Evolution of Reproductive Behavior

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ABSTRACT Behaviors associated with reproduction are major contributors to the evolutionary success of organisms and are subject to many evolutionary forces, including natural and sexual selection, and sexual conflict. Successful reproduction involves a range of behaviors, from finding an appropriate mate, courting, and copulation, to the successful production and (in oviparous animals) deposition of eggs following mating. As a consequence, behaviors and genes associated with reproduction are often under strong selection and evolve rapidly. Courtship rituals in flies follow a multimodal pattern, mediated through visual, chemical, tactile, and auditory signals. Premating behaviors allow males and females to assess the species identity, reproductive state, and condition of their partners. Conflicts between the “interests” of individual males, and/or between the reproductive strategies of males and females, often drive the evolution of reproductive behaviors. For example, seminal proteins transmitted by males often show evidence of rapid evolution, mediated by positive selection. Postmating behaviors, including the selection of oviposition sites, are highly variable and Drosophila species span the spectrum from generalists to obligate specialists. Chemical recognition features prominently in adaptation to host plants for feeding and oviposition. Selection acting on variation in pre-, peri-, and postmating behaviors can lead to reproductive isolation and incipient speciation. Response to selection at the genetic level can include the expansion of gene families, such as those for detecting pheromonal cues for mating, or changes in the expression of genes leading to visual cues such as wing spots that are assessed during mating. Here, we consider the evolution of reproductive behavior in Drosophila at two distinct, yet complementary, scales. Some studies take a microevolutionary approach, identifying genes and networks involved in reproduction, and then dissecting the genetics underlying complex behaviors in D. melanogaster. Other studies take a macroevolutionary approach, comparing reproductive behaviors across the genus Drosophila and how these might correlate with environmental cues. A full synthesis of this field will require unification across these levels.

KEYWORDS Drosophila; adaptation; genetics; chemoreception; multigene family; gene–environment interaction; natural variation; selection; mating; courtship; song; postmating behaviors; fitness; pheromones; wing spots; seminal proteins; FlyBook

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Drosophila melanogaster, as well as other members of the genus Drosophila, has served as a model system in genetics, evolution, and development for over 100 years, and as an important model in behavioral studies for nearly as long. Some of the earliest comparative studies were done by A. H. Sturtevant (1915), and examined courtship behaviors and sexual recognition between closely related Drosophila species. Subsequent work by Dobzhansky (1946), Spieth (1947), and others further developed Drosophila as not only a model for courtship and mating behavior, but as one of the key experimental systems responsible for establishing the biological species concept (Mayr 1982), and our understanding of how species diversify and evolve (Coyne and Orr 1989, 1997).

Behaviors are more than simply the expression of the nervous system; they include complex interactions with various biotic and abiotic factors. Studies on Drosophila behavior have benefited from a diverse range of experimental approaches. For example, geneticists correlate gene expression and gene interaction studies with complex behavioral phenotypes. Ecologists elucidate the underlying biotic and abiotic stimuli giving rise to complex behaviors. Evolutionary biologists reconstruct the history of the behavior and the genes underlying those behaviors. A comprehensive approach, incorporating aspects of several disciplines, yields the most robust, and we would argue, biologically relevant and interesting results.

In this article, we focus on the evolution of reproductive behaviors in Drosophila. Classical genetic and genomic studies on D. melanogaster have had a significant impact on our understanding of behavior through powerful tools that are available to genetically dissect the molecular and neural pathways, and networks that underlie complex behaviors. The genus Drosophila is an exceptional model system for understanding the evolution of behavior, including reproductive behaviors. The melanogaster species subgroup consists of nine species, divided into four different species complexes. Two of these, the melanogaster and simulans complexes, are extensively studied models of species formation. The melanogaster complex consists of a single species, D. melanogaster, and is sister to three sibling species: D. simulans, D. mauritiana, and D. sechellia. There is extensive literature relating to behaviors, in their ecological settings, of multiple species in the genus Drosophila, allowing a comparative approach that can help determine how various behaviors may have evolved over a macroevolutionary scale spanning > 60 MY and in response to a diverse set of selection pressures (including, but not limited to, environmental conditions, host plant chemistry, predation pressures, and reproductive isolation). Sequeined genomes exist (Drosophila 12 Genomes Consortium et al. 2007; Song et al. 2011; Miller et al. 2019; Yang et al. 2018) for > 20 species, permitting comparative evolutionary studies on the genes that underlie behaviors. At the same time, the genus Drosophila contains a major genetic model system, D. melanogaster, that has been the subject of study by many laboratories for many behaviors, and their neural and genetic underpinnings, providing a framework from which to
consider variation within and between Drosophila species. The community has assembled a series of resources that allow evolutionary studies at both the micro- and the macro-level. Resources for the microlevel include the Drosophila Synthetic Population Resource, a population of recombinant inbred lines derived from an advanced intercross population of eight founder strains (King et al. 2012; Long et al. 2014), and the D. melanogaster Genetic Reference Panel (DGRP), a publicly available resource of 205 fully sequenced lines that harnesses naturally occurring variants for the dissection of complex traits, including behaviors (Mackay et al. 2012; Huang et al. 2014), and can identify candidate genes by association that can be tested genetically. The macrolevel is represented by the existence and study of many species whose phylogenetic relationships are known, and for which genomes, natural biology, and descriptive studies are available. Finally, the advent of clustered regularly interspaced short palindromic repeats (CRISPR)/Cas technologies (e.g., Jinek et al. 2012; Bassett et al. 2013; Gratz et al. 2013) allows one to toggle back-and-forth between the behavioral consequences of pathways that are known in D. melanogaster and ways that they can be modulated based on results from other species.

Selective forces act on genetic variation within a population. Natural selection can change allele frequencies of mutations that improve or reduce fitness. Thus, a precondition for any behavior to evolve is that there is genetic variation for the behavior within the population. The DGRP and other resources mentioned above have revealed extensive natural variation for behavioral phenotypes (Brown et al. 2013; Harbison et al. 2013; Swarup et al. 2013; Appel et al. 2015; Arya et al. 2015; Ayroles et al. 2015; Garlapow et al. 2015; Shorter et al. 2015; Carbone et al. 2016; Rohde et al. 2017; Wu et al. 2018a), including reproductive behaviors (Turner et al. 2013; Gaertner et al. 2015). The genetic architectures underlying behaviors are often complex, allowing very fine-grained effects when selective forces result in the redistribution of allele frequencies leading to modifications of the genetic networks that orchestrate the behavior. Gene-by-gene and gene-by-environment interactions among genes or their networks are prominent hallmarks of behavioral phenotypes (Fedorowicz et al. 1998; Sambandan et al. 2006, 2008; Rollmann et al. 2007, 2008; Yamamoto et al. 2008, 2009; Kent et al. 2009; Zwarts et al. 2011; Huang et al. 2012; Swarup et al. 2012, 2013; Shorter et al. 2015; He et al. 2016).

This review leans heavily on the D. melanogaster literature for an understanding of the genetic mechanisms underlying specific reproductive behaviors and their variation. We then attempt to integrate this genetic information in a phylogenetic context to discuss how such behaviors may have arisen across the genus Drosophila. The obvious caveat here is that there is a large disconnect between what we know in D. melanogaster and the diverse behaviors that are observed across the genus. This is because behaviors, particularly those relating to reproduction, are among the most rapidly evolving traits. Accurately and completely reconstructing changes that may have taken place over relatively short time periods millions of years ago is a challenging analytical problem. Here, we present hypotheses for how divergent reproductive behaviors may have evolved, including given the genes known from studies of D. melanogaster. We will conclude with a Perspectives section summarizing the current understanding in the field and suggesting future studies.

**Drosophila Reproductive Behavior**

Adult Drosophila perform a series of behaviors linked to survival and fitness. Male and female Drosophila must locate mating substrates, most of which are also associated with feeding resources. Once in the mating arena, flies must identify conspecifics and, in some cases, compete for access to partners. The latter often encompasses aggressive behaviors to defend feeding and mating opportunities. Once mating has been successful, females must locate a suitable location to oviposit. Both biotic and abiotic stimuli impact reproductive behaviors. For example, social conditions can affect reproduction (Krupp et al. 2008).

**Courtship and mating**

A diverse array of behaviors in the genus Drosophila are associated with reproduction, including those that occur prior to mating, such as male–male aggression and various courtship displays by both males and females, and ones taking place following intromission, such as male guarding and changes in female remating behavior. Many of these behaviors often require specialized morphologies of genitalia, forelegs, mouthparts, and wings; modifications to those structures evolve with changes in those behaviors (Tanaka et al. 2009, 2015). Likewise, several molecules are associated with reproductive behaviors, including seminal proteins and pheromones, and these too evolve with the behaviors [Swanson et al. (2001) and Haerty et al. (2007); reviews include Swanson and Vacquier (2002), and Panhuis et al. (2006); the neurogenetics of female D. melanogaster reproductive behaviors has been reviewed by Laturney and Billeter (2014)]. Mating and courtship behaviors, as well as associated reproductive traits, tend to evolve rapidly, in part because of their importance in species isolation mechanisms, and in part because of sexual conflicts between males (sperm competition), and between the “interests” and strategies of females and males (Swanson and Vacquier 2002; O’Grady and Markow 2012). These behaviors include aggressive male–male competition, sexual selection acting on a range of characteristics, and sexual antagonism between conspecific males and females resulting in a coevolutionary arms race. The cost of sexual selection was demonstrated by an elegant laboratory evolution study in which single D. melanogaster females were mated with single males for 47 generations. Removal of sexual selection over many generations of forced monogamy resulted in decreased intermale aggression and increased
resistance of females to male-induced postmating effects (Holland and Rice 1999).

Behaviors, because of their rapid evolution and highly plastic nature, can quickly establish barriers between species. The diversity of reproductive behaviors observed among Drosophila species is an important component of this isolation in this genus (Markow and O’Grady 2005, 2006, 2008; O’Grady and Markow 2012). Traditionally, such barriers can arise among premating, mating, postmating prezygotic, and zygotic behaviors, depending on when they occur during mating and reproduction. Premating isolation mechanisms can help individuals identify conspecifics, thus preventing matings between different species. Mating barriers are important, so individuals do not waste valuable resources (e.g., gametes and energy) on nonproductive matings. However, should interspecies matings occur, postmating prezygotic and zygotic barriers can decrease the reproductive success of the cross-species mating.

Premating and mating

Premating behaviors allow males and females to recognize and assess partners to determine whether the species, reproductive state, and condition of the partner are appropriate for and conducive to mating. Courtship rituals in Drosophila follow a multimodal pattern, mediated through visual, chemical, tactile, and auditory signals, and consist of a sequence of-oriented, genital licking, courtship song through wing vibration, and attempted copulation (Hall 1994; reviewed in Sokolowski 2001; Figure 1). Courtship behaviors include locating a potential mate by using visual or olfactory cues, and communicating with and assessing the potential mate by olfactory, auditory, and visual cues during courtship. Behaviors during mating also are important for reproductive fitness (Markow and O’Grady 2008) and are variable. These include copulation duration as well as interactions that determine whether gametes will be present for fertilization. Although the courtship ritual of D. melanogaster males was once considered a defined stereotypical progression of behavioral elements, studies using the DGRP showed extensive heritable variation in courtship patterns (Gaertner et al. 2015). Selection acting on variation in any of the sensory inputs that drive the component behaviors of this multimodal courtship ritual can lead to a reproductive isolation barrier as a scaffold for incipient speciation.

