

ANIMAL CULTURE

Cultural flies: Conformist social learning in fruitflies predicts long-lasting mate-choice traditions

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Despite theoretical justification for the evolution of animal culture, empirical evidence for it beyond mammals and birds remains scant, and we still know little about the process of cultural inheritance. In this study, we propose a mechanism-driven definition of animal culture and test it in the fruitfly. We found that fruitflies have five cognitive capacities that enable them to transmit mating preferences culturally across generations, potentially fostering persistent traditions (the main marker of culture) in mating preference. A transmission chain experiment validates a model of the emergence of local traditions, indicating that such social transmission may lead initially neutral traits to become adaptive, hence strongly selecting for copying and conformity. Although this situation was suggested decades ago, it previously had little empirical support.

Researchers increasingly acknowledge that cultural traditions exist in nonhuman animals, including chimpanzees (1), orangutans (2), cetaceans (3), meerkats (4), and birds (5). However, thus far, examples have been limited to higher vertebrates. Exploration of this question in other taxa requires a transferable definition of culture. The typical criterion of culture is generally that transferred traits must be socially acquired and spread to others repeatedly (6).

Here, we propose a definition focusing on the properties of social learning. Integrating with previous studies, we define animal culture as phenotypic variation that is inherited through a form of social learning (i.e., learning from others) (criterion 1) (1, 5–9). Cultural inheritance will occur if social learning occurs across age classes (minimally, from older to younger individuals) (criterion 2) (9, 10), is maintained over the long term to be copied (criterion 3) (11), produces trait-based copying (criterion 4) (12), and incorporates repair or reinforcement mechanisms (13) [e.g., conformity (5, 14, 15) or information digitalization (16)] (criterion 5). Lastly, to connect this mechanistic definition with classical definitions focusing on the sole

existence of behavioral variation across populations, we tested whether the observed cognitive properties can generate local traditions spanning over generations (the most notable marker of culture) (13, 17). Cultural inheritance then makes cultural variation subject to selection and evolution.

We tested this multicriterion definition in fruitflies, which are known to have the capacity to socially learn sexual preferences from the observation of copulating conspecifics (18–20).

To test criterion 1 of social learning, we used the “speed-learning” design (Fig. 1) (20), testing whether, after watching a single demonstrator female choosing between two males of contrasting phenotypes, an observer female shows a bias for the male of the phenotype she saw being chosen during the demonstration. This two-step protocol involves a demonstration in a tube device (fig. S1) during which an observer female separated by a glass partition can watch a demonstrator female freely choosing between

one green and one pink male, immediately followed by a mate-choice test during which the observer female chooses to copulate with one of two new males, one of each color. The partition was transparent glass (informed females) or opaque white paper (uninformed control females).

The social learning index quantifying the learned bias toward the male of the color preferred during demonstrations (see S1.4 in the supplementary materials) differed between informed and uninformed replicates [generalized linear mixed model (GLMM), Wald χ^2 test; $n = 127$; $\chi^2_1 = 5.115$; $P = 0.024$] (Fig. 1B). Uninformed observer females chose in a way that did not differ from random (binomial test; $n = 63$; $P = 1$). Informed females mated preferentially with new males of the color they saw being chosen during the demonstration (binomial test; $n = 64$; $P = 0.002$) (Fig. 1B) whatever the color chosen during the demonstration (GLMM, Wald χ^2 test; $n = 127$; $\chi^2_1 = 0.0112$; $P = 0.916$). Thus, observer females learned to prefer the male of the color that was favored during demonstrations, exhibiting social learning and fulfilling criterion 1.

To satisfy criterion 2 of transmission across age classes, socially learned traits must be transmitted vertically or simply from older to younger individuals (9, 10). We replicated in tubes the horizontal informed treatment of criterion 1 (in which both females were 3 days old) as a positive horizontal control and compared it with an across-age-class treatment in which demonstrator females were 11 days older (i.e., of an age similar to that of the flies’ parents) (Fig. 2).

Both treatments were biased in favor of the male of the color that copulated during demonstrations (binomial tests; $n = 65$, $P = 0.025$, and $n = 63$, $P = 0.011$ for horizontal and across-age-class treatments, respectively) (Fig. 2). We found no difference between horizontal and across-age-class trials (GLMM, Wald χ^2 test; $n = 128$; $\chi^2_1 = 0.0555$; $P = 0.814$), showing that social transmission was equally efficient in the two contexts and thus fulfilling criterion 2.

To satisfy culture criterion 3, that of durability, learned preferences must be maintained

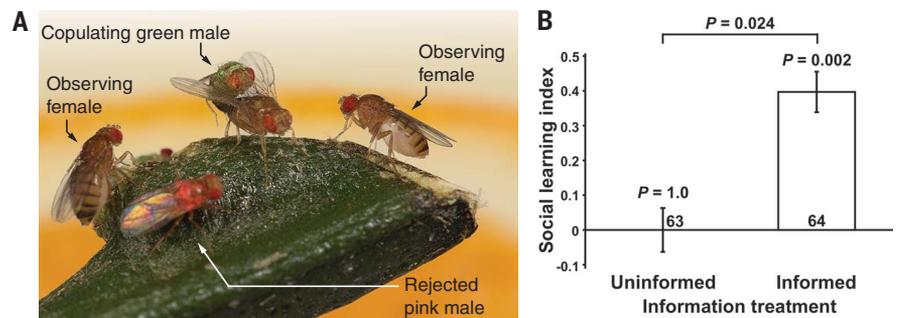


Fig. 1. Criterion 1 of social learning. (A) A situation of mate-copying in which two females watch a copulating green male while a pink male is rejected. (B) Social learning index of informed versus uninformed observer females. Positive social learning indices reveal preference for the male color chosen during demonstrations, whereas zero reveals random choice. P values above bars, binomial tests of departure from random choice; error bars, SEM.

