



Mate copying in *Drosophila melanogaster* males

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To assess potential mates' quality individuals can observe sexually interacting conspecifics. Such social information use is called mate copying and occurs when observer individuals witnessing sexual interactions of conspecifics later show a mating preference for mates that were seen mating. Most studies have focused on female mate copying, as females are usually the choosy sex. However, much less is known about the existence of male mate copying, probably because of the usual strong asymmetry in sex roles. Mate copying has been documented in female *Drosophila melanogaster*, and here we report on experimental evidence for mate copying in males of this species in which females can actively reject males and prevent copulation. As mate choice implies high costs for males we assumed that they perform mate copying as well. We created two artificial female phenotypes by randomly dusting females with green or pink powders, and virgin naïve observer males were given the opportunity to see a demonstrator male choosing between a pink and a green demonstrator female. Immediately afterwards, observer males were given the choice between two new females, one of each colour. To circumvent the difficulty of determining actual male mate preference, we used two complementary indices of male mate choice, both of which provided evidence for male mate copying. Informed observer males showed a bias towards females of the colour they saw being chosen during demonstrations, while uninformed males chose randomly between pink and green females. This suggests that male fruit flies can also perform mate copying. Although significant, our results in males were less clear-cut than in females in previous studies. However, like females, *D. melanogaster* males can mate copy based on a single observation. The importance and generality of such mate copying abilities in nature, and their potential impact on the evolution of *Drosophila* and probably other invertebrates, need further exploration.

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To evaluate potential mates, animals of a wide range of taxa have been shown to use public information (i.e. information that is accessible to all individuals; Wagner & Danchin, 2010). In particular, individuals can assess the quality of potential mates from their mating performance. One form of such social learning is mate copying (Danchin, Giraldeau, Valone, & Wagner, 2004; Gibson & Höglund, 1992; Wade & Pruett-Jones, 1990) which occurs when an observer uses the mating performance of potential mates to develop a sexual preference. Typically, in mate copying, an observer shows a mating preference for mates that it saw being preferred by conspecifics (Pruett-Jones, 1992; reviewed in; Vakirtzis, 2011). Mate copying can be either at the individual level (individual-based

copying, Bowers, Place, Todd, Penke, & Asendorpf, 2012) or at the phenotypic level (trait-based copying, Bowers et al., 2012). In the former case, the preference is only for the specific individual that was seen successfully attracting a partner, while in the latter case the preference is for any potential mate of the same phenotype.

Mate copying can be a fast and safe strategy to gather integrative information about the quality of potential mates (Westneat, Walters, McCarthy, Hatch, & Hein, 2000; reviewed in; Witte & Nöbel, 2011). It has been experimentally demonstrated in several species of birds (Galef & White, 1998; Gibson, Bradbury, & Vehrencamp, 1991; Höglund, Alatalo, Gibson, & Lundberg, 1995; Knier et al., 2015), mammals (Bowers et al., 2012; Galef, Lim, & Gilbert, 2008; Waynforth, 2007), fish (reviews in Danchin et al., 2004; Witte, Knier, & Kureck, 2015) and one insect species (*Drosophila melanogaster*; Dagaëff et al., 2016; Mery et al., 2009; Monier, Nöbel, Isabel, & Danchin, 2018). Most published experiments tested the effect of positive social information (i.e. success in attracting mates), but it has also been shown that the negative

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social information provided by the rejection of a potential mate can also elicit mate copying with observers tending to reject previously rejected individuals (Witte & Ueding, 2003).

Most studies have reported on female mate copying as females are usually considered the choosier sex (Trivers, 1972) because they usually invest more in the production of each descendant (Kokko & Johnstone, 2002). However, although males are expected to be less choosy than females, they may still benefit from distinguishing between receptive and nonreceptive females to avoid courting reluctant females or the costs of sperm production and/or parental care (reviews in Dewsbury, 1982; Kokko & Jennions, 2008; Nakatsuru & Kramer, 1982). Accordingly, evidence is accruing that males also can have mating preferences (reviewed in Verzijden et al., 2012). Nevertheless, studies of mate copying in males remain relatively rare and often concern species in which males invest significantly in reproduction. For instance, male mate copying has been shown in the deep-snouted pipefish, *Syngnathus typhle*, a sex role-reversed species, in which males but not females mate-copy (Widemo, 2006). Similarly, in the darter *Etheostoma flabellare*, a species in which males invest in reproduction by guarding the nest site and caring for developing eggs, males but not females show mate copying (Moran, von Ende, & King, 2013). However, male mate copying can also exist in species without parental care, and in which both males and females perform mate copying. This is the case in three fish species, the sailfin molly, *Poecilia latipinna* (Witte & Ryan, 2002), the Atlantic molly, *Poecilia mexicana* (Bierbach, Kronmarck, Hennige-Schulz, Stadler, & Plath, 2011; Heubel et al., 2008) and the darter *Etheostoma zonale* (Moran et al., 2013).

