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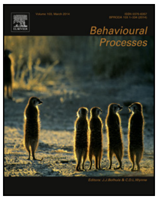

Mate-choice copying in *Drosophila melanogaster*: Impact of demonstration conditions and male–male competition

M. Germain[a,b,c,*], S. Blanchet[b,d], A. Loyau[e,f,g], É. Danchin[b,c]

[a] Université de Lyon, F-69000, Lyon; Université Lyon 1; CNRS, UMR5558, Laboratoire de Biométrie et Biologie Evolutive, F-69622 Villeurbanne, France
[b] CNRS, Université Paul Sabatier, ENFA, UMR5174 EDB (Laboratoire Évolution & Diversité Biologique), 118 route de Narbonne, 31062 Toulouse, France
[c] Université de Toulouse, UMR5174 EDB, F-31062 Toulouse, France
[d] CNRS, 09200 Moulis, France
[e] Helmholtz Centre for Environmental Research—UFZ, Department of Conservation Biology, Permoserstrasse 15, 04318 Leipzig, Germany
[f] Helmholtz Centre for Environmental Research—UFZ, Department of System Ecotoxicology, Permoserstrasse 15, 04138 Leipzig, Germany
[g] ECOLAB, Université de Toulouse, CNRS, INPT, UPS, France

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ABSTRACT

Individuals of many species, including invertebrates, have been shown to use social information in mate choice, notably by extracting information from the mating performance of opposite sex conspecifics, a process called “mate-choice copying” (MCC). Here, we performed four experiments with *Drosophila melanogaster* to investigate two aspects of MCC methodology: whether (*i*) providing positive and negative social information simultaneously or sequentially during the demonstration phase of the protocol, and (*ii*) male–male competition during the mate-choice test, affect MCC. We found that the simultaneous provision of positive and negative information during demonstrations hampered female MCC performance, compared to the sequential provision of information. This can be interpreted in two alternative, yet not exclusive, ways: (*i*) attentional mechanisms may restrict the focus of the brain to one source of information at a time, and/or (*ii*) the shorter duration of demonstrations in the simultaneous protocol may have not permit full social learning use and may explain the non-detection of MCC in that protocol. Moreover, we did not detect any significant effect of male–male competition on female choice. This study thus provides further evidence for MCC in *D. melanogaster* and expands on the necessary methodology for detailed studies.

1. Introduction

Organisms need to continually assess environmental cues to increase the accuracy in their appraisal of the environment (Wagner and Danchin, 2010). This allows them to adaptively adjust their behaviour to current conditions (Dall et al., 2005; Danchin et al., 2004). However, sources and types of information available in the environment are diverse, both qualitatively and quantitatively, and it is still not clear how these numerous sources of information are perceived and processed, especially when they are contradictory. One intuitive view is that the more information available, the better the decision should be. For example, in the context of mate choice, multiple sources of information (i.e. signals or cues) have been shown to provide a more accurate estimation of the overall quality of a potential mate (Candolin, 2003; Moller and Pomiankowski, 1993; Scheuber et al., 2004). However, an increasing number of studies suggest that a too high information flow may hamper learning (Dukas and Real, 1993; van Swinderen, 2007; Weiss and Papaj, 2003), which may be rooted in two different constraints that are not mutually exclusive: a perception/sensory constraint and/or a processing constraint.

Processing constraints may result from a limited rate of information being processed by the brain (the “limited attention” hypothesis: Dukas, 2002; Dukas and Kamil, 2000). Alternatively, an organism may only attend to a subset of the available sources of information. For instance, organisms may filter out sources of information by focusing only on portions of the visual field at any given moment (spatially selective attention: Sareen et al., 2011), or by attending to only one or a few stimuli and ignoring the others (stimulus selective attention: Dukas, 2002). Sensory constraint, limited attention, and/or selective attention may strongly affect the simultaneous execution of two or more cognitive tasks; organisms

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[*] Corresponding author at: Laboratoire de Biométrie et de Biologie Evolutive, UMR 5558, CNRS—Université Claude Bernard, 43, Boulevard du 11 Novembre 1918, 69622 Villeurbanne, France.
E-mail address: marion.germain@univ-lyon1.fr (M. Germain).

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are therefore expected to sort the different sources of information, focusing on the most relevant ones (Dukas, 2002).

According to Wagner and Danchin (2010), detectable information can be divided into two broad categories. First, organisms may interact directly with the physical environment thereby obtaining non-social information (Blanchet et al., 2010; Wagner and Danchin, 2010). Second, organisms may acquire social information by monitoring others interacting with their abiotic or biotic environment, including conspecifics (Bonnie and Earley, 2007; Dall et al., 2005; Wagner and Danchin, 2010). Social information may offer unique benefits by providing information about the quality and temporal predictability of the environment (Valone and Templeton, 2002), allowing organisms to make informed decisions (Reed et al., 1999).