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(11). Multiple spaced training (a series of training phases separated by resting periods) (21) in invertebrates and vertebrates leads to a stable form of long-lasting memory (22). We transposed this long-term memory protocol in *Drosophila* (23) to our visual social learning. After watching five conditioning demonstrations spaced by 15- to 30-min resting periods, observer females were tested for social long-term memory 24 hours later.

Informed spaced-trained flies displayed unusually high mate-copying after 24 hours (binomial test; $n = 62$; $P < 0.0001$) (Fig. 3A), but the uninformed ones did not (binomial test; $n = 65$; $P = 0.457$) (Fig. 3A). To confirm that this social long-term memory depends on de novo protein synthesis, a third group of spaced-trained flies fed an inhibitor of protein synthesis (cycloheximide) was tested in parallel. The cycloheximide treatment disrupted social long-term memory [$n = 65$, $P = 0.804$ (binomial test); $n = 192$, $\chi^2_1 = 15.6934$, $P = 0.0004$ (GLMM for the three treatment groups tested after 24 hours, Wald χ^2 test)] (Fig. 3A). Furthermore, cycloheximide-treated observer females tested shortly after a single demonstration did not differ from the horizontal control for criterion 2 [GLMM for informed cycloheximide-treated females versus non-cycloheximide-treated females of the horizontal control (Fig. 2); $n = 131$; $\chi^2_1 = 0.016$; $P = 0.898$ (Fig. 3B)] and showed significant mate-copying (binomial test; $n = 66$; $P = 0.036$) (Fig. 3B). Thus, the cycloheximide treatment did not impair mate-copying (21, 23). In a complementary experiment, we found that the high average learning index 24 hours after a spaced training (0.55, corresponding to a mate-copying index of 0.78) was produced by both spaced training and the 24-hour delay (see fig. S2). Thus, flies built de novo protein synthesis-dependent durable memory, meeting criterion 3.

Criterion 4 states that copying must be trait based (11, 12). In all the experiments described above [and most previous experiments (18, 20, 24, 25)], mate-choice tests used new green and pink males, suggesting that observer females learned to prefer any male of the same color. However, observer females may have confounded test males with demonstration males of the same color. To test whether females learned to prefer males of a given color, we used very different looking mutant males during mate-choice tests to rule out the possibility that observer females confounded test males with demonstrator males. Demonstrations involved a wild-type demonstrator female freely choosing between one green and one pink wild-type male, whereas males (green and pink) used in mate-choice tests were either both wild type (controls) or both curly-winged or white-eyed mutants (two experimental treatments). In a previous study, we showed that flies exhibit mate-copying with wild-type and curly-winged males instead of color variants, demonstrating that the flies do distinguish these genetic variants (26).

In all three treatments, during the mate-choice test observer females preferred males of the same color as the one chosen during demon-

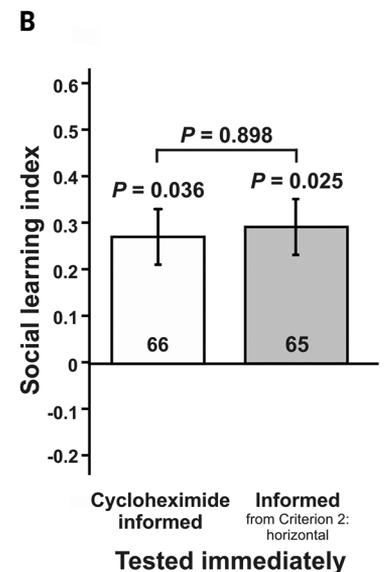
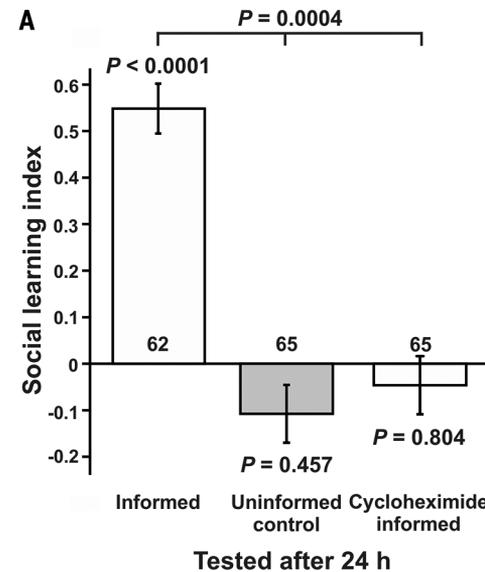
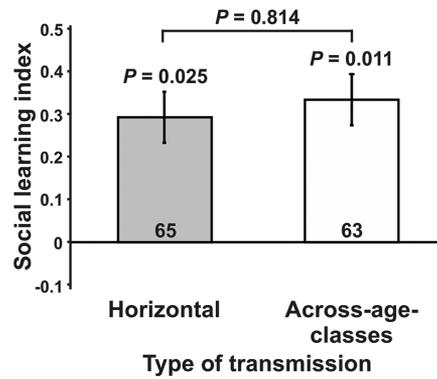


