Original Article

Female fruit flies copy the acceptance, but not the rejection, of a mate

Sabine Nöbel,[a,b,*,ᵒ] Magdalena Monier,[b,*] Laura Fargeot,[c] Guillaume Lespagnol,[b] Etienne Danchin,[b,†,ᵒ] and Guillaume Isabel[c,†,ᵒ]

[a]Université Toulouse 1 Capitole and Institute for Advanced Study in Toulouse (IAST), Toulouse, France
[b]Laboratoire Évolution & Diversité Biologique (EDB), UMR5174, CNRS, IRD, Université Toulouse III Paul Sabatier, 118 route de Narbonne, F-31062 Toulouse Cedex 9, France[c]Centre de Recherches sur la Cognition Animale (CRCA), Centre de Biologie Intégrative (CBI), CNRS UMR 5169, Toulouse, France

Acceptance and avoidance can be socially transmitted, especially in the case of mate choice. When a *Drosophila melanogaster* female observes a conspecific female (called demonstrator female) choosing to mate with one of two males, the former female (called observer female) can memorize and copy the latter female's choice. Traditionally in mate-copying experiments, demonstrations provide two types of information to observer females, namely, the acceptance (positive) of one male and the rejection of the other male (negative). To disentangle the respective roles of positive and negative information in *Drosophila* mate copying, we performed experiments in which demonstrations provided only one type of information at a time. We found that positive information alone is sufficient to trigger mate copying. Observer females preferred males of phenotype A after watching a female mating with a male of phenotype A in the absence of any other male. Contrastingly, negative information alone (provided by a demonstrator female actively rejecting a male of phenotype B) did not affect future observer females' mate choice. These results suggest that the informative part of demonstrations in *Drosophila* mate-copying experiments lies mainly, if not exclusively, in the positive information provided by the copulation with a given male. We discuss the reasons for such a result and suggest that *Drosophila* females learn to prefer the successful males, implying that the underlying learning mechanisms may be shared with those of appetitive memory in non-social associative learning.

Key words: ***Drosophila melanogaster*, mate copying, observational learning, social learning**.

INTRODUCTION

Social learning allows an individual to learn about its environment at a lower cost than with a trial-and-error tactic, potentially affecting fitness positively (Boyd and Richerson 1995). Acceptance, as well as avoidance, can be transmitted through social learning. Allen (2019) provides a nice review showing how widespread social learning is in the animal kingdom and that it appears in various contexts. For example, Norway rat pups socially learn to avoid poisoned food by copying their parent's diet and avoiding other food types (Galef and Clark 1971). Such kind of learning can exist especially in animals with prolonged maternal care (Mirza and Provenza 1990) or in social insects, where social information helps finding new foraging areas and synchronizing nest activities (Chittka and Leadbeater 2005; Leadbeater and Chittka 2007; Avarguès-Weber et al. 2018). Other examples for social information

use can be found in nine-spined sticklebacks in a foraging context (Duffy et al. 2009), in three spined sticklebacks for predator avoidance (Hogan and Laskowski 2013) or choosing nest material in zebra finches (Guilette et al. 2016). Social information is also used in non-social insects like fruit flies, notably in mate choice (Mery et al. 2009; Sarin and Dukas 2009). Mate choice being a major fitness impacting decision, it is thus no surprise that animals often use multiple information sources for mate choice (Danchin et al. 2004).

Studying social learning in the model species *Drosophila melanogaster* has the major benefit that we can build on broad knowledge about the underlying learning processes. Like in no other animal, the mechanisms of (non-social) learning have been extensively studied for the last decades in several forms and sensory modalities in direct associative learning (Quinn et al. 1974; Tempel et al. 1983; Wolf and Heisenberg 1991; Tully et al. 1994; Schwaerzel et al. 2003; Isabel et al. 2004; Aso et al. 2010; Vogt et al. 2014, 2016; Cognigni et al. 2018; Aso et al. 2019). Direct associative learning occurs when the animal experiences the association between conditional (produces a reaction only after the

Address correspondence to S. Nöbel. E-mail: Sabine.noebel@iast.fr.
*Co-first authors
†Co-senior authors

subject has learned to associate it with a given outcome) and unconditional stimuli (causes an automatic, spontaneous response without any prior learning on the part of the subject). On the contrary, indirect associative learning involves a demonstration and no direct experience of the stimulus association. Typically, social learning is an indirect form of learning (Olsson et al. 2007) in which a focal individual observes a demonstrator—or teacher—experiencing the association between a cue and a reward or punishment. The mechanisms of social learning in general, and social learning in insects in particular, are now under investigation (Burke et al. 2010; Munger et al. 2010; Debiec and Olsson 2017; Kavaliers et al. 2017; Allsop et al. 2018; Loureiro et al. 2019), with valuable recent advances on the social learning model of dialect transmission in *Drosophila* species (Kacsoh et al. 2018, 2019). However, we are still far from understanding these mechanisms thoroughly. In particular, the question of the extent of the overlap between pathways of social learning and the better studied direct associative learning remains poorly explored (Heyes 1994; Heyes and Pearce 2015; Leadbeater and Dawson 2017).

