

Public Versus Personal Information for Mate Copying in an Invertebrate

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Summary

Organisms require information to make decisions about fitness-affecting resources, such as mates. Animals may extract “personal information” about potential mates by observing their physical characteristics or extract additional “public information” by observing their mating performance [1]. Mate copying by females [2–6] is a form of public information use that may reduce uncertainty about male quality, allowing more adaptive choices [2]. Experimental studies have produced evidence that female mate copying occurs in several species of fish [3], birds [5–7], and mammals [8], including humans [9]. We report the first evidence that a female invertebrate can exploit public information to select mates. In a first experiment, *Drosophila melanogaster* female prospectors increased their time in the attraction zones of poor-condition males, but not of good-condition males, after having observed them with a model female. This suggests that females appraised prospective mates by exploiting

public information and did so mainly when it contrasted with personal information. In a second experiment, prospector females preferably mated with males of the color type they had previously observed copulating over males of the rejected color type, suggesting that female *Drosophila* can generalize socially learned information. The complexity of *Drosophila* decision-making suggests an unprecedented level of cognition in invertebrates. Our findings have implications for evolution given that socially learned mate preferences may lead to reproductive isolation, setting the stage for speciation [10].

Results and Discussion

Mate-copying experiments implicitly create an inconsistency between personal and public information about male attractiveness. Several recent studies have provided evidence that invertebrates can use public information to make decisions about foraging and predation [11]. However, lacking are experiments that determine whether invertebrates are capable of exploiting public information to choose mates. We chose *Drosophila melanogaster* to study mate copying in an invertebrate because its sexual behavior is well known [12–17] and its ability to learn [18–20] and memorize food location [18, 21–23] provides the potential for females to also learn and retain information about male quality. We performed two complementary experiments to examine whether fruit flies can exploit and generalize public information under multiple conditions. In experiment 1, we manipulated personal and public information to examine their relative roles in male attractiveness (Figure 1A). We did this by creating two male phenotypes that markedly contrasted in condition [24], which in nature may reveal differences in genetic and sperm quality that may affect female fitness [25]. In experiment 2, we created two colored male phenotypes that were unrelated to male quality (Figure 1B), allowing us to further examine whether the manipulation of male attractiveness affects copulation success.

Experiment 1

We created two contrasting male fly phenotypes, those in good and poor condition, by raising them in rich food medium comprising 100% of standard nutrients or poor medium comprising 25% of standard nutrients, respectively [24]. Each fly was used only once. In our setup (Figure 1A), the female prospector was recorded as being or not being in one of the two attraction zones, which comprised the perforated lids containing each male. Our experiment comprised three one-day steps [3, 7, 8] and three treatments, each replicated 24 times. The pretest measured unmanipulated male attractiveness, revealing personal information use that was measured as the percentage of time that female prospectors spent on the lids of the males’ containers. The treatment entailed the enclosure of a model female with one of the two males and manipulated public information about the two males’ relative attractiveness. Because time may affect male attractiveness and because the experiment lasted three days, we included a control treatment with no model female. The posttest recreated the situation of the pretest for

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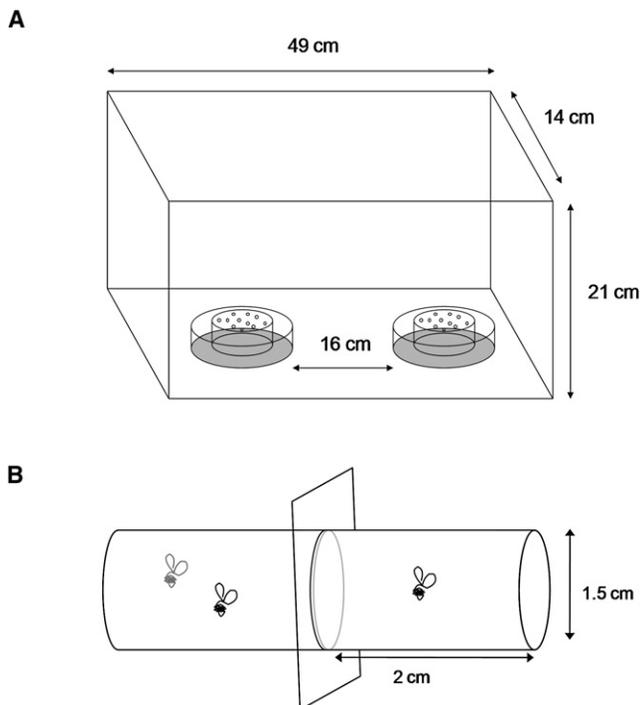


