect interactions), and processing of the signals.

Pheromone biosynthesis appears to use one or a few enzymes that convert the products of normal primary metabolism into pheromones (Tillman et al., 1999). For example, pheromones arise from isoprenoid biosynthesis, or by the transformation of amino acids or fatty acids (Field et al., 2000). Three hormonal messengers regulate production of pheromones by insects: juvenile hormone III, ecdysteroids, and a neuropeptide called PBAN (pheromone-biosynthesis-activating neuropeptide). The antennae contain olfactory organs (sensillae) that mediate pheromone perception. Some receptor neurons on the antennae appear to respond to one particular chemical (specialist neurons), but others appear to respond to a number of compounds (generalist neurons). Pheromones often are perceived with other chemicals, including plant volatiles.

The detection of pheromones and other chemicals by insects involves proteins (**odorant binding proteins, OBPs**) that carry the compounds from the surface of the antennal sensilla through the sensillum lymph to the receptors and the olfactory neurons (Krieger and Breer, 1999). The odorant-binding proteins (which includes pheromone-binding proteins) are small, soluble proteins concentrated in the sensillum lymph (Christophides et al., 2000). Analysis indicates binding proteins of unrelated species have low levels of amino-acid sequence similarity. It appears there has been gene duplication and divergence of odorant-binding protein genes (Christophides et al., 2000).

11.5.6 Neurobiochemistry of Drosophila

Molecular neurobiology is concerned about how the nervous system controls behavior at the molecular level. What are the biochemical substrates of behavior? A molecular-genetic approach using *Drosophila* is providing interesting answers for both insects and mammals, although *D. melanogaster* has approximately 250,000 neurons while humans have approximately 10^{12} neurons. For example, a potassium-channel gene family was cloned first from *Drosophila,* and subsequently from humans and mice, using probes from *Drosophila*. The *Shaker*$^+$, *Shal*$^+$, *Shab*$^+$ and *Shaw*$^+$ subfamilies of the K-channel gene family have been found in the Chordata, Arthropoda, and Mollusca, suggesting the ancestral K-channel gene gave rise to these subfamilies by the Cambrian radiation (Salkoff et al., 1992).

A number of enzymes and receptors are involved in neurobiology, including receptors for neurotransmitters and hormones, ion-channel proteins and associated signal-transduction components, brain-specific protein kinases, enzymes for transmitter synthesis, neuropeptide-processing enzymes, neuron-specific growth factors and their receptors, inhibitors of neuronal growth, glial-specific growth factors and their receptors, proteins associated with memory, neuronal cytoskeleton and axonal-transport proteins, and others not listed here or yet to be identified. A major endeavor in molecular neurobiology involves establishing the primary structure of all categories of proteins involved in nerve-signal reception and transmission.

The nervous system receives information about its internal and external environment, processes this information, and produces an appropriate response. The signaling of nerve cells depends on the electrical status of their outer membranes. Nerve cells maintain a potential difference across the membrane with the inside of the cell negative relative to the outside of the cell. The resting nerve cell also maintains concentration gradients of sodium (Na), calcium (Ca), and potassium (K) ions. Na and Ca ions are at a relatively high concentration outside the cell while K-ion levels are relatively high inside the cell. Signaling involves a change in the resting-membrane potential brought about by charge transfers carried by ionic fluxes through gated pores formed by transmembrane proteins called **channels**.

Ion-channel proteins catalyze the transmembrane flow of ionic charge by forming narrow, hydrophilic pores through which ions can diffuse (Miller, 1991). **Ion channels** must open or close rapidly in response to biological signals (= gating). Furthermore, the open pore is generally selective and will determine which ions will permeate and which will not (**ionic selectivity**). Thus, a specific channel will permit K but not Na to pass.

Stimuli from the environment are perceived by specialized nerve cells (sensory cells). Each type of sensory cell responds to a particular stimulus such as light, sound, touch, heat, or chemicals. These sensory cells transform and amplify the energy provided by a stimulus into an electrical signal (= sensory transduction). **Sensory transduction** is probably due to an alteration in the ionic permeability of the sensory-cell membrane, which causes a depolarization of the membrane from its resting level. The amplitude and duration of this departure generally increases logarithmically with the intensity of the stimulus. This signal is local and is not transmitted along the nerve cell; however, it acts as a stimulus to the axon and if depolarization increases over a threshold level, the signal will trigger a change in **action potential** in the axon. Action potentials are all-or-nothing electrical impulses that propagate without distortion or attenuation along the entire length of an axon.

