



Drosophila mate copying correlates with atmospheric pressure in a speed learning situation



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ARTICLE INFO

Article history:

Received 6 February 2016

Initial acceptance 8 March 2016

Final acceptance 20 July 2016

Available online 6 October 2016

MS. number: 16-00105R

Keywords:

Drosophila melanogaster

mate choice

observational learning

personal information

social information

social learning

Mate choice can strongly affect fitness in sexually reproducing organisms. A form of mate choice is mate copying, in which individuals use information about potential mates by copying the mate choice of other individuals. While many studies have documented mate copying, little is known about the effect of environmental conditions on this behaviour. Here, we report the first evidence that *Drosophila melanogaster* females can acquire a sexual preference for one male characteristic after witnessing a single mate choice event (i.e. speed learning). We also found that mate copying was correlated with air pressure and air pressure changes, so that females copied far more when air pressure was high and increasing, i.e. in good and improving weather conditions. These results reveal a quick social observational learning and highlight the potential importance of meteorological conditions for mate copying, a trait potentially driving reproductive isolation.

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Mate choice has important fitness consequences as it is a major driver of sexual selection (Verzijden et al., 2012). To select a suitable mate, individuals need to assess potential partners by collecting information about them. Such information can be acquired either by trial-and-error tactics or innate rules, i.e. using private information, or by monitoring other individuals with similar requirements, i.e. using inadvertent social information (Danchin, Giraldeau, Valone, & Wagner, 2004; Danchin & Wagner, 2010). In particular, the mating performance of potential mates provides public information on their quality (Nordell & Valone, 1998), and females of many species develop mating preferences that are affected by such public information (Westneat, Walters, McCarthy, Hatch, & Hein, 2000). This behaviour is called mate choice copying or more simply mate copying.

In their simplest form, mate copying experimental designs encompass two sequential phases: a demonstration followed by a test. During the demonstration, a naïve female (called the observer female) is allowed to witness two males with contrasting phenotypes, only one being chosen for copulation by another female (called the demonstrator female). During the preference test, the observer female preference is assessed by either the relative amount of time she spends near the two males or actual copulation with one of them. By copying the mate choice of others, females can also generalize their preference for any other male with similar traits (Bowers, Place, Todd, Penke, & Asendorpf, 2012), implying that mating preference may be transferred socially between individuals within populations (horizontal transmission) and across generations (vertical transmission; Bowers et al., 2012; Danchin et al., 2004).

Mate copying has been mainly reported in vertebrates (see Galef & White, 2000; Vakirtzis, 2011 for reviews) and, to our knowledge, only two studies have investigated its occurrence in invertebrates, both of them on *Drosophila*. Mery et al. (2009) provided evidence of mate copying in *Drosophila melanogaster*. In this study, the design

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differed from those used in vertebrates: the observer female witnessed not an actual choice between two males, but instead the behaviour of six females sequentially mating with one male phenotype and rejecting the other (Fig. 1). By contrast, Auld, Punzalan, Godin, and Rundle (2009) found no evidence of mate copying in *Drosophila serrata*. These contrasting outcomes may result from the difference between the experimental protocols as well as the species used. In addition, external parameters may impact copying and mating behaviours. For example, in guppy, *Poecilia reticulata*, mating behaviour is correlated with both the quantity and the spectral composition of the lighting conditions (Archard, Cuthill, & Partridge, 2009). In insects, mating behaviour has been shown to be impacted by atmospheric pressure (Ankney, 1984; Austin, Guglielmo, & Moehring, 2014; McFarlane, Rafter, Booth, & Walter, 2015; Pellegrino et al., 2013).

Here, we investigated whether *D. melanogaster* females can acquire a sexual preference for one male characteristic after witnessing a single mate choice event. We also examined the effects of external parameters, especially air pressure, on mate copying. We had long noticed that the extent of mate copying seemed to be correlated with weather conditions during the experiment. As we controlled temperature, light and humidity, we postulated that the main external parameter potentially explaining a weather effect should involve air pressure. This seemed coherent with the fact that a change in weather, in particular the arrival of heavy rains or storms, can have serious fitness consequences for small animals such as insects (Wellington, 1946) but can also be relatively well predicted by monitoring air pressure. Good weather is usually associated with high air pressure, whereas rain mostly happens in low air pressure conditions (Ahrens, 2009). Air pressure change also needs to be considered: a rapid drop indicates an approaching storm or heavy winds (Ahrens, 2009). Even though the influence of weather on animal behaviour has been observed by humans for centuries (Aristotle, trans. 1883, e.g. XXVII:46), it has been investigated only in a few studies in mammals (Paige, 1995), birds (Breuner, Sprague, Patterson, & Woods, 2013; Metcalfe, Schmidt, Bezner Kerr, Guglielmo, & MacDougall-Shackleton, 2013), fish (Heupel, Simpfendorfer, & Hueter, 2003) and insects (Ankney,

1984; Austin et al., 2014; McFarlane et al., 2015; Pellegrino et al., 2013). In particular, mating behaviour has been shown to be affected by air pressure changes in the cucumber beetle, *Diabrotica speciosa*, the true armyworm moth, *Pseudaletia unipuncta*, and the potato aphid, *Macrosiphum euphorbiae* (Pellegrino et al., 2013). In *D. melanogaster*, only two studies have focused on the influence of air pressure on the prevalence of sexual behaviour (Ankney, 1984; Austin et al., 2014). Ankney (1984) found that *Drosophila* mating frequency decreases in low air pressure conditions. Austin et al. (2014) found an effect of air pressure change on *D. melanogaster* courtship and mating frequency: in decreasing air pressure some flies showed reduced mating activity, whereas others increased it. But the effects of air pressure on other aspects of sexual behaviour such as mate choice or, more generally, on cognitive abilities, have never been investigated.

Here, we report the results of two experiments followed by a correlational analysis of the potential role of air pressure on fruit fly sexual behaviour. Experiment 1 investigated whether *Drosophila* females can perform mate copying in a protocol similar to those traditionally used in studies of mate copying in vertebrates. To this aim, we performed and compared two experimental designs of mate copying. The first design (adapted from Mery et al., 2009) involved six apparent female choices in a sequence (long demonstration protocol, Fig. 1). The second design involved a single live demonstration of one female choosing between two males of contrasting phenotypes (short demonstration protocol, Fig. 1). Very little is known about the ecology of *Drosophila* in the wild (Reaume & Sokolowski, 2006), and it is uncertain whether *Drosophila* females have the opportunity to experience sequential demonstrations of mate choice in nature as in the long demonstration protocol. Thus, in addition to bridging the gap with vertebrate studies, the rationale for our short demonstration protocol was that if *Drosophila* females were able to perform mate copying in speed learning situations, then our confidence that they can perform mate copying in nature would be greatly increased. The short demonstration protocol thus tested whether young virgin females can acquire a preference for a given male phenotype after the observation of one female choosing and copulating with one of two