Fig. 3. Criterion 3 of durable social learning. (A) Long-term memory in mate-copying 24 hours after demonstrations. (B) Cycloheximide-treated females within the usual protocol of a single live demonstration immediately followed by the mate-choice test showed mate-copying similar to that of the non-cycloheximide-treated females of the horizontal-transmission group in Fig. 2. *P* values above bars, binomial tests of departure from random choice; error bars, SEM.

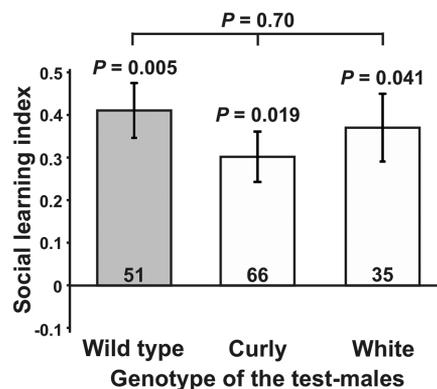


Fig. 4. Criterion 4 of trait-based copying. Social learning indices according to the genotype of the males used during mate-choice tests. Demonstrations involved a wild-type demonstrator female freely choosing between one green and one pink wild-type male. We previously showed that females clearly distinguish wild-type from curly-winged genetic variants (26). *P* values above bars, binomial tests of departure from random choice; error bars, SEM.

strations, with similar social learning indices (GLMM, genotype effect; $n = 152$; $\chi^2_2 = 0.714$; $P = 0.70$) (Fig. 4), despite their contrasting and distinguishable (26) genotypes relative to those of demonstrator males. Females performed trait-based copying, therefore meeting criterion 4.

Criterion 5 concerns the existence of a repair mechanism such as a conformist bias (an exaggerated tendency to copy the majority) (5, 14, 27, 28). To test this, we used a new device we called “the hexagon” (fig. S1B). By introducing already-copulating pairs along with a

Fig. 5. Criterion 5 of conformist mate-copying. The nine demonstration treatments with various proportions of demonstrator females copulating with the pink males (x axis). Level of majority, proportion of the most commonly chosen male color during demonstrations; error bars, SEM; P values above or below bars, binomial tests of departure from random. The four treatments with a majority of pink (P) demonstrations differed from controls ($n = 348$; $P < 0.0004$). So did the four treatments with a majority of green (G) demonstrations ($n = 333$; $P = 0.042$), and these two blocks differed from each other ($n = 513$; $P < 0.0001$).