Here, we focus on a form of observational social learning called mate copying. It has been described in several vertebrate and invertebrate species [reviewed in Varela et al. (2018)]. Mate copying occurs when after observing the mate choice of demonstrator individuals the choice of the observer individuals (usually females) is biased towards either the specific male chosen during the demonstration (individual-based mate copying) or towards males of similar phenotypes (trait-based mate copying; Bowers et al. 2012). The latter form of mate copying can strongly affect evolution (Agrawal et al. 2001; Witte et al. 2015) as it can considerably amplify sexual selection of male traits. Trait-based mate copying has been described and studied in the fruit fly *Drosophila melanogaster* for a decade (Mery et al. 2009; Dagaeff et al. 2016; Danchin et al. 2018; Monier et al. 2018; Nöbel et al. 2018; Monier et al. 2019).

A first question concerns the stimuli eliciting mate copying. Previous work in fish identified some important factors affecting mate copying, like the age and experience of the demonstrator. For instance, female guppies (*Poecilia reticulata*) preferentially copy the choice of larger, older, and more experienced females over smaller, young demonstrator females (Amlacher and Dugatkin 2005; Vukomanovic and Rodd 2007). Similarly, the quality of demonstrator females affects mate copying in the sailfin molly *Poecillia latipinna* (Hill and Ryan 2006). Females show a higher preference for a given male after a demonstration in which it was close to a high quality (conspecific) female, whereas they show a lower preference for a specific male after a demonstration showing it with a low-quality (a heterospecific) female (Hill and Ryan 2006). Furthermore, sailfin molly females copy the rejection of a potential mate by demonstrator females (Witte and Ueding 2003). In other social contexts, rejection of poorly performing demonstrators has been observed (Mason et al. 1984; Forsman and Seppänen 2011), demonstrating the possibility of active rejection based on social cues.

In a mate-copying experiment in *D. melanogaster*, the demonstration classically involves a female choosing between two males of contrasting phenotypes (randomly and artificially dusted in pink or green) in front of a naïve observer female (Dagaeff et al. 2016; Danchin et al. 2018; Monier et al. 2019). In such circumstances, observer females simultaneously gather positive information for the successfully mating male A and negative information for the rejected male B. Here, we modified this protocol in order to provide only one kind of information about male attractiveness (positive or negative) at a time.

Based on previous results, we predicted that flies receiving only positive information would copy the choice of the demonstrator. This assumption is based on the observation that mate-copying scores did not differ significantly between trials in which observer females watched the courtship plus the copulation during the demonstration versus trials in which the observer female only saw the copulation (Dagaeff et al. 2016). However, when a demonstrator female rejects a male, this can occur for various reasons. It can indeed be due to the poor quality of the courting male (Dugatkin and Godin 1993; Dale and Slagsvold 1996), but it can also occur for reasons independent from male quality, like the refractory period of females after a previous mating (Bussel et al. 2014; Laturney and Billeter 2014; Gorter et al. 2016). Thus, rejection does not necessarily carry information about male quality and should be more difficult to interpret by observer females, which could lead them to ignore the sole rejection of a male. Moreover, in the wild, females may be more likely to observe a copulating pair than a female rejecting a potential suitor, as copulation lasts for twenty minutes or more, while a rejection event can last no longer than a few seconds when the female decamps or escapes. Subsequently, we hypothesized that the ability to grasp social information from acceptance rather than rejection cues would be more ecologically relevant. However, there was still the possibility that the rejection of a male provides enough information for observer females to avoid this male phenotype.

METHODS

Fly maintenance

Wild-type Canton-S *Drosophila melanogaster* were raised in 30 mL vials on a corn flour-agar-yeast-medium at 25 ± 0.1 °C and $63.8 \pm 0.4\%$ relative humidity, in an artificial 12:12 h light/dark cycle. Newly emerged, virgin flies (males and females) were collected several times a day and sexed without anesthesia by gentle aspiration using a glass pipette, tubing, and gauze. They were kept in unisex groups of 7 females and 15 males and used for the behavioral experiments when 3–5 days old. For the experiments, males were dusted with artificial green (Shannon Luminous Materials, Inc. #B-731) and pink (BioQuip Products, Inc. #1162R) powders, and left in a food vial for 20–30 min to allow them to clean the excess powder before being transferred to the experimental setup. All males were randomly assigned to one color.

Animal welfare

Our study involved a population of *D. melanogaster* that have been maintained exclusively under laboratory conditions for hundreds of generations. The current study did not require ethical approval and complied with French laws regarding animal welfare. Whenever possible, however, we adhered to the guidelines for the treatment of animals in behavioral research. We handled flies by gentle aspiration without anesthesia to minimize damage and discomfort. After the experiments individuals were euthanized in a freezer at −20 °C.