The generation and propagation of an action potential alters ionic conditions within the cell. When axonal membranes are depolarized, Na channels open and allow Na ions to flow down their gradient into the cell, producing the depolarizing phase of an action potential. Within milliseconds after the Na channels open, they are inactivated, but at about the same time the membrane depolarization activates K channels, and the reciprocal K flow repolarizes the cell and restores the membrane-resting potential. During the course of an action potential, the Na currents in one region of the axon membrane cause the depolarization and firing of an action potential in an adjacent region of the membrane so the action potential is propagated along the full length of the axon.

The electrical signal is transmitted between cells at special sites called **synapses**, which occur between two nerve cells as well as between nerve cells and effectors such as muscle cells. The signal is relayed by a chemical neurotransmitter packaged in membrane-bound vesicles. When an action potential reaches the presynaptic terminal, the depolarization activates Ca channels in the presynaptic membrane and the subsequent influx of Ca ions leads to the release of neurotransmitter. The neurotransmitter diffuses to the postsynaptic cell and interacts with specific receptors on that cell surface. Receptors are activated in response to binding of the specific neurotransmitter molecules.

Acetylcholine (ACh) is the major neurotransmitter in the central nervous system of *Drosophila* and other insects. Choline acetyltransferase (ChAT) is the biosynthetic enzyme, and acetylcholinesterase (AChE) is the degradative enzyme. AChE terminates synaptic transmission by rapidly hydrolyzing acetylcholine (Fournier et al., 1989).

The acetylcholinesterase gene (*Ace*) from *Drosophila* is 34 kb in size and is split into 10 exons, with the splicing sites of the two last exons precisely conserved among *Drosophila* and vertebrate cholinesterases (Fournier et al., 1989). The deduced mature *Ace* transcript is 4.2 kb long. A gene for an acetylcholine receptor subunit has been identified, and the amino-acid sequence of AChR shares similarity with vertebrate sequences.

Two types of ion channels, permeable to sodium (Na) or potassium (K) ions, are responsible for membrane electrical phenomena. Multigene families encode the Na and K channels. Mutated genes that affect Na channels include *nap*^{ts} (no-action-potential, temperature-sensitive) and *para* (paralytic) (Salkoff et al., 1987; Kernan et al., 1991). The *para*^{ts} is a temperature-sensitive mutation that causes instantaneous paralysis of adults at 29°C and of larvae at 37°C (Loughney et al., 1989). Mutations of several different genes (*Shaker*, *Shal*, *Shab*, and *Shaw*) alter K currents (Covarrubias et al., 1991). One of the best-studied mutations is *Shaker* and *D. melanogaster* carrying the *Shaker* allele shake their legs when anesthetized with ether (Papazian et al., 1987). Flies in which the *Shaker* gene is deleted still have K currents and Butler et al. (1989) isolated three additional family members, *Shab*$^{+}$, *Shaw*$^{+}$, and *Shal*$^{+}$. These four genes define four K-channel subfamilies in *Drosophila* and homologous genes isolated from vertebrates all appear to fall into one of these four subclasses.

Other K-channel mutants, including *eag* (Warmke et al., 1991) and a calcium-activated K channel gene (*slo*), have been isolated. Gaba-aminobutyric acid (GABA), is a major inhibitory neurotransmitter in the insect nervous system. The synthesis of GABA is controlled by the enzyme glutamic acid decarboxylase (GAD).

11.5.7 Divergent Functions of Est-6 and Est-5 in Two Drosophila Species: A Cautionary Tale of Homologs

Evolutionary changes in gene regulation can be important in macroevolutionary change and species divergence. One case study involves the *esterase-6* gene in *D. melanogaster* and its homolog (*esterase 5*) in *D. pseudoobscura* (Brady and Richmond, 1990). Both influence behavior in *D. melanogaster* but have very different functions, indicating sequence similarity may not be equivalent to behavioral homology.

Esterase-6 (*Est-6*) influences male-mating speed and rate of remating by *D. melanogaster* females. Fast- and slow-variants of esterase-6 protein are produced in natural populations of *D. melanogaster*. More esterase-6 protein is produced in adult males than in females. The enzyme is transferred to females during the first two to 3 min of the 20-min copulation. Enzyme activity in females can be detected up to 2 h after mating and influences the timing of remating by females. Males transfer a substance that is converted in the females' reproductive tract by esterase 6 into a pheromone that serves as an **antiaphrodiasiac**. The antiaphrodiasiac reduces sexual attractiveness and receptivity of females, reducing the likelihood she will remate. Because sperm from the most recent male takes precedence in fertilizing a female's eggs, this behavior encourages monogamy (Richmond et al., 1986).

Est-6 also influences the rate of mating by *D. melanogaster* males. Males with the slow variant of the protein require 10.2 min to achieve copulation, while males with the faster variant require 5.7 min. Once the *Est-6* gene was cloned, it was used as a probe to identify homologous genes in related species (Brady and Richmond, 1990), and *Est-5* was isolated from *D. pseudoobscura*. *Est-5* in *D. pseudoobscura* is expressed, surprisingly, in the eyes and hemolymph. Despite these different expression patterns, *Est-6* and *Est-5* have similar proteins, transcripts, and DNA sequences.