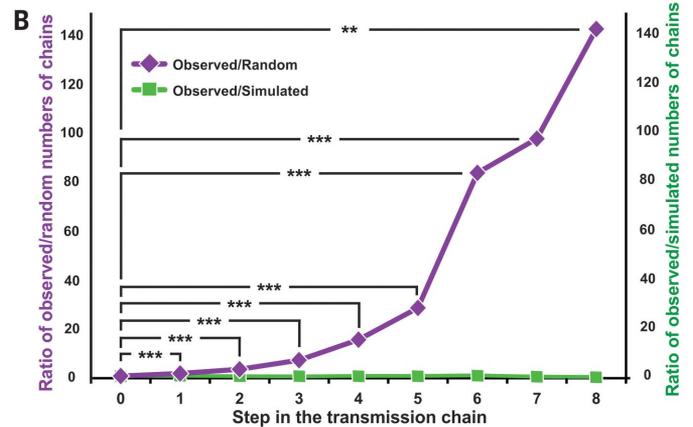
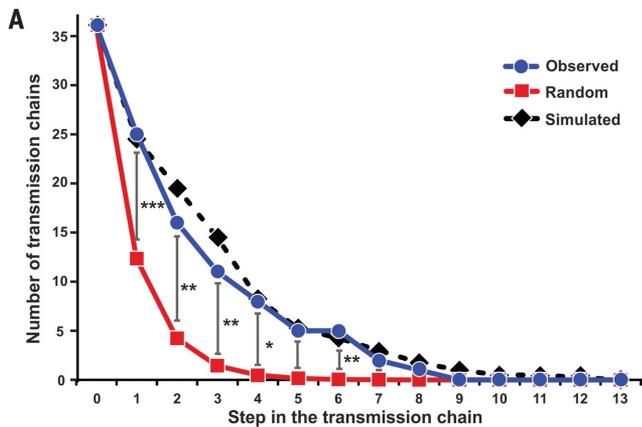
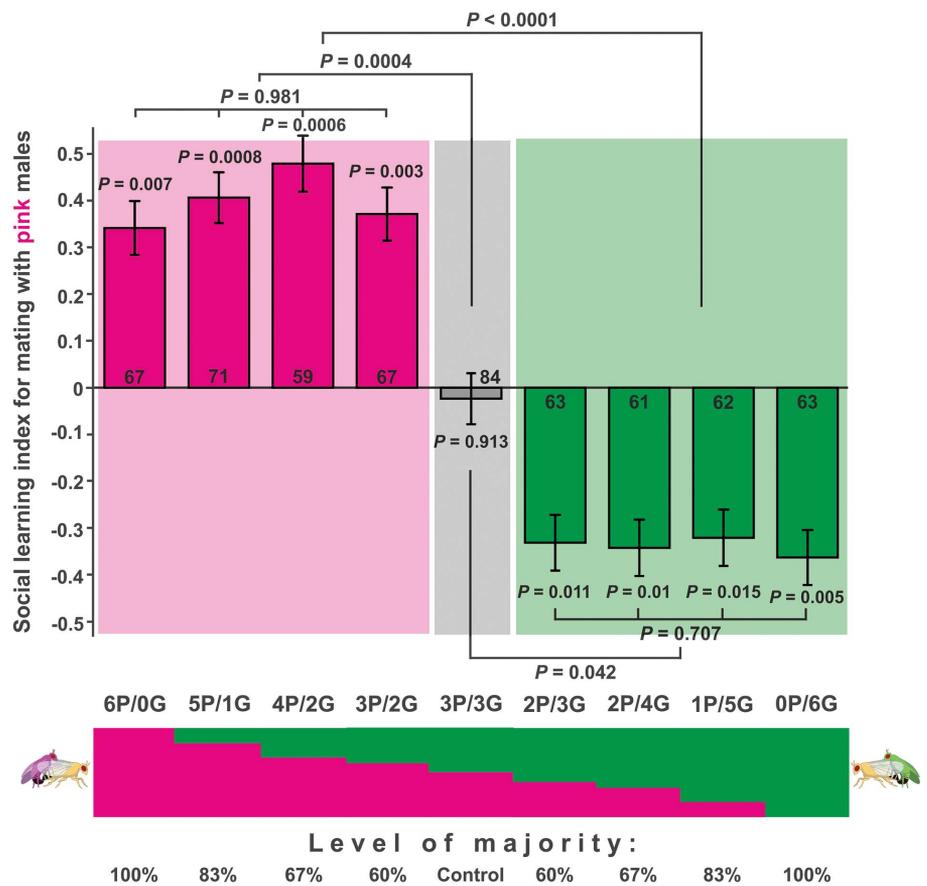


Fig. 6. Transmission chain in which observer females of one step became demonstrators for the next step. (A) Number of the 36 chains that kept the initial preference for the indicated number of steps. Both experimental and simulation data involved only six observer females, which explains the relatively short persistence of the population preference (arrow in Fig. 7B). For each step, asterisks indicate the significance of the pairwise binomial test between the observed number of chains reaching that step (blue) and the number expected under random choice (red). Simulated data (black) were obtained under conditions mimicking experimental chains (see S1.5 and S1.6). Asterisks indicate the significance of the binomial test between

the observed and expected numbers of chains reaching the step in view of the number that reached the previous step. (B) Ratio of the observed number of chains reaching the indicated step to either the number predicted under random choice (purple) or the number produced by simulations (green). Asterisks indicate the significance of the binomial test between the observed number of chains reaching that step and the number predicted by chance from the initial number of 36 chains under the null hypothesis that females choose randomly (i.e., binomial test against a probability of 0.3438^x , where x is the step number) (see S1.5 and table S2). * $P < 0.05$; ** P value < 0.01 ; *** $P < 0.001$. More information is provided in S1.5 and table S2.

Fig. 7. A model of local tradition emergence in *D. melanogaster*.

