Male *Drosophila melanogaster* learn to prefer an arbitrary trait associated with female mating status

Machteld N. VERZIJDEN[1,4*], Jessica K. ABBOTT[2], Anne C. von PHILIPSBORN[3], Volker LOESCHCKE[4]

[1] Aarhus Institute for Advanced Studies, Aarhus University, Denmark

[2] Department of Biology, Section for Evolutionary Ecology, Lund University, Sweden

[3] Dandrite, Department of Molecular Biology and Genetics, Aarhus University, Denmark

[4] Department of Bioscience, Section for Genetics, Ecology and Evolution, Aarhus University, Denmark

Abstract   Although males are generally less discriminating than females when it comes to choosing a mate, they still benefit from distinguishing between mates that are receptive to courtship and those that are not, in order to avoid wasting time and energy. It is known that males of *Drosophila melanogaster* are able to learn to associate olfactory and gustatory cues with female receptivity, but the role of more arbitrary, visual cues in mate choice learning has been overlooked to date in this species. We therefore carried out a series of experiments to determine: 1) whether males had a baseline preference for female eye color (red versus brown), 2) if males could learn to associate an eye color cue with female receptivity, and 3) whether this association disappeared when the males were unable to use this visual cue in the dark. We found that naïve males had no baseline preference for females of either eye color, but that males which were trained with sexually receptive females of a given eye color showed a preference for that color during a standard binary choice experiment. The learned cue was indeed likely to be truly visual, since the preference disappeared when the binary choice phase of the experiment was carried out in darkness. This is, to our knowledge 1) the first evidence that male *D. melanogaster* can use more arbitrary cues and 2) the first evidence that males use visual cues during mate choice learning. Our findings suggest that that *D. melanogaster* has untapped potential as a model system for mate choice learning [*Current Zoology* 61 (6): 1036–1042, 2015].

Keywords   Mate choice, Learning, Male, Drosophila, Visual trait

Being able to distinguish between mates that are receptive to courtship and those that are not can help an individual to avoid wasting time and energy. Thus, cues that indicate receptivity are especially valuable to males in deciding which females to court and eventually mate with. Understanding how individuals make decisions in mate choice is important in order to understand the dynamics of sexual selection and reproductive isolation (Verzijden et al., 2012). Mate choice decision-making can be aided by learning from experience, and many species have shown to learn some aspects of their mate choice (Dukas, 2006; Gailey et al., 1982; Kozak and Boughman, 2009; Magurran and Ramnarine, 2004; Svensson et al., 2010; ten Cate and Vos, 1999; Verzijden et al., 2012). In particular, when learning which females are receptive to their courtship, males of several species show associative learning between particular cues and female mating status. For instance, *D. melanogaster* males learn to associate an pheromone, *cis*-vaccenyl

acetate (cVA), with mated females, and will subsequently reduce courtship to females that carry this pheromone (Ejima et al., 2007; Keleman et al., 2012). Similarly, male rove beetles discriminate unreceptive females by cuticular hydrocarbons (Schlechter-Helas et al., 2012). Such learning is not restricted to within-species variation: in several species, males can also learn to stop courting heterospecific females after rejection from such females (Dukas, 2004, 2008; Magurran and Ramnarine, 2004).

This learning behavior is a valuable field of study for a number of reasons. First, it is a useful model for how the interaction between experience and genetic predispositions can explain variation in animal behavior (Bateson and Laland, 2013). Second, because mate choice directly influences the genetic composition of the next generation, it is important for evolutionary processes such as sexual selection and speciation (Verzijden et al., 2012). Third, learned mate choice allows us to study the

underlying cellular mechanismsofthis behavior, by mapping the interplay between brain regions, the function of individual neurons (e.g. Datta et al., 2008; Keleman et al., 2012) and even epigenetic cellular processes (e.g. Kramer et al., 2011). *Drosophila* species have been used as a model organism to great advantage within all three of these research aims.