Mate choice is a major fitness-affecting decision in sexually reproducing organisms. Classical sexual selection theories assume that females have genetically heritable preferences (Agrawal, 2001; Kirkpatrick and Ryan, 1991) and make independent choices, meaning that the choice of a given female is not context dependent and does not depend on the choices of other females (Alonzo, 2008; Wade and Pruett-Jones, 1990; Westneat et al., 2000). However, it has been demonstrated in many vertebrates and few invertebrates that females can extract information about male quality by observing the male's mating performance and use this information to develop a preference for a given male, or male phenotype (Candolin, 2003; Danchin et al., 2004; Galef and White, 2000; Mery et al., 2009; Qvarnström, 2001; Witte and Noltemeier, 2002). This process is called mate-choice copying (hereafter MCC), and over the last two decades increasing attention has been devoted to exploring possible social influences on the development of mate preferences leading to non-independent mate choice (Alonzo, 2008; Losey et al., 1986; Pruett-Jones, 1992; Stohr, 1998; Westneat et al., 2000). Many studies have presented convincing evidence for MCC in a wide array of taxa, including humans (e.g. birds: White and Galef, 1999; fish: Witte and Ryan, 2002; mammals: Galef et al., 2008; humans: Waynforth, 2007, and even one non-social insect species (*Drosophila melanogaster*: Dukas, 2005; Mery et al., 2009).

In species where mating preference can be tested only once (e.g. because of the reluctance of females to copulate twice), MCC can be investigated using a two-phase experimental protocol. In the first phase (the demonstration), a naive female (called observer female) can gather social information about potential mates by observing them interacting with another female. A male copulating while being observed provides positive social information for its ability to attract mates and secure copulation, with male rejection providing negative social information. Demonstration methodology can vary greatly, for instance, by being performed sequentially (i.e. one male is observed at a time, as in Mery et al., 2009) or simultaneously (e.g. several demonstrations running jointly: Auld et al., 2009; Loyau et al., 2012; Witte and Ryan, 2002 and see Vakirtzis, 2011 for a review).

In the second phase (the mate-choice test), the preference of the observer female is assessed by offering her the "choice" between the same males she observed during the demonstration (MCC). Alternatively, the female can be presented with two other males of the same contrasting phenotypes as those used during demonstration. This latter protocol tests whether the observer female learnt the general rule of preferring males of a given phenotype, which corresponds to a generalised version of MCC. During this mate-choice test, males sometimes cannot interact, thus avoiding male–male competition. The female's interest and willingness to mate with a particular male is then measured as the time she spends close to that male (i.e. latency: White and Galef, 1999). Even though this measure has been widely used in MCC experiments (Dugatkin and Godin, 1992; Frommen et al., 2009; Galef and White, 1998; Godin et al., 2005; Witte and Ryan, 2002), it has also been widely criticised as it remains an indirect measure of female mate preference (see Walling et al., 2010 for a review). Authors thus recommend using actual copulation tests whenever possible.

Here we report on four experiments that aimed to test the impact of two aspects of the demonstration protocol on the capacity of females to perform MCC in *D. melanogaster*. We created two male phenotypes by dusting them with green or pink powders. We first tested whether a simultaneous demonstration limits female ability to perform and generalise MCC, compared with a sequential demonstration protocol as described above. The sensory constraint/limited attention framework (Clark and Dukas, 2003; Dukas, 2002; Schmieder et al., 2012) predicts more efficient MCC when negative and positive social information about male attractiveness is provided sequentially.

Second, we intended to analyse the potential confounding effects of the male–male competition occurring during the mate-choice test on MCC. Male–male competition may limit observer female access to a given male, or information on male competitiveness may confuse her. Therefore, we manipulated the level of male–male competition during the mate-choice test by randomly assigning half of the observer females to one of two treatments: (*i*) females were offered the choice between a green and a pink male (competition situation) and (*ii*) females were randomly put with a green or a pink male (absence of competition). We expected MCC to be stronger in the absence of male–male competition, which we assessed by measuring the latency between the beginning of the test and the onset of the copulation.

2. Methods

2.1. Biological model

We used a stock of *D. melanogaster*, initiated from wild flies caught in Chavroche (Allier, France). Flies were caught by Frederic Mery in the village of Chavroche (France). Flies were kept in 9.5 × 2.5 cm population vials containing fly medium consisting of ethanol, an antifungal (methyl 4 hydroxy benzoate), *agar agar*, brewer's yeast, maize flour and water. The population was housed in a controlled environment room, at 23 °C and approximately 60% humidity with a 12:12 h light:dark cycle.

Virgin males and females were collected daily, within the first eight hours after emergence. Virgin flies were sexed and sorted into groups of 5 males and 5 females, which were kept in distinct vials containing food medium until experiments. All individuals used in the experiments were virgin and three days old.

2.2. Set up

The set up was based on Experiment 2 of Mery et al. (2009) and consisted of a three-compartment apparatus. Each compartment was made of a transparent plastic tube (3.5 cm × 1 cm), which were aligned in series on a frame (Fig. 1 extracted from Loyau et al. (2012)). Compartments were separated by a thin transparent glass partition (microscope cover glass), so that a fly placed in the central compartment could watch conspecifics interacting in the peripheral compartment(s).