Behavioral assay

Experiments were conducted in a double plastic tube device (Dagaeff et al. 2016) of 0.8×3 cm each, separated by a transparent partition composed of a microscopy cover slide of 1.6×1.6 cm.

Experiments had three distinct phases (stage 1, stage 2, stage 3) that each lasted 30 min. First and second, an observer female watches two demonstrations ("stage 1" and "stage 2") successively providing different information about male phenotypes. The order of stage 1 and stage 2 alternated from one trial to the next. The third phase (stage 3) followed and consisted in a mate-choice test where observer females could choose between the same types of males (Figure 1). We conducted three different treatments: a control treatment in which observer females received positive and negative information; a rejection treatment providing only negative information; an acceptance treatment providing positive information only (Figure 1).

In the control treatment we provided both acceptance and rejection of a male during the demonstration. To be treated like the other observer females and the observer female in the control setting was alone during stage 1, and for stage 2 we introduced in the other compartment a virgin demonstrator female, plus a pink and a green male (Figure 1). The observer female could thus see the courtship and the actual choice of the demonstrator female. Trials in which the demonstrator female did not copulate within 30 min of the demonstration were discarded. After the demonstrator copulation ended demonstrator flies were removed.

In the rejection treatment, stage 1 consisted of a 30 min presentation of a single male, pink or green (alternating from one trial to the next; Figure 1). This ruled out a potential novelty effect (i.e., the discovery of a new male color during the test),

which could occur if the observer female had only seen one male color before the mate-choice test. As that male was alone, this did not provide any social information about its attractiveness (Allain, Nöbel, Isabel, Danchin unpublished data). In stage 2, a male of the opposite color was presented together with a demonstrator female that recently mated (Figure 1). As recently mated *D. melanogaster* females actively reject courting males (Barnes et al. 2008; Kimura et al. 2015) for several days after copulation (Markow 2002), these trials provided negative information for the rejected male. As there seems to be no visual cues indicating the previous mating status (Aranha and Vasconcelos 2018) and direct contact between observer female and demonstrators was prevented by the glass separation, we assumed that observer females were not able to detect the demonstrator female's previous mating status. To ensure that the female really had access to negative information in the rejection treatment, we checked that the male courted the female and were rejected effectively, and we discarded trials in which the male did not court the demonstrator female.

In the acceptance treatment, again observer females could observe a single male in stage 1 and then in stage 2 they observed a copulation with a male of the opposite color. Trials in which the demonstrator female did not copulate within 30 min of the demonstration were discarded. After the demonstrator copulation ended, demonstrator flies were removed.
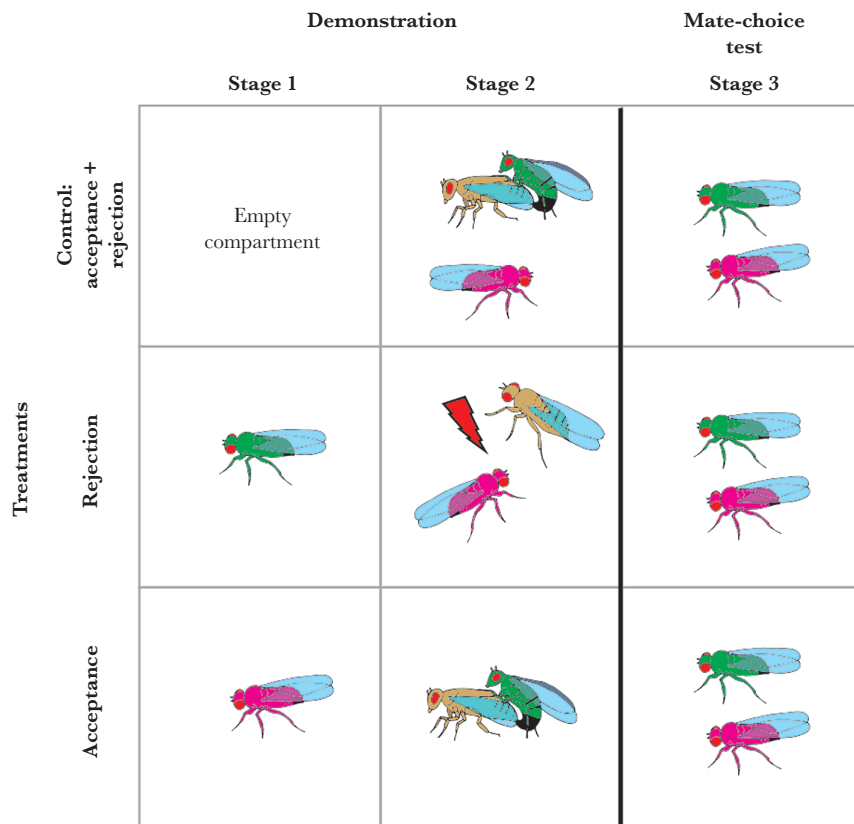

Figure 1
Demonstrations presented to observer female in each of the three treatments. Each demonstration lasted 30 min. All female received both demonstrations, either stage 1 then stage 2, or stage 2 then stage 1, with the order of these demonstrations alternatively from one trial to the next. We also did the same demonstrations with reversed colors.