Drosophila melanogaster males are known to learn aspects of their mating behavior. They learn to suppress courtship towards females that are unreceptive, either because they are unable to mate (immature) (Ejima et al., 2005), unwilling to mate because they were recently mated (Ejima et al., 2007), or unwilling to mate because they are of another species (Dukas, 2004, 2008). Males will suppress their courtship behavior in general for several hours after rejection. This is usually mediated through a short-term memory of the experience (Siegel and Hall, 1979). When rejection occurred repeatedly over the course of a training period of several hours, males will form a long-term memory, in which they learn to associate female rejection behavior with olfactory cues of the females (Ejima et al., 2005; Ejima et al., 2007; Griffith and Ejima, 2009a). Males will then continue to suppress courtship to females with a similar pheromone profile. For instance, immature virgin females have a different pheromone profile than mature virgin females, and mated females emit pheromones that are either present in the male sperm, or are transferred by males upon close contact during mating (Ejima et al., 2007). Species also differ in pheromone profiles, which probably facilitates species discrimination by males after experience (e.g. Blows and Allan, 1998; Dyer et al., 2014; Jallon and David, 1987).

Studies on the proximate factors involved inmale courtship learning have focused on olfactory/gustatory memories. However, male courtship is not only guided by olfactory cues, visual and tactical cues are also used, and male courtship only ceases if all three modalities are impaired (Krstic et al., 2009). In fact, female *D. melanogaster* have shown preferences for visual traits (Katayama et al., 2014), and vision is an important sensory modality in courtship (Griffith and Ejima, 2009b), indicating that visual traits are commonly evaluated in mate choice. *Drosophila* species show intra- and interspecific variation, in several visual cues, such as abdominal pigmentation (Matute and Harris, 2013), eye color, and wing inference patterns (Shevtsova et al., 2011), yet it has to date not been shown that males can learn to associate female receptivity to mating with a visual trait. This may be because visual traits are typically unrelated

to mating status (although immature individuals are paler), they are less likely to change in short time spans, whereas pheromone profiles are a direct and honest cue (Ejima et al., 2007). However, from an ecological point of view - i.e. outside of the laboratory mating settings of most studies on male mate choice learning in *D. melanogaster* - offspring from one female are likely to emerge within a short time span, and be within a relatively short distance from each other. They are likely to share any distinguishing visual traits with, such as the degree of abdominal pigmentation (e.g. Gibert et al., 2004b; Gibert et al., 1998) and eye color (Nitasaka et al., 1995) and are likely to have the same mating status, at least shortly after emergence. Integrating cues from several sensory modalities, such as vision and smell, would potentially aid males in rapid decision making in which female to court and which not (Griffith and Ejima, 2009a). Furthermore, male *Drosophila* learn to avoid heterospecific females (Dukas, 2004, 2008, 2009), and this learning too could be based on visual differences between species (e.g. Gibert et al., 2004a; Llopart et al., 2002).

Here we ask if a male is able to learn to associate a trait, which is initially arbitrary in relation to the female's receptivity, with her willingness to mate. The trait in question is the eye color: brown or red. Wild-type eye color of *Drosophila* is red, but brown eye color is a single gene mutation, found in the wild in several species (Aparisi and Najera, 1990; Ashadevi and Ramesh, 2000; Nitasaka et al., 1995). By giving males experience with both red-eyed and brown-eyed females that are either immature virgins – and thus unable and unwilling to mate, and mature virgins – that are willing to mate, we challenged males to associate the mating status of females with their eye color.

1   Materialsand Methods

We used a large ($N_e > 1800$) outbred *D. melanogaster* population, LH$_m$ (Rice et al., 2005), and a replicate population, LH$_m$-*bw*, which has all brown-eyed individuals, caused by the recessive *bw* marker, but otherwise the same genetic background as the main LH$_m$ population. To ensure that the LH$_m$ and the LH$_m$-*bw* populations were genetically equally heterogenous, we crossed the two populations for 10 generations prior to this experiment. The LH$_m$ populations are maintained on a 14-day cycle at 25°C, with a 12:12 hour light/dark. Males were collected and separated from females under light CO_2 anesthesia, within 3 hours of emergence from their pupae, and kept with 20 individuals / vial with 7 ml food.