2.3. Creating male phenotypes

We created two artificial male phenotypes by randomly dusting males in green or pink powders, as in Mery et al. (2009). This allowed us to create two contrasting male phenotypes that were not a result of genetic variation. Males were dipped into 0.0012 g of powder (pink or green) for 15 s and transferred into a clean tube containing food medium for 30 min. During this time, males cleaned themselves and removed excess powder. After 30 min, males displayed normal behaviour and could then be used

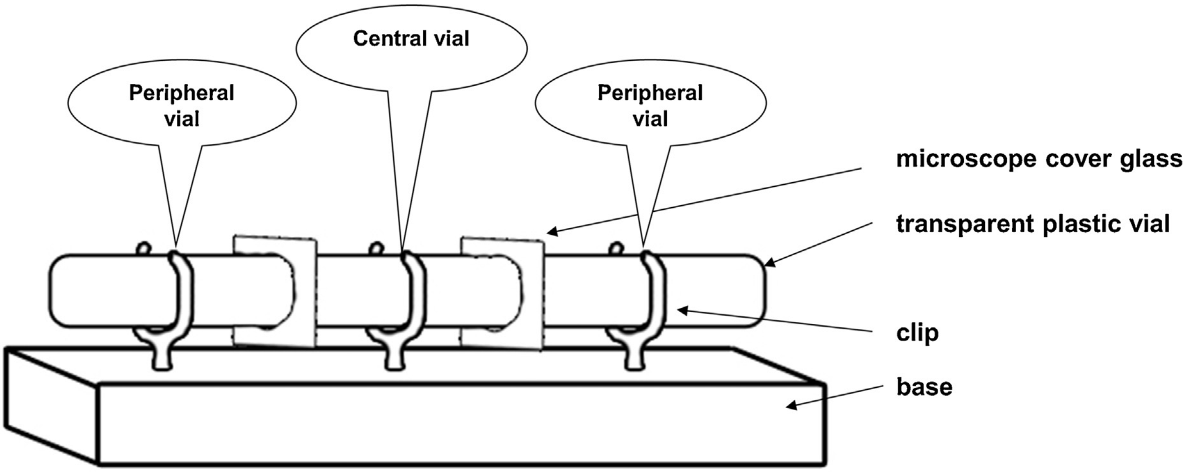

Fig. 1. Experimental set-up.

as demonstrators or potential mates. In order to know whether females would be able to distinguish an individual dusted with green powder from and an individual dusted with pink powder, we characterized the pink and green powder. Indeed, it is known that flies are very good at discriminating colours with no overlap in their spectra and this was confirmed experimentally (Brembs and de Ibarra, 2006). We found that the spectra of the green and pink powder did not overlap suggesting that females would be able to distinguish males dusted with one or the other colour. We also found that both green and pink powders were very bright, allowing therefore a discrimination of both phenotypes by females.

2.4. Experimental design

Four complementary experiments manipulated how social information was presented during demonstrations and allowed or prevented male–male competition during the mate-choice test phase. All experiments were conducted in the same room with controlled environmental and lighting conditions.

2.4.1. Experiment 1: sequential demonstrations, male–male competition

Experiment 1 replicated Mery et al. (2009) Experiment 2 (Fig. 2). A virgin observer female ($n=61$) was first introduced into the central compartment where she could watch social interactions occurring in one of the peripheral compartments. The demonstrations were as follows: a pink (or green) male was introduced in a peripheral compartment with a virgin demonstrator female. In the vast majority of cases, this led to a copulation providing positive social information on that male's mating success to the observer female. Replicates in which copulation did not occur were discarded. Demonstrators were left in the plastic tube until the end of copulation, after which they were immediately removed. This avoided the provision of contradictory information about a given male phenotype, as recently mated *Drosophila* females actively reject copulations (Barnes et al., 2008). Forty-five minutes after the beginning of the demonstration, a new green (or pink, in any case the opposite phenotype to the first demonstration male) male was introduced into the opposite peripheral compartment with a recently mated female for 45 min. In that case, the rejection of the male by the recently mated female provides negative social information on that male's mating success. This sequence of positive and negative demonstration was repeated three times, each time with different male and female demonstrators (i.e. six pairs of demonstrators used) for a total of 4.5 h (i.e. 45 min repeated six times). Observer females thus saw three positive social interactions for one male colour phenotype alterning with three negative interactions for males of the second phenotype. The mate-choice test