Throughout the courtship process, females assess male quality [see Laturney and Billeter (2014) for review]. For example, D. melanogaster females tend to prefer larger males (Markow and O’Grady 2006). Also, in D. melanogaster, females appear to prefer “successful” males: a male is more likely to be selected as a mate by a female who observes him mating with another female (Mery et al. 2009). Communication between flies about male quality has been suggested (Danchin et al. 2010). As a result of this assessment process, females are either receptive to mate, or they decamp and leave the courtship arena. Females can reject males by flying away or, particularly if the female has mated previously, by kicking males away or extruding the ovipositor to block the male’s access (Connolly and Cook 1973). Males persist in attempting to mate but learn from the experience: D. melanogaster male virgins who have been repeatedly rejected become less likely to mate (Siegel and Hall 1979).

Courtship behaviors in D. melanogaster [reviewed in Sokolowski (2001); Figure 1] contain all the major components of courtship seen in most other species in the genus. While courtship and mating is a continual process, and difficult to divide into discrete units, we can distinguish three broad categories of this composite behavior: mate location, display, and copulation. All species in the genus Drosophila perform these three actions during the courtship process, although the relative order, duration, and importance of each vary between species.

Mate location

D. melanogaster, and most other members of the genus Drosophila, encounter mates in close proximity to feeding resources. Therefore, mate location in most species is tied to the long-distance volatile plumes produced by microbes and associated decomposing substrates (Grosjean et al. 2011; Becher et al. 2012). This eliminates the need for species-specific volatile sex pheromones to broadcast mate location. Instead, a suite of compounds, many of which are components of decomposing host plants, evokes strong responses in specific olfactory sensory neurons (Laissue and Vosshall 2008), leading to aggregation or mate finding. This behavior is described further in the Oviposition section.

While most Drosophila species locate mates using food-related cues, with both courtship display and copulation occurring at the feeding site, many endemic Hawaiian Drosophila utilize a location separate from the feeding and oviposition substrate for courtship and copulation (Spieih 1966, 1984; Figure 2). Males aggregate to engage in competitive displays to entice visiting females that are surveying prospective mating partners. This “lek behavior” is unique to Hawaiian Drosophila. Lek behavior correlates with a high degree of male–male aggression, with competitions between males lasting > 20 min (Spieih 1984). Within some rainforest habitats, individual trees can serve as arenas for multiple Hawaiian Drosophila species, creating a spatially partitioned multispecies lek (Bell and Kipp 1994).

“Orienting” using visual and tactile displays

Once a mate is located, the first step in a successful mating is for males and females to be properly positioned so they can mate [reviewed in Sokolowski (2001); Figure 1]. This is generally referred to as orienting, where individuals of both sexes undergo a complex series of behaviors across visual, auditory, chemical, and tactile modalities to position themselves in preparation for mating. Some of these are stereotypical species-specific behaviors, while others are situational and do not necessarily occur in all mating events. Canalized, repeated display elements are considered part of the mating ritual while other, more opportunistic behaviors can be
considered as a prelude to mating itself. *D. melanogaster* males will often chase females in their initial approach, eventually ending up in front of the female or slightly to her side [reviewed in Sokolowski (2001)]. This is primarily a visual display but can also include tactile aspects in which males use their forelegs to tap females either on their heads or forelegs. Both of these areas of the female contain high concentrations of chemosensory cells, which may sense pheromonal cues (Miyamoto and Amrein 2008). Thus, this behavior by males allows females to smell or taste potential mates (discussed below). Females, in turn, assess and respond to the male, either by being receptive to the mating display or by decamping and leaving the mating area.

While both sexes may be involved in signaling during reproduction, males are the primary signalers. Wings are important in orientation and males will often hold them out to their sides [reviewed in Sokolowski (2001)], perpendicular to the plane of the body, thus enlarging their visual footprint. Depending on the species, wings can range from completely clear ("hyaline"; e.g., *D. melanogaster*) to lightly shaded ("infuscated") near the cross veins, to possessing distinct apical spots or having more extensive patterns of spots, stripes, and/or darkened areas of the wings (e.g., *D. biarmipes*) (Markow and O'Grady 2006; Figure 2). Males with patterned wings often wave them during visual displays to females.

Wing patterning can play an important role in species recognition. It has been suggested that it provides visual stimulation by accentuating the visibility of wing vibrations during courtship (Shevtsova et al. 2011) and, thus, may have gained an evolutionary advantage and spread to fixation in some species in which they have appeared (Fuyama 1979; Hegde et al. 2005). Indeed, many *Drosophila* species, including the agricultural pest *D. suzukii*, have independently evolved wing pigmentation spots in some males (Figure 2; True et al. 1999; Gompel et al. 2005; Prud'homme et al. 2006;
reported that noninvasive elimination of the wing spot of species of the suzukii group—D. biarmipes, D. suzukii, and D. subpulchrella—did not affect mating success. As noted above, different mechanisms have been used in different lineages to result in wing spots. D. biarmipes has acquired a transcription factor-binding site for the Engrailed transcriptional regulator through modification of a cis-regulatory element at the yellow locus (Gompel et al. 2005; Prud’homme et al. 2006). This results in the appearance of a male-specific pigmented spot on the wing. Multiple wing spots are present on the wings of D. guttifera as a consequence of a different regulatory mechanism that results in Wingless-mediated regulation of pigment deposition (Werner et al. 2010).

Visual displays are part of the mating behaviors of most Drosophila species. These displays include a variety of behaviors often referred to as mating “dances,” and can include wings, forelegs, and mouth parts (Markow and O’Grady 2008). Mating dances are species-specific, and distinct from the situational movements used to optimize the positions of females and males for mating. Some species, such as D. affinis, are so reliant on visual displays that courtship is abolished in darkness (McRobert and Tompkins 1987). One endemic Hawaiian species, D. clavistetae, has a particularly unique display involving both visual and chemosensory cues. Males of this species possess elongated setae on the tips of their abdomens, extending anteriorly from the cerci. These setae are used as part of the mating display: when the abdomen is everted over the top of the male’s head, the setae are extended, and a droplet of fluid is secreted and displayed in front of the female. The female then touches this droplet, obtaining taste and/or smell signals (Spieth 1966, 1974).

Another lineage of Hawaiian species, the antopocerus group, has elongated antennae that are densely packed with chemosensory setae on the dorsal surface and devoid of setae on the ventral surface. These antennae, along with modified setae on the male forelegs, are displayed to females during courtship. Spieth (1968) described the unique “lunge” behavior performed by this group. The elongated first and second antennal segments of the male forcibly spread the female’s wings, with the ventral surface of the third antennal segment sliding along the posterior surface of the female wing. These antennal modifications correlate with a dimorphism in olfactory centers of the brain, suggesting a role in host or mate location mediated by chemosensation, in addition to mediating the physical interaction during courtship (Kondoh et al. 2003).

**Courtship song**

Another important mating cue is the mating “song,” usually produced by the male. Drosophila mating “songs” are composed of a combination of sounds generated by vibrating or “clanking” the wings, and/or by drumming the forelegs on the substrate, rather than being a true vocalization (Fabre et al. 2012; Mazzoni et al. 2013). Different Drosophila species have evolved distinct courtship songs, and natural genetic variation in courtship song within species bears testimony

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**Figure 2** Phylogenetic distribution of reproductive behaviors and associated morphologies in species groups of Drosophila. All terminal taxa represent species groups [for definitions see Markow and O’Grady (2005) and O’Grady and DeSalle (2018)]. AMC indicates the antopocerus-modified tarsus clade; PNA indicates the picture wing-nudidrosophila clade. Open squares denote missing information. Characters that are polymorphic within a group show multiple colors. Sexual dimorphism (present, green with horizontal line; absent, black). Dimorphic characters, observed in males, include wings (W), forelegs (L), mouthparts (M), and head broadening (H). Wing spreading (present, green with horizontal line; absent, black). Wing pigment (present, green with horizontal line; absent, black). Reproductive maturity in males and females (0–5 days, red with diagonal line; 6–10 days, green with horizontal line; 11–15 days, orange with vertical line, > 15 days, blue with dot). Sperm size (< 6 mm (short), red with diagonal line; > 6 mm (giant), blue with dot). Ovariole numbers (< 25, red with diagonal; > 25, blue with dot). Female remating frequency (frequent, red with diagonal line; infrequent, blue with dot). Copulation duration (< 20 min, red with diagonal line; 20–60 min, green with horizontal line; > 60 min, orange with vertical line). Insemination reaction (none, red with diagonal line; moderate, green with horizontal line; strong, orange with vertical line).

Edwards et al. 2007; Werner 2015), and experiments with D. biarmipes showed that males with wing spots mate faster and have greater mating success than males without wing spots (Hegde et al. 2005). However, Roy and Gleason (2019)
to the plasticity and evolvability of this courtship component (Gleason 2005; Arthur et al. 2013; Ding et al. 2016). Males of most species sing to females, sometimes with as many as four distinct songs (Markow and O’Grady 2006).

*D. melanogaster* creates its courtship song through wing vibrations, which give rise to distinctive pulse song and sine song components with characteristic rhythms, frequencies, and interpulse intervals (Kyriacou and Hall 1980). While the sine song is a regularly fluctuating song, the pulse song reflects irregular bursts of sound waves. These differences are the result of how the songs are produced and have a functional role in courtship (Kyriacou and Hall 1982; Ritchie et al. 1999; Kowalski et al. 2004; Talyn and Dowse 2004; Tomaru et al. 2004). Kyriacou and Hall (1982) showed that *D. melanogaster* females and females of its closely related species *D. simulans* mate most readily when exposed to their species-specific songs, with their characteristic interpulse intervals and oscillations. Ritchie et al. (1999) confirmed that *D. melanogaster* females mate most quickly when stimulated by songs typical of their own species rather than songs of *D. simulans* or *D. sechellia*. Talyn and Dowse (2004) showed that the pulse song rather than the sine song stimulates female mating. Recently, Clemens et al. (2018) reported a second pulse song in *D. melanogaster*. They also found that visual feedback from females influenced male song choice and that male song selection had an impact on female response (e.g., receptivity vs. rejection). This male display also includes a complex visual component, with males ranging in position from directly in front of females to perpendicular to the female and vibrating the wings to produce the song [reviewed in Sokolowski (2001)]. The visual component continues as males position themselves directly behind the female.

The rhythmicity of the song appears to be regulated by genes associated with the regulation of circadian activity [see Dubowy and Sehgal (2017) for a review of circadian rhythms in *Drosophila*]. The *per* gene was one of the first loci implicated, based on analysis of mutants, in affecting the rhythmicity of the courtship song (Kyriacou and Hall 1980). In addition to circadian rhythmicity genes, genes identified in studies on other traits have been correlated with courtship song characteristics. For example, a study of 27 natural lines in *D. melanogaster* identified five polymorphisms at the cacophony (cac) locus that appeared to segregate under neutral selection and were associated with variation in interpulse interval, pulse amplitude, and cycles per pulse (Peixoto and Hall 1998; Peixoto et al. 2000). Finally, QTL mapping studies of courtship song qualities demonstrated that the song is a highly polygenic trait (Gleason 2005). QTL mapping studies on recombinant inbred lines of *D. melanogaster* showed variance that exceeded the variance observed in the parental lines, indicative of epistasis (Gleason et al. 2002). However, QTL studies have only rarely (e.g., Ding et al. 2016) identified causal polymorphisms associated with variation in courtship song parameters.

Song production requires a neural circuit that enables manifestation of the courtship song; this circuit likely evolves with the song. Male-specific neuronal differentiation of the song circuit has been attributed to genes that comprise the sex-determination pathway. Neurons in the brain that express *fruitless*, designated P1 and PIp10 neurons, initiate the onset of courtship song [for reviews on the *fruitless*-expressing neuronal circuit that controls courtship behavior, see Yamamoto and Koganezawa (2013) and Yamamoto et al. (2014)]. The descending PIp10 neuron drives activity of thoracic motor neurons to shape the courtship song (von Philipsborn et al. 2011). In addition, regulation of sexual differentiation by *doublesex* results in malespecific expansion of dendritic arborizations of thoracic interneurons (TN1A neurons), which drive activity of the hgl1 wing motor neuron (Shirangi et al. 2016). Studies using noninvasive imaging have identified at least seven motor neurons for the generation of courtship song that are distinct from motor neurons used to power flight (O’Sullivan et al. 2018).