Figure 1. Experimental Setups

(A) Experiment 1. The setup comprised a transparent Plexiglas box that contained two 10 cm Petri dishes containing nutritional medium, in the center of which was placed a 4 cm transparent container that enclosed a male, with a perforated lid that allowed visual, acoustic, and olfactory interactions. The attraction zones comprised the perforated lid of the transparent container that enclosed demonstrator males.

(B) Experiment 2. A virgin female prospector was introduced into a tube (right side) and was given the opportunity to observe a pair of flies (a colored male and either a virgin or a previously mated female) through a thin glass partition that was either transparent or opaque, permitting or preventing the female prospector from observing the pair. The glass partition prevented olfactory cues.

measurement of changes in male attractiveness after the treatment. We performed two additional controls that replicated the experiment. In the first, we replaced males with females to control for potential group size effects. In the second control, we introduced prospector females at the posttest to prevent them from seeing male-female interactions, to exclude potential effects on male behavior of their having been with a model female.

In the pretest (Figure 2), naive females preferred good condition males ($F_{1,61} = 4.08$, $p = 0.048$), suggesting that female *Drosophila* may use personal information to infer male quality. If females also use public information, then they are expected to increase the time spent on the lid of the male that had previously been with a model female. When housed with a model female, poor condition males increased their attractiveness (i) between the pre- and the posttest compared to the no-model control treatment (Figure 3: interaction pretest-posttest \times phenotype, $F_{1,57} = 6.08$, $p = 0.016$; and Figure 4: points C versus B, $F_{1,40} = 5.08$, $p = 0.029$), and (ii) across treatments (solid line in Figure 4: $F_{1,61} = 10.01$, $p = 0.002$). The changes in the attractiveness of good condition males were unaffected by whether or not the males had been with a model female (Figure 4, comparison of points X and Y, $F_{1,41} = 0.01$, $p = 0.92$). Consequently, the trends in the changes in male attractiveness differed significantly among treatments and male

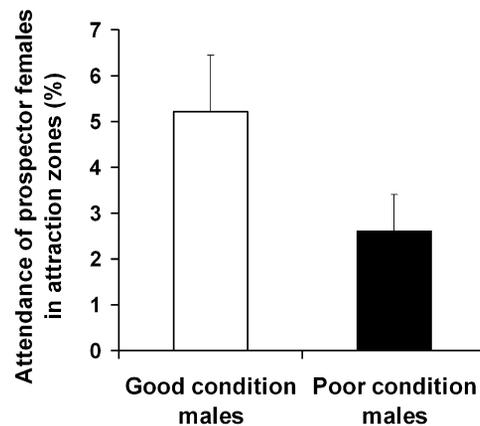


Figure 2. The Relative Attractiveness of Good and Poor Condition Males during the Pretest in Experiment 1

Females assigned to the three treatments did not behave differently during the pretest, that is, before any manipulation (the SAS generalized linear mixed procedure [GLIMMIX], with replicate nested within treatment as random effect: $F_{2,61} = 1.99$, $p = 0.147$). Male attractiveness was estimated as the mean percentage of scans in which prospector females were recorded in an attraction zone for the good or poor condition males. Females spent more time on the lids of the good condition males (GLIMMIX, with prospectors as a random effect: $F_{1,61} = 4.08$, $p = 0.048$). Prospector females were recorded in attraction zones (representing 1.9% of potential space) more often than by chance (7.83% and 14.44% of the scans in the pre- and posttest, respectively, $p < 0.0001$), suggesting that the presence of female prospectors on the lids of either male probably reflected mating preference. Bars represent means \pm SE; $n = 72$ for each bar.

phenotypes (general linear mixed models [GLMM], interaction treatment \times phenotype: $F_{1,61} = 8.28$, $p = 0.0055$; Figure 4).