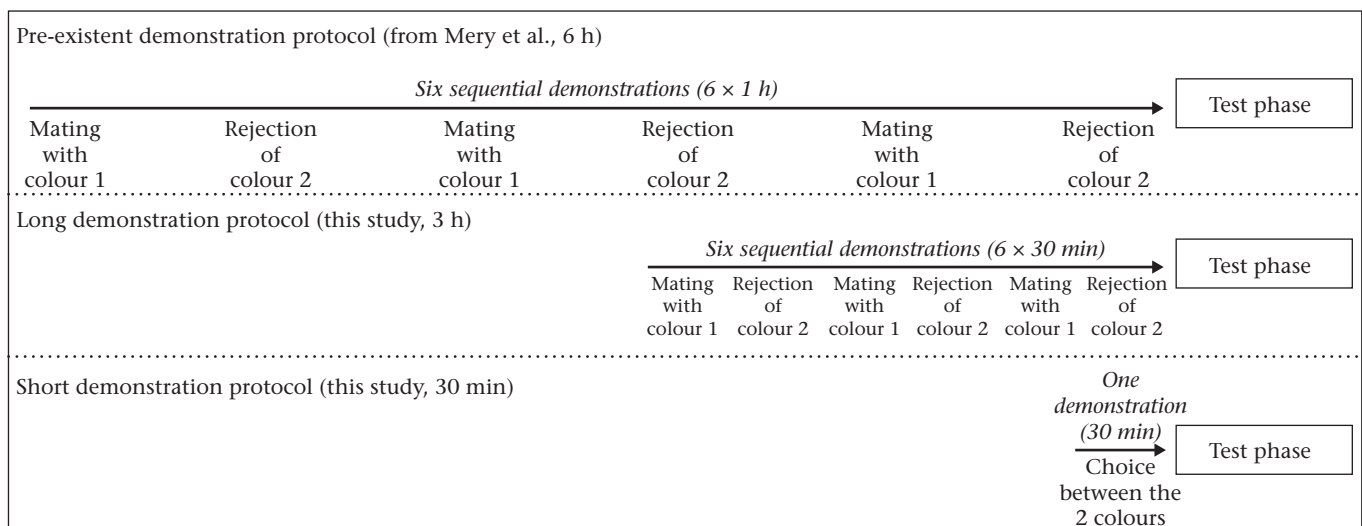


Figure 1. *Drosophila* mate copying protocols: the first was used in the second experiment of Mery et al. (2009); the other two were used in this study. Our long demonstration protocol followed that of Mery et al., except that each demonstration step lasted for 30 min instead of 1 h. Both consisted of a sequence of demonstrations involving one virgin female mating with a male of one colour, followed by a demonstration with a recently mated female rejecting the male of the other colour. These two steps were repeated three times for a total of 6 h in Mery et al.'s study versus 3 h in our long design. The short demonstration protocol involved only one live demonstration of one female freely choosing between two differently coloured males (experiment 1), or a female already copulating with a male of one colour plus a male of the opposite colour next to them (experiment 2). This shorter demonstration phase lasted 30 min. In both protocols, the preference test was run just after the demonstration and the colour of the male with which the observer female copulated was recorded and used to estimate its preference.

males with contrasting phenotypes. Experiment 2 tested whether, in the short protocol, the sole observation of an ongoing copulation is sufficient to elicit mate copying or whether the observation of male courting performance is necessary. Finally, we used a correlational approach on the experimental data to analyse the effect of natural air pressure, as well as its change across experimental days, on mate copying performances.

METHODS

Fly Maintenance

We used 1445 individuals of the common laboratory Canton-S strain of *D. melanogaster*. Flies were raised in 8 ml vials containing a standard wheat flour–agar–yeast medium. Raising and experimental conditions were: 25 ± 1 °C, with a 12:12 h light:dark cycle and $60 \pm 5\%$ humidity during the first experiment and $60 \pm 10\%$ humidity during the second. To keep humidity constant, three independent automatic humidifiers were placed in different locations in the experimental room; each had a hydrostat that was set to 60%. Flies were sorted without anaesthesia within 6 h after emergence and kept in unisex groups of six individuals before experiments. All *Drosophila* used for the experiments were 3 or 4 days old. Fly manipulations were performed by gentle aspiration. Experiments consisted only of matings; no other disturbance or harmful manipulations were made. Once the copulation ended, individuals were put in a vial and were euthanized in a freezer shortly after.

General Procedures

The two artificial male phenotypes were created by dusting males with green or pink powders (Mery et al., 2009). To have as little difference in our coloured phenotypes as possible, we took two males from a raising vial and allocated randomly one to the green and the other to the pink colour. Males were then placed in food vials to clean the excess of dust for 30 min. Then, for the next replicate, we took two males from another vial and so on.

Experiments took place in double plastic tubes separated by a thin glass partition that could be either opaque (controls) or transparent (Fig. 2). The light came from above and was equally distributed so that the flies were not attracted to a specific point. In all preference tests, as observer females courted by only one of two males were not in a position to choose mates, we only kept replicates in which both males courted the female and discarded the others. Before starting the experiment, we first tested whether there was an innate preference for one male colour. To do that, one virgin observer female was placed in the tube with a male of each colour for 30 min during which we recorded the colour and number of males courting the female (i.e. if none, one or both males

displayed wing vibration or ‘singing’, Sokolowski, 2001), as well as the copulation duration and colour chosen.

We found that naïve virgin females copulated with green and pink males in a way that did not differ from the absence of choice ($N = 63$ trials, 30 copulations with the pink male and 33 with the green male; chi-square test green versus pink: $\chi^2_1 = 0.127$, $P = 0.722$). This is consistent with other experiments using these powders (Mery et al., 2009; Pavković-Lučić, Lučić, Miličić, Tomić, & Savić, 2014).

Experiment 1: Repeated Versus Single Demonstration

At the beginning of the experiment, one virgin observer female was placed in one compartment of the tube, demonstrations taking place in the other compartment.

Two types of protocols were run in parallel: one with long demonstrations, inspired from the protocol of the previous study of mate copying in fruit flies (Mery et al., 2009) but with shorter demonstrations (6×30 min instead of 6×1 h in Mery et al., 2009), and one with a single live demonstration (Fig. 1).

The long demonstration protocol consisted of one demonstration of a virgin female mating with a male of one colour for 25–30 min (Fig. 1). As virgin females readily accept copulation, this provided positive information about this male phenotype to observer females. In *D. melanogaster*, copulations last 20 min on average (Pavković-Lučić et al., 2014). So a 30 min demonstration ensured that copulation had the time to start and to last long enough to inform the observer female. Immediately after the copulation ended (i.e. possibly before the end of the 30 min demonstration), individuals were removed from the tubes so that the demonstrator female never had the opportunity to thereafter reject or remate with the male it had just mated with. For the next 30 min, the demonstration involved another male of the other colour together with a recently mated female. As mated females actively reject every male for several hours (Van Vianen & Bijlsma, 1993), this demonstration provided negative information about that male colour phenotype. This combination of two demonstrations was repeated three times in a sequence (Fig. 1). For each replicate, we visually checked that virgin females did mate with the males and that already mated females did refuse mating. If these conditions were not fulfilled, we discarded the trial (5% of the total number of replicates).

For the control group of the long protocol, the same protocol was performed with an opaque instead of a transparent partition separating the tubes (Fig. 2), so that the observer female could not see the demonstration.