These two types of demonstrations resemble the classic aversive or appetitive conditioning in drosophila, where a conditioned stimulus (CS) (e.g., an odor) is paired with an unconditioned one (US) (e.g., an electric shock) and another CS (a different odor) that is not associated with the US. In the present experiment, the male colors are the CS, and the acceptance or rejection of the demonstrator females are the social US. So, in the mate-choice test (stage 3, Figure 1), observer females will be able to choose between the colors that were associated or not with the US. For this third phase we used two new virgin males, one of each color. Then the partition separating males and female was removed, beginning the mate-choice test. During that test, we recorded the color of the male showing first wing extension ("singing") as the beginning of courtship and if the other male sang as well and the color of the male chosen for copulation. No threshold was applied to courtship duration, even 1 second courtship was considered as courtship event. Demonstrations and test were performed by the same experimenter. As in previous studies (Dagaeff et al. 2016; Danchin et al. 2018; Monier et al. 2018; Nöbel et al. 2018; Monier et al. 2019), we only kept trials in which the two males courted the observer female before copulation as this constitutes a reliable indicator that females were really in a position to choose. This gave us 196 trials over the 585 for which we initiated a demonstration.

Mate-copying index

For each trial, we computed a mate-copying score as a binomial variable taking the value 1 when the observer female mated with a male of the color preferred (or rejected) by the demonstrator female, and 0 in the opposite case. We then calculated the mate-copying index for each group as the mean of mate-copying scores. Mate-copying indices significantly above 0.5 indicate that observer females were biased in their mate choice towards the color preferred or not rejected by the demonstrator, and thus reveal mate copying.

Statistical analyses

We analyzed the data using the version 4.0.2 of the R software (R Core Team 2020). For each treatment, we measured the difference from random choice with a binomial test. We then ran GLMM (generalized linear mixed model) with binary logistic regression (package lme4, Bates et al. 2015) on the data of the three treatments in order to see if treatment and normalized air pressure (air pressure at the beginning of the trial minus mean air pressure in the whole data set) was significantly related mate-copying scores. Air pressure was added to the model because it was found that mate-copying scores are sensitive to this weather variable (Dagaeff et al. 2016). Experiments were performed by LF and SN; thus, we included the experimenter in the starting model as fixed effect. We also included a random block effect to account for the non-independence of the set of six trials trained and tested in parallel in the same observation box. We used Wald chi-square tests implemented in the ANOVA function of the car package (Fox and Weisberg 2011) to test the significance of fixed effects. The starting model included three fixed effects (treatment, normalized air pressure, and experimenter) and an interaction between treatment and experimenter, and the final model was obtained through a backward selection approach, removing the interactions and the experimenter as fixed effect as they were non-significant. Finally, we did two-by-two comparisons between groups using Pearson's chi-squared test with Yates' continuity correction and Holm's correction for multiple comparisons.

RESULTS

We measured mate-copying scores after a demonstration showing a female either accepting a male (in the absence of a rejected male), rejecting a male (in the absence of an accepted male), or accepting one male and rejecting the other as a control treatment. Control observer females that received both positive and negative information during the demonstration copied the choice of the demonstrator (binomial test, $N = 65$, $P = 0.006$, left bar of Figure 2). Females that received only positive information, by watching a demonstrator female accepting copulation with a male, also copied the demonstrators choice (binomial test, $N = 65$, $P = 0.00005$; right bar of Figure 2). Contrastingly, females that only saw a male being rejected by a female (negative information) did not develop a preference for the opposite phenotype, suggesting that they did not avoid mating with the male of the phenotype that they saw being rejected by the demonstrator female (binomial test, $N = 66$, $P = 0.268$, central bar of Figure 2). Mate-copying scores of the three treatments differed significantly (GLMM, Wald χ^2 test, $N = 196$, $\chi^2_2 = 14.462$, $P = 0.0007$), while normalized air pressure had no effect (GLMM, Wald χ^2 test, $N = 196$, $\chi^2_1 = 0.953$, $P = 0.329$). Finally, in pairwise post hoc χ^2 tests, we found a significant difference between the acceptance and rejection treatment groups ($N = 130$, $\chi^2_1 = 13.355$, $P = 0.0008$) and between the control and the rejection treatment ($N = 131$, $\chi^2_1 = 7.457$, $P = 0.0126$), but not between the control and acceptance treatments ($N = 131$, $\chi^2_1 = 0.605$, $P = 0.437$). Thus, in our experimental conditions, positive information for a certain phenotype, but not negative information, appeared sufficient to
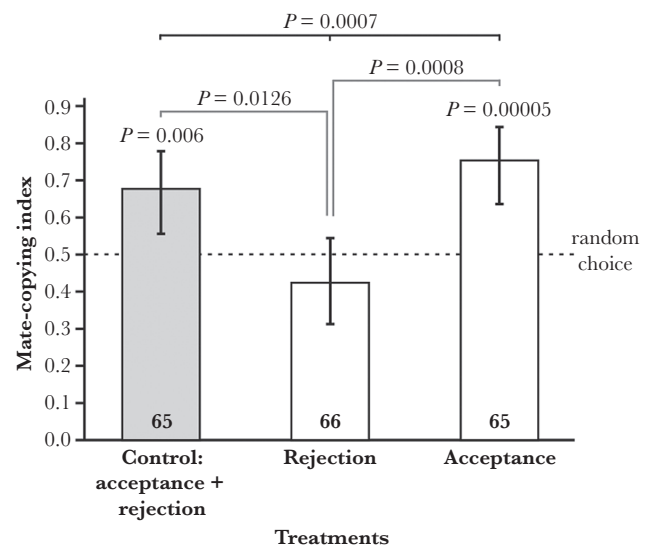