When *Est-5* from *D. pseudoobscura* was cloned into a *P* element vector and introduced into *D. melanogaster*, its activity and pattern of expression matched those of *D. pseudoobscura*, implying that regulatory elements had been conserved since their divergence 20–46 mya. Brady and Richmond (1990) speculated the enzyme in their common ancestor had a more-extensive expression pattern. After their divergence, regulatory mutations may have occurred that enhanced *Est-5* expression in the eyes of *D. pseudoobscura*, while mutations in *Est-6* led to increased expression in male ejaculatory ducts of *D. melanogaster*. Thus, *DNA sequence similarity may not always predict function in diverse species*.

11.5.8 Courtship Behavior in Drosophila

Mating behavior of *D. melanogaster* is stereotypical, with a fixed sequence of actions under genetic control. Courtship involves visual stimuli, acoustic signals, and pheromones (Hall, 1994; Yamamoto et al., 1997; Goodwin, 1999; Savarit et al., 1999; Greenspan and Ferveur, 2000; Ganter et al., 2011). Male courtship behavior involves six elements in the following order: orienting → following → wing vibration → licking → attempting to copulate → copulation.

A cascade of regulatory genes control sexual differentiation in *Drosophila* (Chapter 10). Their expression determines all aspects of maleness and femaleness in the soma and central nervous system. The genes also influence courtship behavior. Sexual behavior is irreversibly programmed during a critical period as a result of activity, or inactivity, of the gene *tra*$^+$. Male behavior is replaced by female behavior when *tra*$^+$ is expressed around the time of puparium formation (Arthur Jr. et al., 1998).

Other genes indirectly affect courtship behavior in *Drosophila*, including genes that involve general behavior, visual behavior, olfaction, learning/memory genes, regulating periodicity of behavior, courtship song mutants, and female receptivity (Hall, 1994). Immonen and Ritchie (2012) analyzed how gene expression changes in response to courtship song in *D. melanogaster*, using microarrays and quantitative PCR, and identified differentially expressed genes, some of which were up-regulated and some of which were down-regulated. Interestingly, some immune-response genes were up-regulated and some down-regulated. One gene identified was *glucose dehydrogenase*, which facilitates sperm storage in mated females, suggesting transcriptional changes associated with mating may begin during courtship, in advance of egg fertilization.

The *fruitless*$^+$ gene is involved both in sex determination and courtship behavior and is active in the central nervous system (Hall, 1994; Ryner et al., 1996; Goodwin, 1999; Baker et al., 2001). Males with a *fruitless* mutation may court both females and males without copulating. Male flies expressing this mutated gene are unable to bend their abdomens in the presence of females because they lack a male-specific Muscle of Lawrence. Some alleles of *fruitless* cause males to be homosexual (they court only males), while others cause males to be bisexual (court both males and females) (Yamamoto et al., 1997).

The *fruitless*$^+$ gene is the first in the sex-determination hierarchy functioning specifically in the central nervous system, affecting nearly all aspects of male sexual behavior (Ryner et al., 1996; Villella et al., 1997; Goodwin et al., 2000). It is ca. 140 kb long and produces a complex array of transcripts using four promoters and alternative splicing; the male-specific transcripts are only expressed in a small fraction of the central nervous system (Goodwin et al., 2000). Ito et al. (2012) showed *fruitless*$^+$ encodes transcription factors that promote male sexual behavior by forming a complex with a transcriptional cofactor.

dissatisfaction$^+$ is necessary for some aspects of *D. melanogaster* courtship behavior and neural differentiation in both sexes. Mutant males are bisexual but attempt to copulate. Males with the *dissatisfaction* phenotype take longer to copulate and females with the *dissatisfaction* phenotype are unreceptive to males and do not lay mature eggs (Goodwin, 1999). Mating behavior of normal females involves the following sequence: stop moving → offer the courting male a chance to lick the female's genitalia → allow males to attempt copulation. Nonreceptive females leave the courting male, and if the male pursues her, she may kick him. Nonreceptive virgin females persistently repel males by lifting their abdomens to block physical contact with males. Nonreceptive fertilized females lower their abdomens, extrude their ovipositors and eggs to repel males. Thus, female receptivity varies with age, diet, hormonal condition and mating experience. A mutation of *spinster*$^+$ affects female receptivity throughout life, and females with the *spinster* phenotype continuously leave, kick, or fend off males (Hall, 1994; Suzuki et al., 1997).