Mating and egg production are costly in *D. melanogaster* females (Chapman, Liddle, Kalb, Wolfner, & Partridge, 1995; Fowler & Partridge, 1989; Partridge, Green, & Fowler, 1987). In this species there is no documented parental care, but males are known to produce large and costly sperm cells (1.91 ± 0.001 mm) and have testes representing up to about 5% of their body mass (Pitnick, 1996); sperm depletion starts after a single copulation event (Demerec & Kaufman, 1941; Lefevre & Jonsson, 1962; Loyau, Blanchet, Van Laere, Clobert, & Danchin, 2012). Males also display elaborate and costly courtship (Spieth, 1974); this plus the transfer of about 4000 large and costly sperm cells (Gilbert, 1981) during the 20 min of a typical copulation (Pavkovic-Lucic, Lucic, Milicic, Tomic, & Savic, 2014) decrease the life span of sexually active males (Cordts & Partridge, 1996; Partridge & Farquhar, 1981). Thus, the asymmetry between male and female investment in each descendant appears much lower in *Drosophila*, and especially in *D. melanogaster*, than in other species. Furthermore, *D. melanogaster* males show adaptive mate choice and mate preference learning (Byrne & Rice, 2006; Dukas, 2004, 2009). For instance, males can distinguish mated from virgin females, direct their courtship more specifically to virgins and learn to associate the pheromone *cis*-vaccenyl acetate (cVA) with mated females (Ejima et al., 2007; Keleman, Vrontou, Krüttner, Yu, Kozaric-Kurtovic & Dickson, 2012). In *Drosophila*, males can also visually associate an arbitrary trait such as eye colour with female mating status (Verzijden, Abbott, von Philipsborn, & Loeschke, 2015).

All these biological characteristics led us to expect mate choice to be partly mutual in *D. melanogaster*. This situation would put males under constraints that are closer to those of females of species with stronger asymmetry in the investment in a single offspring. We could thus expect them to adopt female-like strategies, and we speculated that they may perform mate copying. The question of whether we could detect it, however, remained open because females of this species often actively reject courting males by kicking, wing fluttering, depression or elevation of the tip of the abdomen, ovipositor extrusion or decamping (Lasbleiz, Ferveur, &

Everaerts, 2006; Spieth, 1974), while the reverse is never observed. This suggested that it might be more difficult for males than females to copulate with their preferred partner, thus hampering our capacity to detect male mate preferences and, thus, male mate copying in this species.

In the current study, following the above considerations, we tested whether mate copying exists in *D. melanogaster* males. We assumed that copying would be trait based rather than individual based (Bowers et al., 2012) because recently mated females show a refractory period, due to proteins transferred by males during copulation (Chapman, Neubaum, Wolfner, & Partridge, 2000; Fricke, Wigby, Hobbs, & Chapman, 2008; Wigby & Chapman, 2005), and actively reject male mating attempts (Barnes, Wigby, Boone, Partridge, & Chapman, 2008; Van Vianen & Bijlsma, 1993). This makes individual-based copying unlikely as it would generate high rejection rates or increase sperm competition risks, except in the case of a last male advantage (Parker & Pizzari, 2010; Parker, 1970; Price, Dyer, & Coyne, 1999). In this context, developing a preference for a specific phenotype (trait-based copying, Bowers et al., 2012) rather than for a specific individual would considerably diminish the risks of sperm competition (Parker & Pizzari, 2010). A trait-based copying strategy would lead males to quickly learn to prefer a given phenotype, thus saving time and energy. This would be particularly true if males can recognize and avoid recently mated females. We know that females can visually distinguish two males of the same colour and avoid those they have recently seen mating (Loyau et al., 2012). Although we do not know whether the converse is true, it seems reasonable that males can also visually distinguish an individual female they just saw copulating from other females of the same phenotype. Alternatively, males might choose the female of the colour they did not see copulating to avoid mating with a recently mated female. This result would imply that males confounded the mated demonstrator female with the test female of the same colour, which would suggest that males use the colour of the female for individual recognition to avoid rejection and sperm competition. Alternatively, males may not copy the mate choice of others, in which case they should mate randomly, ignoring any source of social information. Based on this reasoning, we explored the existence of male mate copying in *D. melanogaster*.

METHODS

Fly Maintenance and General Procedures

We used the common laboratory Canton-S strain of *D. melanogaster*. Flies were raised in 30 ml vials containing 8 ml of a standard corn meal–agar–yeast medium at 25 ± 1 °C and $60 \pm 5\%$ humidity with a 12:12 h light:dark cycle.

Flies were sexed and sorted without anaesthesia by gentle aspiration within 6 h after emergence and kept in unisex groups of seven individuals per vial before experiments. All adult experimental flies were virgin and 3 or 4 days old after emergence. Experiments were conducted under the same conditions as the breeding (12 h daylight, 25 ± 1 °C, $60 \pm 5\%$ relative humidity). We created two artificial female phenotypes by randomly dusting females with green or pink powders (Mery et al., 2009), which created two contrasting phenotypes independent of any genetic variation. All flies were used only once as recently mated females reject further copulations for several hours (Barnes et al., 2008; Van Vianen & Bijlsma, 1993).