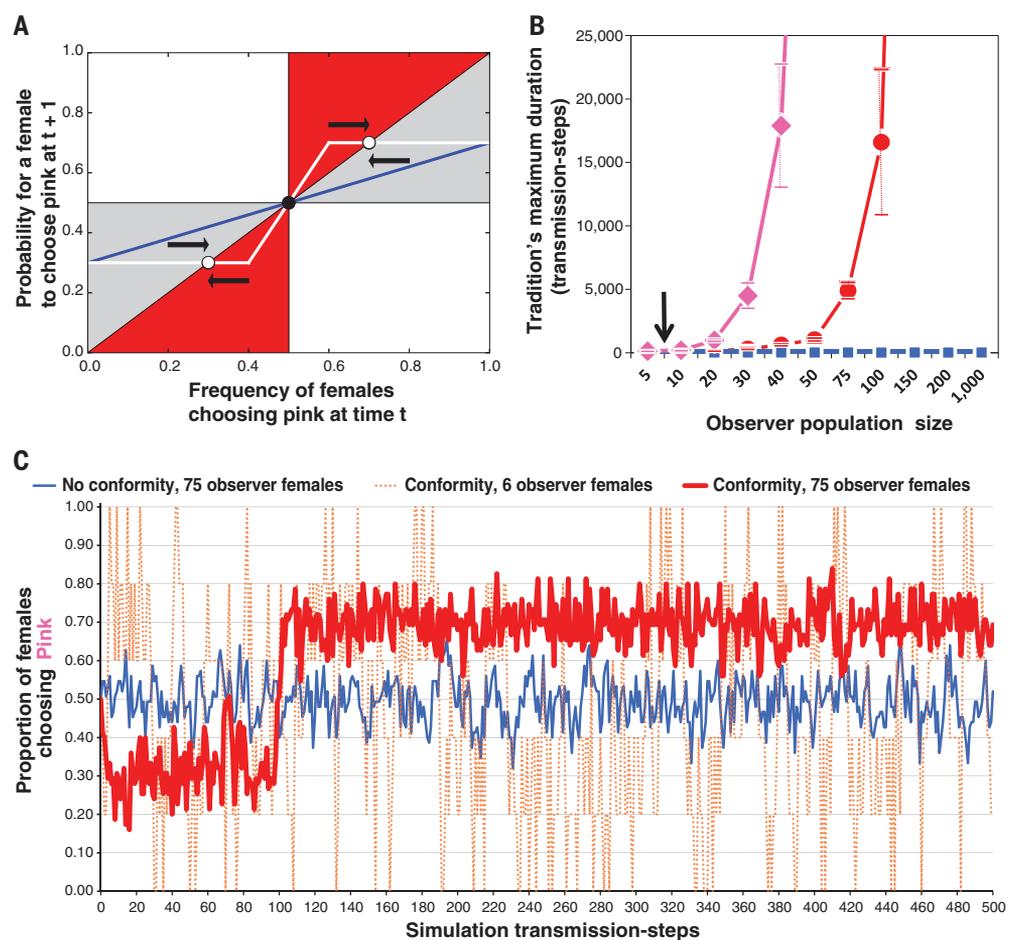
(A) Areas of the set of possible population mating preference response functions to demonstrations. Red areas, areas where observer females copy at a higher rate than the majority (i.e., exaggerate the choice of the majority), driving the population toward a preference for one color (white dots); gray areas, areas where observer females copy at a lower rate than the majority, driving the population toward the stable equilibrium of no preference (black dot); white areas, zones of anticonformity (a bias for males of the most commonly nonpreferred phenotype); blue line, without conformity; white line, the conformity function documented in Fig. 5.

(B) Effect of observer female number and mate-copying index on mean maximum population preference durations during five simulations of 100,000 transmission steps. Blue, simulations without conformity, as in the blue line of (A); red, simulations with conformity, as in the white line of (A), and with the mate-copying index set at 0.68 (i.e., the average in all experiments but long-term memory); pink, same simulations but with a mate-copying index of 0.78, as observed in long-term memory; vertical bars, SDs (most are too small to be visible); arrow, situation of the transmission chain performed in this study (Fig. 6). With transmission steps occurring every 24 hours (Fig. 3), the maximum tradition durations reported with conformity would correspond to thousands of *Drosophila* generations.

(C) Simulated dynamics over 500 transmission steps. Blue, dynamics without conformity [as in the blue line of (A)] with 75 observer females; red, dynamics with conformity with 75 observer females; orange, simulations with conformity and only six observer females, leading to frequent population preference shifts (this mimics the parameter of the transmission chain of Fig. 6). More results are shown in S1.6 and figs. S4 to S6.

male of the other color in each peripheral compartment of the hexagon, we manipulated the proportion of demonstrator females copulating with green or pink males (x axis of Fig. 5). We set up four situations with a majority of females copulating with pink males and four with a majority copulating with green males (100, 83, 67, and 60%), as well as one control in which three females copulated with green males and three with pink males.

As expected, control observer females did not build any mating preference (gray in Fig. 5), and replicates in which all six demonstrator females copulated with the same male color revealed strong mate-copying as in the above-described experiments. More surprisingly, as long as one male phenotype was chosen more often than the other (range, 100 to 60%), observer females learned to prefer males of the most commonly chosen phenotype (every color bar in Fig. 5 significantly differed from zero). We found no significant difference among the four treatments with a majority for one color [for the proportion of females copulating with pink as a class factor



or a continuous effect, $n = 264$ and $P = 0.981$ or 0.813 , respectively (Fig. 5, pink block); for green, $n = 249$ and $P = 0.707$ or 0.920 , respectively], indicating that social learning did not decline as the proportion of the most commonly chosen male decreased down to only 60%. Thus, mate-copying followed a step function, with females learning equally well to prefer the most commonly chosen male color whatever the level of majority (Fig. 5), revealing strong conformity in mate-copying and fulfilling criterion 5.

Fisher (29) speculated that by mating with their preferred males, females produce offspring that may inherit both the male trait and the mating preference in association, triggering the Fisher runaway process (29). In a theoretical population with A and B male phenotypes and in which more females mate with A males, females choosing B males will have sons of the non-preferred phenotype and daughters that will learn to prefer A males (as we show here). This will strongly diminish the fitness of nonconformist females (here choosing B males), thus favoring conformist females (here choosing A males). Con-

sequently, as soon as even the slightest detectable mating preference emerges within a population, conformist females are favored, as they transmit the most attractive trait to their male descendants while potentially culturally transmitting the preference for that same male trait to their daughters and/or to younger females.