Both *D. melanogaster* and *D. simulans* females produce contact pheromones, which consist of cuticular hydrocarbons that elicit wing displays by males

(Ferveur, 1997). These signals have a low volatility, act at a very short distance (a few mm) and are perceived by contact rather than smell. Flies from a given strain, sex, and age produce a reproducible pattern of cuticular hydrocarbons, the biochemical pathway of which is under genetic control. The most important hydrocarbons involved are 7-tricosene and 7-pentacosene. One mutation, *Ngbo*, influences the ratios of 7-tricosene and 7-pentacosene in *D. simulans*. Another, *kete*, reduces the amount of 7-tricosene and all other linear hydrocarbons but does not affect the ratio (Ferveur and Jallon, 1993). Flies homozygous for both *kete* and *Ngbo* have reduced viability and fertility, perhaps because they have very little 7-tricosene.

Experiments were conducted to eliminate all known cuticular hydrocarbons in *D. melanogaster* to determine how mating behavior would be modified (Savarit et al., 1999). The results were surprising; contrary to expectation that *D. melanogaster* females lacking cuticular pheromones would induce no courtship by males, such females remained attractive. Additional analysis indicated undetermined pheromone(s), probably also cuticular hydrocarbons, were present on both control and transgenic flies. Savarit et al. (1999) suggested that the newly discovered pheromones represent ancestral substances in *D. melanogaster* and its sibling species.

A male-biased gene family, *takeout*$^+$, affects male courtship behavior in *D. melanogaster*, as well as other aspects of its biology (Vanaphan et al., 2012). This gene family is conserved across more than 350 million years of insect evolution, and may have evolutionarily conserved sex-specific roles in male-mating behavior among all insects.

11.5.9 Speciation Genes in Drosophila and Other Insects

Changes in sexual behavior can result in reproductive isolation between populations, leading to speciation. Studies of sexual behavior in *Drosophila* species have led to different conclusions about the number of genes involved in speciation by modified sexual behavior (Doi et al., 2001; Ting et al., 2001; Arbuthnott, 2009).

Analyses of speciation genetics usually involve crossing pairs of related species that do not normally mate, but will do so under laboratory conditions when given no choice. The progeny of such “interspecific” crosses then are examined to determine what phenotypes are related to reproductive isolation. Reproductive isolation can be due to sterility of the hybrids (**postmating isolation**) or differences in mate preference (contributing to **premating isolation**). Study of postmating isolation mechanisms indicate a number of genes (loci) are involved.

Premating isolation is thought to be a common cause of speciation caused by divergence in male sexual signals and female preferences. As a result, **assortative**

mating occurs, with individuals preferring to mate with individuals who resemble themselves. Ting et al. (2001) studied the sexual isolation of two populations of *D. melanogaster* (M and Z forms). Z females strongly prefer Z males over M males due to at least four loci on chromosome III that influence male behavior and at least three loci that influence female behavior, suggesting this premating isolation has a multigenic basis.

By contrast, Doi et al. (2001) used *D. ananassae* and its sibling species *D. pallidosa* to analyze sexual isolation. These species are almost completely isolated, but *ananassae* females no longer discriminate strongly against *pallidosa* males if the males are prevented from singing their songs (by removing their wings) or if females are prevented from hearing them (by removing their ears). This suggests divergence in male song patterns and associated female preferences underlies their sexual isolation. The genetic basis of the preference of *ananassae* females for *ananassae* males appears to be a single dominant gene.

The divergence of acoustic signals alone appears to explain the isolation between the *ananassae* and *pallidosa* species, but the basis of mate choice in the M and Z forms of *D. melanogaster* appears to involve different signals, which are probably determined by multiple genes. The histories of these populations could explain the different isolation mechanisms. M and Z forms of *D. melanogaster* appear to have diverged in the same geographic area (**sympatric speciation**), but *ananassae* and *pallidosa* may have evolved while isolated geographically (**parapatric species**). Butlin and Ritchie (2001) concluded analyses of additional populations and species are required to resolve how many behavioral genes are involved in *Drosophila* speciation.

Arbuthnott (2009) reviewed studies on reproductive isolation in insects, and concluded the analysis method was important in the conclusions reached; if the behavior analyzed actually consisted of several behaviors (i.e., "mating behavior" consists of male signaling, female reception, female rejection, and others), then the conclusion was likely that multiple genes underlie reproductive isolation. If, however, specific behaviors were studied, many examples (25 of 36, or 69%) were found in which one or a few genes are involved. However, when reproductive isolation evolved rapidly by changes in behavior (mate signaling), postzygotic isolating mechanisms may not have developed.