These findings suggest that female *Drosophila* used public information, in that they modified their behavior after witnessing the apparent choices of model females. The treatment in which the model female was enclosed with the good condition male created a consistency between personal and public information, whereas these two types of information were inconsistent when the model female was housed with the

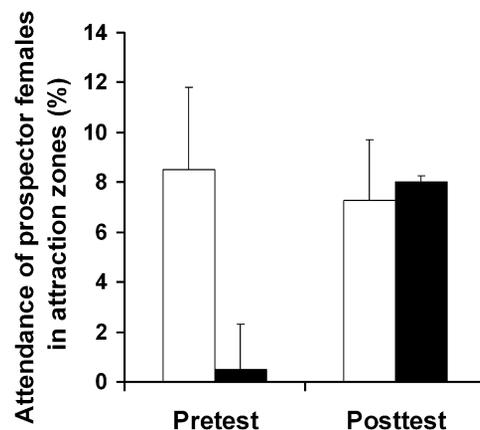


Figure 3. Mean Percentage of Time Spent by Female Prospectors on the Lids of the Good and Poor Condition Males in the Treatment in which the Model Female Was Placed with the Poor Condition Male

Open bars: good condition males; black bars: poor condition males. The interaction of the pretest-posttest \times phenotype ($F_{1,57} = 6.08$, $p = 0.016$) indicates that females significantly increased their time in the attraction zones of the poor (but not good) condition males after the treatment. Bars represent means \pm SE; $n = 24$ for all bars.

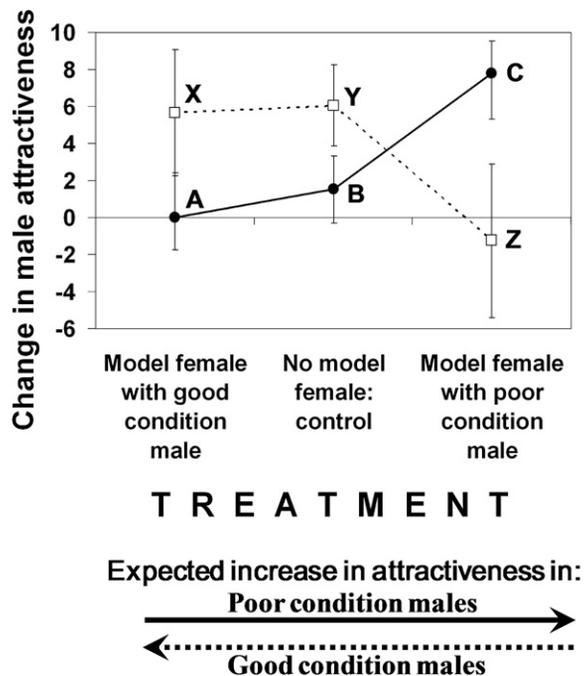


Figure 4. Effect of the Manipulation of Public Information on Male Attractiveness According to Male Phenotype

We manipulated public information by enclosing a model female with either a poor or a good condition male, and we included a no-model control treatment. The y axis shows the changes in male attractiveness from the pretest (day 1) to the posttest (day 3) that followed the manipulation on day 2. Each point represents the mean (\pm SEM) change in the males' attractiveness, determined by the time that 24 female prospectors spent in the corresponding attraction zones. A total of 72 sets of flies were used for the three treatments. Open squares and dashed line: attractiveness of good condition males. Black circles and thick line: attractiveness of poor condition males. Letters near points are used in the text in reference to specific comparisons. Note that most points are positive, indicating that on average, male attractiveness increased from the pre- to the posttests.

poor condition male. Females apparently reacted to this inconsistency by increasing their time spent near the poor condition, though apparently attractive, male. Interestingly, housing a model female with the good condition male did not significantly increase its attractiveness relative to the no-model control (Figure 4, points X–Y, $p = 0.92$), suggesting that prospectors used public information mainly when it was inconsistent with personal information.