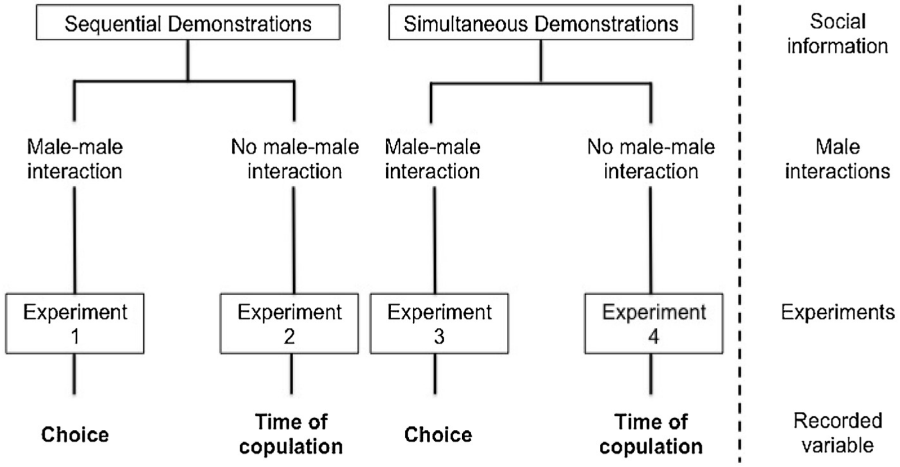

Fig. 2. Experimental plan.
The four experiments allowed us to manipulate both the demonstration treatment, i.e. the way we provided social information to observer females (sequential versus simultaneous demonstrations) and the possibility for male–male competition during the mate-choice test.

happened straight after demonstrations with the same observer female. To do this, we introduced two fresh virgin males, a pink and a green, together in one peripheral compartment. The test began with the removal of the glass partition between the compartments. We then recorded the colour of the male copulating with the observer female. Males were allowed to court the observer female and compete, which could limit the free expression of the observer female's choice. In parallel, we conducted control replicates in which we tested female mate choice ($n = 33$) after fake demonstrations of three green and three pink demonstrator males without females (everything else being equal), providing no social information.

2.4.2. Experiment 2: sequential demonstrations, no male–male competition

Experiment 2 was similar to Experiment 1 except that male–male competition was absent during the mate-choice test. Only one pink (or green) male was introduced with the observer female. We then compared the latency to copulation (i.e. time separating the onset of the mate-choice test to that of the copulation). While this situation may, at first, seem to be a no choice situation, the female still has the choice to either mate with the proposed male or resist male display. We thus expected latencies to copulation to be negatively related to the strength of the preference for a given male phenotype (pink or green), as shown in previous studies (reviewed by Narraway et al., 2010). Observer females ($n = 126$) were randomly assigned to copulate with a green or a pink male. Here again, in parallel, we conducted control replicates ($n = 65$), in which females received no social information during the demonstrations.

2.4.3. Experiment 3: simultaneous demonstrations, male–male competition

Experiment 3 was similar to Experiment 1 except that positive and negative demonstrations occurred in each of the peripheral compartments simultaneously, and observer females ($n = 65$) could receive negative and positive information simultaneously. Since the aim was only to modify the amount of information at a time without modifying the experimental design, the demonstration phase only lasted 2.25 h (i.e. three repetitions of 45 minutes of simultaneous positive and negative demonstrations). The mate-choice test did not differ from that of Experiment 1 (male–male competition). As previously, control females ($n = 33$) received no social information during the demonstrations and performed independent mate choice.

2.4.4. Experiment 4: simultaneous demonstrations, no male–male competition

In Experiment 4 ($n = 122$, plus $n = 63$ control replicates), demonstrations were performed as in Experiment 3 (i.e. simultaneously), but the mate-choice test was performed as in Experiment 2 (i.e. introduction of a single male, no male–male competition).

Together, these experiments manipulated two parameters: the amount of information provided at a time (sequential versus simultaneous demonstrations), and male–male competition (presence or absence) during the mate-choice test (Fig. 2).

2.5. Statistical analyses

First, we investigated, within each experiment, the use of social information in the context of mate choice. We analysed female preference (i.e. female mate choice for Experiments 1 and 3 and latency to copulation for Experiments 2 and 4) for each experiment independently, using generalised linear mixed models (GLMM, library ‘lme4’, lmer and glmer functions) in R software 3.0.3 (R Core Team, 2014). We used a Binomial distribution of error terms when the response variable was the female mate choice between the pink and green male during the mate-choice test (i.e. Experiments 1 and 3). For Experiments 2 and 4, the continuous response variable (i.e. the latency to copulation) was square root transformed to improve normality prior statistical analysis and we used a Gaussian distribution of error terms. For Experiments 1 and 3, the explanatory variable included in the models, as a fixed effect, was the male phenotype associated with the positive social information during the demonstrations (i.e. green phenotype, pink phenotype, or no phenotype in controls). For Experiments 2 and 4, the fixed effects included in the models were the male phenotype associated with positive social information (i.e. green phenotype, pink phenotype, or no phenotype in controls), the male phenotype offered for the mate-choice test (i.e. green or pink), as well as the one-way interaction between the two variables. To account for possible non-independence of data, experiment date was included as a random effect in all models. We removed all non-significant terms using a backward selection procedure, starting with interaction terms when relevant (i.e. for Experiments 2 and 4). The day at which each a series of replicates was done was included as a random effect on the intercepts only to account for a potential non-independence between days of experimentation. The random effect was kept in all models; we only removed backward fixed effects. In addition, we explored the significant differences between the experimental groups (positive information pink versus positive information green, positive information pink versus controls, positive information green versus controls) using GLMMs.