11.5.10 Personality in Insects: Tribolium confusum, Apis mellifera, Acyrthosiphon pisum, and Pyrrhocoris apterus

Individuals of many animal species have personality, i.e., they have consistent differences in behavior across time, situations, and/or contexts (Wolf et al., 2007, 2008; Nakayama et al., 2012). Personality in insects was recognized relatively and as an issue relevant to ecology and evolution. Insect personality can complicate molecular analyses of behavior if this is not recognized. The evolution of animal personalities is not well understood, but Wolf et al. (2007) argue personalities can be given an adaptive explanation based on the trade-off between current and future reproduction that can result in polymorphic populations when some individuals emphasize future fitness and others are less likely to take risks. Wolf et al. (2008) further argue that "…the benefits of responsiveness are frequency-dependent; that is, being responsive is advantageous when rare but disadvantageous when common… Second, positive-feedback mechanisms reduce the costs of responsiveness; that is, responsiveness is less costly for individuals that have been responsive before." Thus, personality variation should affect population ecology and dynamics.

Insect species documented to have personalities include *Tribolium confusum, Apis mellifera, Acyrthosiphon pisum* and *Pyrrhocoris apterus*. Individuals of the confused flour beetle, *T. confusum*, exhibit consistent differences in walking activity and death-feigning behavior (a possible mechanism to escape predation). Selection for higher or lower frequencies and longer or shorter durations of death feigning resulted in two genetically distinct strains and Nakayama et al. (2012) showed lower activity levels were the result of lower brain dopamine levels. Administration of caffeine to low-activity strains resulted in decreased durations of death feigning and increased activity.

Honeybee workers exhibit differences in scouting behaviors, with some more adventurous than others (Liang et al., 2012). Adventurous scouts for food and nest sites had extensive differences in brain gene expression (catecholamine, glutamate, and gama-aminobutryic acid signaling). Octopamine and glutamate treatments increased scouting activity, but dopamine-antagonist treatment decreased it. Interestingly, "Our results demonstrate intriguing parallels between honey bees and humans in novelty-seeking behavior. Although the molecular mechanisms that produce this behavioral variation are similar, it is unknown whether both species inherited them from a common ancestor or evolved them independently" (Liang et al., 2012).

Honey bees vary individually in their social behavior and a genetic analysis indicated they have related genes for autism spectrum disorder (similar to those in humans) (Shpigler et al., 2017). These genes are expressed in mushroom bodies, suggesting deep conservation of genes have occurred for this pathology rather than evolutionary convergence.

The pea aphid *Acyrthosiphon pisum* has differential escape responses to predator attack (dropping or not dropping off the plant) (Schuett et al., 2011). Different aphid clones expressed different phenotypes (dropper, nondropper, and inconsistent). Individuals within a clone dropped or did not drop. Some clones were consistent over repeated trials, others contained both consistent and inconsistent individuals, one clone failed to produce droppers. Individuals repeated their escape response in six trials over five days of adult life. When individuals were reared under different conditions, they were consistent in their tendency to drop and in the repeatability of their behavior. Thus, clonal individuals expressed personality variation.

The short- and long-winged individuals of the firebug *Pyrrhocoris apterus* (Heteroptera: Pyrrhocoridae) exhibit consistent behaviors over time and across contexts, indicating they have personalities. Winged females were "…braver and more exploratory." These data support the model of Wolf et al. (2007) that "…individuals choose different strategies to find the balance between present and future reproduction. In the case of firebug, it is known there are differences in the behavior of brachypterous and macropterous individuals, for example, in the higher walking activity and lowered mating propensity of macropterous individuals."

11.5.11 Transition From Blood Feeding to Obligate Nonbiting in a Mosquito

Bradshaw et al. (2017) evaluated the pitcher plant mosquito *Wyeomyia smithii*, which has populations that blood feed in one region and are obligate nonfeeders in other parts of the range, using both selection and molecular tools. Differential selection on blood feeding within a polymorphic population resulted in feeding and nonbiting females within seven generations. Gene expression was analyzed in the two types to determine what genes might be involved in the transition to nonbiting (which has potential pest-management implications). The results indicated biting and nonbiting females had different metabolic pathways, as well as an up-regulation of visual over odorant reception. Additional analyses may identify methods for transforming biting mosquitos that are vectors of disease into nonbiters.

11.6 Symbionts and Insect Behavior

Discovery of the effects of symbionts on insect behavior required molecular tools to identify and quantify their role. Markov et al. (2009) found mating preference of *D. melanogaster* depends, in part, on whether both males and females are infected with *Wolbachia*. Assortative mating depended on genotype, infection status, and a combination of genotype and infection status. Apparently mating choice can "…involve testing the partner for degree of genetic or biochemical similarity with self, based on chemoreception with possible immune system components" and, in this case, *Wolbachia* was a significant component.