The short demonstration protocol consisted of a single demonstration of one female placed with two males, one of each colour for 25–30 min depending on the copulation duration (Fig. 1). The copulation of the demonstrator female with one of the males provided positive information for that male phenotype and

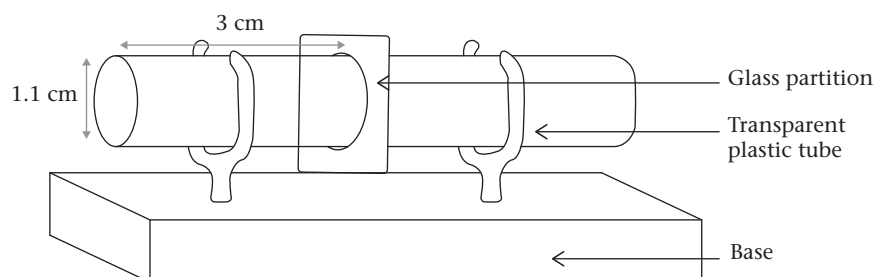


Figure 2. Experimental device. The observer female was randomly placed on one side while the demonstration occurred on the other. A partition was placed between the demonstrator and observer flies: this was either transparent (experimental group: informed females) or opaque (control group: uninformed females).

negative information for the other male phenotype. In the control group, the demonstration consisted of two males, one of each colour, but without any female so that the observer female watched the artificial male phenotypes without receiving any information about their attractiveness. This allowed observer females of the control group to get used to these artificial phenotypes for the same amount of time as experimental females. (In the control with an opaque partition (long protocol), the females had not seen any coloured male until the preference test, while the experimental (informed) females did. This new control avoided differences between informed and uninformed treatments as uninformed females (controls) had never seen coloured males before.)

The preference test immediately followed each demonstration. The test males were previously coloured using the same protocol as those used for the demonstration males. The males used in tests were not those used in demonstrations, came from different vials and were not powdered at the same time as the demonstrator males. Thus, a new pair of males of each colour was placed with the observer females for 30 min. During the preference test, we recorded colour and number of males (i.e. zero, one or two) courting the female (i.e. displaying wing vibration or 'singing', Sokolowski, 2001). For 90 replicates of the long protocol and all the replicates of the short protocol, we also recorded courtship latency (i.e. the time between the insertion of the two males and the first wing vibration by one of the males), copulation time and mating latency (i.e. the time between the first courtship and the beginning of copulation).

When mate copying was the variable to explain, we performed analyses only on those replicates in which both males had courted the female (i.e. displaying wing vibration at least once) and discarded all the others. For the long demonstration protocol, we kept 125 replicates out of 543 trials (23%); for the short demonstration protocol, we kept 159 replicates out of 472 trials (34%). We used all replicates to detect any weather effect on sexual behaviour in general, by testing the effect of climatic parameters on the proportion of discarded trials and on courtship latency, copulation time and mating latency (1015 replicates, see Appendix Table A1).

Experiment 2: Demonstration with an Ongoing Copulation

In the short demonstration protocol, observer females could gather information from two components of behaviour: male courtship and/or actual copulation. To distinguish between the effects of these two sources of information we carried out an additional experiment. It replicated the short demonstration protocol, except that in the treatment group the observer female was shown a demonstrator female already mated with a male of a given phenotype, while a male of the other phenotype was placed next to them. Consequently, the observer female could not gather any information about males other than their copulating success. In this second experiment we first put virgin females in tubes with males of the desired colour. Once one mating started, the two flies were gently placed on the demonstration side of the experimental apparatus (Fig. 2), to which we added a male of the opposite colour. This triad mimicked a situation in which the demonstrator female had chosen one male phenotype over the other. This demonstration lasted 20 min on average depending on the copulation duration. The preference test was performed later on, following the same protocol as in previous experiments. The control group involved exactly the same procedure but with an opaque partition during the demonstration. As for experiment 1, we only kept replicates in which both males courted the female (80 replicates out of 180 trials, i.e. 44% for the experimental group; 80 replicates out of 250 trials, i.e. 32% for the control group). For more information on the number of replicates used in experiment 2 see Appendix Table A1.

Mate Copying Index

For a given replicate, a mate copying score was defined as 1 when the observer female copulated with the male of the phenotype preferred during the demonstration and 0 in the opposite case. The mate copying index for a given treatment was the mean of mate copying scores for that situation, and quantified female learning. Values around 0.5 indicate random choice by observer females, while values above 0.5 represent mate copying.

Effect of External Parameters

To analyse the role of meteorological conditions on mate copying, we included two climatic parameters. As we controlled light, temperature and humidity in the experimental room, we speculated that any meteorological effect would mostly come from air pressure. We obtained the pressure data from a nearby weather station in the Toulouse airport that records barometric conditions every 30 min and checked that these measurements were highly correlated with those taken by our own laboratory station at the beginning of each replicate (see Appendix). We then examined the effects of (1) current air pressure at the onset of the experiment and (2) its change during the 6 h preceding the start of each replicate. The temporal change in air pressure was calculated as the slope of a linear regression to values of air pressure during the 6 h preceding the experiment, a time span used in a previous study about the influence of air pressure in insects (Pellegrino et al., 2013). The inclusion of these two parameters in the statistical models explaining the mate copying score allowed us to test their significance on the mate copying index. See the Appendix for details of the pressure distribution on the experimental days.

Statistical Analyses

All statistical analyses were performed with the R software, version 3.1.2 (R Core Team, 2014). Mate copying scores were analysed by a generalized linear mixed model with binary logistic regression with the package lme4 (Bates, Maechler, Bolker, & Walker, 2014). A Wald chi-square post hoc analysis then tested whether the observed proportions differed from random choice (package RVAideMemoire, Hervé, 2015). All models included the date as a random effect to control for a potential day effect. For the control of the short demonstration protocol, as no phenotype was preferred during the demonstration, the mate copying index was the proportion of pink chosen during the preference test for the pink demonstration and the proportion of green chosen during the preference test for the green demonstration. To test the effects of air pressure on mate copying, we pooled green and pink demonstrations and used a generalized linear model including demonstration colour, current air pressure, air pressure change and their interaction. When one term was nonsignificant we removed it from the model. Courtship latencies and time between the first wing vibration and copulation were analysed using a linear model followed by a type III ANOVA.

Fig. 5 in the Results was created using the R packages plot3D (Soetaert, 2014) and rgl (Adler et al., 2014).

RESULTS

Experiment 1: Repeated Versus Single Demonstration

When we analysed the results of the whole data set including the two types of protocols, we found the mate choice of the observer females depended on the choice of the demonstrator females (logistic regression, Wald test: uninformed, i.e. control,

versus informed females: $\chi^2_1 = 10.261$, $P = 0.001$, $N = 284$; Fig. 3) but not on the type of protocol (logistic regression, Wald test: short versus long demonstration protocol for informed females: $\chi^2_1 = 0.320$, $P = 0.572$, $N = 199$), nor on the interaction between these two factors (logistic regression, Wald test: $\chi^2_1 = 0.155$, $P = 0.694$, $N = 284$; Fig. 3). In both protocols, observer females mated preferentially with the male of the colour phenotype they saw being chosen by the demonstrator females during the demonstration phase, regardless of the colour preferred during the demonstration phase (long demonstration protocol: demonstration with pink, Wald chi-square test: $\chi^2_{41} = 2.917$, $P = 0.006$, $N = 42$; demonstration with green, Wald chi-square test: $\chi^2_{41} = 2.092$, $P = 0.043$, $N = 42$; pink versus green demonstration, logistic regression, Wald test: $\chi^2_1 = 0.529$, $P = 0.467$, $N = 84$; short demonstration protocol: demonstration with pink, Wald chi-square test: $\chi^2_{66} = 2.502$, $P = 0.015$, $N = 67$; demonstration with green, Wald chi-square test: $\chi^2_{47} = 2.240$, $P = 0.030$, $N = 48$; pink versus green demonstration, logistic regression, Wald test: $\chi^2_1 = 0.002$, $P = 0.965$, $N = 115$; Fig. 3; see the Appendix for details of replicates in which only one male courted the observer female).