The gene responsible for brown eyes (bw^1) is part of a pathway that also involves production of serotonin and dopamine, neuromodulators that are important in experiencing reward and memory formation (Krstic et al., 2013). For this reason, we only used red-eyed males. At the same time as males were collected, we also collected virgin females and separated them by eye color-with 40 individuals/vial with 7 ml food. 48 hours after initial collection, we again collected virgin females, within 2 hours of emergence, and kept them by eye color for another 2 hours after collection.

1.1   The training phase

We then set up vials in which we had either: 40 mature virgin females (> 48 hours after emergence) with red eyes, and 40 immature females with brown eyes (up to 4 hours after emergence), or 40 mature virgin females with brown eyes and 40 immature females with red eyes. We then introduced 20 virgin males to each vial, thus having a sex ratio of 1:4 in each vial. We chose a highly female-biased sex ratio in order to ensure that all males would have ample opportunity to gain experience with females, and reduce the possible effects of male-male competition. Males were then allowed to interact with the females for up to 1.5 hours, until mating ceased. Vials were inspected for mating pairs every 15 minutes. Only matings with mature females were observed. All individuals were then anesthetized and males were separated from the females, and kept in a fresh vial. The females were discarded.

1.2   The testing phase

After 24 hours, males were individually placed in a vial with 2 mature virgin females, one red eyed and one brown eyed, which were collected in the initial collection round (i.e. > 40 hours after emergence), and were observed for 30 minutes. Each minute, males were inspected if they were courting (orientation, wing extension and following) red or brown-eyed females, until they were mated with one of the females. Duration of the mating was then also scored (one minute precision). After that all individuals were discarded.

We tested 53 naïve males for a baseline preference for eye-color. We then tested 83 males that experienced brown eyed mature virgin females in the training phase and 78 males that experienced red eyed mature virgin females in the training phase.

After obtaining positive results of the above training (see results below), we proceeded to test if the males were truly choosing the females based on their eye color, and repeated the experiment with final testing phase in a dark room under dim red light from a 15 W dark room safelight (model 4018 Kaiser Fototechnik GmbH & Co. KG, Buchen, Germany). The light emission in the spectrum below 600 nm of these lights is negligible, whereas *D. melanogaster* light sensitivity is negligible above 600 nm (Schnaitmann et al., 2013), meaning that the flies are effectively blind under these conditions. Such light conditions have previously been successfully used to observe *D. melanogaster* courtship without visual stimuli (Joiner and Griffith, 2000). These light conditions made it impossible to distinguish red-eyed and brown-eyed females for human observers as well, thus we were not able to note which of the two females the males were courting. When a male mated a female, the vial was taken out of the dark room and the eye color of the female was scored.

1.3   Statistical analysis

Chi square tests were used to test if males had a baseline preference for eye color, had a preference of eye color after training, and finally if they had a preference for eye-color genotype in darkness. In order to test if males courted females of one eye color more than the other, paired *t*-tests were performed. All statistical analyses were done in *R* (version 3.1.3).

2   Results

2.1   Baseline preference test

Naïve males showed no baseline preference for either female eye-color. They mated equally with brown-eyed and red-eyed females. Of 53 males tested, 26 mated with red-eyed females, 24 mated with brown-eyed females (3 males did not mate): $\chi^2 = 0.08$, $df = 1$, $P = 0.777$. Males did not court females with brown or red eye color more often ($t_{53} = 0.072$, $P = 0.943$), neither did they mate significantly faster or longer with either eye color (latency to mate: $t_{43.77} = -0.421$, $P = 0.676$; duration of mating: $t_{47.85} = -0.493$, $P = 0.624$) (Table 1).