All experiments took place in double plastic tubes separated by a thin glass partition in the informed experimental treatment or opaque white cardboard in the uninformed treatment (control as this prevented observer females from gathering any visual information during the demonstration phase). Each mate-copying

experiment consisted of two phases: a demonstration followed by a mate choice test. Demonstrations consisted of a single male placed with two virgin females, one of each colour, for 30 min on one side of the tubes, and a naïve observer male on the other side of the tubes, separated by the partition. The copulation of the demonstrator male with one of the coloured females provided information about which coloured female was preferred and which was rejected. As copulation lasts approximately 20 min in *D. melanogaster* (Pavkovic-Lucic et al., 2014), the observer male received sufficient information about the colour preferred by the demonstrator male. Informed and uninformed treatments differed only in the type of partition. After copulation ended, we removed the demonstrator male and both females and started the mate choice test immediately by inserting a new pair of coloured, virgin females into the demonstration part of the tube. Then, we removed the partition and the observer male was allowed to choose between the two new females for 30 min. During that time, we recorded which female he first courted (i.e. displaying a wing vibration or ‘singing’ (Sokolowski, 2001)), and whether he started to court the other female. We also recorded the colour of the female he copulated with. All replicates were run in blocks of six trials with cardboard barriers between experimental set-ups to prevent information exchange between the flies and disturbance from the surroundings.

First-court and Mate-copying Indices

Because females are expected to be the choosier sex, to measure the observer male choice we defined two indices of mate copying. First, we defined the first-court score for each tested observer male as 1 when it directed its first courtship towards the female phenotype that copulated during the demonstration and 0 in the other case. Second, as in previous studies, we defined the mate-copying score as 1 when the observer male copulated with the female of the phenotype preferred during the demonstration and 0 in the other case. The first-court (or mate-copying) index for a given treatment was the mean of the first-court (or mate-copying) scores for that experimental condition. They both quantified the preference of the observer males, the mate-copying index being more under female control as females accept or reject copulation, while we assumed that the first-court index was less under female control and thus better revealed male preference. The latter avoided cases in which males first courted their preferred female, but were then rejected, so that they finally copulated with the other female. Differences in these indices between the informed and uninformed experimental groups allowed us to measure the effect of social learning. First-court and mate-copying indices around 0.5 indicated random choice by observer males, while values above 0.5 revealed mate copying.

Statistical Analysis

As we expected patterns of mate copying to be weaker in males than females, we a priori decided to triple the sample size relative to equivalent studies on female mate copying. All statistical analyses were performed with the R software (version 3.3.3; R Core Team, 2017). For each treatment, the difference from random choice was tested with a binomial test. Mate-copying and first-court scores were analysed in a generalized linear mixed model (GLMM) with binary logistic regression with the package lme4 (Bates, Maechler, Bolker, & Walker, 2015). All models included air pressure as a fixed effect as previous studies in *D. melanogaster* showed that weather affects social learning in females (Dagaëff et al., 2016). We also included the colour of the female copulating in the demonstration as a fixed effect, as well as ‘block’, i.e. the six simultaneously performed trials, as a random factor to account for

their nonindependence. Block and air pressure did not carry the same kind of information as there were several blocks per day with or without variation in air pressure. Furthermore, ignoring these two effects led to the same conclusions. All starting models included interactions between the fixed effects. Significance of fixed effects was tested using Wald chi-square tests implemented in the ANOVA function of the car package (Fox & Weisberg, 2011). We applied a stepwise backward selection method using *P* values to simplify our model, by starting from the full model containing all main effects plus their interactions and dropping nonsignificant terms one by one, starting with the highest order interaction down to the main effect and only keeping the significant effects. We also used the Akaike information criterion (AIC, Akaike, 1969) to check that our model reduction method led to the model with the lowest AIC value as recommended (Anderson & Burnham, 2002).

Ethical Note

Our study involved populations 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 ASAB/ABS Guidelines for the treatment of animals in behavioural research. We handled flies by gentle aspiration without anaesthesia to minimize damage and discomfort. After the experiments individuals were euthanized in a freezer at -20°C .

RESULTS

We tested 396 observer males in total, of which 381 courted at least one female during the mate choice test and 366 copulated. Among the 396 trials, 15 were discarded because the observer male did not interact with the females during the mate choice test. Final sample sizes were thus 381 for first-court and 366 for mate-copying indices.

We first used the free choices of the uninformed demonstrator males between the two stimulus female phenotypes to test whether *D. melanogaster* males showed any innate preference for one of the colours. In the demonstration ($N = 396$), demonstrator males chose randomly between the two female phenotypes (191 males (48.2%) copulated with the pink and 205 (51.8%) with the green female; binomial test: $N = 396, P = 0.514$).

To test for the significance of mate copying, we built a global statistical model accounting for the effect of information (informed versus uninformed observer males) and the colour of the copulating female in the demonstration (green versus pink) on either the first-court or the mate-copying scores.

First-court Scores

When using the colour of the female that the observer male first courted during the mate choice test as an indicator of the observer male's preference, both the second-order interaction of information * female phenotype (GLMM with Wald χ^2 : $N = 381, \chi^2 = 4.36, P = 0.606$) and the female phenotype as a main effect (GLMM with Wald χ^2 : $N = 381, \chi^2 = 3.575, P = 0.059$) were nonsignificant, although the latter was close to significance. The selected model contained the effect of information (informed versus uninformed observer males; GLMM with Wald χ^2 : $N = 381, \chi^2 = 5.226, P = 0.022$; Fig. 1a). Uninformed observer males, which had no opportunity to watch the copulation occurring during demonstrations because of the opaque partition, chose randomly (binomial test: $N = 190, P = 0.942$; among the 190 uninformed observer males, 94 (49.5%) first courted the female of the colour that copulated