Like many reproductive traits, mating songs evolve rapidly. Examples of rapid mating song evolution are seen within the *melanogaster* species group, where females distinguish and prefer the song from their species over that of other, even closely related, species. Moreover, males of most species in this group have two distinct courtship songs. *D. yakuba* generates two types of pulse song, one generated by wing vibration as in *D. melanogaster* and the other, termed “clack song,” which results from clapping both wings together behind the back (Demetriades et al. 1999). Inhibition of the descending PIp10 neurons by tetanus toxin results in loss of the clack component of the song, and optogenetic activation of the PIp10 neuron results in the production of clack song. Production of the clack song is dependent on the level of light intensity (Ding et al. 2019). In *D. yakuba*, under low light only clacks are produced, but under high light intensity, clacks followed by a pulse song are observed, indicating that PIp10 neurons can access both pulse and clack song circuits in an environment-dependent manner. Whereas electrophysiological properties of the PIp10 neurons are conserved between *D. yakuba* and *D. melanogaster*, a neuroanatomical comparison between these species showed significant quantifiable differences in the song circuit, with about a twofold increase in the mesothoracic triangle of *D. yakuba* compared to *D. melanogaster* (Ding et al. 2019). The mesothoracic triangle is a triangular-shaped structure between the dorsal pro- and mesothoracic ganglia in the ventral nerve cord, which represents a neural circuit associated with courtship behavior (Yu et al. 2010). An elegant study comparing the courtship songs of the closely related species *D. mauritiana* and *D. simulans* combined a classical QTL mapping approach with CRISPR technology to identify a transposable element insertion in the *slowpoke* (*slo*) gene that accounts for differences in the sine song between these two species. This gene encodes a calcium-activated potassium channel that is expressed in neurons and muscles; molecular variation at this
locus may affect muscle movements necessary to generate the courtship song (Ding et al. 2016).

Males of species in the repleta group produce either one or two songs. This shows a phylogenetic distribution: taxa with one song are in one clade and those with two are in another [reviewed in Markow and O’Grady (2005)]. The range of variation is wider in the obscura species group, where species have either one or two songs (Markow and O’Grady 2005). In yet another variation, D. subobscura has entirely lost an auditory display; it does not sing during courtship. Song characteristics in the willistoni species group are even more variable. While D. nebulosa does not sing, D. paulistorum has two distinct songs (Ritchie and Gleason 1995; Gleason and Ritchie 1998). Two other species, D. insularis and D. willistoni, utilize three songs during courtship. Interestingly, two species in the species group, D. tropicalis and D. equinoxialis, have independently evolved a fourth song. The virilis species group also shows a pattern of independent gains of courtship song (Ritchie and Gleason 1995; Gleason and Ritchie 1998). The ancestral condition is a single song, with four unrelated species each having independently evolved a second song [reviewed in Markow and O’Grady (2005)]. The high degree of variability in auditory display is likely a reflection of how important these behaviors are, not only for females to assess male quality, but also to identify conspecific males. However, songs are constrained against being too variable. Any male with a song outside the tolerances of a conspecific female may not have the opportunity to mate (Ritchie and Kyriacou 1994; Ritchie and Gleason 1995; Klappert et al. 2007).

Evolution of courtship song requires coevolution of the production of the song by the male and receptivity to that particular song by the targeted female. Whereas most studies have focused on the production of courtship song by the male, fewer studies have focused on the female’s perception of the song. Sound is perceived by mechanosensory antennal neurons in Johnston’s organ, which project to the antennal mechanosensory and motor center (AMMC), the first synaptic relay in the brain. This relay filters incoming information to preserve the spacing of song pulses (Tootoonian et al. 2012). Recordings from central neurons that innervate the AMCC of D. melanogaster and D. simulans did not show differences in responses to their pulse songs, despite the fact that each species has evolved a unique song (Tootoonian et al. 2012), and behavioral studies show that each species responds specifically to its species-specific song (Kyriacou and Hall 1982; Ritchie et al. 1999). Thus, details of how the spectral characteristics of the courtship song are represented in the female brain and the neural basis of their species divergence that results in species-specific female behaviors in response to male songs remain to be elucidated.

Evolution of courtship song may be driven by female preference (Hoikkala et al. 1998; Yukilevich et al. 2016). Females of D. montana use courtship song to evaluate the genetic quality of the courting male and they prefer a courtship song with short sound pulses at high frequency (Hoikkala et al. 1998). Therefore, males with longer sound pulses would mate less frequently and, as a consequence, songs with such pulses would become less common in the population.

Variation in courtship song can lead to sexual isolation. This is especially evident in the striking diversity of courtship songs among the > 500 species of Drosophila that have evolved on the Hawaiian Islands. Some species have acquired aspects of their courtship songs that are reminiscent of the complex pulse rhythm observed in crickets (D. cyrtoloma) or the high carrier frequency seen in cicadas (D. fasciculissetae). Others, like D. silvestris, have songs more similar to the courtship song of D. melanogaster, but use abdominal rather than wing vibrations to generate the song (Hoy et al. 1988).

Females of some species, such as members of the virilis species group, respond to males with a song of their own, indicating receptivity to courtship (Satokangas et al. 1994; LaRue et al. 2015). While little is currently known about why female songs evolved in this group, several lines of evidence suggest that it might be a way to recognize conspecific individuals and, in closely related species, to provide a barrier to interspecific matings. Male responses to female songs in virilis species range from singing and the licking of female terminalia to completely stopping courtship. Interestingly, male genitalia in the virilis group show little variation, suggesting that morphology is most likely not the way in which conspecific individuals recognize one another, and that pre-mating barriers are weak or lacking in this group. Evidence from forced interspecific matings in the laboratory shows that fertile hybrids are obtained between almost all species pairs (Throckmorton 1982). This suggests that there are few post-mating barriers in this group. Thus, female songs in the virilis group (Satokangas et al. 1994) may have arisen to prevent interspecific matings in a morphologically homogeneous group of genetically closely related taxa.

**Evolution of pheromone-mediated courtship signals**

In addition to male wing displays, courtship and mating in Drosophila species are guided by chemical signals exchanged between the male and female [reviewed in Venard and Jallon (1980), Jallon (1984), Ferveur (1997, 2005), and Billette and Wolfner (2018)].

**Cuticular hydrocarbons:** Cuticular hydrocarbons protect against desiccation, and some components of the cuticle have been coopted as contact pheromones (Chung and Carroll 2015). Evolution of pheromonal communication requires co-evolution of the production and perception of the pheromonal profiles of the interacting partners, and shifts in chemical communication, like changes in the courtship song, can establish reproductive barriers. Thus, evolution of differences in the hydrocarbon profile and response could lead to reproductive isolation. Male mate choice based on discrimination of female cuticular hydrocarbons has been implicated as the driving source for reproductive isolation between D.
bers and D. sechellia (Shahandeh et al. 2018). D. melano-
aster, D. simulans, and D. sechellia evolved different sen-
sitivities, and behavioral responses, to the aggregation
pheromones (Z)-8-tetradecenoic acid and (Z)-7-tetradeceno-
acid (Mast et al. 2014). Responses to these compounds are
mediated by neurons that express ppk23 and ppk29 (Mast
et al. 2014; Seeholzer et al. 2018). In adults, ion channels of
the pickpocket family are expressed in fruitless (fru)-expressing
neurons on the legs that respond to pheromones by mediat-
ing inhibition of courtship between males, while promoting
male–female interactions (Thistle et al. 2012; Toda et al.
2012). In addition to ppk23, ppk25 also promotes conspecific
courtship in D. simulans (Ahmed et al. 2019).

The cuticular hydrocarbon profile is sexually dimorphic in
D. melanogaster (Antony and Jallon 1982; Jallon and David
1987; Grillet et al. 2006; Dembeck et al. 2015) and other
Drosophila species, including D. sechellia (Jallon and David
1987; Gleason et al. 2009) and D. erecta (Jallon and David
1987), but sexual dimorphism in hydrocarbon profile has not
been observed in their closely related sister species D. simu-
lan (Gleason et al. 2009). D. melanogaster males produce
7-tricosene as their principal cuticular pheromone, whereas
females produce 7,11-dienes, such as 7,11-heptacosadiene.
Analysis of DGRP strains revealed extensive variation in cu-
ticular hydrocarbon profiles (Dembeck et al. 2015). Variation
in hydrocarbon profiles is also evident among species of the
genus Drosophila. Among the three closely related sister spe-
cies D. simulans, D. mauritiana, and D. sechellia, the predo-
ninant male hydrocarbon in D. sechellia is 6-tricosene (Coyne
1996a). Females of D. simulans predominantly produce
7-tricosene, the pheromone characteristic of D. melanogaster
males (Coyne 1996b). The predominant compound of the
cuticular hydrocarbon profile of D. sechellia females is
7,11-heptacosadiene, whereas 7-tricosene is prevalent in the
cuticular hydrocarbon profile of females of D. mauritiana
(Coyne and Charleworth 1997). Thus, distinct cuticular hu-
drocarbon compositions have evolved among closely related
species within the melanogaster group.

D. melanogaster males are stimulated by the 7,11-
heptacosadiene produced by the females, but this same phero-
mone inhibits courtship in males of its sister species D. simu-
lan (Coyne et al. 1994; Marcillaci et al. 2005b; Seeholzer et al.
2018). Evolutionary reorganization of the neural
route that regulates the activity of P1 neurons, which is
implicated in mediating courtship song (von Philippsborn et al.
2011), is responsible for the switch in the activation and sup-
pression of courtship in response to 7,11-heptacosadiene be-
 tween D. melanogaster and D. simulans (Seeholzer et al. 2018).

QTL mapping studies on hybrids derived from closely re-
lated species, such as D. melanogaster and D. simulans, or
D. mauritiana and D. sechellia, have implicated loci on the
third chromosome associated with generating differences in
cuticular hydrocarbon composition among species (Coyne
et al. 1994; Coyne 1996a,b; Coyne and Charleworth 1997;
Gleason et al. 2005) and sensing different pheromonal signa-
tures (McMahon et al. 2002). Subsequent studies associated
members of the desaturase gene family with sex-specific ex-
pression and the evolution of differences in cuticular phero-
mones between Drosophila species (Marcillaci et al. 2005a; Legende et al. 2008; Bousquet et al. 2009; Shirangi et al.
2009; Keays et al. 2011; Pardy et al. 2019).

The desaturase gene family, which is located on the third
chromosome in D. melanogaster, has evolved through re-
peated gene duplications and diversification, with evidence
of purifying selection of daughter genes (Keays et al. 2011). A
transposon in the Desat1 gene of D. melanogaster reduced the
production of unsaturated hydrocarbons in both sexes, and
mutant males could not discriminate sex pheromones of con-
 trol flies and vice versa (Marcillaci et al. 2005a). Expression of
DesatF has been correlated with the production of long-chain
dienes across the Drosophila phylogeny. Evolutionary analy-

ses have identified multiple independent inactivations and
changes in sex-specificity of this gene. These studies also
identified the acquisition or loss of a functional binding site
for the sex-determining transcriptional regulator Doublesex
in evolutionary transitions between the acquisition and loss
of sexual dimorphism of gene expression of DesatF (Shirangi
et al. 2009).