In a second step, to better delineate the experimental conditions under which mate choice copying is more likely to occur, we used a meta-analytic framework (Garamszegi et al., 2009). Indeed, the previously used null hypothesis significance testing allowed us to investigate, within each experiment, the use of social information in the context of mate choice. However, one of the limits of the inferential approach is that it does not allow a comparison of experiments with different type of response variables (i.e. choice or latency), preventing us to estimate the strength of the effects of the investigated parameters (social information and male–male competition) (Nakagawa and Cuthill, 2007). The meta-analytic framework is therefore particularly powerful in our case to better delineate (i.e. with a higher accuracy) the experimental conditions, which are likely to increase the likelihood of observing copying. For each experiment, we calculated a mean effect size and the 95% confidence interval. Finally, a mean effect size was calculated to compare (*i*) experiments with sequential and simultaneous demonstrations (Experiments 1 and 2 versus Experiments 3 and 4), and (*ii*) experiments with and without male–male competition in the mate-choice test (Experiments 1 and 3 versus Experiments 2 and 4) (see online Supplementary information 1). We therefore quantitatively evaluated the effect of the demonstration treatment and the effect of male–male competition on social learning and MCC efficiency.

3. Results

In Experiment 1, we found that females gathered social information during the demonstrations and this influenced their subsequent mate choice (i.e. MCC) (GLMM, $n = 94$, $\chi^2{}_2 = 8.79$, $P = 0.012$; Fig. 3a). Indeed, in contrast to controls, observer females copulated more often with the male phenotype that mated than with the male phenotype that was rejected during demonstrations. However this impact of social information was only significant when the positive information was provided by green males (post-hoc tests: green versus control: $n = 65$, $\chi^2{}_2 = 4.44$, $P = 0.035$, pink versus control: $n = 62$, $\chi^2{}_2 = 0.85$, $P = 0.36$).