Figure 2

Mate-copying index in the three treatments. Observer females of the control treatment received positive and negative information; those of the rejection treatment received only negative information; those of the acceptance treatment received positive information only. The dashed line represents the expectations under random choice. Sample sizes are indicated at the bottom of each bar. Error bars are Agresti–Coull intervals. P-values above histograms are those of the corresponding binomial tests, and those above horizontal bars are either those of pairwise post hoc χ^2 tests (grey), or of the GLMM among the three treatments (black).

elicit a mate copying behavior that is as efficient as when both types of information are provided simultaneously.

DISCUSSION

Our goal was to disentangle the role of positive and negative information during the observation of binary mate-choice decisions in *D. melanogaster* in order to evaluate its ecological relevance. We found that females, that received positive information only or positive and negative information at the same time, learned and copied the choice of the demonstrator females, as in previous studies (Dagaeff et al. 2016; Danchin et al. 2018; Nöbel et al. 2018; Monier et al. 2019). We further found no significant difference in the learning capacities of females of these two treatments. In contrast, females receiving only negative information did not significantly avoid the color they saw being rejected, which differs from a previous study in fish (Witte and Ueding 2003). Thus, positive information appears sufficient to elicit mate copying after one demonstration in fruit flies.

The absence of mate copying in the rejection treatment suggests that one demonstration containing rejection(s) of a male is not sufficient to elicit avoidance behavior in the observer females. This may be because a female can reject a male for reasons that are independent from its quality, like the female being non-receptive (Connolly and Cook 1973; Neckameyer 1998), as this is the case in our study. Alternatively, it may be that observer females were less interested in negative demonstrations as they did not involve copulation, in which case the negative result would simply result from a lack of interest in the demonstrations. Or it could be that the solitary male and the rejected male were evaluated in the same way, and thus, no preference was developed.

A recent study of aversive olfactory memory in *Drosophila* showed that an initially neutral stimulus can become attractive to fruit flies under some circumstances—the "safety memory" (Jacob and Waddell 2020). Briefly, after a multiple spaced training with sequences of conditioned stimuli (CS) simultaneously with an aversive cue (CS+) followed by another CS without reinforcement (CS−), Jacob and Waddell conclude that the individuals display both a CS + avoidance and an approach movement towards the CS- when later given the choice between the CS + and CS− odors. Thus, in our design, a sequence of several rejections (showing first a male of phenotype A rejected by a female and then a single male of another phenotype B, repeated several times) might elicit aversive learning for phenotype A leading to a choice for the male phenotype B. Interestingly, in the fruit fly larva, appetitive but not aversive olfactory stimuli support associative gustatory learning (Hendel et al. 2005). Opposite to what we observe in fruit fly females, female sailfin mollies (*Poecillia latipinna*) copy the rejection of a male (Witte and Ueding 2003). However, the setup used in that study was quite different from ours, as the rejection demonstration consisted of a sequence of four 12-min video of four different females escaping from a courting male, so that the rejection cue seemed much stronger than in the present study that only involved a single demonstrator female. Similarly, in humans, women, but not men, decrease their interest for a relationship to a demonstrator after watching a speed-dating video in which the demonstrator and a potential partner showed mutual lack of interest (Place et al. 2010). This can indicate that beyond the effect of the experimental conditions, different species use different social cues for mate copying. However, the motivations to reject a partner are way less studied than for building specific mating preferences.

A last alternative can be that in nature newly emerged females do not see older females choosing between only two males, but rather see females choosing among many males to copulate with one of them. The fact that the former chooses that specific male is informative in itself but the fact that she rejected all other potential male does not reveal much information about all the non-selected males. This purely statistical fact may explain the absence of an effect of seeing only a rejection.