African D. melanogaster show different cuticular hydrocar-
bon profiles from those of the cosmopolitan D. melanogaster
strains that are typically used in laboratories. African
D. melanogaster females produce high levels of 5,9-dienes
and low levels of 7,11-heptacosadiene relative to the levels
produced by their cosmopolitan counterparts (Ferveur et al.
1996; Legendre et al. 2008). Variation at the Desat2 locus has
been implicated in this difference (Dallerac et al. 2000), spe-
cifically a 16-bp deletion in the 5′ regulatory region of Desat2
in African D. melanogaster relative to cosmopolitan strains
(Takahashi et al. 2001). An elegant gene replacement study of
Desat2 alleles between a Zimbabwe population and cos-
mopolitan D. melanogaster showed that the cosmopolitan
Desat2 allele also contributed to cold resistance and starva-
tion resistance, suggesting a connection between ecological
adaptation and reproductive isolation (Greenberg et al.
2003). A similar demonstration connecting pheromone-pro-
ducing enzymes with ecological adaptation and reproductive
isolation comes from a temperature selection study of a D.
melanogaster population from the Comoro islands. There, a
few generations of selection at higher temperature resulted
in an increase of 7-pentacosene at the expense of 7-tricosene
and concomitant increased resistance to desiccation, and par-
tial sexual isolation between selected and nonselected strains
(Bontonou et al. 2013). Significant species-specific differences
in cuticular hydrocarbon composition have also been
documented for the closely related sibling species of the de-
sert-dwelling cactophilic D. repleta group, D. mojavensis, D.
arizonae, and D. navojoa. It has been suggested that such
differences occur early in the evolution of new species
(Endes and Jackson 2001).

A comprehensive survey of cuticular hydrocarbon variation
in the DGRP showed that 17 DGRP lines contain the functional
Desat2 allele thought to be unique to African and Caribbean
D. melanogaster females, and accordingly produce a high 5,9-heptacosadiene to 7,11-heptacosadiene ratio. Thus, this Desat2 allele is also segregating among cosmopolitan D. melanogaster populations (Dembeck et al. 2015). Furthermore, genome-wide association analyses with the DGRP identified 305 and 173 genes in females and males, respectively, associated with variation in cuticular hydrocarbon profiles, of which 24 candidate genes, associated with fatty acid metabolism, were causally validated (Dembeck et al. 2015). Thus, variation in cuticular hydrocarbon composition is highly polygenic and is mediated by variation in the activities of enzymes that control cuticular hydrocarbon biosynthesis (Yew and Chung 2015). Variation in pheromonal communication can result from sexually dimorphic variation in the activity of desaturases that form double bonds (Labeur et al. 2002; Houot et al. 2010) and elongases that extend hydrocarbon chains (Cher temps et al. 2007; Ng et al. 2015).

Variation in pheromone-mediated chemical communication is in part due to the ratio of female-characteristic vs. male-characteristic cuticular hydrocarbons. Mutations in Darkener of Apricot (Doa) interfere with sex-specific splicing of doublesex pre-mRNA in D. melanogaster, resulting in the masculinization and feminization of female and male cuticular hydrocarbon profiles, respectively, along with disruption of associated courtship behavior (Fumey and Wicker-Thomas 2017).

Cuticular hydrocarbon profiles of D. melanogaster show extensive environmental plasticity (Rajpurohit et al. 2017). The composition of cuticular hydrocarbons can be modulated by time of day and social environment in D. serrata (Chenoweth et al. 2010; Gershman et al. 2014) and D. melanogaster (Kent et al. 2008; Krupp et al. 2008). Expression of cuticular hydrocarbons in males of the Australian species D. serrata increased when more females than males were present and resulted in greater male mating success (Gershman and Rundle 2017). Transcription of Desat1 in oenocytes, which are the pheromone-producing cells, is under circadian control, leading to fluctuations in the accumulation of cuticular hydrocarbons over the course of a day (Krupp et al. 2008). Cuticular hydrocarbon profiles are also modified as a result of mating and aging (Everaerts et al. 2010). This may involve the epigenetic modulation of gene expression, since aging and mating are accompanied by extensive changes in histone modifications (Zhou et al. 2014). Thus, environmental plasticity, genotype-by-environment interactions, and plasticity in the epigenetic landscape of the genome provide a rich palette for natural selection and the evolution of behavior.

cis-vaccenyl acetate: The pheromone 11-cis-vaccenyl acetate (cVA) regulates courtship behavior and intermale aggression, and attracts females to oviposition sites (Ha and Smith 2006; Kurtovic et al. 2007; Wang and Anderson 2010). It is made in the male’s ejaculatory bulb and transferred to females during mating, where it is found in the mating plug that forms within the bursa (uterus) of the female and decreases her attractiveness. However, this inhibitory effect on attractiveness also requires transfer to the female of cuticular hydrocarbons (rubbed off from her mate during mating), particularly 7-tricosene, made by the male’s oenocytes. These two hydrocarbons are most potent as a blend: a female with reproductive tract cVA and cuticular 7-tricosene is particularly unattractive to males (Laturney and Billeter 2016). A few hours postmating, females eject their mating plugs and with it the cVA that they received from their mates. This changes their hydrocarbon blend, making them somewhat more attractive to males, as compared to immediately after mating when they have both 7-tricosene and cVA.

Odorant-binding proteins (OBPs) are a diverse group of small proteins, some of which are thought to bind hydrophobic odorants and present them to their membrane-bound chemoreceptors (Sun et al. 2018). The LUSH OBP, which was originally identified as a carrier for short-chain alcohols (Kim et al. 1998), serves as a transporter of cVA to the OR67d receptor, mediating courtship behavior and intermale aggression (Ha and Smith 2006; Kurtovic et al. 2007; Wang and Anderson 2010). The OR67d receptor is expressed in trichoid sensillae of the T1 class (van der Goes van Naters and Carlson 2007) and activation of the T1 circuitry promotes courtship behaviors (Ronderos and Smith 2010). However, cVA also interacts with the OR65a receptor suppressing intermale aggressive behavior (Liu et al. 2011) and inhibiting courtship (Lebreton et al. 2014). Thus, it appears that multiple neural circuits activated by cVA regulate intermale aggression and courtship through balanced integration between the activities of both neural projections, which could conceivably be modulated by environmental factors. OBP69a has also been implicated in mediating effects of cVA on social behavior (Benzur et al. 2018).

Dekker et al. (2015) showed that the agricultural pest species D. suzukii does not produce cVA, and in this species the T1 neural projection has regressed. However, D. suzukii has an intact OR67d receptor, and application of cVA to D. suzukii males reduced mating (Dekker et al. 2015). In the absence of an intact T1 neural projection it is possible that this effect is mediated via the OR65a receptor.

Elimination of a functional microRNA, miR-124, which influences the sex-determination pathway in D. melanogaster by limiting expression of the female-specific form of transfor mer, resulted in a change in pheromone profile in males. These males produced less cVA and more pentacosenes, which are attractive to males (Siwicki et al. 2005). This altered pheromone composition promoted male–male courtship with a concomitant reduction in male reproductive success (Weng et al. 2013).

Variations in Premating and Mating Behaviors Across the Genus Drosophila

Near the end of the courtship ritual, D. melanogaster males lick the female’s genitalia with their mouthparts, prior to curling their abdomens and initiating copulation (Sokolowski 2001). Close interactions like tapping and licking may facilitate the use of cuticular hydrocarbons, or short peptides, for species
remate roughly every 22 hr in the wild (Giardina and can be an important factor in reproductive Drosophila. Quent mating would increase this risk.

risky behavior, opening both participants to higher incidences of sperm competition. Mating itself is a risky process, leading to the death or sterility of heterospecific zygotes.

Evolution of male mating advantages

It is to a male's advantage that his mate does not copulate with other males, to protect his sperm investment in the female. If the female's attractiveness decreases after mating, the chance of the male's sperm being competed by a rival's also decreases. Males can use their bodies, sperm, and/or secretions from their reproductive glands to physically or chemically block the female reproductive tract, reducing the chance that another male will mate with the female prior to fertilization and the start of oviposition.

Mate-guarding behavior

Following copulation, males of most Drosophila species depart, leaving the female to feed, oviposit, or mate again. Males of two species of the cactophilic D. repleta species group, D. pegasa and D. mainlandi, have independently evolved a mate-guarding behavior (Wasserman et al. 1971). Males of these species guard females by remaining on the back of the female and riding around on her while she feeds. While genitalia are not in contact for much of this time, his presence physically blocks other males from copulation, not only allowing for time for insemination but also providing the opportunity for a second copulation by the guarding male. D. pegasa, for example, will ride on females for extended periods of time, often > 8 hr (Gronlund et al. 2002), and sometimes for as long as 14 hr. Wasserman et al. (1971) reported “chains” of up to four males riding on the backs of females.

Chemical mate guarding

In some species, males can prevent their mates from remating by chemical or physical means. This is mediated by molecules in the seminal fluid that are transferred during mating. In D. melanogaster, transfer of seminal fluids and sperm initiates at ~5–7 min after coupling (Lung and Wolfner 1999; Gilchrist and Partridge 2000) and continues during the 15–20-min mating. Several different forms of mate guarding can occur.

First, among the seminal secretions that are transferred are components of the mating plug, a physical plug that forms within the mated female. In D. melanogaster at least, many of these components come from the male's ejaculatory bulb, but some come from his accessory glands. The mating plug has been suggested to serve several functions in D. melanogaster, such as possibly forming a scaffold along which sperm can move (Avila et al. 2015a and b). But from the perspective of
this article, which concerns reproductive behaviors, its most important function is to contain cVA, thus decreasing the female’s attractiveness as described above (Laturney and Billeter 2016).

Second, seminal secretions “guard” the female by decreasing her receptivity to remating; these are described in the next section.

Finally, whereas the two examples above concern mate guarding within the male’s own species, in certain interspecies matings insemination can result in the formation of a hard plug, the “insemination reaction” in the bursa of the female. This plug is generated by a chemical reaction between contributions of males (sperm and other seminal proteins) and females (Markow and Ankney 1988). The insemination reaction blocks the reproductive tract and prevents other males, even conspecifics, from copulating with a mated female. The hard plug formed as a result of the insemination reaction is different from a mating plug that forms in the bursa of a female during normal (intraspecific) matings.

**Postmating Behavioral Changes in Drosophila Females: Competition Between the Sexes**

Postmating behaviors have been well characterized in *D. melanogaster*. Behaviors are different between virgin and mated females. First, a mated female is less attractive to courting males. During the first hours postmating, this is due to a change in her pheromonal profile due to molecules transferred from the male, as described above. However, even after expelling the mating plug along with excess unstored sperm by grooming it off with her legs, females' mating propensity still remains low, even though she is less unattractive in a pheromonal sense (as described above).

Rather, at this stage, a *D. melanogaster* female’s receptivity to remating has decreased. Mated females actively reject males, by kicking them off or extruding the ovipositor. These responses have been attributed to the action of a peptide made in the male *D. melanogaster*’s accessory gland and transferred in his seminal fluid (Baumann 1975; Chapman et al. 2003; Liu and Kubli 2003). This 36-amino acid “sex peptide” binds to a G protein-coupled receptor called SPR (Sex Peptide Receptor; Yapici et al. 2008), and potentially to at least one more protein (Haussmann et al. 2013), to exert its action. A small number of neurons that innervate the female’s reproductive tract are sufficient for the loss of receptivity after mating (Häsemeyer et al. 2009; Rezával et al. 2012, 2014; Yang et al. 2009); at least some of these express SPR. During storage, the sex peptide is cleaved to release its C-terminal active region from the sperm. This released region binds to its receptor, keeping the female’s mating receptivity down. Viewed from the perspective of the previous section in terms of benefits to the male of decreasing the chance of his mate’s remating, the sex peptide provides the long-term chemical guarding once the male-derived hydrocarbon pheromones are gone.

In the absence of the sex peptide, copulation itself can decrease female receptivity through the activation of mechanosensitive neurons (Shao et al. 2019). These neurons communicate with a pair of lateral sensory ascending neurons in the abdominal ganglion that project to neurons expressing myoinhibitory peptides in the brain. Even though sex peptide transferred from the male does not contribute to this particular aspect of the decreased female receptivity, SPR is essential for mediating the behavioral switch, suggesting that an endogenous SPR ligand is released during copulation (Shao et al. 2019).