Animal culture is classically studied through the existence of local traditions (1–4, 10). To study the capacity of the observed mate-copying in the fruitfly to generate persistent population preferences (i.e., traditions), we performed 36 transmission chains in hexagons in which the six observer females of one step were used as the six freely choosing demonstrators of the following step. A chain started with a demonstration where all six females chose the same male color and ended when the preference for the initially induced population preference became $\leq 50\%$. In this experiment, because the population had only six observer females, the frequency of shifts from a majority of females (four or more) choosing one color at one step to a majority of females choosing the other was high (0.2936) (S1.5 and

table S2). Nonetheless, experimental chains lasted much longer than predicted by chance, as revealed by Fig. 6A, in which the blue curve (representing observed behavior) is significantly higher than the red curve (representing behavior predicted by chance alone). These significant differences at each step accumulated so that the observed number of chains that reached the eighth step was 142 times as high as the number predicted by chance (Fig. 6B; more details are in S1.5). Thus, because of the effect of mate-copying, transmission chains lasted far longer than expected on the basis of chance alone.

The transmission chain experiment indicates that the social learning capacities in *Drosophila melanogaster* have the potential of stabilizing a preference for an arbitrary male phenotype at the scale of a population. However, for practical reasons, we cannot perform experiments under different conditions—for instance, under such conditions as large populations. Thus, to explore theoretically the effects of key parameters documented in testing criteria 1 to 5, we built a dynamical model of culture recapitulating our results. This model simulated a sequence of transmission steps in which observer females of one step became the demonstrators of the following step, as in our transmission chain experiment.

In infinitely large populations, the model is deterministic. A graphical analysis shows that nonconformist observer females (gray areas in Fig. 7A) always adopt an attenuated preference at each transmission step, leading initial preference for one male phenotype to fade away toward the black dot of Fig. 7A and generating dynamics as in the blue curve of Fig. 7C. Contrastingly, conformist learning [usually modeled with response functions entirely within the red areas of Fig. 7A, e.g., as in (27)] reinforces any initial preference, making population preference persistent. In *Drosophila*, however, parts of the response function (white line of Fig. 7A) are outside of this red area, making it difficult to extrapolate results from previous models.

In finite populations, the model is stochastic and cultural drift occurs, much as genetic drift occurs in finite populations. In small populations, odds that 50% or more of the flies make a copying “error” just by chance can be high, each time leading to a cultural shift in the population mating preference (Fig. 7C and figs. S4 to S6) and thus forbidding the establishment of a local tradition. For instance, with a mate-copying index of 0.68 (our observed average) (see S1.6) and six observer females, the probability that at each step at least half of the females choose the incorrect color by chance is 0.2936 (calculation in S1.5). This probability drops rapidly with increasing population size down to <0.001 and <0.0001 with 80 and 114 observer females, respectively (see S1.5). With a mate-copying index of 0.78 (as observed in long-term memory), odds that 50% or more observer flies make a copying error by chance are 0.139 for six flies and drop below 0.001 and 0.0001 with only 32 and 48 observer females, respec-

tively. Thus, the bigger the population and the higher the mate-copying index, the less frequently cultural shifts will occur and the longer the local population preference will persist, making it a cultural tradition.

To study this phenomenon, we built an individual-based model using the fruitfly response function (as in the white line of Fig. 7A). Simulations under the conditions of our transmission chain experiment (six observer flies, the initial preference at 100%, and ending when the initial preference drops to 50% or below) provided distributions of chain durations that closely matched the observed ones (black curve of Fig. 6A), which validates our model. In view of this validation, we then used this model to explore the effect of sets of parameters that would make transmission chain experiments infeasible for the capacity of the documented social learning function to produce persistent population preferences and thus cultural traditions. The form of conformity observed in *Drosophila* elicited long-lasting local preference (red line, Fig. 7C) corresponding to the stable equilibria of the infinite population model, with tradition stability strongly depending on both the population size and the mate-copying index [Fig. 7B and figs. S4 to S6; see also (30)]. For instance, these traditions potentially lasted for more than 100,000 transmission steps with 150 observer flies and a mate-copying index of 0.68 (red curve in Fig. 7B). The same result was obtained with only 50 observer females with a mate-copying index of 0.78 (the value observed with long-term memory) (pink curve in Fig. 7B). These population sizes are well below those observed in nature (S1.6). With one step representing 1 day (as suggested in Fig. 3), this would mean that traditions would theoretically last for thousands of fruitfly generations (see S1.5).

Culture used to be considered to be limited to humans. However, the range of species showing patterns of local variation in behavior akin to traditions now includes several mammals and birds (1–4). In this study, we found that fruitfly females express strong social learning (criterion 1) across age classes (criterion 2) that is memorized for sufficient time to be copied (criterion 3) and is trait-based (criterion 4) and conformist (criterion 5). With a model parameterized with the properties documented in our experiments, we found that these social learning properties can foster persistent local traditions in mating preference in populations of sizes common in nature. We have shown that population mate preference is maintained in transmission chains for longer than expected on the basis of chance in a way that closely matches our model predictions. Our lab experiments thus can be seen as a proof of concept in the lab that *D. melanogaster* has all the cognitive capacities and dispositions to transmit female mating preferences culturally across generations in ways that can elicit potentially long-lasting traditions of preferring an arbitrary male phenotype. This suggests that the taxo-

nomical range of culture may be much broader than ever before envisioned.