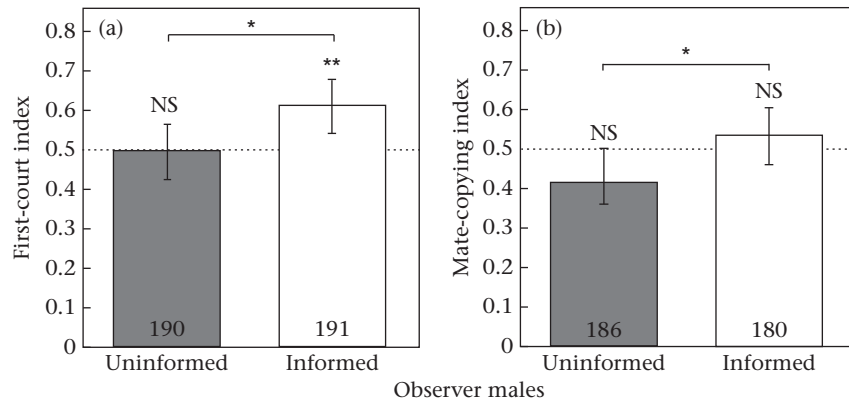


Figure 1. (a) First-court and (b) mate-copying indices of uninformed versus informed observer males. The first-court index is the mean of the first-court scores for each replicate which was coded as 1 when the observer male directed its first courtship towards the female of the colour that copulated during the demonstration and 0 in the other case. The mate-copying index was calculated analogously as the mean of the mate-copying scores (coded as 1 when the observer male copulated with the female of the phenotype preferred during the demonstration, and 0 in the other case). Sample sizes are given within the bars. Dotted lines mark random choice. * $P < 0.05$; ** $P < 0.01$ from binomial tests of departure from random choice. Vertical lines: Agresti–Coulil confidence intervals. Apart from binomial test results provided above the bars, statistics account for the potential confounding effects of air pressure as a fixed effect and block as a random effect.

during the demonstration versus 96 (50.5%) with the other colour). In contrast, the first choice of informed observer males differed significantly from random choice in that they first courted the female phenotype they had seen copulating in the demonstration more often than by chance (binomial test: $N = 191$, $P = 0.002$; 117 (61.3%) of the informed observer males first courted the female of the colour that copulated during the demonstration; the remaining 74 (38.7%) observer males first courted the female of the other colour; Fig. 1a).

Mate-copying Scores

When taking copulation as an indicator of the observer males' preference (Fig. 1b), the second-order interaction was nonsignificant (GLMM with Wald χ^2 : $N = 366$, $\chi^2 = 0.606$, $P = 0.436$), and the colour of the female that copulated in the demonstration did not affect the mate-copying scores (GLMM with Wald χ^2 : $N = 366$, $\chi^2 = 0.067$, $P = 0.796$). There was a weak but significant difference between informed and uninformed observer males (GLMM with Wald χ^2 : $N = 366$, $\chi^2 = 4.004$, $P = 0.045$; Fig. 1b), suggesting a trend in male mate copying although the preference of each group did not differ from random (binomial tests: informed observer males: $N = 180$, $P = 0.412$; 96 (53.3%) males copulated with the female of the colour that copulated during the demonstration, versus 84 (46.7%) with females of the other colour; uninformed observer males: $N = 186$, $P = 0.067$; 80 (43%) males copulated with the female of the colour that copulated during the demonstration, versus 106 (57%) with the other colour; Fig. 1b).

Table 1
Mate preferences of observer males measured as first-court or mate-copying index

	Number of males that courted first the female of the colour	Number of males that copulated with the female of the colour		P
		Chosen during demonstration	Rejected during demonstration	
	N	N	N	
Chosen during demonstration	N	117	98	0.220
Rejected during demonstration	N	55	126	< 0.0001
	P	< 0.0001	0.071	

P values indicate binomial post hoc tests.

When analysing links between first-court and actual copulation (Table 1) we found a significant difference between the two indices (chi-square test: $N = 396$, $\chi^2 = 23.1$, $P < 0.0001$). More specifically, after first courting the female of the colour that was chosen during the demonstration, observer males copulated with that female phenotype in 54.4% of cases (post hoc binomial test: $N = 215$, $P = 0.220$; 117 of 215 cases). This suggested that males that seemed to have developed a preference for females of the colour chosen during the demonstration in the end only showed a slight and nonsignificant tendency to copulate with that female, probably because they were often constrained by the female's choice. In contrast, males that first courted the female of the colour rejected during the demonstration in the end copulated significantly less often with the female of the colour chosen during the demonstration (post hoc binomial test: $N = 181$, $P < 0.0001$; 30.4% of cases, 55 of 181; Table 1). Inversely, when observer males copulated with the female of the same colour as in the demonstration, they had first courted the female of the preferred colour in 68% of cases (binomial test: $N = 172$, $P < 0.0001$; 117 of 172) against only 43.8% when they had first courted the female of the rejected colour (binomial test: $N = 224$, $P = 0.071$; 98 of 224; Table 1).

DISCUSSION

Mate copying has mainly been documented in females of many taxa (except for some fish species). Here, we provide the first evidence for male mate copying in *D. melanogaster* using females artificially dusted with green or pink powders. We found that during the free male mate choice of demonstrations and again in the control group of uninformed observer males, males did not show any innate preference for green or pink females. In contrast, observer males that had the opportunity to witness a demonstration showed a detectable bias for females of the colour chosen during the demonstration.