Interestingly, *D. melanogaster* females normally remain less receptive to remating for 10–14 days, for as long as they contain sperm. This is because the sex peptide binds via its N-terminus to sperm, allowing it to be stored in females long-term (Peng et al. 2005). Indeed, this has been posited as a reason for an unusual feature of *Drosophila* sperm: they are very long in some species. Sperm size varies widely across the genus *Drosophila* (Pitnick et al. 1995; Gage 2012), from species with relatively short sperm of ~1 mm in length (e.g., *D. melanogaster*) to species with giant sperm that approach 6 cm in length (*D. bifurca*; Figure 2). Peng et al. (2005) suggest that this great length has arisen because longer sperm can hold more sex peptide, permitting longer (or better) dosing of the female. While this is an attractive hypothesis, it is not clear whether receptivity-regulating seminal proteins in all species with long sperm bind to those sperm. The potential advantage of long sperm for holding sex peptide(s) is balanced by cost: it is energetically demanding to make such long sperm, so species like *D. bifurca* with very long sperm invest in length over sperm numbers.

Several other behaviors change in *D. melanogaster* females after mating, most also due to the action of the sex peptide. Many of these can be understood in terms of increased egg production by mated females. Sex peptides cause mated females to eat more (Carvalho et al. 2006), which provides more resources for their egg production; their diet also changes to more protein-rich (Ribeiro and Dickson 2010; Vargas et al. 2010) and their guts grow and increase their absorptive capacity (Cognigni et al. 2011; Apger-McLaughon and Wollner 2013; Reiff et al. 2015). Receipt of the sex peptide also causes a decrease in females’ siesta sleep (Isaac et al. 2010), presumably giving them more opportunities to move around and lay eggs. Finally, female–female aggression increases postmating (Bath et al. 2017), again as a result of the action of the sex peptide. Presumably this increased aggression aims to increase a female’s access to limited food resources.

Mated females also ovulate at a higher rate, but this is regulated by a different seminal protein, ovulin (Heifetz et al. 2000). Ovulin is a 264-amino acid protein (Monsma and Wollner 1988) that is also made in the male’s accessory gland and transferred to females, where it is proteolytically processed (Park and Wollner 1995). Through an as yet unknown receptor and cellular target, ovulin causes an increase in octopaminergic signaling in the female’s reproductive tract (Rubinstein and Wollner 2013). In turn, this relaxes the muscles that wrap the female’s oviduct, permitting the passage of...
an oocyte for ovulation (Rubinstein and Wolfrner 2013; Mattei et al. 2015). Another muscle-based behavior in the reproductive tract involves the storage of sperm from the mating. *Drosophila* sperm are long, and movement to their storage sites appears to be assisted by contractions and relaxations of the bursa or uterus (Adams and Wolfrner 2007), and potentially by a scaffold provided by the mating plug (Avila et al. 2015b) that coagulates inside the female. These uterine contractions and relaxations are triggered by the action of other seminal proteins, such as the glycoprotein Acp36DE (Avila and Wolfrner 2009).

Finally, mated *D. melanogaster* females show changes in their oviposition site behaviors (Becher et al. 2012). Oviposition site selection includes evaluating sites for ones with low likelihoods of parasites and high likelihoods of food. Females also tend to lay eggs where other females lay; cVA ejected with the mating plug can form part of the cue to attract other females to the site of oviposition (e.g., Bartelt et al. 1985; Lebreton et al. 2012; Billiter and Levine 2015). ILP7-expressing neurons are important in the selection of egg-laying sites by females (Yang et al. 2008). Also, females are attracted to acetic acid, and avoid UV light, when they are laying eggs. Their attraction to acetic acid is not triggered by mating per se but rather by the stretching of the females' reproductive tract as eggs pass through the tract (indirectly, there can be a mating effect, as mating increases egg production and ovulation; e.g., Heifetz et al. 2000, 2001). Some *ppk* mechanosensory neurons that innervate the lateral and common oviducts (tract-tiling *ppk* neurons) are involved in sensing that eggs are present in the reproductive tract (stretching the tract); these are different from the *ppk* neurons that are reported to mediate the response to the sex peptide. The aversion to UV appears to be mediated by bitter-sensing neurons in the female's proboscis, via certain isoforms of the dTRPA1 channel (Guntur et al. 2017).

Seminal proteins delivered by the male often show evidence of rapid evolution under positive selection (e.g., Haerty et al. 2007). The sex peptide is found in many, but not all *Drosophila* species (*D. mojavensis* and *D. grimshawi* appear to lack it) and not outside of Drosophilidae (Tsuda et al. 2015; Tsuda and Aigaki 2016). Its receptor, SPR, is found in most insects (Yapici et al. 2008), likely because of this protein's ancestral role as a receptor for myoinhibitory peptides (Kim et al. 2010; Poels et al. 2010). The sex peptide gene occurs in more than one copy in several *Drosophila* species (Cirera and Aguadé 1997, 1998). Tsuda et al. (2015) showed that *D. melanogaster* sex peptide can induce postmating responses and binds to the oviduct in all *melanogaster*-group species tested, but did not induce postmating responses in females of the *obscura* or *willistoni* groups, or *D. virilis*, and injection of conspecific sex peptides did not either. This suggested to Tsuda and Aigaki (2016) that the induction of postmating responses via sex peptide/SPR interaction has uniquely evolved in the *melanogaster* group (Tsuda and Aigaki 2016). The function of the sex peptide in the other *Drosophila* species remains unknown.

Ovulin is also very rapidly evolving at the primary sequence level and is almost impossible to recognize, even in *D. pseudoobscura* (Clark et al. 1995). The seminal protein Acp36DE also shows evidence of rapid evolution (Wagstaff and Begun 2005). Such rapid evolution is also evident in mammalian seminal proteins (e.g., Dean et al. 2011) and occurs against a backdrop of conserved molecular functions in seminal fluid (Mueller et al. 2004). For example, there are proteases and protease inhibitors in the seminal fluids of all species examined (insects and mammals), but their primary sequences can be very different (reviewed in Laflamme and Wolfrner (2013)). This rapid evolution may be driven by conflicts between the interests of individual males, and/or between the reproductive strategies of males and females. In terms of the former, sperm competition occurs in *D. melanogaster* females, which can mate three to five times in nature (Reinhart et al. 2015). In this case, there will be selection for molecules, including better and new ones, that can improve or enhance the chance that a male's sperm will be stored, or will compete well against sperm of other males. In terms of the latter, the changes that are caused in mated females by seminal proteins from the male may be more beneficial from his perspective than they are from hers.

Decreasing remating can be beneficial to a male in preventing competition from rivals’ sperm, but it may be less advantageous for a “choosy” female who can benefit from the chance to select among the sperm of different males. Similarly, increasing feeding and egg production, and decreasing the female’s sleep may be beneficial to the male in terms of enhancing the fertility of the mating, but it may decrease the female’s health and longevity; indeed, mating, and the sex peptide in particular, decreases the longevity of mated *D. melanogaster* females (Chapman et al. 1995; Wigby and Chapman 2005). Thus, there may be selection on females to develop resistance to male seminal proteins or other modulators, causing strong selection pressure for males to evolve more potent versions of those proteins, or new ones that can be coopted for their functions. In this light, it is interesting that ovulin appears to act at the very top of the ovulation-regulatory cascade, turning up octopaminergic signaling rather than tweaking the details of octopamine’s action. It is conceivably easier to evolve a new switch to turn up an existing, conserved, physiological pathway than to modify the components of that pathway (Kirschner and Gerhart 1998; Hoke et al. 2019). In a somewhat analogous argument, although the sex peptide is a novel molecule, it functions as a new ligand for a conserved, previously existing receptor; SPR is originally the receptor for myoinhibitory peptides. In addition, we can hypothesize that rapid evolution of inducers of postmating behaviors may help in species isolation. In such cases, if heterospecific matings occur despite premating isolation mechanisms, having highly species-specific molecules that induce postmating responses in females may contribute to decreasing the reproductive success of the heterospecific pair.
Oviposition

Oviposition behavior in *Drosophila* can be divided into two phases: (1) finding the oviposition site or “host plant selection,” and (2) the act of physically inserting the egg into the selected substrate (Jaenike 1985, 1990). The former requires chemosensory cues that are interpreted via the olfactory and gustatory system. The latter involves a series of characteristic movements leading up to and including the placement of the egg, and may be correlated with special morphological adaptations depending on the physical structure and stage of decomposition of the oviposition site.

Host plant selection

With a few exceptions, most *Drosophila* species are saprophagous and rely on microbes (i.e., yeasts and bacteria) to break down plant material upon which they feed. Some species, like *D. melanogaster*, are generalists that have been reared successfully from decomposing fruits, fungi, and flowers of many different plant species, as well as a range of other substrates (e.g., decomposing animal matter). However, the majority of species in the genus *Drosophila* display a degree of fidelity in oviposition site selection in or on a given host plant taxon (Throckmorton 1975; Magnacca et al. 2008). This is likely due to the fact that flies are targeting microbes that have a characteristic niche on a specific plant or group of plants (Ort et al. 2012). Interestingly, microbes are not the only drivers in this system, as it is clear that plant secondary compounds also play a role in the selection of an oviposition site, either as deterrents or as attractors [reviewed in Barker and Starmer (1982)]. For example, volatile profiles of necrotic cacti species (and many other plants) are highly attractive to some *Drosophila* species. However, once *Drosophila* arrive on a substrate, they may find that toxic plant compounds make that substrate unsuitable. Therefore, when assessing a given substrate, *Drosophila* must make decisions based on a complex calculation involving microbial community profile, plant species, and stage of decay of a substrate (Yang et al. 2008; Guntur et al. 2017). Since the microbial community is linked to the host plant and decay condition, we will refer to this behavior broadly as host plant selection.

Most *Drosophila* species fit along a spectrum from oviposition generalists, where a single species of fly may use many different plants for egg laying and development, to oviposition specialists that lay eggs on only a single plant species. O’Grady and Markow (2012) recognized four broad categories when discussing oviposition preference in *Drosophila*. “Broad generalists” can utilize a wide range of resources spread across feeding guilds. *D. melanogaster* is an example of a broad generalist and will oviposit in nearly any decomposing fruit, flower, or fungus.

“Substrate generalists” feed on a single type of host plant, but can utilize a wide range of unrelated host species of similar types. Members of the *tripunctata* species group fall into this category; they oviposit on fungi but, rather than lay eggs on a single fungal species, they oviposit on and develop in many different unrelated fungal species (Throckmorton 1975).

“Substrate specialists” utilize a single substrate type from a single clade of host plant. For example, the cactophilic *repleta* species group oviposits on only a single family of plant, Cactaceae (Oliveira et al. 2012). However, within this single family there are many different host species, and various *repleta*-group species may be generally attracted to and utilize many. Hawaiian *Drosophila* are also categorized as substrate specialists (Magnacca et al. 2008) since they oviposit on a single substrate from a single plant lineage (e.g., leaves of Araliaceae).

The most narrowly defined class of specialists are the “true specialists.” These species use only a single type of substrate from a single species of host plant. *D. sechellia*, which uses only the noni fruit (*Morinda citrifolia*) for oviposition, is an example of a specialist species (Jones 2005). *D. pachea*, a species that only uses senita cactus (*Pachycereus schottii*), is another (Lang et al. 2012).