Finally, our results suggest that in the classical *Drosophila* mate-copying design, the rejected male shown in the demonstration may not constitute the prominent cue triggering learning in the observer female. Moreover, the presentation of a male of the opposite color together with the copulating pair in the classical demonstration might even constitute a distractive stimulus, as indirectly suggested by Germain et al. (2016 experiment 3). In nature, females may observe copulations longer than rejection as copulations likely last for more than 30 min (Markow 2000), while rejections are brief and thus far less prominent (Gromko and Markow 1993). It is thus possible that our result is explained by the fact that *D. melanogaster* females evolved an ability to gather social information from the most easily detectable and reliable social cues. Alternatively, females might pay attention to rejection events too but might have difficulties in interpreting them or distinguishing them from other neutral information, such as solitary males.

Our finding that the acceptance of a male by the demonstrator female is the most relevant cue to elicit full mate copying by the observer female suggests that it involves networks of appetitive learning neurons and mechanisms rather than the aversive pathway. Several authors suggested that social learning in many contexts can have an associative explanation (e.g., Munger et al. 2010 ; Avarguès-Weber et al. 2015; Heyes and Pearce 2015; Leadbeater and Dawson 2017). For mate copying, this has yet to be proven. At the moment, asocial learning, like olfactory associative direct learning, is way better understood. Here the pairing between a conditioned stimulus (CS; for instance, odor A) and an appetitive US (sucrose) leads flies to prefer odor A over B even in the absence of any reward (Tempel et al. 1983) through the association of odor A to the reward (Schultz et al. 1997). In our social learning paradigm, we can speculate that the relevant cues eliciting learning are the color of the copulating males in association with the successful mating. Hence, the copulating pair would mediate the appetitive US, while male color would constitute the CS (Avarguès-Weber et al. 2015). Under this hypothesis, it would be interesting to study whether mate-copying mechanisms resemble those of visual, appetitive, associative learning, given that its neural bases are now well-understood (Vogt et al. 2014, 2016).

More generally, understanding how social learning works can only help sharpening our view on the evolution of the different types of learning, opening the way to new theories about the evolution of behavior, cognition, and culture in invertebrates.

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REFERENCES

Agrawal A. 2001. The evolutionary consequences of mate copying on male traits. Behav Ecol Sociobiol. 51:33–40.

Allen JA. 2019. Community through culture: from insects to whales: how social learning and culture manifest across diverse animal communities. Bioessays 41:e1900060.

Allsop SA, Wichmann R, Mills F, Burgos-Robles A, Chang CJ, Felix-Ortiz AC, Vienne A, Beyeler A, Izadmehr EM, Glober G, et al. 2018. Corticoamygdala transfer of socially derived information gates observational learning. Cell 173:1329–1342.e18.

Amlacher J, Dugatkin LA. 2005. Preference for older over younger models during mate-choice copying in young guppies. Ethol Ecol Evol. 17:161–169.

Aranha MM, Vasconcelos ML. 2018. Deciphering Drosophila female innate behaviors. Curr Opin Neurobiol. 52:139–148.

Aso Y, Ray RP, Long X, Bushey D, Cichewicz K, Ngo TT, Sharp B, Christoforou C, Hu A, Lemire AL, et al. 2019. Nitric oxide acts as a cotransmitter in a subset of dopaminergic neurons to diversify memory dynamics. eLife. 8:e49257.

Aso Y, Siwanowicz I, Bracker L, Ito K, Kitamoto T, Tanimoto H. 2010. Specific dopaminergic neurons for the formation of labile aversive memory. Curr Biol. 20:1445–1451.

Avarguès-Weber A, Lachlan R, Chittka L. 2018. Bumblebee social learning can lead to suboptimal foraging choices. Anim Behav. 135:209–214.

Avarguès-Weber A, Lihoreau M, Isabel G, Giurfa M. 2015. Information transfer beyond the waggle dance: observational learning in bees and flies. Front Ecol Evol. 3:24.

Barnes AI, Wigby S, Boone JM, Partridge L, Chapman T. 2008. Feeding, fecundity and lifespan in female Drosophila melanogaster. Proc R Soc Lond B. 275:1675–1683.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. J Stat Soft. 67:1–48

Bowers RI, Place SS, Todd PM, Penke L, Asendorpf JB. 2012. Generalization in mate-choice copying in humans. Behav Ecol. 23:112–124.

Boyd R, Richerson PJ. 1995. Why does culture increase human adaptability? Ethol Sociobiol. 16:125–143.

Burke CJ, Tobler PN, Baddeley M, Schultz W. 2010. Neural mechanisms of observational learning. Proc Natl Acad Sci USA. 107:14431–14436.

Bussell JJ, Yapici N, Zhang SX, Dickson BJ, Vosshall LB. 2014. Abdominal-B neurons control Drosophila virgin female receptivity. Curr Biol. 24:1584–1595.

Chittka L, Leadbeater E. 2005. Social learning: public information in insects. Curr Biol. 15:R869–R871.

Cognigni P, Felsenberg J, Waddell S. 2018. Do the right thing: neural network mechanisms of memory formation, expression and update in Drosophila. Curr Opin Neurobiol. 49:51–58.

Connolly K, Cook R. 1973. Rejection responses by female Drosophila melanogaster: their ontogeny, causality and effects upon the behaviour of the courting male. Behaviour 142–166.