In *D. melanogaster* both sexes face high costs of reproduction and there is accruing evidence for mate preference learning in this species (Chapman et al., 1995; Cordts & Partridge, 1996; Ejima et al., 2007; Fowler & Partridge, 1989; Keleman et al., 2012; Partridge & Farquhar, 1981; Partridge et al., 1987; Verzijden et al., 2015). Previous authors have insisted on the necessity of controlling for female behaviour to identify individual male reproductive consistency in *D. melanogaster* (Balaban-Feld & Valone, 2017). To

exclude female influence during our mate choice tests we could have used decapitated females (as in Ejima et al., 2007; Tan et al., 2013). However, this might have led us to measure the behaviour of males that were willing to court/copulate with nonresponding (dead) females. Using fixed females (e.g. tethered with silk), on the other hand, would have diminished the females' mobility but would probably not have prevented males from being rejected by females by kicking, wing fluttering, depression or elevation of the tip of the abdomen, ovipositor extrusion or decamping (Lasbleiz et al., 2006; Spieth, 1974). Thus, to avoid the effect of female behaviour on male mate copying, we used a second measure of male preference as male courtship in this species involves wing flapping ('singing', Lasbleiz et al., 2006; Spieth, 1974), which is easily observed (e.g. Tan, Løvlie, Pizzari, & Wigby, 2012; Tan et al., 2013; Verzijden et al., 2015). This made it possible to record the colour of the female towards which observer males first wing-flapped and we used this to indicate the observer males' preference, independently from whether females accepted them. This constituted an estimate of male mate choice in a more natural situation with freely moving flies. We thus used it as another index of male mate preference in addition to the usual mate-copying index which is based on copulation.

As expected, mate preference learning appeared stronger when we used courtship rather than copulation as a measure. This is in accordance with other studies investigating male mate choice (Verzijden et al., 2015). We found that the first-court index revealed a bias towards the female of the colour that successfully mated during the demonstration, as did the usual mate-copying index, although to a lesser extent. Both supported the existence of male mate copying. Informed observer males (but not uninformed ones) more often first courted the female of the phenotype that was chosen during the demonstration, and this pattern differed from that predicted by chance. Using the mate-copying index, we found a similar trend with a significantly higher tendency to copulate with the female of the colour chosen during the demonstration in the informed than the uninformed group, although neither differed from random chance. This might be because uninformed observer males slightly tended to copulate more often with females of the colour not preferred by demonstrator males (Fig. 1b). This last trend was unexpected as (1) males had no information about which female copulated during the demonstration and (2) females in the mate choice test were new, naïve females ignorant of the previous demonstration. The difference in the results obtained with the two estimates of preference further suggests that, in *Drosophila*, copulation is more under female control, which is consistent with our observation and expectations.

More generally, there is well-documented asymmetry in the sex roles of many species that would make males less likely to mate-copy and that would also considerably constrain male mate choice in general (Arnqvist & Rowe, 1995; Cordts & Partridge, 1996; Davies, Hartley, Hatchwell, & Langmore, 1996; Krebs & Davies, 1997; Partridge & Farquhar, 1981; Partridge et al., 1987). Sex differences in parental investment per descendant naturally leads the sex that invests in offspring the most (usually females) to become the choosy sex, while the other sex is expected to readily accept mating opportunities. In such species, females have more latitude to mate with their preferred partner (even if they face some degree of sexual harassment which might hamper their choice; Carazo, Tan, Allen, Wigby, & Pizzari, 2014; Løvlie & Pizzari, 2007; Plath, Makowicz, Schlupp, & Tobler, 2007) and males may have less choice over with whom they copulate, making it more difficult to detect mate copying in males than females. In this context, it is noticeable that apart from the specific case of sex role-reversed species, all reported cases of male mate copying exclusively concern fish species (positive results in fish: Bierbach et al., 2011;

Frommen, Rahn, Schroth, Waltschyk, & Bakker, 2009; Heubel et al., 2008; Moran et al., 2013; Widemo, 2006; Witte & Ryan, 2002; negative results in birds: Kniel et al., 2015; White & Galef, 2000). In fish the between-sex asymmetry in investment in offspring is often considerably diminished, or even sometimes reversed, making it a priori easier to detect male mate copying.

None the less, in some species, male mate copying may be an efficient way of gathering integrative information about potential mates' quality, thus reducing the costs of mate choice (Gibson & Höglund, 1992; Westneat et al., 2000; reviewed in Witte & Nöbel, 2011). Theoretical considerations suggested that females with poor discriminating ability for male quality should benefit most from mate copying (Nordell & Valone, 1998; Stöhr, 1998) and the same might be true for males, notably males using a trait-based copying strategy rather than an individual-based copying strategy (Bowers et al., 2012). Developing a preference for a specific phenotype rather than for a specific individual would considerably reduce the risks of sperm competition (Parker & Pizzari, 2010), risks that are inherent to individual-based copying. As females in the mate choice test were green and pink virgins, our experiment suggests that males probably performed trait-based rather than individual-based copying even though we did not explicitly test it. Furthermore, trait-based mate copying is of special interest because learning to prefer females of a given phenotype rather than a specific female may potentially lead to the establishment of persistent local traditions in mate preference (Danchin et al., 2018), which in turn may affect sexual selection differentially across populations, thus setting the stage for speciation.