Female ovipositor and egg morphologies often vary with preferred host sites. This has been studied extensively in the Hawaiian *Drosophila* and Scaptomyza (Craddock and Kambyssellis 1990; Kambyssellis et al. 1995). Species of *Scaptomyza* tend to have simple, fleshy ovipositors without many bristles or even sclerotization (the darkening of the cuticle that indicates cross-linking of proteins and structural strength). These taxa oviposit in flowers or on the surfaces of leaves, effectively “dumping” eggs, rather than inserting them into the host substrate (Kambyssellis et al. 1995). The respiratory filaments of the eggs of these species are correspondingly very short or completely absent, reflecting the abundance of available oxygen and the relatively dry oviposition substrates. In contrast, females of the *Drosophila picture wing* species group possess large, strong ovipositors with many bristles (Kambyssellis et al. 1995). These species insert their eggs deep into rotting wood or other decomposing plant tissue. Respiratory filaments in these species are elongated, so eggs can access oxygen during development. In the species just discussed, ovipositor length, oviposition depth, and respiratory filament length are highly correlated.

Attraction to or repulsion from oviposition sites

Chemical recognition features prominently in how *Drosophila* select host plants for feeding and oviposition. The ability to feed on a food source that is repellent to competing species reduces competition, and ensures a reliable niche for a species that is adapted to and specialized on a specific plant. For example, the noni plant, *M. citrifolia*, produces hexanoic and octanoic acid that are repellent to most *Drosophila* species (Amlou et al. 1998; Jones 2005). However, *D. sechellia* is attracted to *Morinda* and, as mentioned above, can successfully oviposit and complete development on this plant. Host plant specialization of *D. sechellia* has arisen through several distinct genetic adaptations, including rapid evolutionary adaptations of its chemoreceptor repertoire (McBride 2007; McBride et al. 2007; Figure 3). Adaptation to *Morinda*
involves expression of two Obp genes, *Obp57d* and *Obp57e*, which are expressed in taste sensilla on the legs. Wild-type expression of these two OBPs results in the avoidance of hexanoic and octanoic fatty acids. Conserved cis-regulatory elements have been identified that govern expression of these OBPs (Tomiioka et al. 2012). Matsuo et al. (2007) reported that a 4-bp CCAT insertion upstream of the *Obp57e* gene in *D. sechellia* prevents its expression, while its open reading frame remains intact. However, Dworkin and Jones (2009) identified a premature stop codon in the *D. sechellia Obp56e* gene resulting in a loss-of-function allele. It is possible that multiple *Obp* alleles have evolved in the *D. sechellia* lineage that have the same end result on host preference behavior, namely the abolition of avoidance of hexanoic and octanoic acid. Deletion of *Obp57e* and *Obp57d* in *D. melanogaster* also resulted in altered behavioral responses to hexanoic acid and octanoic acid (Matsuo et al. 2007). Furthermore, studies on interspecies hybrids between *D. melanogaster Obp57d/e* knockout flies and *D. simulans* or *D. sechellia*, shifted oviposition site preferences of the hybrid offspring to that of *D. simulans* or *D. sechellia*, respectively (Matsuo et al. 2007).

The olfactory system of *D. melanogaster* has been well characterized. Olfaction is mediated through odorant receptors expressed by olfactory sensory neurons in sensilla on the third antennal segment. Each neuron expresses a single odorant receptor from among the chemoreceptor repertoire and axons of olfactory sensory neurons that express the same receptor converge on output neurons in the antennal lobes, forming spherical structures of neuropil: glomeruli. Combinatorial activation of olfactory sensory neurons is translated in a spatial and temporal pattern of glomerular activity that encodes the concentration and quality of the odor [reviewed by Joseph and Carlson (2015)].

Dekker et al. (2006) reported femtogram-level sensitivity of *D. sechellia* antennae to the host plant odorant methyl hexanoate with an approximately threefold overrepresentation of neurons responding to this odorant, compared to *D. melanogaster*, and a concomitant increase in volume of the glomerulus to which they project. Ibba et al. (2010) reported that the A neuron of the ab3 sensillum responds to hexanoate esters and projects to its corresponding enlarged DM2 glomerulus, whereas ab3B neurons respond to 2-heptanone, another volatile compound from the *Morinda* fruit, and also project to an enlarged glomerulus. Subsequently, Prieto-Godino et al. (2017) studied acid-sensing circuitry, and found that a single amino acid change in the IR75b receptor in *D. sechellia* in ac3 sensilla confers sensitivity to hexanoic acid and results in attraction to this odorant. This switch in odorant response profile is associated with concomitant expansion of the DL2d glomerulus, which receives projections from IR75b-expressing neurons. However, higher-order circuits appeared unaffected (Prieto-Godino et al. 2017). In addition to the roles of OBPs, OBPs and IR75b in mediating host preference in *D. sechellia*, gene expression studies identified several other genes that showed extensive upregulation in *D. sechellia* compared to its sister species *D. simulans*, including *Or22a* (Kopp et al. 2008) *Obp50a*, *Or85c*, and *Ir84a* (Shiao et al. 2015).

*D. erecta*, a close relative of *D. melanogaster*, has evolved host specialization for screw pine fruits (*Pandanus sp.*). Similar to the situation with *D. sechellia* and *M. citrifolia* volatiles, the number of olfactory sensory neurons that respond to 3-methyl-2-butenyl acetate, a volatile produced by the *Pandanus* fruits, has increased in *D. erecta* with corresponding enlarged

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**Figure 3** Lineage-specific gene loss and gain in the Or family. Diagram that illustrates gene-loss events (red slashes) and duplications (blue dots) in the *melanogaster* subgroup, including *D. simulans* (sim), *D. sechellia* (sec), *D. melanogaster* (mel), *D. yakuba* (yak), and *D. erecta* (ere). Generalist lineages are shown by solid black lines and specialist lineages by dotted lines. The timing of events was inferred via parsimony (modified from McBride et al. 2007, with permission).
glomeruli in the antennal lobe. Exposure to this odorant induces oviposition in D. erecta, but not D. melanogaster, females, providing yet another example of an exquisitely tuned olfactory-mediated host plant relationship (Linz et al. 2013).

Another well-studied example of behavioral adaptation in Drosophila comes from the cactophilic species D. mojavensis, which feeds and oviposits on decomposing cactus in Arizona, the Mojave Desert, Baja California, the Sonoran Desert, and Catalina Island off the coast of California. Different races of D. mojavensis have diversified in terms of preferences for the different types of cacti endemic at each location (Newby and Etges 1998). Electrophysiological and behavioral studies have shown olfactory adaptations to volatiles from different cacti (Date et al. 2013), and RNA-sequencing analyses have revealed differential expression of members of the Or gene family between the different populations (Crowley-Gall et al. 2016). The geographical separation and different behavioral niches among the D. mojavensis populations provide a scenario for incipient speciation (Pfeiler et al. 2009). Indeed, analyses of genome sequences between D. mojavensis and its close relatives, D. arizonae and D. navojoa, showed fixed inversion differences in three of their six chromosomes (Sanchez-Flores et al. 2016).

From an evolutionary perspective, specialization is beneficial because it reduces interspecific competition for resources. However, specialization can also be risky if a host plant or other resource becomes scarce in the future. Understanding the genetic changes behind specialization is important because it yields insight into the mechanisms of specialization and helps in understanding how flexible a given taxon might be in the face of changing environmental factors. The studies above on D. sechellia, D. erecta, and some species of cactophilic Drosophila highlight some of the recent progress made on the evolution and genetics of specialization in Drosophila.

**Adaptation to fresh fruit**

Unlike most Drosophila species, D. suzukii females are attracted to ripening fruit for oviposition. This adaptation has resulted in D. suzukii, often referred to as “spotted-wing Drosophila,” emerging as a major agricultural pest over the last decade as it has spread from its native range in Asia to Europe and North America (Walsh et al. 2011). Infestation of fresh fruit requires both morphological and chemical adaptations. D. suzukii females possess an enlarged, heavily serrated ovipositor relative to other taxa in the melanogaster species group. This ovipositor enables them to penetrate the skin of ripe fruit, such as strawberries, cherries, and cane fruits (Mitsui et al. 2006; Atallah et al. 2014; Cini et al. 2014).

Evolutionary changes in the olfactory receptor repertoire have also occurred that predispose D. suzukii to selecting fresh fruit as an oviposition substrate (Keesey et al. 2015). Comparisons of the Or gene repertoire of D. suzukii with those of its closely related species D. biarmipes and D. taka hashii showed duplications of Or23a and Or67a in D. suzukii, with evidence for positive selection at the Or67a locus. In addition, Or74a, Or85a, and Or98b were pseudogenized in D. suzukii (Hickner et al. 2016). Furthermore, behavioral studies show that D. suzukii is attracted to the odor of ripe strawberry and that oviposition behavior on ripe fruit is reduced when the common odorant coreceptor gene, Orco, is knocked down by RNA interference or eliminated through CRISPR deletion (Karageorgi et al. 2017). However, it should be noted that in the DGRP, pseudogenes of chemoreceptors segregate in the population along with their intact counterparts apparently shielded from selection through functional redundancy; therefore, segregation of intact counterparts of pseudogenes in the D. suzukii population cannot be excluded.

**Leaf mining and herbivory**

Although saprophagy is the major lifestyle of most drosophilid species, a few have adapted to novel oviposition substrates. One such adaptation is the evolution of true herbivory observed in many species of the genus Scaptomyza. This involves oviposition on living plant material and the subsequent development of larvae miners in the interstitial space between the top and bottom layer of leaves. The evolution of this behavior has required a suite of morphological and chemical changes. Scaptomyza flav a oviposits, and its larvae feed, on plants of the family Brassicaceae, e.g., Arabidopsis thaliana (Whiteman et al. 2011; Goldman-Huertas et al. 2015). Evolutionary analysis of the odorant receptor repertoire of S. flav a shows stepwise loss or pseudogenization of receptor genes that respond to short-chain aliphatic esters, commonly found in yeast, and duplication at the Or67b locus has resulted in three paralogs with evidence for positive selection (Goldman-Huertas et al. 2015). Interestingly, the D. mel anogaster OR67b receptor also responds to the green-leaf volatile (Z)-3-hexenol (Galizia et al. 2010), suggesting a role for attraction to leaf volatiles in nonherbivorous Drosophila.

**Carnivory**

While most Drosophilidae species are attracted to rotting plant and fungal matter as oviposition substrates, there are a few with more specialized and unusual ecological niches (Ashburner 1981). Several of these are in the genus Scaptomyza, a lineage that arose on Hawaii and has since escaped to colonize all continental landmasses except Antarctica. There are endemic Hawaiian Scaptomyza in the subgenus Titanochaeta that are predators on spider eggs sacs (Wirth 1952). The female fly oviposits in the eggs and the larvae develop in the sac by eating the spider eggs, all while being guarded by the mother spider. The larval development of Titanochaeta remains unknown, but the sister lineage, the subgenus Engiscaptomyza, larviposits rather than laying eggs (Throckmorton 1975). This is quite common in some flies, particularly those that are predators or parasitoids. Members of Engiscaptomyza are suspected to be predators on endemic Hawaiian land snails, an ecological niche that might require an extremely short or nonexistent egg phase. Other parasitic drosophilid species, including members of the genus Cacoxenus, oviposit in bee nests (Ashburner 1981). Another unusual oviposition site used by three unrelated species of
Drosophila is the green gland of giant land crabs (Carson 1974). This has arisen in parallel on islands of the South Pacific and Caribbean. The genetic underpinnings of the evolutionary trajectories that have given rise to these diverse ecological specializations in the genus Drosophila remain yet to be explored.

**Perspectives**

With its multiple sequenced and characterized species, multiple interspecies lines for examining intraspecies variation, and a vast array of publicly available resources, Drosophila offer unique opportunities for investigating the evolution of complex behaviors, such as reproductive behaviors, at both micro- and macroevolutionary scales. Much is known about the diversity of mating strategies, and ecological adaptations among members of the genus have been extensively documented. Yet, we still know little about the genetic basis of the evolutionary forces that drive these adaptations. The ability for one-way hybridization between several closely related sister species (e.g., D. melanogaster, D. simulans, D. sechellia, and D. mauritiana) provides opportunities to assess the relationship between the diversification of reproductive behaviors and speciation.