For the long demonstration protocol, in controls with an opaque glass partition (preventing the observer female from gathering visual information about the mating success of the two male phenotypes; uninformed 1, Fig. 3), no preference was detected (22 copulations with a pink male and 19 with a green male; Wald chi-square test: $\chi^2_{40} = 0.462$, $P = 0.646$, $N = 41$; pink versus green demonstration, logistic regression, Wald test: $\chi^2_1 = 0.141$, $P = 0.707$, $N = 41$) and results of control and treatment groups differed significantly (logistic regression, Wald test: long demonstration protocol versus control: $\chi^2_1 = 6.518$, $P = 0.011$, $N = 125$). The control of the short demonstration protocol was slightly different: observer females were shown one green and one pink male without a demonstrator female during the demonstration phase (uninformed 2, Fig. 3). As in the control of the long demonstration protocol (using an opaque partition), no preference for a phenotype was detected (21 copulations with a pink male and 23 with a green male, Wald chi-square test: $\chi^2_{43} = -0.298$, $P = 0.767$, $N = 44$; pink versus green demonstration, logistic regression, Wald test:

$\chi^2_1 = 0.182$, $P = 0.670$, $N = 44$), and again controls and treatments differed significantly (logistic regression, Wald test: short protocol versus control: $\chi^2_1 = 4.716$, $P = 0.030$, $N = 159$). Finally, the absence of an effect of the long versus short protocol suggests that observer females acquire equivalent mating preferences under the two protocols implying that female *D. melanogaster* can copy a mate preference even after witnessing a single live demonstration, a situation we call 'speed learning'.

Experiment 2: Demonstration with an Ongoing Copulation

In this design, again, observer females mated preferentially with the male of the colour phenotype they had seen copulating with the demonstrator females during demonstrations (demonstration with pink, Wald chi-square test: $\chi^2_{39} = 2.425$, $P = 0.020$, $N = 40$; demonstration with green, Wald chi-square test: $\chi^2_{39} = 2.703$, $P = 0.010$, $N = 40$; pink versus green demonstration, logistic regression, Wald test: $\chi^2_1 = 0.005$, $P = 0.943$, $N = 80$; Fig. 4). Moreover, there was no significant influence of the demonstration duration (which varied according to copulation duration) on the mate copying index (logistic regression, Wald test: $\chi^2_1 = 0.002$, $P = 0.964$, $N = 80$). In the control group with an opaque partition (uninformed females), no mate copying was detected (demonstration with pink, Wald chi-square test: $\chi^2_{41} = -0.305$, $P = 0.762$, $N = 42$; demonstration with green, Wald chi-square test: $\chi^2_{37} = -0.956$, $P = 0.345$, $N = 38$; pink versus green demonstration, logistic regression, Wald test: $\chi^2_1 = 0.245$, $P = 0.621$, $N = 80$, Fig. 4), and controls and treatments differed significantly (logistic regression, Wald test: uninformed control versus informed flies: $\chi^2_1 = 10.566$, $P = 0.001$, $N = 160$). Thus, information extracted from the sole observation of an ongoing copulation was sufficient to elicit a mating preference in the observer females.

Effect of External Parameters

We explored possible covariation of the mate copying index with small remaining variations in temperature and humidity. In experiment 1, for both protocols, we did not find any significant

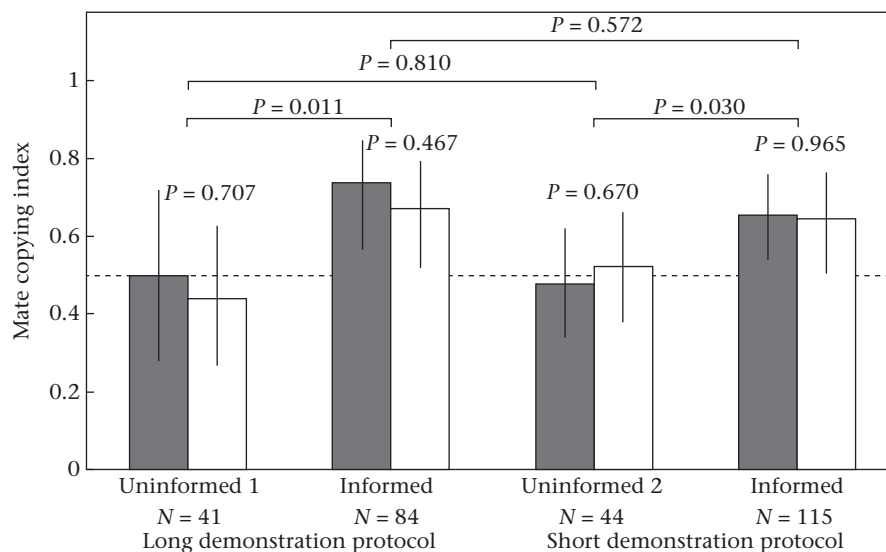


Figure 3. Mate copying index according to the demonstration protocols (see Fig. 1). The mate copying index was the proportion of observer females that copied the choice of demonstrator females. The two protocols differed in their demonstration durations (3 h versus 30 min) and demonstration type (sequential versus simultaneous). For each protocol, grey bars show the mate copying index of flies that saw a demonstration in which the pink male was preferred and white bars the mate copying index of flies that saw the green male being chosen. In the controls, the observer flies did not see any demonstrator female choice either because the partition between compartments was opaque (uninformed 1, $N = 41$) or because there were only two males without any female in the demonstration compartment (uninformed 2, $N = 44$). P values are given for comparisons between two conditions. Vertical bars: 95% Agresti–Coull confidence intervals; horizontal dashed line: expected value under random choice.

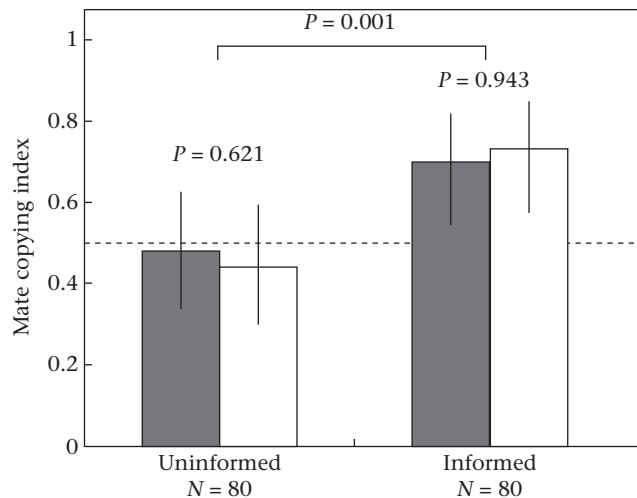


Figure 4. Mate copying index in experiment 2. In this experiment, demonstrations consisted of an already formed pair plus a male of the other phenotype so that the observer female only saw copulation but not the preceding courtship. In this design, the colour chosen during the demonstration was thus entirely controlled by the experimenters. Grey bars: mate copying index of flies that saw a demonstration in which the pink male was preferred; white bars: mate copying index of flies that saw the green male being chosen. In controls, the observer flies did not see any demonstrator female choice because the partition was opaque (uninformed, $N = 80$). P values are given for comparisons between two conditions. Vertical bars: 95% Agresti–Coul confidence intervals; horizontal dashed line: expected value if males were chosen randomly.

correlation of the mate copying index either with temperature (logistic regression, Wald test: long demonstration protocol: $\chi^2_1 = 0.04$, $P = 0.842$, $N = 84$; short demonstration protocol: $\chi^2_1 = 0.099$, $P = 0.753$, $N = 115$) or with humidity (logistic regression Wald test: long demonstration protocol: $\chi^2_1 = 0.874$, $P = 0.350$, $N = 84$; short demonstration protocol: $\chi^2_1 = 1.47$, $P = 0.225$, $N = 115$). Similar results were found in experiment 2 (logistic

regression, Wald test: temperature: $\chi^2_1 = 3.572$, $P = 0.059$, $N = 80$; humidity: $\chi^2_1 = 1.588$, $P = 0.208$, $N = 80$).