Our results support the existence of male mate copying in *D. melanogaster*, which provides further evidence for the existence of mate copying in this species. The ability to copy may be an adaptation to the naturally crowded conditions existing on rotten pieces of fruit to which males and females are attracted both as sources of food and egg-laying sites (Keeseey et al., 2016; Rodrigues et al., 2015). In these aggregations, copulations are common and surrounding flies can watch the mate choice of other males, thus setting the stage for mate copying in natural situations.

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References

- Akaike, H. (1969). Fitting autoregressive models for prediction. *Annals of the Institute of Statistical Mathematics*, 21, 243–247.
- Anderson, D. R., & Burnham, K. P. (2002). Avoiding pitfalls when using information-theoretic methods. *Journal of Wildlife Management*, 66, 912–918.
- Arnqvist, G., & Rowe, L. (1995). Sexual conflict and arms races between the sexes: A morphological adaptation for control of mating in a female insect. *Proceedings of the Royal Society B: Biological Sciences*, 261, 123–127.
- Balaban-Feld, J., & Valone, T. J. (2017). Identifying individual male reproductive consistency in *Drosophila melanogaster*: The importance of controlling female behaviour. *Behavioural Processes*, 142, 84–90.
- Barnes, A. L., Wigby, S., Boone, J. M., Partridge, L., & Chapman, T. (2008). Feeding, fecundity and lifespan in female *Drosophila melanogaster*. *Proceedings of the Royal Society B: Biological Sciences*, 275, 1675–1683.

- Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48.
- Bierbach, D., Kronmarck, C., Hennige-Schulz, C., Stadler, S., & Plath, M. (2011). Sperm competition risk affects male mate choice copying. *Behavioral Ecology and Sociobiology*, 65, 1699–1707.
- Bowers, R. L., Place, S. S., Todd, P. M., Penke, L., & Asendorpf, J. B. (2012). Generalization in mate-choice copying in humans. *Behavioral Ecology*, 23, 112–124.
- Byrne, P. G., & Rice, W. R. (2006). Evidence for adaptive male mate choice in the fruit fly *Drosophila melanogaster*. *Proceedings of the Royal Society B: Biological Sciences*, 273, 917–922.
- Carazo, P., Tan, C. K. W., Allen, F., Wigby, S., & Pizzari, T. (2014). Within-group male relatedness reduces harm to females in *Drosophila*. *Nature*, 505, 672–675.
- Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. F., & Partridge, L. (1995). Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature*, 373, 241–244.
- Chapman, T., Neubaum, D. M., Wolfner, M. F., & Partridge, L. (2000). The role of male accessory gland protein Acp36DE in sperm competition in *Drosophila melanogaster*. *Proceedings of the Royal Society B: Biological Sciences*, 267, 1097–1105.
- Cordts, R., & Partridge, J.-G. J. (1996). Courtship reduces longevity of male *Drosophila melanogaster*. *Animal Behaviour*, 52, 269–278.
- Dagaëff, A.-C., Pocheville, A., Nöbel, S., Loyau, A. L., Isabel, G., & Danchin, E. (2016). *Drosophila* mate copying correlates with atmospheric pressure in a speed learning situation. *Animal Behaviour*, 121, 163–174.
- Danchin, E., Giraldeau, L.-A., Valone, T. J., & Wagner, R. H. (2004). Public information: From nosy neighbors to cultural evolution. *Science*, 305, 487–491.
- Danchin, E., Nöbel, S., Pocheville, A., Dagaëff, A.-C., Demay, L., Alphand, M., et al. (2018). *Cultural flies: conformist social learning in fruit-flies predicts long-lasting mate-choice traditions* (Submitted manuscript).
- Davies, N. B., Hartley, I. R., Hatchwell, J. B., & Langmore, N. E. (1996). Female control of copulations to maximize male help: A comparison of polyandrous alpine accentors, *Prunella collaris*, and dunlocks, *P. modularis*. *Animal Behaviour*, 151, 27–47.
- Demerec, M., & Kaufman, B. P. (1941). Time required for *Drosophila* males to exhaust the supply of mature sperm. *American Naturalist*, 75, 366–379.
- Dewsbury, D. A. (1982). Ejaculate cost and male choice. *American Naturalist*, 119, 601–610.
- Dukas, R. (2004). Male fruit flies learn to avoid interspecific courtship. *Behavioral Ecology*, 15, 695–698.
- Dukas, R. (2009). Dynamics of learning in the context of courtship in *Drosophila persimilis* and *D. pseudoobscura*. *Animal Behaviour*, 77, 253–259.
- Ejima, A., Smith, B. P. C., Lucas, C., van der Goes van Naters, W., Miller, C., Carlson, J. R., et al. (2007). Generalization of courtship learning in *Drosophila* is mediated by *cis*-vaccenyl acetate. *Current Biology*, 17, 599–605.
- Fowler, K., & Partridge, L. (1989). A cost of mating in female fruitflies. *Nature*, 338, 760–761.
- Fox, J., & Weisberg, S. (2011). *An {R} Companion to Applied Regression* (2nd ed.). Thousand Oaks, CA: Sage Publishing.
- Fricke, C., Wigby, S., Hobbs, R., & Chapman, T. (2008). The benefits of male ejaculate sex peptide transfer in *Drosophila melanogaster*. *Journal of Evolutionary Biology*, 22, 275–286.
- Frommen, J., Rahn, A. K., Schroth, S., Waltschky, N., & Bakker, T. C. M. (2009). Mate-choice copying when both sexes face high costs of reproduction. *Evolutionary Ecology*, 23, 435–446.
- Galef, B. G., Lim, T. C. W., & Gilbert, G. S. (2008). Evidence of mate choice copying in Norway rats, *Rattus norvegicus*. *Animal Behaviour*, 75, 1117–1123.
- Galef, B. G., & White, D. J. (1998). Mate-choice copying in Japanese quail, *Coturnix coturnix japonica*. *Animal Behaviour*, 55, 545–552.
- Gibson, R. M., Bradbury, J. W., & Vehrencamp, S. L. (1991). Mate choice in lekking sage grouse revisited: The roles of vocal display, female site fidelity, and copying. *Behavioral Ecology*, 2, 165–180.
- Gibson, R. M., & Höglund, J. (1992). Copying and sexual selection. *Trends in Ecology & Evolution*, 7, 229–232.
- Gilbert, D. G. (1981). Ejaculate esterase 6 and initial sperm use by female *Drosophila melanogaster*. *Journal of Insect Physiology*, 27, 641–650.
- Heubel, K. U., Hornhardt, K., Ollmann, T., Parzefall, J., Ryan, M. J., & Schlupp, I. (2008). Geographic variation in female mate-copying in the species complex of a unisexual fish, *Poecilia formosa*. *Behaviour*, 145, 1041–1064.
- Höglund, J., Alatalo, R. V., Gibson, R. M., & Lundberg, A. (1995). Mate-choice copying in black grouse. *Animal Behaviour*, 49, 1627–1633.
- Keesey, I. W., Koerte, S., Retzke, T., Haverkamp, A., Hansson, B. S., & Knaden, M. (2016). Adult frass provides a pheromone signature for *Drosophila* feeding and aggregation. *Journal of Chemical Ecology*, 42, 739–747.
- Keleman, K., Vrontou, E., Krüttner, S., Yu, J. Y., Kurtovic-Kozaric, A., & Dickson, B. J. (2012). Dopamine neurons modulate pheromone responses in *Drosophila* courtship learning. *Nature*, 489, 145–150.
- Kniel, N., Dürler, C., Hecht, I., Heinbach, V., Zimmermann, L., & Witte, K. (2015). Novel mate preference through mate-choice copying in zebra finches: Sexes differ. *Behavioral Ecology*, 26, 647–655.
- Kokko, H., & Jennions, M. D. (2008). Parental investment, sexual selection and sex ratios. *Journal of Evolutionary Biology*, 21, 919–948.
- Kokko, H., & Johnstone, R. A. (2002). Why is mutual mate choice not the norm? Operational sex ratios, sex roles and the evolution of sexually dimorphic and monomorphic signalling. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 357, 319–330.
- Krebs, J. R., & Davies, N. B. (1997). *Behavioural Ecology: an Evolutionary Approach* (4th ed.). U.K.: Blackwell: Oxford.
- Lasbleiz, C., Ferveur, J.-F., & Everaerts, C. (2006). Courtship behaviour of *Drosophila melanogaster* revisited. *Animal Behaviour*, 72, 1001–1012.
- Lefevre, G., & Jonsson, U. B. (1962). Sperm transfer, storage, displacement, and utilization in *Drosophila melanogaster*. *Genetics*, 47, 1719–1736.
- Løvlie, H., & Pizzari, T. (2007). Sex in the morning or in the evening? Females adjust daily mating patterns to the intensity of sexual harassment. *American Naturalist*, 170, E1–E13.
- Loyau, A., Blanchet, S., Van Laere, P., Clobert, J., & Danchin, E. (2012). When not to copy: Female fruit flies use sophisticated public information to avoid mated males. *Scientific Reports*, 2, 768.
- Mery, F., Varela, S. A. M., Danchin, E., Blanchet, S., Parejo, D., Coolen, I., et al. (2009). Public versus personal information for mate copying in an invertebrate. *Current Biology*, 19, 730–734.
- Monier, M., Nöbel, S., Isabel, G., & Danchin, E. (2018). Effects of a sex ratio gradient on female mate-copying and choosiness in *Drosophila melanogaster*. *Current Zoology*, 64, 251–258.
- Moran, R. L., von Ende, C. N., & King, B. H. (2013). Mate choice copying in two species of darters (Percidae: *Etheostoma*). *Behaviour*, 150, 1255–1274.
- Nakatsuru, K., & Kramer, D. L. (1982). Is sperm cheap? Limited male fertility and female choice in the lemon tetra (Pisces, Characidae). *Science*, 216, 753–754.
- Nordell, S. E., & Valone, T. J. (1998). Mate choice copying as public information. *Ecology Letters*, 1, 74–76.
- Parker, G. A. (1970). Sperm competition and its evolutionary consequences in the insects. *Biological Reviews*, 45, 525–567.
- Parker, G. A., & Pizzari, T. (2010). Sperm competition and ejaculate economics. *Biological Reviews*, 85, 897–934.
- Partridge, L., & Farquhar, M. (1981). Sexual activity reduces lifespan of male fruit-flies. *Nature*, 294, 580–582.
- Partridge, L., Green, A., & Fowler, K. (1987). Effects of egg production and of exposure to males on female survival in *Drosophila melanogaster*. *Journal of Insect Physiology*, 33, 745–749.
- Pavkovic-Lucic, S., Lucic, L., Milicic, D., Tomic, V., & Savic, T. (2014). Mating success and copulation duration in *Drosophila melanogaster* flies having different mating experience: A brief experimental note. *Journal of BioScience & Biotechnology*, 153–159. special edition.
- Pitnick, S. (1996). Investment in testes and the cost of making long sperm in *Drosophila*. *American Naturalist*, 57–80.
- Plath, M., Makowicz, A. M., Schlupp, I., & Tobler, M. (2007). Sexual harassment in live-bearing fishes (Poeciliidae): Comparing courting and noncourting species. *Behavioral Ecology*, 18, 680–688.
- Price, C. S. C., Dyer, K. A., & Coyne, J. A. (1999). Sperm competition between *Drosophila* males involves both displacement and incapacitation. *Nature*, 400, 449–452.
- Pruett-Jones, S. (1992). Independent versus nonindependent mate choice: Do females copy each other? *American Naturalist*, 140, 1000–1009.
- R Core Team. (2017). *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Rodrigues, M. A., Martins, N. E., Balancé, L. F., Broom, L. N., Dias, A. J., Fernandes, A. S. D., et al. (2015). *Drosophila melanogaster* larvae make nutritional choices that minimize developmental time. *Journal of Insect Physiology*, 81, 69–80.
- Sokolowski, M. B. (2001). *Drosophila*: Genetics meets behaviour. *Nature Reviews Genetics*, 2, 879–890.
- Spieth, H. T. (1974). Courtship in *Drosophila*. *Annual Review of Entomology*, 19, 385–405.
- Stöhr, S. (1998). Evolution of mate-choice copying: A dynamic model. *Animal Behaviour*, 55, 893–903.
- Tan, C. W. K., Løvlie, H., Greenway, E., Goodwin, S. F., Pizzari, T., & Wigby, S. (2013). Sex-specific responses to sexual familiarity, and the role of olfaction in *Drosophila*. *Proceedings of the Royal Society B: Biological Sciences*, 280, 20131691.
- Tan, C. W. K., Løvlie, H., Pizzari, T., & Wigby, S. (2012). No evidence for precopulatory inbreeding avoidance in *Drosophila melanogaster*. *Animal Behaviour*, 83, 1433–1441.
- Trivers, R. L. (1972). *Parental investment and sexual selection* (Vol. 136). Cambridge, MA: Biological Laboratories, Harvard University.
- Vakirtzis, A. (2011). Mate choice copying and nonindependent mate choice: A critical review. *Annales Zoologici Fennici*, 48(2), 91–107.
- Van Vianen, A., & Bijlsma, R. (1993). The adult component of selection in *Drosophila melanogaster*: Some aspects of early-remating activity of females. *Heredity*, 71, 269–276.
- Verzijden, M. N., Abbott, J. K., von Philipsborn, A. C., & Loeschke, V. (2015). Male *Drosophila melanogaster* learn to prefer an arbitrary trait associated with female mating status. *Current Zoology*, 61, 1036–1042.
- Verzijden, M. N., ten Cate, C., Servedio, M. R., Kozak, G. M., Boughman, J. W., & Svensson, E. I. (2012). The impact of learning on sexual selection and speciation. *Trends in Ecology & Evolution*, 27, 511–519.
- Wade, M. J., & Pruett-Jones, S. G. (1990). Female copying increases the variance in male mating success. *Proceedings of the National Academy of Sciences USA*, 87, 5749–5753.
- Wagner, R. H., & Danchin, E. (2010). A taxonomy of biological information. *Oikos*, 119, 203–209.
- Waynforth, D. (2007). Mate choice copying in humans. *Human Nature*, 18, 264–271.