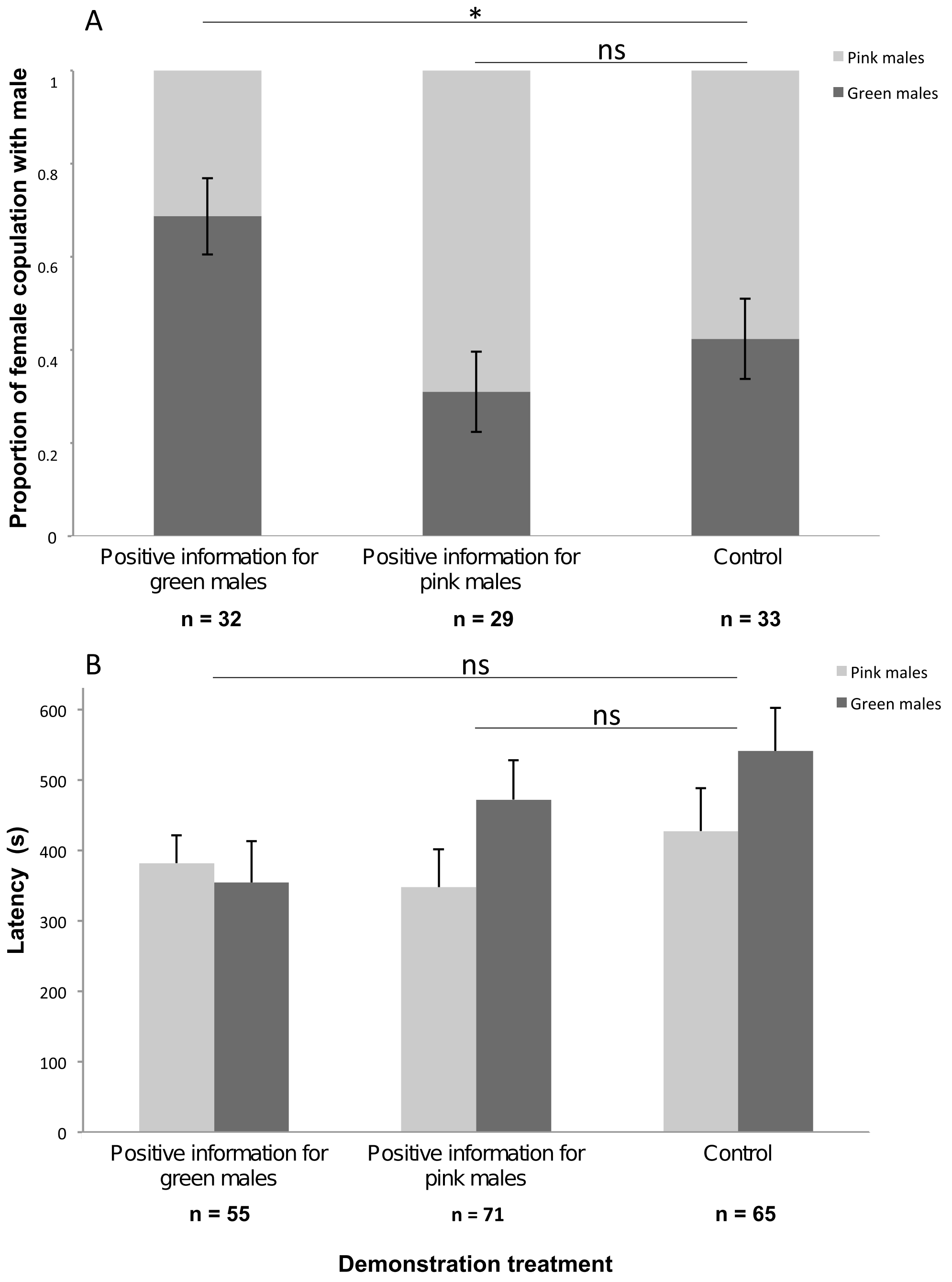

Fig. 3. Mate-choice in Experiments 1 and 2 (sequential demonstrations).
(a) Female choice during the mating test in Experiment 1 in which positive and negative demonstrations were provided sequentially and females could choose between a green and a pink male during the copulation test (in the presence of male-male competition). The proportion of females that to mated with pink and green males is reported for each treatment. (b) Latency before copulation during the mating test in Experiment 2 in which positive and negative demonstrations were provided alternatively and females were presented with a single male of one or the other phenotype so that they were not in a position to choose between males (no male-male competition). The latency of the copulation for each phenotype in each treatment is reported.

In Experiment 2, the latency to copulation did not differ when the observer female was given the opportunity to copulate with the male phenotype that successfully copulated versus the phenotype rejected during the demonstrations (GLMM, interaction between the male phenotype associated with positive social information and male phenotype offered for the mate-choice: $n = 191$, $\chi^2_2 = 4.34$, $P = 0.12$; Fig. 3b).

In Experiments 3 and 4 (positive and negative information provided simultaneously), it appeared that females did not perform MCC and ignored the social information provided during the demonstrations for their subsequent mate choice. In Experiment 3, in which male–male competition occurred, pink and green males were equally chosen during mate-choice tests in controls as well as in situations in which observer females were allowed to collect social information (GLMM, male phenotype associated with positive social information: $n = 98$, $\chi^2_2 = 1.09$, $P = 0.58$, Fig. 4a). Similarly, in Experiment 4, which did not allow male–male competition, the interaction between the male phenotype associated with positive information and the male phenotype offered for the mate-choice test was not significant (GLMM, $n = 185$; $\chi^2_2 = 2.11$, $P = 0.35$, Fig. 4b). Latency to copulation was therefore similar between pink