2.2   Preference tests under normal light conditions.

Males mated more often with the female with the eye-color that was associated with the mature females in the training phase, and this effect was similar in the two treatments: 60 % of the red-eye trained males mated with red-eyed females, and 59% of the brown-eyed trained males mated with the brown-eyed females (Fig. 1): $\chi^2 = 4.7196$, $df = 1$, $P = 0.0298$. All but six of the 161 males we trained mated.

Prior to mating, males also courted the female with the eye color associated with the mature females in the training phase twice as often: $t_{77} = 3.412$, $P = 0.001$ for males trained with mature red-eyed females; $t_{82} = -3.026$, $P = 0.003$ for males trained with mature brown-

eyed females (Table 1).

Males that mated with the 'preferred' eye color did not mate faster than males that mated with the other eye color ($t_{59.22}$ = 1.7788, P = 0.080 for males trained with mature brown-eyed females; males trained with mature red-eyed females: $t_{72.33}$= -0.043, P = 0.966). The duration of the mating was longer for males trained with brown-eyed mature females, when mating with brown-eyed females than for those mating with red-eyed females: $t_{77.87}$ = -3.652, P = 0.0005. However, there was not a similar trend for males trained with red-eyed mature females ($t_{70.89}$ = -0.32, P = 0.750) (Table 1).

2.3 Preference test under dark conditions

We tested 112 males under dark conditions, that were collected and trained identically (under light conditions) as described above, 55 trained with mature brown-eyed females and immature red-eyed females, and 59 males trained with mature red-eyed females and immature brown-eyed females. Males mated equally often with red-eyed females as with brown-eyed females: χ^2 = 0.0321, df = 1, P = 0.8578, and this effect was similar in the two treatments: 48 % of the males trained with mature red-eyed females mated with red-eyed females, and 49% of the males trained with mature brown-eyed females mated with the brown-eyed females (Fig. 1).

3 Discussion

After experience with mature and immature virgins, males preferred to mate with females with the eye color that was associated with the mature females. Naïve males showed no preference for female eye color, and males preferred females with brown and red eye colors similarly according to their experience. We thus conclude that males learned to associate the female eye color trait with their mating status and associated receptivity to mate. The eye color trait of the females is gene-rally unrelated to their mating status, and thus in that sense arbitrary. Only through training with mature and immature females with different eye colors did males learn to prefer this trait, generalizing from their previous experience with mating with mature females and rejection from the immature females. Immature *D. melanogaster* females show different behavior towards courting males than mature virgin females (Dukas and Scott, 2015, this issue). These behavioral cues are presumably associated with the success of their courtship attempt and, in this case, with the eye color of the females.

The exact mechanism behind this learned preference for eye color is not entirely clear from these experi-
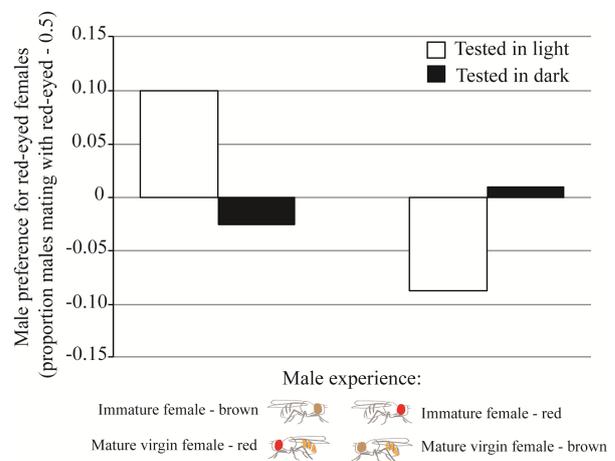

Fig. 1 After either of the two training treatments (immature brown-eyed females + mature virgin red-eyed females vs immature red-eyed females + mature virgin brown-eyed female), males were given the choice between two equally mature virgin females, and preferred the female with the eye color associated with the mature virgin females in the training phase, indicated by the white bars deviating from the zero-line. This preference was not present under dark conditions