Population and quantitative genetic studies that use well-annotated whole-genome data sets to identify genes and genetic networks associated with different elements of reproductive behaviors, across multiple species, may provide insights as to how these behaviors have evolved and diverged among various lineages within the genus Drosophila. It will be important to examine the evolution of behaviors from the contexts of trade-offs, adaptations to the social and abiotic environment, and responses to different selective pressures. Since behaviors are expressions of the nervous system, neurobiological studies are necessary to identify adaptations at the level of functional neural circuits that accompany genomic adaptations. It is here of interest to note that a study of the visual and olfactory structures among 62 Drosophila species showed that expansion of visual structures occurred at the expense of the size of the third antennal segment, which mediates olfaction, and vice versa (Keesey et al. 2019). This trade-off could be accounted for by a shared larval developmental blueprint, the eye–antennal imaginal disc, and could affect bias of sensory input for the identification of hosts and mates (Keesey et al. 2019).

Comparative studies within and integrating genetic/genomic, behavioral, ecological, and neurobiological approaches across multiple Drosophila species may provide insights into the selective forces that led to the evolution of reproductive behavior, the ultimate determinant of fitness.

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**Literature Cited**

Adams, E. M., and M. F. Wolfner, 2007 Seminal proteins but not sperm induce morphological changes in the Drosophila melanogaster female reproductive tract during sperm storage. J. Insect Physiol. 53: 319–331. https://doi.org/10.1016/j.jinsphys.2006.12.003

Ahmed, O. M., A. Avila-Herrera, K. M. Tun, P. H. Serpa, J. Peng et al., 2019 Evolution of mechanisms that control mating in Drosophila males. Cell Rep. 27: 2527–2536.e4. https://doi.org/10.1016/j.celrep.2019.04.104

Amlou, M., B. Moreteau, and J. R. David, 1998 Genetic analysis of Drosophila sechellia specialization: oviposition behavior toward the major aliphatic acids of its host plant. Behav. Genet. 28: 455–464. https://doi.org/10.1023/A:1021689312582

Antony, C., and J.-M. Jallon, 1982 The chemical basis for sex recognition in Drosophila melanogaster. J. Insect Physiol. 28: 873–880. https://doi.org/10.1016/0022-1910(82)90101-9

Apper-McGlaughon, J., and M. F. Wolfner, 2013 Post-mating change in excretion by mated Drosophila melanogaster females is a long-term response that depends on sex peptide and sperm. J. Insect Physiol. 59: 1024–1030. https://doi.org/10.1016/j.jinsphys.2013.07.001

Appel, M., C. J. Scholz, T. Müller, M. Dittrich, C. König et al., 2015 Genome-wide association analyses point to candidate genes for electric shock avoidance in Drosophila melanogaster. PLoS One 10: e0126986. https://doi.org/10.1371/journal.pone.0126986

Arthur, B. J., T. Sunayama-Morita, P. Coen, M. Murthy, and D. L. Stern, 2013 Multi-channel acoustic recording and automated analysis of Drosophila courtship songs. BMC Biol. 11: 11. https://doi.org/10.1186/1741-7007-11-11

Arya, G. H., M. M. Magwire, W. Huang, Y. L. Serrano-Negron, T. F. C. Mackay et al., 2015 The genetic basis for variation in olfactory behavior in Drosophila melanogaster. Chem. Senses 40: 233–243. https://doi.org/10.1093/chemse/bjv001

Ashburner, M., 1981 Entomophagous and other bizarre Drosophilidae, pp. 395–425 in The Genetics and Biology of Drosophila, Vol. 3a, edited by M. Ashburner, H. L. Carson, and J. N. Thompson. Academic Press, New York.

Atallah, J., L. Teixeira, R. Salazar, G. Zaragoza, and A. Kopp, 2014 The making of a pest: the evolution of fruit-penetrating ovipositor in Drosophila suzukii and related species. Proc. Biol. Sci. 281: 20132840. https://doi.org/10.1098/rspb.2013.2840

Avila, F. W., and M. F. Wolfner, 2009 Acp36DE is required for uterine conformational changes in mated Drosophila females. Proc. Natl. Acad. Sci. USA 106: 15796–15800. https://doi.org/10.1073/pnas.0904029106

Avila, F. W., L. K. Sirot, B. A. LaFlamme, C. D. Rubinstein, and M. F. Wolfner, 2011 Insect seminal fluid proteins: identification and function. Annu. Rev. Entomol. 56: 21–40. https://doi.org/10.1146/annurev-ento-120709-144823
Coyne, J. A., and H. A. Orr, 1989 Patterns of speciation in Drosophila. Evolution 43: 362–381. https://doi.org/10.1111/j.1558-5646.1989.tb04233.x

Coyne, J. A., and H. A. Orr, 1997 'Patterns of speciation in Drosophila' revisited. Evolution 51: 295–303.

Coyne, J. A., A. P. Crittenden, and K. Mah, 1994 Genetics of a phenomenonal difference contributing to reproductive isolation in Drosophila. Science 265: 1461–1464. https://doi.org/10.1126/science.8073292

Crandall, K. S., M. P. Kambysellis, 1990 Vitellogenin protein diversity in the Hawaiian Drosophila. Biochim. Genet. 28: 415–432. https://doi.org/10.1007/BF02401429

Crowley-Gall, A., P. Date, C. Han, N. Rhodes, P. Andolfatto et al., 2017 Circadian rhythms and sleep in Drosophila 12 Genomes Consortium; Clark, A. G., M. B. Eisen, D. R. Smith, C. M. Bergman, et al., 2007 Evolution of genes and genomes on the Drosophila phylogeny. Nature 450: 203–218.

Dobzhansky, Th., 1946 Complete reproductive isolation between two morphologically similar species of Drosophila. Ecology 27: 205–211. https://doi.org/10.2307/1932895

Drosophila 12 Genomes Consortium; Clark, A. G., M. B. Eisen, D. R. Smith, C. M. Bergman, et al., 2007 Evolution of genes and genomes on the Drosophila phylogeny. Nature 450: 203–218.

Dubowy, C., and A. Sehgal, 2017 Circadian rhythms and sleep in Drosophila melanogaster. Genetics 205: 1373–1397. https://doi.org/10.1534/genetics.115.185157

Dworkin, I., and C. D. Jones, 2009 Genetic changes accompanying the evolution of host specialization in Drosophila sechellia. Genetics 181: 721–736. https://doi.org/10.1534/genetics.108.093419

Edwards, K. A., L. T. Doescher, K. Y. Kaneshiro, and D. Yamamoto, 2007 A database of wing diversity in the Hawaiian Drosophila. PLoS One 2: e487. https://doi.org/10.1371/journal.pone.000487

Etges, W. J., and L. L. Jackson, 2001 Euppticular hydrocarbon variation in Drosophila mojavensis cluster species. J. Chem. Ecol. 27: 2125–2149. https://doi.org/10.1023/A:10122032232876

Eversaerts, C., J. P. Farine, M. Cobb, and J. F. Verger, 2010 Drosophila cuticular hydrocarbons revisited: mating status alters cuticular profiles. PLoS One 5: e9607. https://doi.org/10.1371/journal.pone.0009607

Fabre, C. B., C. Hedwig, G. Conduit, P. A. Lawrence, S. F. Goodwin et al., 2012 Substrate-borne vibratory communication during courtship in Drosophila melanogaster. Curr. Biol. 22: 2180–2185. https://doi.org/10.1016/j.cub.2012.09.042

Fedorowicz, G. M., J. D. Fry, R. R. H. Anholt, and T. F. C. Mackay, 1998 Epistatic interactions between smed-impaired loci in Drosophila melanogaster. Genetics 148: 1885–1891.

Ferveur, J. F., 1997 The phenomenonal role of cuticular hydrocarbons in Drosophila melanogaster. Bioessays 19: 353–358. https://doi.org/10.1002/bies.950190413

Ferveur, J. F., 2005 Cuticular hydrocarbons: their evolution and roles in Drosophila phenomenonal communication. Behav. Genet. 35: 279–295. https://doi.org/10.1007/s10519-005-3220-5

Ferveur, J. F., M. Cobb, H. Boukella, and J. M. Jallon, 1996 World-wide variation in Drosophila melanogaster sex pheromone: behavioural effects, genetic bases and potential evolutionary consequences. Genetica 97: 73–80. https://doi.org/10.1007/BF00132583

Fumey, J., and C. Wicker-Thomas, 2017 Mutations at the Darkener of Apricot locus modulate pheromone production and sex behavior in Drosophila melanogaster. J. Insect Physiol. 98: 182–187. https://doi.org/10.1016/j.jinsphys.2017.01.005

Fuyama, Y., 1979 A visual stimulus in the courtship of Drosophila suzukii. Experientia 35: 1327–1328. https://doi.org/10.1007/BF01963987

Gaertner, B. E., E. A. Ruedi, L. J. McCoy, J. M. Moore, M. F. Wolfer et al., 2015 Heritable variation in courtship patterns in Drosophila melanogaster. G3 (Bethesda) 5: 531–539. https://doi.org/10.1534/g3.114.014811

Gage, M. J. G., 2012 Complex sperm evolution. Proc. Natl. Acad. Sci. USA 109: 4341–4342. https://doi.org/10.1073/pnas.1208727109

Galizia, C. G., M. Strach, A. Nissler, and S. Ma, 2010 Integrating heterogeneous odor response data into a common response model: a DoOR to the complete olfactome. Chem. Senses 35: 551–563. https://doi.org/10.1093/chemse/bjp042

Garlapow, M. E., W. Huang, M. T. Yarboro, K. R. Peterson, and T. F. C. Mackay, 2015 Quantitative genetics of food intake in Drosophila melanogaster. PLoS One 10: e0138129. https://doi.org/10.1371/journal.pone.0138129

Gershman, S. N., and H. D. Rundle, 2017 Crowd control: sex ratio affects sexually selected cuticular hydrocarbons in male Drosophila serrata. J. Evol. Biol. 30: 583–590. https://doi.org/10.1111/jeb.13028

Gershman, S. N., E. Toumishey, and H. D. Rundle, 2014 Time flies: time of day and social environment affect cuticular hydrocarbon sexual displays in Drosophila serrata. Proc. Biol. Sci. 281: 20140821. https://doi.org/10.1098/rspb.2014.0821

Giardina, T. J., A. G. Clark, and A. C. Fiumera, 2017 Estimating the evolution of h oest specialization in Drosophila melanogaster. The re-
activity in the post-mated female. Proc. Biol. Sci. 277: 65–70. https://doi.org/10.1098/rspb.2009.1236

Jaenike, J., 1985 Parasite pressure and the evolution of amanitin tolerance in Drosophila. Evolution 39: 1295–1301. https://doi.org/10.1111/j.1558-5646.1985.tb05695.x

Jaenike, J., 1990 Host specialization in phytophagous insects. Annu. Rev. Ecol. Syst. 21: 243–273. https://doi.org/10.1146/annurev.es.21.110190.001331

Jallon, J.-M., 1984 A few chemical words exchanged by Drosophila during courtship and mating. Behav. Genet. 14: 441–478. http://doi.org/10.1007/BF01065444

Jallon, J.-M., and J. R. David, 1987 Variation in cuticular hydrocarbons among the eight species of the Drosophila melanogaster subgroup. Evolution 41: 294–302.