In experiment 1, for the long demonstration protocol, there was no significant effect of the demonstration colour (logistic regression, Wald test: $\chi^2_1 = 0.429$, $P = 0.513$, $N = 84$) and no significant effect of current or change in air pressure on the mate copying index (logistic regression, Wald test: $\chi^2_1 = 0.006$, $P = 0.937$; $\chi^2_1 = 0.684$, $P = 0.408$, respectively, $N = 84$). However, for the short demonstration protocol, the interaction term between current air pressure and air pressure change covaried with the mate copying index (logistic regression, Wald test: $\chi^2_1 = 6.629$, $P = 0.010$, $N = 115$) and similarly there was no effect of the demonstration colour (logistic regression, Wald test: $\chi^2_1 = 0.172$, $P = 0.678$, $N = 115$). Replicates performed in high and increasing air pressure led to significantly higher mate copying indices than those in other conditions. We then investigated the effects of air pressure and air pressure change on other aspects of mating behaviour, including replicates from the control groups and discarded replicates. We did not find any significant effect of current air pressure or air pressure change (logistic regression, Wald test: $\chi^2_1 = 1.004$, $P = 0.316$; $\chi^2_1 = 0.582$, $P = 0.445$, respectively, $N = 1015$) on the number of failed copulations in both protocols. However, current air pressure and air pressure change covaried with courtship latency, i.e. the time until the first wing vibration in the preference test (ANOVA: $F_{1,244} = 9.977$, $P = 0.002$; $F_{1,244} = 4.303$, $P = 0.039$, respectively; protocol effect: $F_{1,244} = 0.089$, $P = 0.766$; demonstration effect: $F_{1,244} = 2.257$, $P = 0.134$; $N = 249$). This latency was significantly shorter in high and increasing pressures (for example, mean latency = 157.9 ± 19.8 s, $N = 51$ when the air pressure was >1013 hPa and increasing, versus 256.03 ± 29.2 s, $N = 36$ when the air pressure was <1013 hPa and decreasing). We did not detect any significant relationship between air pressure and the time between the first wing vibration and copulation in the preference test (ANOVA: current air pressure: $F_{1,246} = 0.0002$, $P = 0.988$; air pressure change: $F_{1,246} = 0.001$, $P = 0.970$; $N = 249$), which is mainly under female control (for example, mean time = 219.5 ± 25.5 s, $N = 51$ when the air pressure was >1013 hPa and increasing, versus 210.4 ± 36 s, $N = 36$ when the air pressure was <1013 hPa and decreasing). The female can indeed accept a courting male by slowing down its walk and allowing the male to mount (Kimura, Sato, Koganezawa, & Yamamoto, 2015) or reject it using various methods such as decamping, kicking the male or extruding its ovipositor (Connolly & Cook, 1973).

In experiment 2, similar results were found, as in the short protocol: the interaction term between current air pressure and air pressure change covaried with the mate copying index (logistic regression, Wald test: $\chi^2_1 = 8.044$, $P = 0.005$, $N = 80$) and there was no effect of the demonstration colour (logistic regression, Wald test: $\chi^2_1 = 0.189$, $P = 0.664$, $N = 80$). Regarding courtship latency in the preference test, there was no significant correlation of current air pressure or air pressure change (ANOVA: $F_{1,157} = 2.935$, $P = 0.089$; $F_{1,157} = 1.262$, $P = 0.263$, respectively; $N = 160$). As in experiment 1, we did not detect any significant correlation between air pressure and time between the first wing vibration and copulation in the preference test (ANOVA: current air pressure: $F_{1,157} = 0.014$, $P = 0.905$; air pressure change: $F_{1,157} = 0.253$, $P = 0.615$, $N = 160$).

We then pooled the data of experiments 1 and 2, obtained with the short demonstration protocol (Fig. 5), and modelled the effect of air pressure on the mate copying index. In the pooled data set, there was no significant effect of the type of experiment (logistic regression, Wald test: $\chi^2_1 = 0.205$, $P = 0.651$, $N = 195$) but there was a significant effect of current air pressure and air pressure change on the mate copying index (logistic regression, Wald test: $\chi^2_1 = 8.972$, $P = 0.003$; $\chi^2_1 = 6.363$, $P = 0.012$, respectively; $N = 195$; Fig. 5).

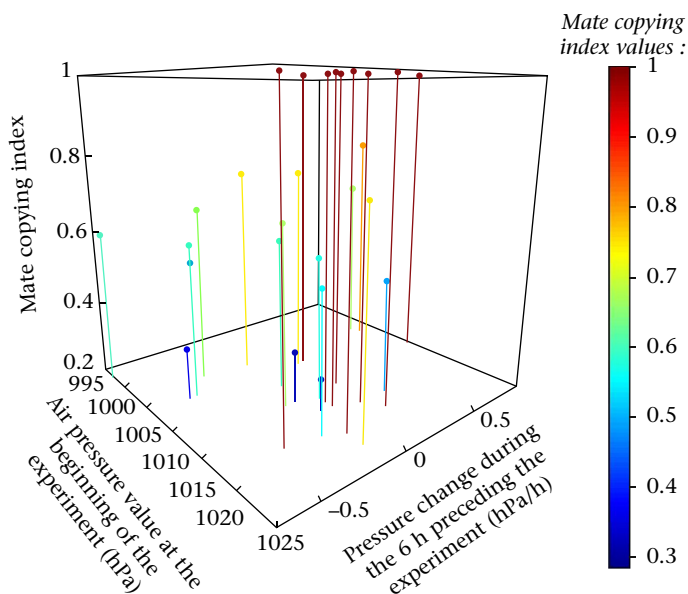


Figure 5. Mate copying index according to current air pressure and its change during the 6 h preceding the experiment in the short demonstration protocol (experiments 1 and 2 pooled, $N = 195$). The mate copying index was the proportion of observer females that copied the choice of demonstrator females. It was calculated for each pair of pressure (hPa) and pressure variation (hPa/h) values.

DISCUSSION

Our study confirms that *D. melanogaster* can perform mate copying as reported in an earlier study (Mery et al., 2009). We further show that *D. melanogaster* females can perform mate copying after witnessing only a single live mate choice by another female, and that the level of mate copying is similar under the long and the two short protocols, even though the quantity of available information differed drastically between them (Fig. 1). This reveals an unsuspected capacity for social learning and mate copying in this species.