Table 1 Courtship and mating behavior of males towards the two types of females

	# courtship displays to red	# courtship displays to brown	Latency (min) to mate with red	Latency (min) to mate with brown	Duration (min) of mating with red	Duration (min) of mating with brown
Naïve males	1.64 ± 1.72 n = 53	1.66 ± 1.91 n = 53	6.23 ± 3.19 n = 26	6.67 ± 4.04 n = 24	15.25 ± 3.94 n = 26	15.9 ± 3.94 n = 24
	ns		*ns*		*ns*	
Males trained with mature red-eyed females	1.82 ± 3.5 n = 78	0.9 ± 2.09 n = 78	7.64 ± 6.47 n = 45	7.7 ± 4.72 n = 30	17.07 ± 4.54 n = 45	17.32 ± 3.71 n = 30
	P = 0.001		*ns*		*ns*	
Males trained with mature brown-eyed females	0.93± 1.64 n = 83	1.9 ± 3.51 n = 83	8.21± 5.81 n = 33	6.04± 4.68) n = 33	15.41± 2.70 n = 47	18.07 ± 4.10 n = 47
	P = 0.003		*ns* (P = 0.08)		P < 0.001	

Mean values ± St dev. Males only mated with one female (after that, observations were stopped), thus the values for latency to mate and duration of mating to red and brown-eyed females are based on different sample sizes than the values for # courtship displays.

ments; however, it has previously been shown that female pheromone profiles that are related to age or mating status act as a conditioned stimulus, and that this learning is associative learning (Griffith and Ejima, 2009a). The eye color trait may similarly be a conditioned stimulus. The neuronal and circuit mechanisms are unknown, as well as the anatomical structures that are involved. However, it is likely that the mushroom body is involved, since visual learning has recently been located in this brain center (Vogt et al., 2014).

We show that males can learn to associate (and prefer) a female trait initially arbitrary to her mating status. It is very likely that the males used the visual (eye color) trait, since males did not distinguish between females under dark conditions. It has been shown that olfaction-impaired male *D. melanogaster* can learn to suppress courtship after rejection by females, but are later unable to distinguish between females in various mating statuses (Ejima et al., 2005). However, this does show that they are able to use non-olfactory sensory information for courtship. Here we show that males with intact olfaction rely on visual information to distinguish between equally mature females, whose eye colors have previously marked female receptivity.

The *bw* gene that is responsible for the brown eye color has pleiotropic effects on the production of neurotransmitters (Krstic et al., 2013), but it is not known if it might also affect the pheromone profile of the individuals, or the behavior of the females. Thus, it is conceivable that males may not have associated the visual eye-color trait with female mating status, but a second unknown trait. However by testing the trained males in the dark, we confirmed that males were not distinguishing between the females without visual cues, strengthening our preliminary conclusion that males indeed used the visual cue under normal light conditions. It is possible that under dark conditions the males did not identify that there were two females, and thus have mated at random. If this were the case, this would imply that males couldn't distinguish between the females based on non-visual cues, which supports our suggestion that the males used eye color to distinguish between the females. Specifically, if red eyed and brown-eyed females differ enough in pheromone profile that males can detect and learn to make associations with these differences, then the males tested in darkness would probably have sensed the two different smells and responded accordingly. Similarly, if eye color had affected mating-relevant behavior, this would have resulted in a mating bias. Instead, the probability of mating with one eye color over the other is equal in both test groups in the dark, as well as under light conditions with the naïve males. Alternatively, it is possible that if there were a pheromone difference between red and brown eye females, males used this to distinguish between them under light conditions, but that the dark conditions, and thus the lack of visual sensory input, may have caused males to switch to different molecular or neurological circuits, bypassing the memory formation that was previously established by training under light conditions (Griffith and Ejima, 2009a; Joiner and Griffith, 2000).