Another example in which symbionts affect behavior of their host involves the nucleopolyhedrosis virus of the gypsy moth *Lymantria dispar* (Hoover et al., 2011). The virus causes infected larvae to climb to the top of their host trees to die

(Hoover et al., 2011). The body contents of the infected larvae become liquefied, and the body bursts, releasing millions of infective virus particles. By contrast, healthy larvae hide in crevices or even climb down the tree to the soil to avoid bird predation. The cause of change in behavior was identified as a gene in the baculovirus (ecdysteroid uridine 5′-diphosphate-glucosyltransferase) that encodes an enzyme that inactivates the molting hormone of gypsy moth larvae. Hoover et al. (2011) inoculated gypsy moth larvae with genetically modified virus containing or lacking the virus gene. As expected, deletion of the gene eliminated climbing behavior of larvae and rescue of the gene (adding the gene or gene product) restored climbing behavior. This behavior was termed an "extended phenotype" because the gene in one organism (the parasite) had a phenotypic effect on another (the host).

Sharon et al. (2010) found gut bacteria played a role in mating preference of *D. melanogaster* reared on different media. Part of a population of *D. melanogaster* was reared on a molasses medium and part on a starch medium. When the two were mixed, flies reared on molasses preferred to mate with molasses flies and vice versa. The mating preference occurred after only one generation and was maintained for 37 generations. Antibiotic treatment eliminated the preference, indicating microbes were responsible. The authors suggest mating preferences were caused by bacteria "…changing the levels of cuticular hydrocarbon sex pheromones." Wong et al. (2017) found flies exposed to *Acetobacter* gut microbes early in life had modified foraging behavior compared to flies with *Lactobacillus* microbes.

As noted in Chapter 4, the facultative symbiont *Hamiltonella defensa* affects the defensive behavior of the pea aphid (Dion et al., 2011). Aphids containing *H. defensa* are protected against parasitoids and, as a result, infected aphids spend less time being aggressive against parasitoids and exhibit fewer escape behaviors. This change in behavior benefited both aphid and symbiont because the aphid was able to feed and survive at a greater rate than uninfected aphids.

11.7 Human Neurodegenerative Diseases and Addictions in *Drosophila*

Drosophila is perhaps unique among eukaryotes in the variety and level of sophistication that can be applied to understand its neurobiology and behavior. As a result, *Drosophila* is being studied to gain knowledge about various neurodegenerative diseases in humans. Modeling diseases in simple invertebrates is attractive because genetics can define cellular cascades mediating disease states such as the death of neurons in Parkinson's disease, the second most common neurodegenerative disorder in humans (Feany and Bender, 2000). Transgenic *Drosophila* containing a mutant form of the human α-synuclein gene exhibit essential features of Parkinson's disease, making it possible to study the function of α-synuclein and determine the underlying pathogenic mechanisms.

The *spongecake* mutant of *Drosophila* shows degenerative changes similar to humans with Creutzfeld-Jakob disease, while the *eggroll* mutant produces changes similar to those seen in humans with Tay-Sachs disease (Min and Benzer, 1997). The *beta-amyloid protein precursor-like* (*Appl*) gene of *Drosophila* encodes a homolog of the human β-amyloid precursor protein which gives rise to β-amyloid, a major component of plaques found in patients suffering from Alzheimer's disease (Luo et al., 1992). Another protein associated with Alzheimer's disease, presenilin, has been found in *Drosophila* and studies suggest it also may be involved in Alzheimer's disease (Fortini and Bonini, 2000). A *Drosophila* homolog was identified for copper/zinc superoxide dismutase in humans; mutants of this gene are implicated in Lou Gehrig's disease (McCabe, 1995; Phillips et al., 1995).

A recessive mutant (*bubblegum*) in *D. melanogaster* causes adult neurodegeneration similar to the human disease adrenoleukodystrophy (ALD), otherwise known as the disease cured in the movie "Lorenzo's Oil" (Min and Benzer, 1999). In ALD, high levels of very long-chain fatty acids are produced that can be lowered by dietary treatment with a mixture of unsaturated fatty acids; feeding ALD flies one of the components, glyceryl trioleate oil, blocked the accumulation of excess very long-chain fatty acids and eliminated the pathology. Thus, *bubblegum* flies provided a model for studying mechanisms of disease and screening drugs for treatment.

Drosophila serves as a model to study the genetics of alcohol abuse and drug addiction in humans (Bellen, 1998; Moore et al., 1998; Andretic et al., 1999; Wolf, 1999; Bainton et al., 2000; Singh and Heberlein, 2000). Alcohol addiction and many types of drug addictions appear to share common mechanisms (Bellen, 1998; Moore et al., 1998). For example, the "dopamine hypothesis" suggests addictive drugs may activate certain areas of the human brain leading to an increase in dopamine neurotransmitter release (Bainton et al., 2000). Elevation of dopamine probably provides a sense of wellbeing, pleasure, or elation resulting in a positive reinforcement. Dopamine is not the only neurotransmitter acting in alcohol abuse; glutamate, serotonin, and GABA also may be involved. Furthermore, four of the five circadian genes (*period*$^+$, *clock*$^+$, *cycle*$^+$, *doubletime*$^+$) in *D. melanogaster* influence the fly's responsiveness to cocaine and suggest a biochemical regulator of cocaine sensitization (Andretic et al., 1999).