Acquiring a Preference in a Speed Learning Context

In the short demonstration protocol of experiment 1, demonstrator females chose freely between two males of contrasting phenotypes; thus observer females were not exposed to a long and possibly artificial sequence of copulations and rejections. One technical drawback of this short demonstration protocol is the impossibility to control the colour chosen during the demonstration phase, so that a minor preference for one colour might distort the copying process. However, no such tendency was detected in our experiments. Moreover, the results of experiment 2, where the choice of the demonstrator female was entirely controlled, corroborated those of experiment 1. The results of these two experiments strongly support the interpretation that, in our set-up, observer female preference was driven by the final choice of the demonstrator female, i.e. by the copulation itself. Too little is known about the use of social information by *D. melanogaster* in laboratory or wild populations to extrapolate these results to situations in which observer females would not be confined close to another mating and at the same time prevented from directly interacting with the demonstrator males. Nevertheless, the fact that observing a single copulation with a male of a given phenotype can induce a preference in the observer female suggests that this kind of observational learning may also occur in nature. Finally, our protocol (like most mate copying protocols) does not enable us to distinguish between females copying mate preference (one male is chosen) and/or mate rejection (one male is rejected; Dagaëff, 2015; Vakirtzis, 2011). To our knowledge, only one study has shown that mate copying based on negative social information alone can also function (Witte & Ueding, 2003). However, we can consider that the use of both positive (acceptance) and negative (rejection) social information in effect reveals mate copying as they both inform on potential mate quality.

Meteorological Effect on Behaviour

Temperature, humidity and photoperiod, but not air pressure, were controlled in our experimental room. We thus suspected air pressure would be responsible for any meteorological effects on *Drosophila* behaviour. We found a strong correlation between the mate copying index and air pressure (current air pressure and recent change) in the short demonstration protocol. Even though we did not detect any effect of air pressure on the occurrence of mating in our experiments, mate copying was more likely in high and increasing air pressure, that is, according to meteorological studies (Ahrens, 2009), in good and/or improving meteorological conditions.

The correlation between air pressure and mate copying was only significant in the short demonstration protocol, although a nonsignificant tendency of the same type was observed in the long protocol. This suggests that the effects of air pressure might be somehow overcome in the long demonstration protocol where positive and negative social information were repeated and thus reinforced for 3 h.

In humans, weather has been shown to influence behaviour and learning. Good weather, high temperatures, air pressure and sunlight improve mood (Keller et al., 2005) but decrease eyewitness memory (Forgas, Goldenberg, & Unkelbach, 2009), while children are more focused on the completion of tasks in stable than variable weather (Ciucci et al., 2012). Moreover, the concentration of university students is negatively affected by an increase in humidity and a drop in air pressure (Howarth & Hoffman, 1984). Very little is known about the effect of weather on cognitive abilities of other animals. Most studies have focused on activity rates which decrease in low air pressure conditions (Malechek & Smith, 1976; Metcalfe et al., 2013; Théau & Ferron, 2000). In insects, for instance, air pressure affects flight activity (Fournier, Pelletier, Vigneault, Goyette, & Boivin, 2005; Rousse, Gourdon, Roubaud, Chiroleu, & Quilici, 2009) and mating behaviour (Ankney, 1984; Austin et al., 2014; Pellegrino et al., 2013). Overall, insects seem to show higher activity levels in good weather (Paige, 1995; Wellington, 1946), that is in high air pressure conditions.

The fact that in our speed learning design mate copying was more prevalent in good or improving meteorological conditions suggests that accounting for air pressure and more generally external conditions might be necessary in studies of insect behaviour. The effect on behaviour may be either direct or indirect, for instance through an effect on activity, potentially impacting information gathering. This latter interpretation seems supported by the fact that in both protocols males started courting more rapidly in good weather conditions and by the fact that the long demonstration protocol seemed to 'overcome' the effect of external conditions. In terms of adaptation, we may speculate that the strong relationship between air pressure and mate copying may be a direct consequence of the predictive value of air pressure to forecast weather. In particular, it is well known that low and declining air pressure indicates the coming of weather conditions that are extremely unfavourable to fruit flies. We can thus imagine that flies have been selected to hide and focus on survival when the air pressure gets too low, which, in turn, should affect their cognition. As a consequence, mate copying (like mating) might be one of the many behavioural patterns that are affected by air pressure, for diverse reasons (be they mechanistic or adaptive). However, we think that it is still premature to claim that this is the correct scenario as, for instance, Ankney (1984) and Austin et al. (2014) found contrasting results regarding the effect of air pressure on the intensity of mating behaviour.

In addition, our results are even more surprising in that the Canton-S strain used in our experiments has now been raised in the laboratory for thousands of generations in conditions where air pressure is no longer associated with weather experienced in the protective laboratory conditions. As our study of the potential effects of air pressure relied on correlative data, it will be particularly valuable in the future to experimentally manipulate air pressure to assess the causal role of this factor on *Drosophila* behaviour and cognitive abilities.

Conclusion

We have shown that *D. melanogaster* can perform mate copying even in a speed learning context, and that this behaviour seems more frequent under good and improving weather conditions. Although little is known about the ecology of *Drosophila* in the wild, we can speculate that matings occur under such good or improving weather conditions, providing females opportunities to copy mate choices of others. The importance of these mate copying abilities in the field, and their potential impact on *Drosophila*'s evolution, needs to be further evaluated.

Acknowledgments

We thank two anonymous referees for their valuable comments which helped us to greatly improve this manuscript. We also thank Thomas Achkar, Mélanie Allain, Amelia Landre and Thibaud Roger for their help in the experiments. This study was supported by the French Laboratory of Excellence project 'TULIP' (ANR-10-LABX-41; ANR-11-IDEX-0002-02) and was funded by the IDEX of the Université Fédérale de Toulouse (Transversality grant, ANR-11-IDEX-0002-02 to G.I. and E.D.) and by the Soc-H2 ANR project (ANR-13-BSV7-0007-01 to E.D.) which also provided part of A.C.D.'s salary. G.I. thanks the CNRS for the support of his Excellence Chair. A.C.D. designed the experiments, carried out experiment 1, performed all statistical analyses and drafted the manuscript. A.P. contributed in analyses and in the writing of the manuscript. S.N. carried out experiment 2. A.L. helped in designing experiment 1 and in the writing of the manuscript. G.I. and E.D. supervised all steps in the process. All authors gave final approval for publication. The authors have no competing financial interests to declare.

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Appendix

Pressure Distribution on Experimental Days

The long demonstration protocol was undertaken on 47 different days distributed over 6 months. The short demonstration protocol was undertaken on 32 (distributed over 5 months for experiment 1) and 10 (distributed over 2 weeks for experiment 2) different days. To forecast weather, it is important to look at the absolute pressure value and the pressure changes. Figs. A1–A4 show how our data are distributed according to these parameters.

Current air pressure at the onset of the experiment and air pressure change during the 6 h preceding the start of each replicate were significantly correlated in experiment 1 ($t_{197} = 2.307$, $r = 0.162$, $P = 0.022$) but not in experiment 2 ($t_{78} = -1.362$, $r = -0.152$, $P = 0.177$).

We also explored the links between latencies during the preference test and mate copying. We found that none of them was significantly related to mate copying. Furthermore, these models also had higher Akaike information criterion (AIC) values than the model with air pressure alone (model with courtship latency: $\chi^2_1 = 0.102$, $P = 0.75$, $AIC = 247.77$; copulation latency: $\chi^2_1 = 0.520$, $P = 0.471$, $AIC = 247.35$; courtship duration: $\chi^2_1 = 1.261$, $P = 0.261$, $AIC = 246.61$; compared to an AIC of 237.22 with air pressure alone).

Air Pressure at the Airport and our Weather Station

We used data from the airport station because it collects the air pressure data continuously and thus even during the night. Our own recording would not have allowed us to estimate the change in air pressure during the 6 h preceding the experiment. Before any analyses, we first compared measures from our own weather

station with those at the airport station (which is located 11 km away) and checked that measurements were highly correlated ($t_{277} = 169.7024$, $r = 0.995$, $P < 2.2e-16$; see Fig. A5).