How can the ability to learn to associate an arbitrary trait to mating status within a species influence the outcome of sexual selection? We expect that this causes the pattern of sexual selection to become more random, both with respect to the traits under selection and the direction of the preferences for those traits. Males that learn to prefer the distinguishing trait of females that are receptive to mating will locally cause a positive frequency dependent effect for females bearing this trait. Such patterns will then be highly transient, since within days, the availability of receptive females can change. Furthermore, we speculate that such memories might last only a number of days (McBride et al., 1999). However, such temporal fluctuations in mate preferences are a possible driver of the maintenance of genetic variation for multiple traits (e.g. Chaine and Lyon, 2008; Lehtonen et al., 2010). Male *Drosophila* also have been shown to learn to reduce courtship towards heterospecific females (Dukas, 2004, 2008; Dukas and Dukas, 2012), and in this case, such associative learning could potentially contribute to increased phenotypic species differentiation, especially if females also employ such learning behavior (Servedio and Dukas, 2013). Interestingly, Morier-Genoud and Kawecki (2015, this issue) showed in a simulation model that when males learn to adjust their courtship effort towards females that are more receptive to them, this could increase their reproductive success, and this learning could strengthen sexual selection in the population by increasing the male fitness variation.

In conclusion, this is the first evidence that male *Drosophila melanogaster* can make mate choice decisions based on learning of an arbitrary visual cue. Given the increasing interest in how learning can influence speciation (Verzijden et al., 2012), and the wealth of knowledge on *Drosophila* neurobiology, this suggests that *D. melanogaster* has untapped potential as a model system for increasing our understanding of the mechanics and evolutionary outcome of mate choice learning.

References

Aparisi ML, Najera C, 1990. Genetic location and biochemical characterization of eye-color mutants from natural populations of *Drosophila melanogaster*. Genome 33: 203–208.

Ashadevi JS, Ramesh SR, 2000. Genetic and biochemical analysis of brown eye mutation in *Drosophila nasuta nasuta* and *Drosophila nasuta albomicans*. Genetica 109: 235–243. doi: 10.1023/a:1017505400086.

Bateson P, Laland KN, 2013. Tinbergen's four questions: An appreciation and an update. Trends in ecology & evolution 28: 712–718. doi: 10.1016/j.tree.2013.09.013.

Blows MW, Allan RA, 1998. Levels of mate recognition within and between two *Drosophila* species and their hybrids. American Naturalist 152: 826–837. doi: 10.1086/286211.

Chaine AS, Lyon BE, 2008. Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the lark bunting. Science 319: 459–462. doi: 10.1126/science.1149167.

Datta SR, Vasconcelos ML, Ruta V, Luo S, Wong A et al., 2008. The *Drosophila* pheromone cVA activates a sexually dimorphic neural circuit. Nature 452: 473–477. doi: 10.1038/nature06808.

Dukas R, 2004. Male fruit flies learn to avoid interspecific courtship. Behavioral Ecology 15: 695–698. doi: 10.1093/beheco/arh068.

Dukas R, 2006. Learning in the context of sexual behaviour in insects. Animal Biology 56: 125–141. doi: 10.1163/157075606777304258.

Dukas R, 2008. Learning decreases heterospecific courtship and mating in fruit flies. Biology Letters 4: 645–647. doi: 10.1098/rsbl.2008.0437.

Dukas R, 2009. Dynamics of learning in the context of courtship in *Drosophila persimilis* and *D. pseudoobscura*. Animal Behaviour 77: 253–259. doi: 10.1016/j.anbehav.2008.10.010.

Dukas R, Dukas L, 2012. Learning about prospective mates in male fruit flies: Effects of acceptance and rejection. Animal Behaviour 84: 1427–1434. doi: 10.1016/j.anbehav.2012.09.010.

Dukas R, Scott A, 2015. Fruitfly courtship: the female prespective. Current Zoology 61: 1008–1014.

Dyer KA, White BE, Sztepanacz JL, Bewick ER, Rundle HD, 2014. Reproductive character displacement of epicuticular compounds and their contribution to mate choice in *Drosophila subquinaria* and *Drosophila recens*. Evolution 68: 1163–1175. doi: 10.1111/evo.12335.