Resistance to ethanol in *D. melanogaster* appears to be determined by multiple genetic components. Singh and Heberlein (2000) analyzed 23 mutant fly strains with different responses to ethanol and the effects of acute ethanol exposure on *Drosophila* locomotor behaviors are "…remarkably similar to those described for mammals." Thus, *Drosophila* "…may pave the way for an in-depth study of the genes involved in acute and chronic effects of ethanol" (Bellen, 1998). Bainton et al. (2000)

showed that, as in mammals, dopaminergic pathways in *Drosophila* play a role in modulating specific behavioral responses to cocaine, nicotine or ethanol.

Drosophila flies can sleep, and they have become a model for understanding sleep in insects and other animals (Harbison et al., 2009a, 2009b; Donlea et al., 2011, 2012; Soshnev et al., 2011; Dubowy and Sehgal, 2017). Flies that are "resting" choose a preferred location, become immobile for periods of up to 157 min at a particular time in the circadian day, and are relatively unresponsive to sensory stimuli. When rest is prevented, the flies tend to rest despite stimulation and exhibit a "rest rebound." In fact, flies subjected to long-term sleep deprivation may die. Drugs that affect sleep in mammals alter "rest" in flies, suggesting conserved neural mechanisms.

"During sleep, an animal cannot forage for food, take care of its young, procreate or avoid the dangers of predation, indicating… sleep must serve an important function," although there is no agreement yet as to its function(s) (Harbison et al., 2009b). Hypotheses proposed for the evolutionary maintenance of sleep include conservation of energy by reduced expenditure of nutrients, restoration of brain glycogen, and maintenance of homeostasis of synapses (Harbison et al., 2009b). Sleep is important in learning and memory (Donlea et al., 2011). Sleep disorders in humans are common, but the genes underlying these disorders are difficult to study. Analysis of *Drosophila* behavior at the molecular level offers promise of elucidating this evolutionarily important aspect of survival, and mutagenesis studies suggest that many genes (perhaps as many as 1000) affect sleep (Harbison et al., 2009a, 2009b). Harbison et al. (2009a) analyzed variation in sleep in 40 inbred lines of *D. melanogaster* and found many variable genes with only a few having large effects. The data suggest, like mammals, regulation of sleep in *Drosophila* is complex and controlled by multiple brain circuits involving sleep duration, waking activity, and number of sleep bouts. Soshnev et al. (2011) found a conserved long noncoding RNA called *yar* affects length of sleep, disruption of sleep, and may regulate sleep by affecting stabilization or translational control of mRNAs.

11.8 High-Throughput Ethomics

The study of insect behavior can be very labor intensive. Common methods of analysis have included videotaping an insect's behavior, then analyzing the tape frame by frame. A camera-based system was developed to automatically quantify individual and group behaviors of *D. melanogaster* and to provide automated analyses of the data obtained (Branson et al., 2009). The system allows analysis of individuals or groups of flies, allowing high-throughput screening. Dankert et al. (2009) developed another automated-monitoring system for analysis of *Drosophila* behavior that provides software for analysis of both single flies and groups of flies. Reiser (2009) hailed the development of these systems that will allow scientists to resolve cellular and molecular underpinnings of behavior. The systems involve video recording of behavior, detection of flies in each frame, assignment of the trajectory, and classification of the behavior into an "ethogram." An ethogram is a catalog of action patterns in an animal's behavioral repertoire. Both systems are freely distributed, and may be useful with insects other than *D. melanogaster*.

11.9 Systems Genetics of Complex Traits in *Drosophila*

The ability to sequence genomes relatively inexpensively has allowed analysis of complex behaviors in *D. melanogaster*. Systems genetics includes the use of *P*-element mutagenesis to identify genes affecting complex behaviors, artificial selection of natural populations to create extreme phenotypes, high-resolution mapping to identify candidate genes corresponding to quantitative trait loci (QTLs), and whole-genome transcriptional profiling to identify networks of interacting genes affecting complex traits (Mackay, 2009).

Mackay (2009) concluded that large numbers of loci affect behaviors in natural populations of *D. melanogaster*, including olfactory-avoidance behavior in response to a single odorant (97 mutations). In studies involving *P*-element insertions into the genome, 17% of the genome was involved in the effects of ethanol on *D. melanogaster*, and 34% of insertions affected locomotor behavior. Whole-genome transcript analyses of lines indicated 530 genes were affected by a single mutation in the *smell-impaired* loci. Mackay concluded: "If a substantial fraction of the genome can affect any single trait, it follows that most genes must be pleiotropic and affect multiple traits."