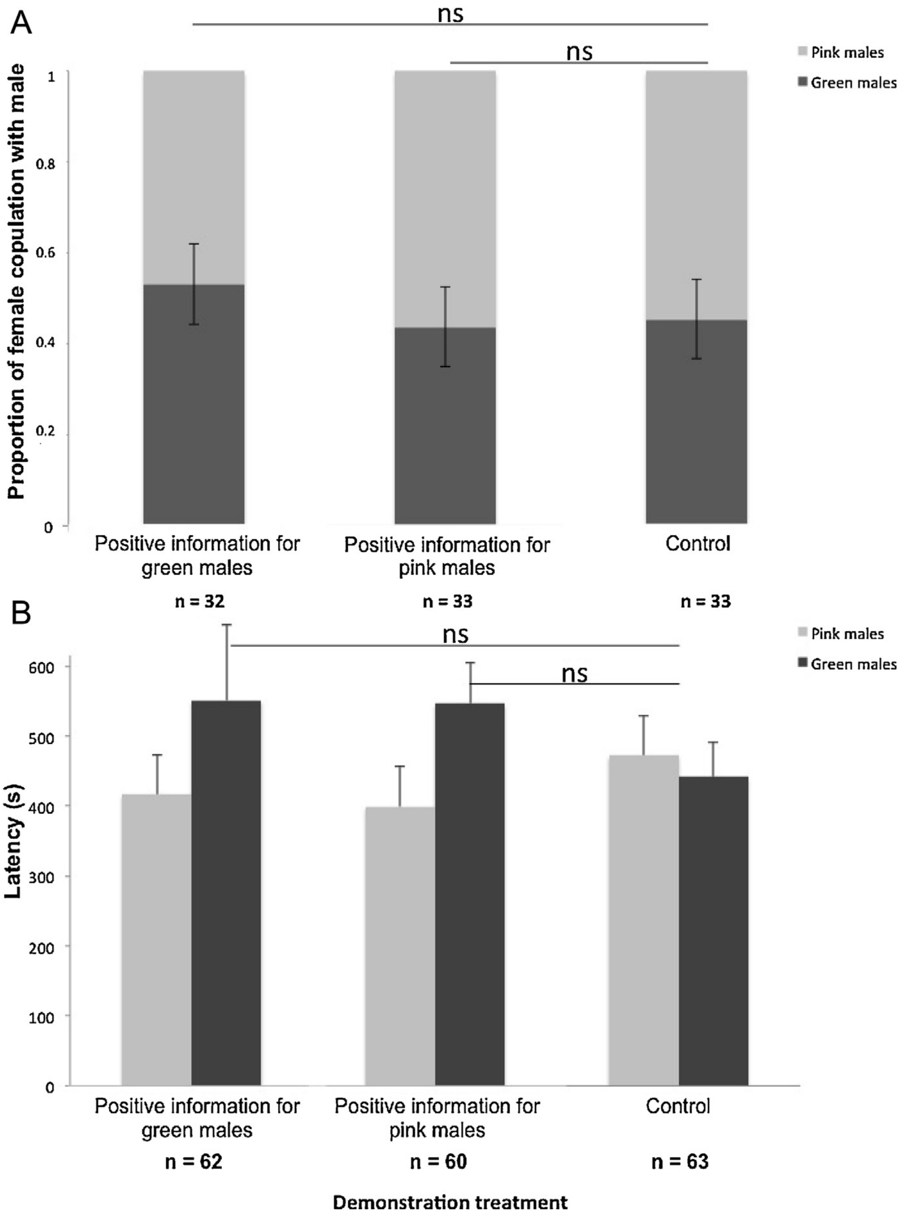

Fig. 4. Mate-choice in Experiments 3 and 4 (simultaneous demonstration).
(a) Female choice during the mating test in Experiment 3, in which positive and negative demonstrations were provided simultaneously and females could choose between a green and a pink male during the copulation test (in the presence of male–male competition). The proportion of females that chose to mate with pink and green males is reported for each treatment. (b) Latency before copulation during the mating test in Experiment 4, in which positive and negative demonstrations were provided simultaneously and females could not choose between males, i.e. a single male of one or the other phenotype was presented (no male–male competition).

and green males in controls and when females observed both male phenotypes successfully copulate.

To summarize, it appears that in protocols providing positive and negative social information sequentially, females seem to use social information in one case and tend to performed MCC (and thus differed from controls), while in protocols providing positive and negative social information simultaneously, females did not use social information and behaved as in controls.

The meta-analysis framework allowed us to evaluate quantitatively both the effect of the demonstration treatment and the effect of male–male competition on MCC in female *D. melanogaster*. This confirms that the use of social information in mate choice differed significantly between experiments with sequential demonstrations (Experiments 1 and 2; Fig. 3) versus simultaneous ones (Experiments 3 and 4; Fig. 4). Females used social information to a lesser extent when demonstrations were provided simultane-

Table 1
Mean effect size and associated 95% confidence interval.

		Mean effect size	95% confidence interval
Quantity of information	Simultaneous demonstrations	0.0849	]0.0435;0.1188[
	Sequential demonstrations	0.1889	]0.1333;0.241[
Male–male interaction	Competition	0.1259	]0.0464;0.1849[
	No competition	0.1577	]0.1184;0.2349[

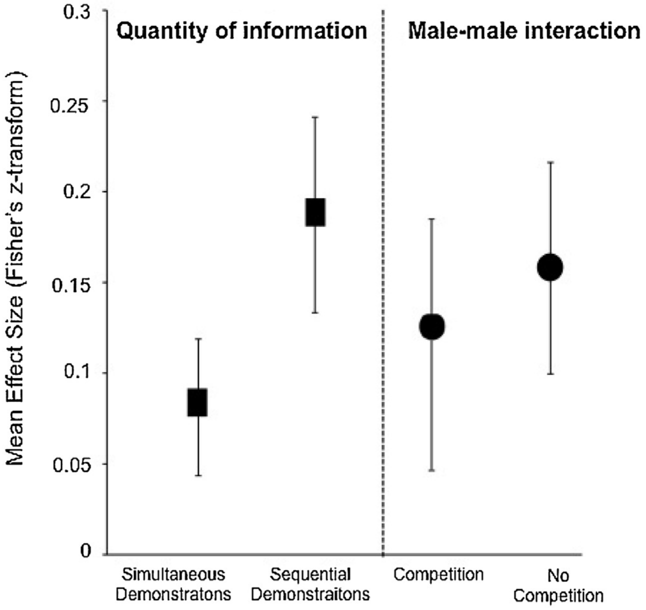

Fig. 5. Effect size (measured as the Fisher's z-transform) measured for the experiments with simultaneous demonstrations and for experiments with sequential demonstrations (left side) and for the experiments with and without male–male competition (right side).

ously, as mean effect sizes were more than double for sequential demonstrations (Table 1). This difference was significant as the 95% confidence intervals did not overlap (Fig. 5). In contrast, no significant difference was detected between experiments with male–male competition present (Experiments 1 and 3) versus absent (Experiments 2 and 4) during mate-choice tests, as revealed by the large overlap of the 95% confidence intervals of effect size (Fig. 5). This suggests that the use of social information in mate choice is not strongly affected by male–male competition.

4. Discussion

In mate-choice copying (MCC), females use social information from male mating performance to develop sexual preferences for a given individual male (simple MCC) or male phenotype (generalised MCC). MCC has already been demonstrated in many species, including the fruit fly, *D. melanogaster* (Mery et al., 2009) in which females were in a situation to learn a general preference for green (or pink) males over pink (or green) males, a form of MCC that is usually dubbed 'generalised MCC'. Here, we tested the impact of two methodological aspects on the results of MCC experiments: (*i*) using sequential versus simultaneous demonstrations of positive and negative social information, and (*ii*) presence or absence of male–male competition during the mate-choice test.