Ejima A, Smith BP, Lucas C, Levine JD, Griffith LC, 2005. Sequential learning of pheromonal cues modulates memory consolidation in trainer-specific associative courtship conditioning. Current Biology: CB 15: 194–206. doi: 10.1016/j.cub.2005.01.035.

Ejima A, Smith BPC, Lucas C, Van Naters WvdG et al., 2007. Generalization of courtship learning in *Drosophila* is mediated by cis-vaccenyl acetate. Current Biology 17: 599–605. doi: 10.1016/j.cub.2007.01.053.

Gailey DA, Jackson FR, Siegel RW, 1982. Male courtship in *Drosophila*: The conditioned response to immature males and its genetic control. Genetics 102: 771–782.

Gibert P, Capy P, Imasheva A, Moreteau B, Morin JP et al., 2004a. Comparative analysis of morphological traits among *Drosophila melanogaster* and D-simulans: Genetic variability, clines and phenotypic plasticity. Genetica 120: 165–179. doi: 10.1023/B:GENE.0000017639.62427.8b.

Gibert P, Moreteau B, David JR, 2004b. Phenotypic plasticity of body pigmentation in *Drosophila melanogaster*: Genetic repeatability of quantitative parameters in two successive generations. Heredity 92: 499–507. doi: 10.1038/sj.hdy.6800449.

Gibert P, Moreteau B, Moreteau JC, David JR, 1998. Genetic variability of quantitative traits in *Drosophila melanogaster* (fruit fly) natural populations: Analysis of wild-living flies and of several laboratory generations. Heredity 80: 326–335. doi: 10.1046/j.1365–2540.1998.00301.x.

Griffith LC, Ejima A, 2009a. Courtship learning in *Drosophila melanogaster*: Diverse plasticity of a reproductive behavior. Learning & Memory 16: 743–750. doi: 10.1101/lm.956309.

Griffith LC, Ejima A, 2009b. Multimodal sensory integration of courtship stimulating cues in *Drosophila melanogaster*. Annals of the New York Academy of Sciences 1170: 394–398. doi: 10.1111/j.1749–6632.2009.04367.x.

Jallon JM, David JR, 1987. Variations in cuticular hydrocarbons among the 8 species of the *Drosophila melanogaster* subgroup. Evolution 41:294–302. doi: 10.2307/2409139.

Joiner MA, Griffith LC, 2000. Visual input regulates circuit configuration in courtship conditioning of *Drosophila melanogaster*. Learning & Memory 7: 32–42. doi: 10.1101/lm.7.1.32.

Katayama N, Abbott JK, Kjaerandsen J, Takahashi Y, Svensson EI, 2014. Sexual selection on wing interference patterns in *Drosophila melanogaster*. Proceedings of the National Academy of Sciences of the United States of America 111: 15144–15148. doi: 10.1073/pnas.1407595111.

Keleman K, Vrontou E, Kruettner S, Yu JY, Kurtovic-Kozaric A et al., 2012. Dopamine neurons modulate pheromone responses in *Drosophila* courtship learning. Nature 489: 145–U210. doi: 10.1038/nature11345.

Kozak GM, Boughman JW, 2009. Learned conspecific mate preference in a species pair of sticklebacks. Behavioral Ecology 20: 1282–1288. doi: 10.1093/beheco/arp134.

Kramer JM, Kochinke K, Oortveld MA, Marks H, Kramer D et al., 2011. Epigenetic regulation of learning and memory by *Drosophila* EHMT/G9a. PLoS Biology 9: e1000569. doi: 10.1371/journal.pbio.1000569.

Krstic D, Boll W, Noll M, 2009. Sensory integration regulating male courtship behavior in *Drosophila*. PLoS ONE 4. doi: 10.1371/journal.pone.0004457.

Krstic D, Boll W, Noll M, 2013. Influence of the white locus on the courtship behavior of *Drosophila* males. PLoS ONE 8. doi: 10.1371/journal.pone.0077904.