- Westneat, D. F., Walters, A., McCarthy, T. M., Hatch, M. I., & Hein, W. K. (2000). Alternative mechanisms of nonindependent mate choice. *Animal Behaviour*, *59*, 467–476.
- White, D. J., & Galef, B. G. (2000). Differences between the sexes in direction and duration of response to seeing a potential sex partner mate with another. *Animal Behaviour*, *59*, 1235–1240.
- Widemo, M. S. (2006). Male but not female pipefish copy mate choice. *Behavioral Ecology*, *17*, 255–259.
- Wigby, S., & Chapman, T. (2005). Sex peptide causes mating costs in female *Drosophila melanogaster*. *Current Biology*, *22*, 316–321.
- Witte, K., Kniel, N., & Kureck, I. M. (2015). Mate-choice copying: Status quo and where to go. *Current Zoology*, *61*(6), 1073–1081.
- Witte, K., & Nöbel, S. (2011). Learning and mate choice. In C. Brown, K. Laland, & J. Krause (Eds.), *Fish cognition and behaviour* (pp. 81–107). Oxford, U.K.: Wiley-Blackwell.
- Witte, K., & Ryan, M. J. (2002). Mate choice copying in the sailfin molly, *Poecilia latipinna*, in the wild. *Animal Behaviour*, *63*, 943–949.
- Witte, K., & Ueding, K. (2003). Sailfin molly females (*Poecilia latipinna*) copy the rejection of a male. *Behavioral Ecology*, *14*, 389–395.