Dagaeff A-C, Pocheville A, Nöbel S, Loyau AL, Isabel G, Danchin E. 2016. Drosophila mate copying correlates with atmospheric pressure in a speed learning situation. Anim Behav. 121:163–174.

Dale S, Slagsvold T. 1996. Mate choice on multiple cues, decision rules and sampling strategies in female pied flycatchers. Behaviour 133:903–944.

Danchin E, Giraldeau LA, Valone TJ, Wagner RH. 2004. Public information: from nosy neighbors to cultural evolution. Science 305:487–491.

Danchin E, Nöbel S, Pocheville A, Dagaeff AC, Demay L, Alphand M, Ranty-Roby S, Van Renssen L, Monier M, Gazagne E, et al. 2018. Cultural flies: Conformist social learning in fruitflies predicts long-lasting mate-choice traditions. Science. 362:1025–1030.

Debiec J, Olsson A. 2017. Social fear learning: from animal models to human function. Trends Cog Sci. 21:546–555.

Duffy GA, Pike TW, Laland KN. 2009. Size-dependent directed social learning in nine-spined sticklebacks. Anim Behav. 78:371–375.

Dugatkin LA, Godin J-GJ. 1993. Female mate copying in the guppy (Poecilia reticulata): age-dependent effects. Behav Ecol. 4:289–292.

Forsman JT, Seppänen J-T. 2011. Learning what (not) to do: testing rejection and copying of simulated heterospecific behavioural traits. Anim Behav. 81:879–883.

Fox J, Weisberg S. 2011. An {R} companion to applied regression. 2nd ed. Thousand Oaks (CA): Sage.

Galef BG, Clark MM. 1971. Social factors in the poison avoidance and feeding behavior of wild and domesticated rat pups. J Comp Physiol Psychol. 75:341–357.

Germain M, Blanchet S, Loyau A, Danchin E. 2016. Mate-choice copying in Drosophila melanogaster: impact of demonstration conditions and male–male competition. Behav Process. 125:76–84.

Gorter JA, Jagadeesh S, Gahr C, Boonekamp JJ, Levine JD, Billeter J-C. 2016. The nutritional and hedonic value of food modulate sexual receptivity in Drosophila melanogaster females. Sci Rep. 6:1–10.

Gromko MH, Markow TA. 1993. Courtship and remating in field populations of Drosophila. Anim Behav. 45:253–262.

Guillette LM, Scott AC, Healy SD. 2016. Social learning in nest-building birds: a role for familiarity. Proc Roy Soc Lond B. 283:20152685

Hendel T, Michels B, Neuser K, Schipanski A, Kaun K, Sokolowski MB, Marohn F, Michel R, Heisenberg M, Gerber B. 2005. The carrot, not the stick: appetitive rather than aversive gustatory stimuli support associative olfactory learning in individually assayed Drosophila larvae. J Comp Physiol A. 191:265–279.

Heyes CM. 1994. Social learning in animals: categories and mechanisms. Biol Rev. 69:207–231.

Heyes C, Pearce JM. 2015. Not-so-social learning strategies. Proc R Soc Lond B. 282:20141709.

Hill SE, Ryan MJ. 2006. The role of model female quality in the mate choice copying behaviour of sailfin mollies. Biol Lett. 2:203–205.

Hogan KE, Laskowski. 2013. Indirect information transfer: three-spined sticklebacks use visual alarm cues from frightened conspecifics about an unseen predator. Ethol. 119:999–1005.

Isabel G, Pascual A, Preat T. 2004. Exclusive consolidated memory phases in Drosophila. Science 304:1024–1027.

Jacob PF, Waddell S. 2020. Spaced training forms complementary long-term memories of opposite valence in Drosophila. Neuron 106:977–991.e4.

Kacsoh BZ, Bozler J, Bosco G. 2018. Drosophila species learn dialects through communal living. PLoS Genet. 14:e1007430.

Kacsoh BZ, Bozler J, Hodge S, Bosco G. 2019. Neural circuitry of social learning in Drosophila requires multiple inputs to facilitate inter-species communication. Commun Biol. 2:309.

Kavaliers M, Matta R, Choleris, E. 2017. Mate-choice copying, social information processing, and the roles of oxytocin. Neurosci Biobehav Rev. 72:232–242.

Kimura K-I, Sato C, Koganezawa M, Yamamoto D. 2015. Drosophila ovipositor extension in mating behavior and egg deposition involves distinct sets of brain interneurons. PLoS One. 10:e0126445.

Laturney M, Billeter J-C. 2014. Neurogenetics of female reproductive behaviors in Drosophila melanogaster. Advanc Genet. 85:1–108.

Leadbeater E, Chittka L. 2007. Social learning in insects—from miniature brains to consensus building. Curr Biol. 17:R703–R713.

Leadbeater E, Dawson EH. 2017. A social insect perspective on the evolution of social learning mechanisms. Proc Natl Acad Sci USA. 114:7838–7845.