Lehtonen TK, Wong BBM, Lindstrom K, 2010. Fluctuating mate preferences in a marine fish. Biology Letters 6: 21–23. doi: 10.1098/rsbl.2009.0558.

Llopart A, Elwyn S, Lachaise D, Coyne JA, 2002. Genetics of a difference in pigmentation between *Drosophila yakuba* and *Drosophila santomea*. Evolution 56: 2262–2277.

Magurran AE, Ramnarine IW, 2004. Learned mate recognition and reproductive isolation in guppies. Animal Behaviour 67: 1077–1082. doi: 10.1016/j.anbehav.2003.10.010.

Matute DR, Harris A, 2013. The influence of abdominal pig-

mentation on desiccation and ultraviolet resistance in two species of *Drosophila*. Evolution 67: 2451–2460. doi: 10.1111/evo.12122.

McBride SMJ, Giuliani G, Choi C, Krause P, Correale D et al., 1999. Mushroom body ablation impairs short-term memory and long-term memory of courtship conditioning in *Drosophila melanogaster*. Neuron 24: 967–977. doi: 10.1016/s0896–6273 (00)81043–0.

Morier-Genoud R, Kawecki, T. 2015. The effect of learning on the evolution of new courtship behavior: a simulation model. Current Zoology 61: 1062–1072.

Nitasaka E, Yamazaki T, Green MM, 1995. The molecular analysis of brown eye color mutations isolated from geographically discrete populations of *Drosophila melanogaster*. Molecular & General Genetics 247: 164–168. doi: 10.1007/bf00705646.

Rice WR, Linder JE, Friberg U, Lew TA, Morrow EH et al., 2005. Inter-locus antagonistic coevolution as an engine of speciation: Assessment with hemiclonal analysis. Proceedings of the National Academy of Sciences of the United States of America 102: 6527–6534. doi: 10.1073/pnas.0501889102.

Schlechter-Helas J, Schmitt T, Peschke K, 2012. Learning individual signatures: Rove beetle males discriminate unreceptive females by cuticular hydrocarbon patterns. Animal Behaviour 84: 369–376. doi: 10.1016/j.anbehav.2012.05.003.

Schnaitmann C, Garbers C, Wachtler T, Tanimoto H, 2013. Color discrimination with broadband photoreceptors. Current Bio-logy 23: 2375–2382. doi: 10.1016/j.cub.2013.10.037.

Servedio MR, Dukas R, 2013. Effects on population divergence of within-generational learning about prospective mates. Evolution 67: 2363–2375. doi: 10.1111/evo.12127.

Shevtsova E, Hansson C, Janzen DH, Kjaerandsen J, 2011. Stable structural color patterns displayed on transparent insect wings. Proceedings of the National Academy of Sciences of the United States of America 108: 668–673. doi: 10.1073/pnas.1017393108.

Siegel RW, Hall JC, 1979. Conditioned-responses in courtship behavior of normal and mutant *Drosophila*. Proceedings of the National Academy of Sciences of the United States of America 76: 3430–3434. doi: 10.1073/pnas.76.7.3430.

Svensson EI, Eroukhmanoff F, Karlsson K, Runemark A, Brodin A, 2010. A role for learning in population divergence of mate preferences. Evolution 64: 3101–3113. doi: 10.1111/j.1558–5646.2010.01085.x.

ten Cate C, Vos DR, 1999. Sexual imprinting and evolutionary processes in birds: A reassessment. Advances in the Study of Behavior, Vol 28 28: 1–31.

Verzijden MN, ten Cate C, Servedio MR, Kozak GM, Boughman JW et al., 2012. The impact of learning on sexual selection and speciation. Trends in Ecology & Evolution 27: 511–519. doi: 10.1016/j.tree.2012.05.007.

Vogt K, Schnaitmann C, Dylla KV, Knapek S, Aso Y et al., 2014. Shared mushroom body circuits underlie visual and olfactory memories in *Drosophila*. Elife 3. doi: 10.7554/eLife.02395.