2D Figures of the Effect of Barometric Pressure on Mate Copying

Figs. A6 and A7 use the same data as Fig. 5 in the main text, but split into two 2D graphs.

Replicates in Which Only One Male Courted the Observer Female

In the study, we only kept replicates in which both males courted the female, as observer females courted by only one of the two males were not in a position to choose between them.

While the decision to court or not is under male control, the time between courtship and copulation is under female control. Thus, one could wonder whether the observer female would accept more quickly a male whose phenotype is similar to that preferred during the demonstration phase.

We found that, in experiment 1, the male of the phenotype preferred during the demonstration phase did not copulate more quickly than the male of the other phenotype (ANOVA: long demonstration protocol: $F_{1,202} = 0.855$, $P = 0.356$, $N = 204$ because 33 latencies were not recorded; short demonstration protocol: $F_{1,169} = 2.045$, $P = 0.155$, $N = 171$). Similar results were found in experiment 2 (ANOVA: $F_{1,80} = 0.063$, $P = 0.803$, $N = 82$ because three beginnings of courtship were not seen).

Number of Replicates in Each Experiment

Table A1 gives the number of replicates used in each experiment.

Table A1

Number of replicates according to the experiment (1 or 2) and the demonstration protocol (short or long)

Experiment type	Protocol type	Group type	Number of courtships during the test
Experiment 1 N=1015	Long demonstration protocol N=543	Control group N=197	Without courtship or mating N=31 With 1 courtship N=125 With 2 courtships N=41
		Experimental group N=346	Without courtship or mating N=25 With 1 courtship N=237 With 2 courtships N=84
	Short demonstration protocol N=472	Control group N=146	Without courtship or mating N=32 With 1 courtship N=70 With 2 courtships N=44
		Experimental group N=326	Without courtship or mating N=40 With 1 courtship N=171 With 2 courtships N=115
Experiment 2 N=430	Short demonstration protocol N=430	Control group N=250	Without courtship or mating N=17 With 1 courtship N=153

(continued on next page)

Table A1 (continued)

Experiment type	Protocol type	Group type	Number of courtships during the test
		Experimental group N=180	With 2 courtships N=80
			Without courtship or mating N=15
			With 1 courtship N=85
			With 2 courtships N=80

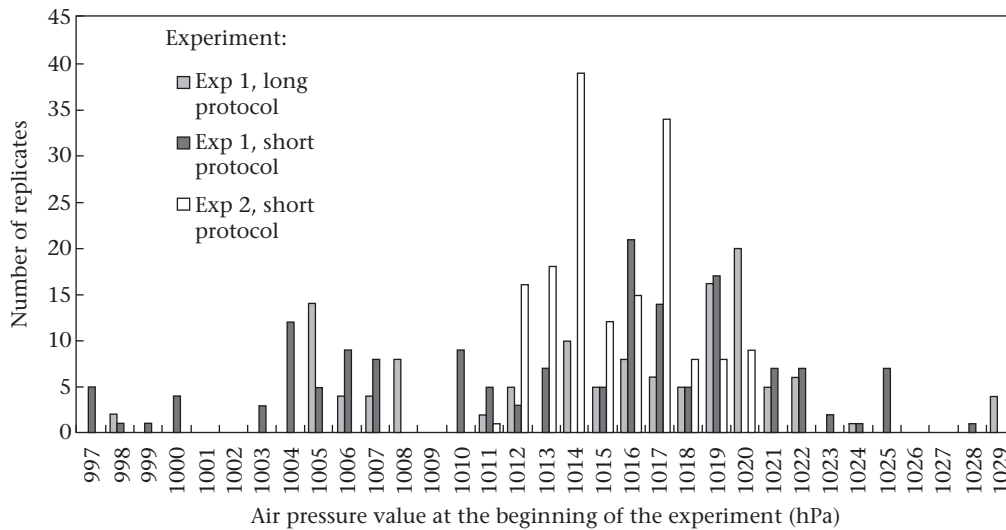


Figure A1. The number of replicates according to the demonstration protocol and the barometric pressure at the beginning of the experiment. The number of replicates corresponds to the number of flies kept for each protocol and experiment.

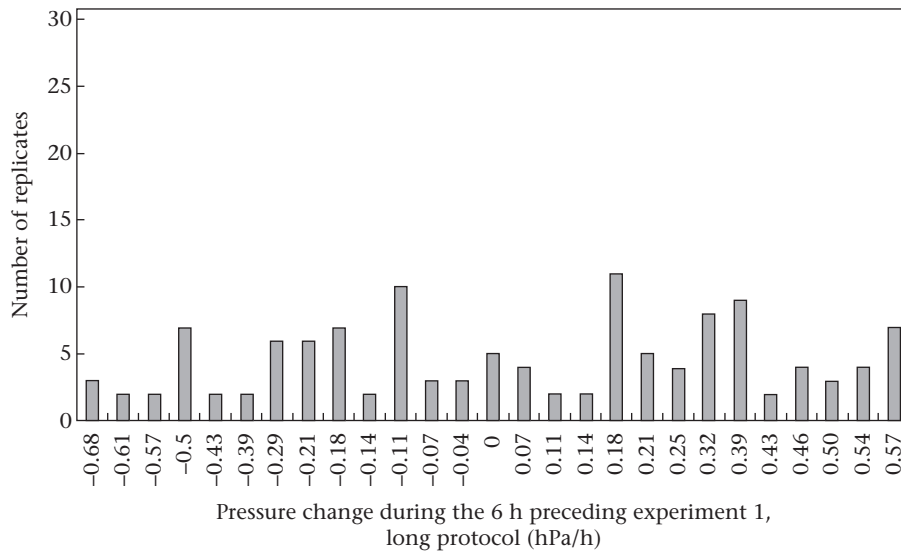


Figure A2. The number of replicates according to air pressure change during the 6 h preceding experiment 1, long protocol. The pressure variation index was calculated as described in the [Methods](#).

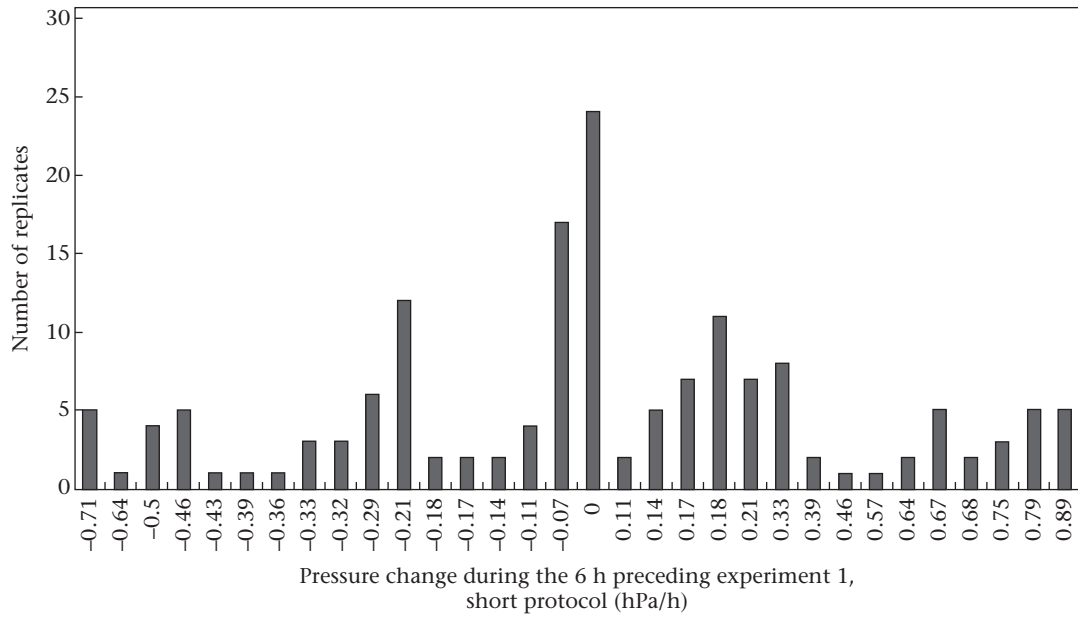


Figure A3. The number of replicates according to air pressure change during the 6 h preceding experiment 1, short protocol. The pressure variation index was calculated as described in the [Methods](#).