An alternative explanation may be that females may have been attracted differentially to groups of two versus one individual, independently of their sex. We addressed this by repeating experiment 1 but replacing the two males by two females in good or poor condition, and we found no significant effect of the number of flies under the lids (same analysis as that of Figure 4, all P s > 0.12). Another alternative explanation could be that, following the treatment, female prospectors may have cued on the possibly increased display rate of poor condition males that had been stimulated by the model female [26, 27]. We thus repeated experiment 1 but introduced the female prospector only at the posttest, so that she had not seen which male had been with the model female. When analyzing this new data set including the three treatments, we found no significant relationships across treatments and according to male phenotype (P s > 0.14). We can thus exclude differential male display rates as an explanation of our findings.

Experiment 1 reveals aspects of how female *Drosophila* use public information when faced with actual differences in male condition. We further examined public information use in a second experiment, in which we created arbitrary male phenotypes that were unconnected to male condition.

Experiment 2

We created two male phenotypes by randomly dusting individuals with green or pink powder. This method allowed us to evaluate the influence of public information on actual male copulation success rather than only on male attractiveness. A virgin prospector female was first introduced into and kept in a small tube ($n = 80$, Figure 1B). A second “demonstration tube” was placed at the end of the tube containing the prospector female, from which it was separated by a thin layer of transparent glass. In alternate trials, a green or a pink colored male and a virgin female were introduced for 1 hr into the demonstration tube. The occurrence of copulation during this period provided positive public information about male attractiveness to the prospector female. The pair of demonstrator flies was then replaced for 1 hr by a new pair comprising a male of the other color and a female that had been with two males for copulation for 2 hr before the experiment. Because female *Drosophila* refuse copulations after recently mating [28], these females provided negative public information. This 2 hr sequence was repeated three times. We visually verified whether virgin females copulated with the males and whether nonvirgin females refused copulation. We analyzed only the trials in which these conditions were fulfilled, which was the case in 96% of the trials. In performing the test, we introduced a dyad of new pink and green males into the demonstration tube and removed the glass partition. We then recorded the color of the male that the prospector female copulated with (Figure 5). As a control, we replaced the transparent partition with an opaque one to prevent visual cues in the observation phase.

Regardless of male color, female prospectors copulated significantly more often with males of the category they had seen successfully mating during the manipulation step (“Visual Cues” in Figure 5, Wald chi-square $Z = 5.9$, $p = 0.01$). Furthermore, this effect disappeared when females were blocked from seeing the other flies during the manipulation ($Z = 0.25$, $p = 0.61$), suggesting that they can extract public information from visual cues for mate-choice copying. These findings did not result from males of one color being more competitive than males of the other color, because females copulated more often with the male type that they saw copulating, regardless of male color (“Visual Cues” in Figure 5).

Females were thus able to use public information to discriminate between two categories of males of equal condition and to select the type that had been manipulated for a more attractive appearance to other females. Furthermore, females were able to discriminate not only between two individual males, as in experiment 1, but also between categories of males. The apparent ability to generalize socially learned stimuli suggests that *Drosophila melanogaster* possesses complex cognitive capacities that resemble stimulus generalization [29, 30].