Jinek, M., K. Chylinski, I. Fonfara, M. Hauer, J. A. Doudna et al., 2012 A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337: 816–821. https://doi.org/10.1126/science.1225829

Jones, C. D., 2005 The genetics of adaptation in Drosophila sechellia. Genetica 123: 137–145. https://doi.org/10.1007/s10709-004-2728-6

Joseph, R. M., and J. R. Carlson, 2015 Drosophila chemoreceptors: a molecular interface between the chemical world and the brain. Trends Genet. 31: 683–695. https://doi.org/10.1016/j.tig.2015.09.005

Kambysellis, M. P., K. F. Ho, E. M. Craddock, F. Piano, M. Parisi et al., 1995 Pattern of ecological shifts in the diversification of Hawaiian Drosophila inferred from a molecular phylogeny. Curr. Biol. 5: 1129–1139. https://doi.org/10.1016/S0003-3472(82)80152-8

Karageorgi, M., P. G. Becker, B. S. Hansson, and P. Witzgal, 2004 Courtship song in Drosophila melanogaster: a differentia effect on male-female locomotor activity. Can. J. Zool. 82: 1258–1266. https://doi.org/10.1139/z04-102

Kopp, A., O. Barmina, A. M. Hamilton, L. Higgins, L. M. McIntyre et al., 2008 Social experience modifies pheromone expression and mating behavior in male Drosophila melanogaster. Curr. Biol. 18: 1373–1383. https://doi.org/10.1016/j.cub.2008.07.089

Kurtovic, A., A. Widmer, and B. J. Dickson, 2007 A single class of olfactory neurons mediates behavioural responses to a Drosophila sex pheromone. Nature 446: 542–546. https://doi.org/10.1038/nature05672

Kyriacou, C. P., and J. C. Hall, 1980 Circadian rhythm mutations in Drosophila melanogaster affect short-term fluctuations in the male’s courtship song. Proc. Natl. Acad. Sci. USA 77: 6729–6733. https://doi.org/10.1073/pnas.77.11.6729

Kyriacou, C. P., and J. C. Hall, 1982 The function of courtship song rhythms in Drosophila. Anim. Behav. 30: 794–801. https://doi.org/10.1016/S0003-3472(82)80152-8

Labeur, C., R. Dallerac, and C. Wicker-Thomas, 2002 Involvement of des1 gene in the control of Drosophila melanogaster pheromone biosynthesis. Genetica 114: 269–274. https://doi.org/10.1023/A:1016223000650

Laflamme, B. A., and M. F. Wolfner, 2013 Identification and function of proteolysis regulators in seminal fluid. Mol. Reprod. Dev. 80: 80–101. https://doi.org/10.1002/mrd.22130

Laisse, P. P., and L. B. Vosshall, 2008 The olfactory sensory map in Drosophila. Adv. Exp. Med. Biol. 628: 102–114. https://doi.org/10.1007/978-0-387-82621-4_7

Lang, M., S. Murat, A. G. Clark, G. Gouppil, C. Blais et al., 2012 Mutations in the neverland gene turned Drosophila paches into an obligate specialist species. Science 337: 1658–1661. https://doi.org/10.1126/science.1224829

LaRue, K. M., J. Clemens, G. J. Berman, and M. Murthy, 2015 Acoustic duetting in Drosophila viridis relies on the integration of auditory and tactile signals. Elife 4: e07277. https://doi.org/10.7554/eLife.07277

Laturney, M., and J. C. Billetter, 2014 Neurogenetics of female reproductive behaviors in Drosophila melanogaster. Adv. Genet. 85: 1–108. https://doi.org/10.1016/B978-0-12-800271-1.00001-9

Laturney, M., and J. C. Billetter, 2016 Drosophila melanogaster females restore their attractiveness after mating by removing male anti-aphrodisiac pheromones. Nat. Commun. 7: 12322. https://doi.org/10.1038/ncomms12322

Lebreton, S., P. G. Becher, B. S. Hansson, and P. Witzgal, 2012 Attraction of Drosophila melanogaster males to food-related and fly odours. J. Insect Physiol. 58: 125–129. https://doi.org/10.1016/j.jinsphys.2011.10.009

Lebreton, S., V. Grab, A. B. Omondi, R. Igenn, P. G. Becher et al., 2014 Love makes smell blind: mating suppresses pheromone attraction in Drosophila females via Or65a olfactory neurons. Sci. Rep. 4: 7119. https://doi.org/10.1038/srep07119
O’Grady, P. M., and, R. DeSalle, 2018 Phylogeny of the genus Drosophila. Genetics 209: 1–25. https://doi.org/10.1534/genetics.117.300583

Oliveira, D. C. S. G., F. C. Almeida, P. M. O’Grady, M. Armella, W. Etes et al., 2012 Monophyly, divergence times, and evolution of host plant use inferred from a revised phylogeny of the Drosophila repleta species group. Mol. Phylogenet. Evol. 64: 533–544. https://doi.org/10.1016/j.ympev.2012.05.012

Ort, B. S., R. M. Bayntay, N. A. Pantoja, and P. M. O’Grady, 2012 Fungal diversity associated with Hawaiian Drosophila host plants. PLoS One 7: e40550. https://doi.org/10.1371/journal.pone.0040550

O’Sullivan, A., T. Lindsay, A. Prudnikova, B. Eral, M. Dickinson et al., 2018 Multifunctional wing motor control of song and flight. Curr. Biol. 28: 2705–2717.e4. https://doi.org/10.1016/j.cub.2018.06.038

Panhuis, T. M., N. L. Clark, and W. J. Swanson, 2006 Rapid evolution of reproductive proteins in alabone and Drosophila. Philos. Trans. R. Soc. Lond. B Biol. Sci. 361: 261–268. https://doi.org/10.1098/rstb.2005.1793

Pardy, J. A., H. D. Rundle, M. A. Bernards, and A. J. Moehring, 2008 Unusual base composition of male cY chromosome in the Drosophila melanogaster melanogaster species group. J. Hered. 99: 117–122. https://doi.org/10.1093/jhered/esn043

Oliveira, D. C. S. G., F. C. Almeida, P. M. O’Grady, and R. H. Anholt, 2006 Dynamic genetic interactions determine odor-guided behavior in Drosophila melanogaster. Genetics 174: 1549–1563. https://doi.org/10.1534/genetics.106.060574
Drosophila sechellia and its siblings. Genes Genet. Syst. 79: 145–150. https://doi.org/10.1266/ggs.79.145
Tomioka, S., T. Aigaki, and T. Matsuo, 2012 Conserved cis-regulatory elements of two odorant-binding protein genes, Obp57d and Obp57e, in Drosophila. Genes Genet. Syst. 87: 323–329. https://doi.org/10.1266/ggs.87.323
Tootoonian, S., P. Coen, R. Kawai, and M. Murthy, 2012 Neural representations of courtship song in the Drosophila brain. J. Neurosci. 32: 787–798. https://doi.org/10.1523/JNEUROSCI.5104-11.2012
True, J. R., K. A. Edwards, D. Yamamoto, and S. B. Carroll, 1999 Drosophila wing melanin patterns form by vein-dependent elaboration of enzymatic prepatterns. Curr. Biol. 9: 1382–1391. https://doi.org/10.1016/S0960-9822(00)80083-4
Tsuda, M., and T. Aigaki, 2016 Evolution of sex-peptide in Drosophila. Fly (Austin) 10: 172–177. https://doi.org/10.1080/19393694.2016.1193655
Tsuda, M., J. B. Peyre, T. Asano, and T. Aigaki, 2015 Visualizing molecular functions and cross-species activity of sex-peptide in Drosophila. Genetics 200: 1161–1169. https://doi.org/10.1534/genetics.115.177550
Turner, T. L., P. M. Miller, and V. A. Cochrane, 2013 Combining genome-wide methods to investigate the genetic complexity of courtship song variation in Drosophila melanogaster. Mol. Biol. Evol. 30: 2113–2120. https://doi.org/10.1093/molbev/msr111
van der Goes van Naters, W., and J. R. Carlson, 2007 Receptors for S6 kinase and serotonin in postmating dietary switch and damage potential. J. Integr. Pest Manag. 2: G1–G5.
Wagstaff, B. J., and D. J. Begun, 2005 Comparative genomics of Drosophila. Mol. Biol. Evol. 22: 818–826. https://doi.org/10.1093/molbev/msi067
Wang, L., and D. J. Anderson, 2010 Identity of function, form and discovery. Lipid Res. 59: 88–105. https://doi.org/10.1016/j.lipres.2015.06.001
Wirth, W. W., 1952 Two new spider egg predators from the Hawaiian Islands (Diptera: Drosophilidae). Proc. Haw. Ent. Soc. 14: 415–417.
Wolfer, M. F., 2002 The gifts that keep on giving: physiological functions and evolutionary dynamics of male seminal proteins in Drosophila. Heredity (Edinb.) 88: 85–93. https://doi.org/10.1038/sj.hdy.6800017
Wu, B., L. Ma, E. Zhang, J. Du, S. Liu et al., 2018a Sexual dimorphism of sleep regulated by juvenile hormone signaling in Drosophila. PLoS Genet. 14: e1007318. https://doi.org/10.1371/journal.pgen.1007318
Yamamoto, A., L. Zwarts, P. Callaerts, K. Norga, T. F. C. Mackay et al., 2008 Neurogenetic networks for startle-induced locomotion in Drosophila melanogaster. Proc. Natl. Acad. Sci. USA 105: 12393–12398. https://doi.org/10.1073/pnas.0804889105
Yamamoto, A., R. R. Anholt, and T. F. Mackay, 2009 Epistatic interactions attenuate mutations affecting startle behaviour in Drosophila melanogaster. Genet. Res. (Camb.) 91: 373–382. https://doi.org/10.1017/S0016672309990279
Yamamoto, D., and M. Koganzewa, 2013 Genes and circuits of courtship behaviour in Drosophila males. Nat. Rev. Neurosci. 14: 681–692. https://doi.org/10.1038/nrn3567
Yamamoto, D., K. Sato, and M. Koganzewa, 2014 Neuroethology of male courtship in Drosophila: from the gene to behavior. J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol. 200: 251–264. https://doi.org/10.1007/s00359-014-0891-5
Yang, C. H., P. Belawat, E. Hafen, L. Y. Jan, and Y. N. Jan, 2008 Drosophila egg-laying site selection as a system to study simple decision-making processes. Science 319: 1679–1683. https://doi.org/10.1126/science.1151842
Yang, C. H., S. Rumpf, Y. Xiang, M. D. Gordon, W. Song et al., 2009 Control of the postmating behavioral switch in Drosophila females by internal sensory neurons. Neuron 61: 519–526. https://doi.org/10.1016/j.neuron.2008.12.021
Yapici, N., Y. J. Kim, C. Ribeiro, and B. J. Dickson, 2008 A receptor that mediates the post-mating switch in Drosophila reproductive behaviour. Nature 451: 33–37. https://doi.org/10.1038/nature06483
Yew, J. Y., and H. Chung, 2015 Insect pheromones: an overview of function, form and discovery. Lipid Res. 59: 88–105. https://doi.org/10.1016/j.lipres.2015.06.001
Yu, J. Y., M. I. Kanai, E. Demir, G. S. X. E. Jefferis, and B. J. Dickson, 2010 Cellular organization of the neural circuit that drives Drosophila courtship behavior. Curr. Biol. 20: 1602–1614. https://doi.org/10.1016/j.cub.2010.08.025
Yukilevich, R., T. Harvey, S. Nguyen, J. Kehlbeck, and A. Park, 2016 The search for causal traits of speciation: divergent female mate preferences target male courtship song, not pheromones, in Drosophila athabasca species complex. Evolution 70: 526–542. https://doi.org/10.1111/evo.12870
Zhou, S., T. F. C. Mackay, and R. R. H. Anholt, 2014 Epistatic and epigenetic responses to mating and aging in Drosophila melanogaster. BMC Genomics 15: 927. https://doi.org/10.1186/1471-2164-15-927
Zwarts, L., M. M. Magwire, M. A. Carbone, M. Versteven, L. Herteleer et al., 2011 Complex genetic architecture of Drosophila aggressive behavior. Proc. Natl. Acad. Sci. USA 108: 17070–17075. https://doi.org/10.1073/pnas.1113877108