Edwards et al. (2009) screened 170 *P*-element insertions for quantitative differences in aggressive behavior from their isogenic control line of *D. melanogaster* and identified 59 mutations in 57 genes that affect aggressive behavior, none of which previously were known. Among the 59 mutations, 32 resulted in increased aggression, and 27 were less aggressive. Edwards et al. (2009) found "Many of the genes affect the development and function of the nervous system… Others affect basic cellular and metabolic processes…" The genes had pleiotropic effects on brain morphology.

Dierick and Greenspan (2006) analyzed aggressive behavior in *D. melanogaster* by selecting populations in a two-male arena assay. After 10 generations, the aggressive lines became more aggressive and after 21 generations, the fighting index increased more than 30-fold. Microarray analysis indicated at least 42 genes were affected, but most of the expression changes were small, with only four genes showing an expression difference greater than twofold. Six genes were analyzed by quantitative PCR and five of the six had expression profiles that matched the microarray results. The differences in results obtained by Edwards et al. (2009) and Dierick and Greenspan (2006) are likely due to differences in experimental design.

11.10 Social Behavior in Bees and Ants

Honey bees and ants are eusocial insects and a great deal of controversy surrounds the evolution of sociality (Nowak et al., 2010; Bloch and Grozinger, 2011). Rapid advances in understanding the social life of the honey bee *Apis mellifera* are being made now the genome has been sequenced. For example, Liang et al. (2012) documented scouting for food and nest sites varies among workers and identified the genes involved using whole-genome microarray analysis and quantitative reverse-transcriptase polymerase PCR. Liang et al. (2012) concluded "Our results demonstrate intriguing parallels between honey bees and humans in novelty-seeking behavior. Although the molecular mechanisms that produce this behavioral variation are similar, it is unknown whether both species inherited them from a common ancestor or evolved them independently." Whitfield et al. (2006) analyzed the process by which workers mature from working in the hive to foraging using gene-expression microarrays of the bee brain.

Jarosch et al. (2011) found how Cape honey bees, *A. mellifera capensis*, modified their biology so workers can reproduce by thelytoky, producing diploid female progeny. The behavior appears to be caused by alternative splicing of a gene homologous to the *gemini* transcription factor of *Drosophila*. It appears that this switch can allow rapid worker ovary activation in Cape honey bees, turning the altruistic worker into a parasite.

Krieger and Ross (2002) identified a single major gene (*Gp-9*, encoding a pheromone-binding protein) that may affect fire ant (*Solenopsis invicta*) workers' ability to recognize queens and regulate their numbers. Some fire ant colonies have multiple egg-laying queens (polygyne form), while others have single queens (monogyne social form). Apparently, colony-queen number is associated with an allele of the *Gp-9* gene.

Lucas and Sokolowski (2009) studied the ant *Pheidole pallidula* and showed differences in major and minor workers are due to the ant *foraging* gene, which encodes a cGMP-dependent protein kinase. Majors, which are larger and defend the nest, have higher protein levels in five cells in the anterior face of the brain, while minors do not. Minors are typically involved in foraging but manipulating the level of the protein increases defense and reduces foraging behavior.

The descriptions above are a small sample of the wealth of information being obtained on the genetics of behavior in social insects. The complete genome sequences of other species will allow even more detailed analysis of behaviors in the future.

11.11 Conclusions

Great advances have been made in understanding the behavior of insects using molecular tools, especially those based on whole-genome sequencing. The ability to sequence genomes of multiple inbred lines of *D. melanogaster*, for example, provided exceptional opportunities to dissect genetic bases of behaviors. Statistical methods have advanced, and methods to evaluate groups of insects by recording their behavior and analyzing the data with computer programs provide new opportunities, as well, to obtain high-throughput data (Buchen, 2009; Walsh, 2009).

However, the report by Vanin et al. (2012) indicating circadian behavior of *D. melanogaster* is different under natural conditions (populations contained in cages outdoors) than in laboratory cages indicates great care must be taken to develop appropriate experimental methods in order to obtain a realistic understanding of an insect's behavior. Another example of the importance of experimental methods is the design of "choice tests." Martel and Boivin (2011) discuss the appropriate design of choice tests in the laboratory and define experimental methods that will distinguish "apparent choice" from "true choice," in which true-choice behavior has to meet three criteria: exploitation of the resource is nonrandom, the chooser makes the same choice even in the absence of a differential response by the resource, and all resources are responded to, even in the absence of choice. Another issue to be resolved is how often gene function is conserved in behavior (Reaume and Sokolowski, 2011). Clearly, the appropriate design and analysis of insect behavior remains a challenge.

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