Our simulations also show that as predicted by theoretical consideration (15, 27, 31), a major characteristic for tradition emergence and maintenance is the existence of a correcting, or repair, mechanism such as trait-based conformity (5, 14, 27, 28, 32), as we empirically and theoretically document here. Conformity alone, however, does not necessarily result in culture and cultural inheritance (28). The fulfillment of a battery of other criteria is also necessary to generate persistent population preferences, eventually leading to cultural traditions. Although we adopted a demanding definition of culture jointly addressing all criteria discussed in the literature, the first explicit test of all these conditions simultaneously involves a nonsocial insect species. Cultural inheritance may thus have been a substantial part of evolutionary processes for extended periods of time.

Our study trait, mate choice, has considerable evolutionary implications, as strong local traditions in mating preference can amplify local sexual selection while hampering gene flow among populations with different traditions, favoring premating reproductive isolation and potentially speciation (33). In this mate-choice context, the Fisher runaway process can lead initially neutral male traits (such as those in our experiments) to quit neutrality as soon as chance generates some detectable statistical preference for one male phenotype. This starts a snowball effect favoring conformist females, a situation that was modeled decades ago (29, 33–36) but that still had little empirical evidence. The tradition then becomes part of the niche to which newcomers have to adapt by copying it (in German, *Gruppenzwang*, or “peer pressure”). Such strong selection for conformity in effect provides a general evolutionary explanation for mate-copying (18, 37) and speed learning (20) because it is essential for females to quickly grasp the local tradition before mating. More generally, our study shows one major way by which culture can affect evolution as it changes the selective social context of every individual.

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Competing interests: The authors declare no competing interests. **Data and materials availability:** Data, materials, associated protocols, and technical details, as well as the simulation code, are available to researchers desiring to replicate or expand studies of *Drosophila* mate-copying. Data and the R code for simulations can be downloaded at Dryad (38). The hexagon device can be purchased from Toulouse Tech Transfer and Paul Sabatier University.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/362/6418/1025/suppl/DC1
Materials and Methods
Figs. S1 to S11
Tables S1 and S2
References (39–41)

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Cultural flies: Conformist social learning in fruitflies predicts long-lasting mate-choice traditions

Etienne Danchin, Sabine Nöbel, Arnaud Pocheville, Anne-Cecile Dagaëff, Léa Demay, Mathilde Alphand, Sarah Ranty-Roby, Lara van Renssen, Magdalena Monier, Eva Gazagne, Mélanie Allain and Guillaume Isabel

Science **362** (6418), 1025-1030.
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Trendsetting flies

Though once believed to be confined to humans, culture has now been demonstrated in many different animal species, from whales to parrots. Most such animals have high levels of cognition, but the basics of transmission and copying could easily occur in less cognitively advanced species. Danchin *et al.* show that mating culture can be passed on in *Drosophila* and model the process by which this occurs (see the Perspective by Whiten). Their results suggest that culture and copying may be much more widespread across the animal kingdom than previously believed.

Science, this issue p. 1025; see also p. 998

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Supplementary Materials for

Cultural flies: Conformist social learning in fruit flies predicts long-lasting mate-choice traditions

Etienne Danchin*†, Sabine Nöbel*, Arnaud Pocheville*, Anne-Cecile Dagaeff, Léa Demay, Mathilde Alphand, Sarah Ranty-Roby, Lara van Renssen, Magdalena Monier, Eva Gazagne, Mélanie Allain, Guillaume Isabel

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S1 Materials and Methods

S1.1 Fly ecology, strains and maintenance

We used the common laboratory Canton-S strain of *Drosophila melanogaster*. Flies were raised in 30 ml vials containing standard corn flour-agar-yeast medium in a room at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and $60\% \pm 5\%$ humidity with a 12:12 h light:dark cycle. Flies were sorted without anaesthesia within 6 h after emergence and kept in unisex groups of 7 individuals for 3 days to ensure virginity (except for criterion 2 where demonstrator females of the across age-classes experimental treatment were kept for 14 days). All flies were wild type, except when specified (i.e. in Criterion 4 where we used coloured males of 2 mutant strains in a Canton-S background in some of the mate-choice tests). Fly manipulations were performed by gentle aspiration using glass pipette, tubing and gaze, allowing us to transfer copulating pairs without them to part.

All flies were 3-day old virgin males and females, except for the across age-classes treatment (Criterion 2) where demonstrator and observer females were 14- and 3-day old respectively. This 11 day age difference corresponds to a generation time at 25°C . All devices were cleaned with alcohol before reuse.

Fruit fly ecology is poorly known. For instance, the common wisdom is that adult survival in nature ranges from 3-4 days up to 2 weeks. The absence of such information is due to the fact that it is impossible to mark individual flies in order to monitor their life history traits. In the lab a small fraction of adult flies can survive up to 50 days. Emergence of new imagoes occurs every day so that natural populations are made of adults of various age classes. Males produce a sex peptide (37) that blocks their mate's libido for several days after copulation during which females actively reject courting males.