Loureiro M, Achargui R, Flakowski J, Van Zessen R, Stefanelli T, Pascoli V, Lüscher C. 2019. Social transmission of food safety depends on synaptic plasticity in the prefrontal cortex. Science 364:991–995.

Markow TA. 2000. Forced matings in natural populations of Drosophila. Am Nat. 156:100–103.

Markow TA. 2002. Perspective: female remating, operational sex ratio, and the arena of sexual selection in Drosophila species. Evolution 56:1725–1734.

Mason JR, Arzt AH, Reidinger RF. 1984. Comparative assessment of food preferences and aversions acquired by blackbirds via observational learning. Auk 101:796–803.

Mery F, Varela SAM, Danchin E, Blanchet S, Parejo D, Coolen I, Wagner RH. 2009. Public versus personal information for mate copying in an invertebrate. Curr Biol. 19:730–734.

Mirza SN, Provenza FD. 1990. Preference of the mother affects selection and avoidance of foods by lambs differing in age. Appl Anim Behav Sci. 28:255–263.

Monier M, Nöbel S, Danchin E, Isabel G. 2019. Dopamine and serotonin are both required for mate-copying in *Drosophila melanogaster*. Front Behav Neurosci. 12:334.

Monier M, Nöbel S, Isabel G, Danchin E. 2018. Effects of a sex ratio gradient on female mate-copying and choosiness in *Drosophila melanogaster*. Curr Zool. 64:251–258.

Munger SD, Leinders-Zufall T, McDouall LM, Cockerham RE, Schmid A, Wandernoth P, Wennemuth G, Biels M, Zufall F, Kelliher KR. 2010. An olfactory subsystem that detects carbon disulfide and mediates food-related social learning. Curr Biol. 20:1438–44.

Neckameyer WS. 1998. Dopamine modulates female sexual receptivity in *Drosophila melanogaster*. J Neurogenet. 12:101–114.

Nöbel S, Danchin E, Isabel G. 2018. Mate-copying for a costly variant in *Drosophila melanogaster females*. Behav Ecol. 29:1150–1156.

Nöbel S, Monier M, Fargeot L, Lespagnol G, Danchin E, Isabel G. 2022. Data from: Female fruit flies copy the acceptance, but not the rejection, of a mate. doi:10.5061/dryad.gtht76hq2.

Olsson A, Nearing KI, Phelps EA. 2007. Learning fears by observing others: the neural systems of social fear transmission. Soc Cog Affect Neurosci. 2:3–11.

Place SS, Todd PM, Penke L, Asendorpf JB. 2010. Humans show mate copying after observing real mate choices. Evol Hum Behav. 31:320–325.

Quinn WG, Harris WA, Benzer S. 1974. Conditioned behavior in *Drosophila melanogaster*. Proc Natl Acad Sci USA. 71:708–712.

R Core Team. 2020. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Sarin S, Dukas R. 2009. Social learning about egg-laying substrates in fruit flies. Proc R Soc Lond B. 276:4323–4328.

Schultz W, Dayan P, Montague PR. 1997. A neural substrate of prediction and reward. Science 275:1593–1599.

Schwaerzel M, Monastirioti M, Scholz H, Friggi-Grelin F, Birman S, Heisenberg M. 2003. Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in *Drosophila*. J Neurosci. 23:10495–10502.

Tempel BL, Bonini N, Dawson DR, Quinn WG. 1983. Reward learning in normal and mutant *Drosophila*. Proc Natl Acad Sci USA. 80:1482–1486.

Tully T, Preat T, Boynton SC, Del Vecchio M. 1994. Genetic dissection of consolidated memory in *Drosophila*. Cell 79:35–47.

Varela SAM, Matos M, Schlupp I. 2018. The role of mate-choice copying in speciation and hybridization. Biol Rev. 93:1304–1322.

Vogt K, Aso Y, Hige T, Knapek S, Ichinose T, Friedrich AB, … Tanimoto H. 2016. Direct neural pathways convey distinct visual information to *Drosophila* mushroom bodies. eLife. 5:e14009.

Vogt K, Aso Y, Hige T, Knapek S, Ichinose T, Friedrich AB, Turner GC, Rubin GM, Tanimoto H. 2014. Shared mushroom body circuits underlie visual and olfactory memories in *Drosophila*. eLife 3:e02395.

Vukomanovic J, Rodd FH. 2007. Size-dependent female mate copying in the guppy (*Poecilia reticulata*): large females are role models but small ones are not. Ethology 113:579–586.

Witte K, Kniel N, Kureck IM. 2015. Mate-choice copying: status quo and where to go. Curr Zool. 61:1073–1081.

Witte K, Ueding K. 2003. Sailfin molly females (*Poecilia latipinna*) copy the rejection of a male. Behav Ecol. 14:389–395.

Wolf R, Heisenberg M. 1991. Basic organization of operant behavior as revealed in *Drosophila* flight orientation. J Comp Physiol A. 169:699–705.