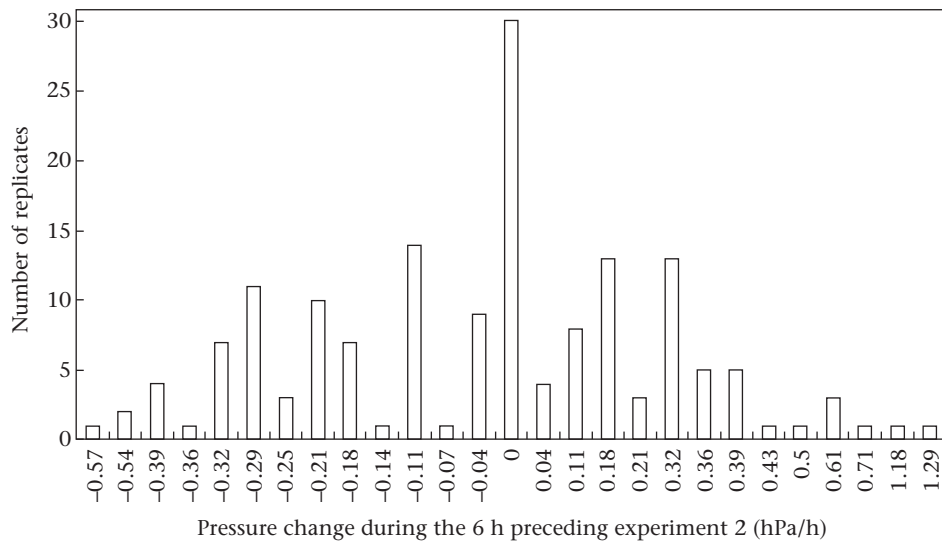


Figure A4. The number of replicates according to air pressure change during the 6 h preceding experiment 2. The pressure variation index was calculated as described in the [Methods](#).

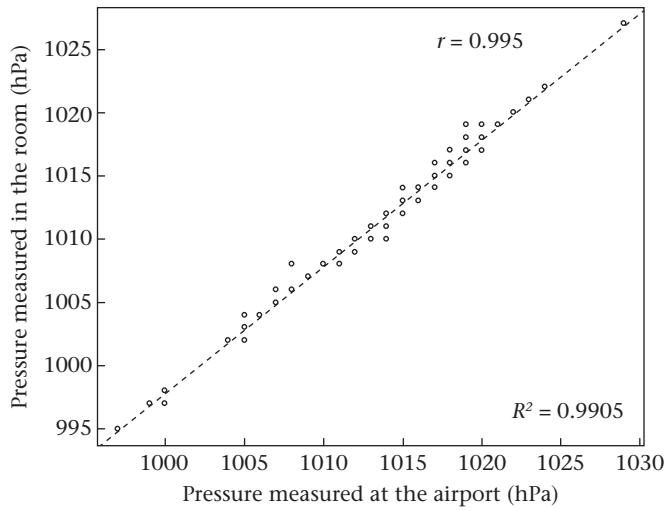


Figure A5. Correlation between the pressure measured in the experimental room and the pressure recorded at the airport.

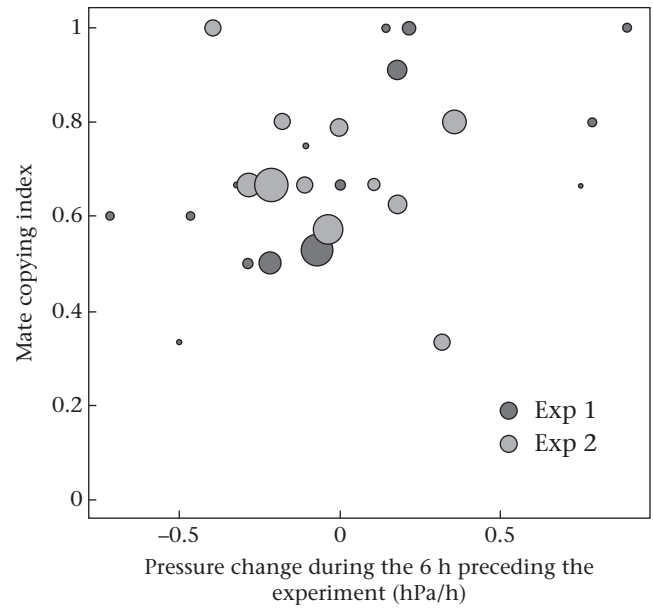


Figure A7. Mate copying index according to air pressure change during the 6 h preceding the experiment in the short demonstration protocol (experiments 1 and 2 pooled, $N = 195$). The mate copying index was the proportion of observer females that copied the choice of demonstrator females. It was calculated for each pressure variation (hPa/h). The dark grey circles represent data of experiment 1 and the light grey circles data of experiment 2. The area of one point is proportional to the number of replicates represented.

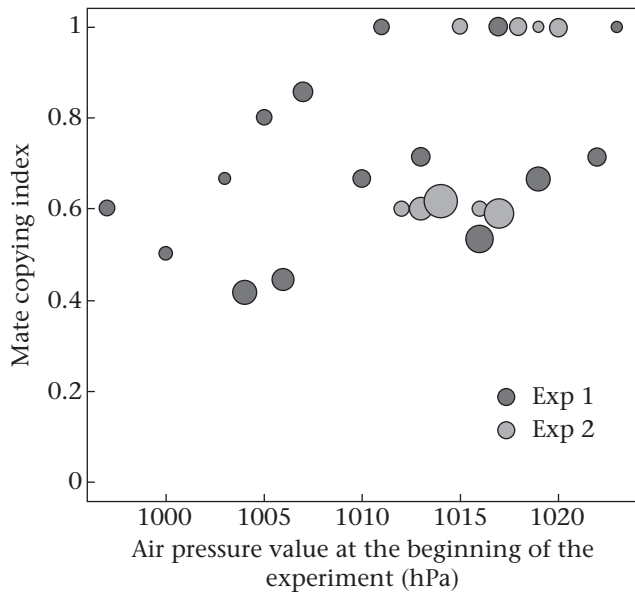


Figure A6. Mate copying index according to current air pressure in the short demonstration protocol (experiments 1 and 2 pooled, $N = 195$). The mate copying index was the proportion of observer females that copied the choice of demonstrator females. It was calculated for each pressure value (hPa). The dark grey circles represent data of experiment 1 and the light grey circles data of experiment 2. The area of one point is proportional to the number of replicates represented.