We found that females fruit flies presented with sequential demonstrations performed generalised MCC, as previously found (Mery et al., 2009). Furthermore, we did not detect any significant MCC under the simultaneous demonstration protocol, suggesting that it could prevent such social learning. This difference may have at least two alternative but non exclusive explanations.

4.1. Simultaneous versus sequential demonstrations

Simultaneous demonstrations may have provided too much information at the same time. However, this interpretation is not supported by the fact that Loyau et al. (2012) found that *Drosophila* females are capable to use social information to avoid mating with the specific male they just watched copulating despite the fact they used two simultaneous contradictory demonstrations. However, in contrast to our study, in that experiment the observer females did not have to extract general rules about male phenotypes but only had to distinguish between two individuals, as the males used in the demonstrations were also used during the mate-choice test. A possibility would thus be that females were confounded by the simultaneous demonstrations so that they were not able to perform a more complex task of generalised social learning. This would fit into the sensory constraint and the limited attention hypotheses that both postulate limited rates of information processing by sensory organs and/or the brain (Dukas, 2002). Such cognitive constraints have been documented in several species including *D. melanogaster* (e.g. Sareen et al., 2011). For instance, when placed in a flight simulator and simultaneously stimulated by left and right stimuli, individuals continuously shift attention between the two sides (Sareen et al., 2011; Tang and Juusola, 2010).

Our results may parallel these previous studies in that visual and/or neurological constraints may have limited information gathering and processing in the simultaneous protocol, as information was provided at the two ends of the experimental set up, physically limiting observer females from watching both demonstrating sides simultaneously. However, observer females might have been able to watch each demonstration sequentially as suggested by Loyau et al. (2012), potentially extracting information from both sides. In that case, the absence of generalized MCC would not result from sensory constraints but rather from limited attention constraints, the brain being unable to process information at the offered rate (e.g. Schmieder et al., 2012). Honeybee workers (*Apis mellifera*) prospecting for food, for instance, cannot evaluate several sources of food simultaneously. Workers process part of the available visual information at a time, performing serial searches to extract relevant information (Spaethe et al., 2006). Neurobiological studies showed that the rate of information processing by the brain is much lower than the rate of information encounter and that attentional mechanisms enable the brain to focus only on a subset of the available information (Chittka and Niven, 2009). As a result individuals can only attend to a small fraction of the information available at any time. To our knowledge, limited attention has not directly been studied in the context of mate choice. Our results suggest that even individuals involved in a single task may fail to gather and/or process several sources of information simultaneously. If this was the case, limited attention may impact social information use in general and mate choice in particular.

Alternatively, the absence of generalised MCC after simultaneous demonstrations may have resulted from a difference in demonstration duration. An unavoidable consequence of our design was that in the simultaneous protocol, females were provided with information for half the duration of demonstrations in the sequential treatment (2.25 h versus 4.5 h respectively). It was not possible to make simultaneous demonstrations lasting for 4.5 h without introducing a new bias, as this would imply providing six pairs of demonstrators instead of three. However, unpublished data strongly suggests that demonstration duration is not really important (Dagaeff et al. unpublished results).

Our negative results under the simultaneous protocol could also explain the absence of MCC reported in the phylogenetically close *D. serrata* (Auld et al., 2009), as that experiment used simultaneous demonstrations at opposite ends of the experimental set up. Overall, our results strongly underline the importance of experimental design in MCC experiments.

4.2. The role of male–male competition

The second part of our experiments tested whether male–male competition could affect MCC by informing females about males competitiveness. The presence or absence of male–male competition during the mate-choice test did not affect MCC significantly. This may have at least two non-exclusive explanations.

First, male–male competition may be unimportant during the mate-choice test in the context of MCC. As we were able to observe clear signs of male–male competition during the mate-choice test, this suggests that established female mate preference is relatively immune to information resulting from male–male competition. Alternatively, the detection of MCC in the presence but not in the absence of male–male competition in case of sequential demonstrations suggests that the use of copulation latency (i.e. the rapidity of observer female choice) as a proxy of female preference was not accurate. Indeed, in Experiment 1, we measured both the phenotype of the chosen male and the copulation latency. We found that females copulated more often with the male phenotype that successfully copulated compared to the one that was rejected during the demonstrations but the associated latency to copulation was not shorter ($n = 94$, $\chi^2_2 = 0.005$, $P > 0.5$). Our results illustrate the need for a better means of eliminating male–male competition when investigating mate choice in *Drosophila*.

In conclusion, our results highlight the importance of the choice of the protocol when investigating MCC. While we did not detect any effect of male–male competition on MCC, we found that females only performed MCC with sequential but not with simultaneous demonstrations. The discovery of MCC in an insect (Mery et al., 2009) as well as the sophisticated use of social information about danger in crickets (Coolen et al., 2005) or about male semen stores in flies (Loyau et al., 2012) came as a surprise and opened the way to further studies on insect cognition. It appears that the use of social information is far more widespread than anticipated and the integration of neurological and ecological research in behavioural studies should further our understanding on how cognitive abilities and constraints influence the evolution of behaviour.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.beproc.2016.02.002.

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