Recent research has shown that manipulating the molecular environment of neurons influences mate choice in *Drosophila* [31]. Here, we demonstrate, in an invertebrate, that the social environment surrounding females may also influence their mate choices. The addition of a novel control in which no public information was provided to the female prospector

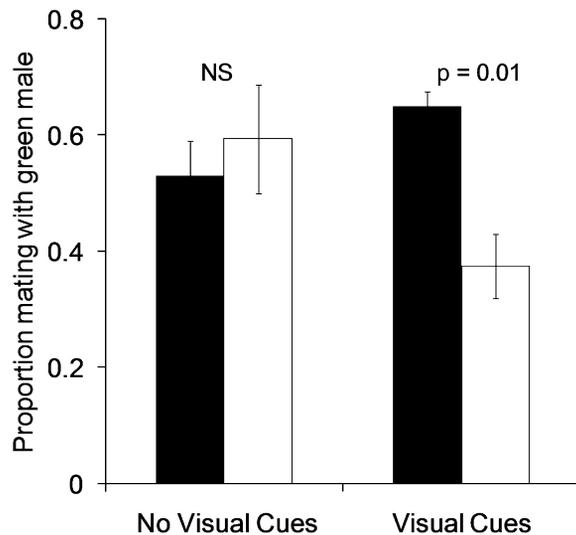


Figure 5. Male Copulation Success According to the Treatment in Experiment 2

We manipulated male attractiveness positively and negatively for green- and pink-colored males by showing females both types of males being either accepted or rejected for copulations. To avoid redundancy, we show the proportion of females that copulated with one male color type: green. Results produced by the pink male color type are the exact reciprocal. Prospector females were previously either in a position of observing the demonstration through a transparent glass partition (visual cue, $n = 80$) or prevented from observing by an opaque glass partition (no visual cues, $n = 64$). White bars indicate when females were provided with positive and negative public information about pink and green males, respectively. Black bars indicate when females were provided with positive and negative public information about green and pink males, respectively. Bars represent means \pm SE. When prospector females were provided with no information about male attractiveness, they showed no preference for either of the two male color types ($t = 0.7$, $p = 0.48$, $n = 32$).

revealed that mate copying by females may be dependent upon the consistency between male condition and public information. This provides the first evidence in any species that mate copying may be a strategy that is used mainly when public information contradicts, rather than supports, personal information.

Experimental Procedures

Experiment 1

We raised flies of the wild-type *DRAVEIL* line in standard culture bottles containing brewer's yeast and maize flour medium, and we maintained them at 25°C on a 12 hr/12 hr day/night cycle. We raised prospector and model females in different culture bottles to avoid kinship effects during mate-choice experiments. All flies were virgins and were maintained separately in tubes.

Analyses

Analyses were performed in SAS. Sample sizes vary because we used only replicates in which females suggested sexual preference by being observed on a male's lid at least once; this differed among treatments. Analyses including all flies produced qualitatively similar results. We calculated the percentage $P_{s,p}$, in which s stands for the step (pre- or posttest) and p for the phenotype (good or poor condition), of times that each female was recorded on each lid separately during the 21 scans per step. We then arcsine-transformed the $P_{s,p}$, (later called " $aP_{s,p}$ "). Next, we calculated the change in the percentage of time that each female had spent on the lid of either male as the difference $aP_{post,p} - aP_{pre,p}$ for each phenotype. Changes in attractiveness were used as the dependent variable in a GLMM, with the replicate number as a random effect, the treatment as a gradient effect, and male phenotype as a class effect. A Gaussian error term was assumed. The

y axis of Figure 4 represents nontransformed changes in percentages of time spent in the attraction zones ($P_{post,p} - P_{pre,p}$). In the experiment ($n = 72$ female prospectors) and the two controls ($n = 144$), 88% of prospectors were recorded at least once in one or both attraction zones. A prevalent pitfall in statistical analyses is "regression toward the mean," which occurs when uncommonly large or small measurements are followed by measurements that are closer to the mean [32]. We corrected for this effect when applicable by using the method provided by Kelly and Price [32]. The corrections did not qualitatively alter the results.

Experiment 2

A base stock population of *Drosophila melanogaster* was derived from flies caught in Chavroche (France) six months before the experiment and raised in a cornmeal medium. All flies were three days old, counted from adult emergence. At emergence from pupae, flies were kept individually in small tubes until the experiment.

Female preference was analyzed through binary logistic regressions, with the color type of the successful male as the dependent variable and the treatment as the covariate. A Wald test was then performed.

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