S1.2 General experimental protocol

All experiments encompassed two phases: a demonstration that lasted for a maximum of 30 minutes during which the observer female was given the opportunity to see a demonstrator female choosing between a green and a pink male. Copulations usually occurred within 10 minutes, implying that the observer female could see the actual copulation for about 20 minutes. Demonstrations ended when the demonstrator pair separated in the tube device, or when the last demonstrator male dismounted in the hexagon device. Demonstrations were followed by a mate-choice test during which each observer female was given the choice between a new green and a new pink male. The rare replicates in which the observer female did not copulate after 30 minutes were discarded. Mate-choice tests ended at the onset of the copulation. Demonstrations unfolded either in tubes or in hexagons, while mate-choice tests were always performed in tubes.

S1.3 Specific protocols

Social long-term memory

We used an experimental design inspired from the one used to study long-term memory in an olfactory aversive conditioning context (22, 40). All observer females were fed on a 1 cm x 2.5 cm piece of Whatman filter paper soaked with 125 μl of 5%-sucrose solution for 17-21 hours before demonstrations. Demonstrations consisted of a sequence of five demonstrations spaced by a 15-30 min resting periods. There were four experimental groups, the first two receiving the sucrose solution and the last two receiving the sucrose solution plus a 35 mM cycloheximide solution (94% purity; Sigma C7698, St Louis, MO, USA) diluted in mineral water (pH \approx 7; Evian, Danone, Paris, France). The first two treatments used a transparent (informed) or opaque (uninformed controls) partition during demonstrations, and mate-choice tests unfolded 24 hours later. The third group followed exactly the same protocol as the informed group, but for the addition of the cycloheximide to the sucrose solution to block the suspected de novo protein synthesis. The fourth group comprised cycloheximide treated observer females that were allowed to watch a single demonstration and that were tested immediately after (short-term memory). Finally, we used the horizontal control of Fig. 2 as a positive control as the latter was performed with the same protocol, but the cycloheximide treatment, and at the same time.

Transmission chain

We performed 36 transmission chains in hexagons. At each step we put 12 observer females in the central arena, which lead to similar social learning (41). At chain initiation, we introduced a trio of one already copulating pair plus an apparently rejected male of the other colour in each peripheral compartment, the six copulating males being of the same colour. After the end of the demonstrations each observer female was individually transferred into tube designs ordered from 1 to 12 and containing one male of each colour, then monitored for courting behaviour. If the tube design number one met our quality criteria (copulation initiated after the 2 males had actively courted the female with characteristic wing-flapping behaviour (39)), the trio of the copulating female plus the other male was transferred into a peripheral chamber of the next hexagon as part of the demonstration for the next set of 12 observer females; if it did not verify this quality criterion, we ignored it and skipped to tube design number 2, and so on until we were able to fill all 6 peripheral compartments of the next hexagon step. Thus, apart from the first step, demonstrations were provided by formerly observer females freely choosing between one green and one pink male. The chain ended when 3 or more of them chose the colour opposite to the one chosen at the chain initiation (preference $\leq 50\%$). To validate our simulation model, we further compared our experimental results to those of 4 batches of 36 simulations under these specific experimental conditions: observer population size = 6, initial preference = 1, mate-copying index of 0.68 (the observed average of 1,737 informed replicates) and a copying function as in the white curve of Fig. 7A.

S1.4 Mate-copying and social learning indices

Every replicate was scored as 1 if during the mate-choice test the observer female chose the male of the colour that was selected during the preceding demonstration, versus 0 in the opposite case. Every observer female being used only once, the Mate-Copying Index (MCI) was the mean of these scores over replicates of one experimental condition and represented the proportion of observer females of that experimental condition that copulated with the male of the colour that copulated during the demonstration. These Mate-Copying Indices are used in Fig. S7 to S11. Values significantly above 0.5 reveal mate-copying.

To illustrate the strength of social learning per se, we used a Social Learning Index (SLI) varying from -1 to +1 and quantifying the difference between observed mate-copying indices and those predicted under random choice (50%):

$$SLI = [\text{NbSame} - \text{NbOther}] / [\text{NbSame} + \text{NbOther}] = 2 * (\text{MCI} - 0.5)$$

where NbSame and NbOther are the number of replicates with a mate-copying score of 1 or 0 respectively. By quantifying the bias relative to random choice induced by the demonstration in favour of the male colour that was preferred during the demonstration, the Social Learning Index quantifies social learning efficiency. For instance, if observer females chose the male of the colour chosen during demonstrations in 70 cases and chose the other male in 30 cases, the Social Learning Index would be $(70-30)/(70+30) = 0.40$. We used SLI to illustrate results in the paper but provide here figures with the mate-copying indices (Fig. S7 to S11).

S1.5 Statistical analyses

All statistical analyses and simulations were performed with the R software (version 3.3.2 2016; packages used: lme4, car, binGroup, binom, as well as DAKS for simulations).

Controlling for potential confounding effects

We first built a data set of all mate-copying replicates and used it to test potential confounding effects (Table S1). In particular, in all previous experiments on social-learning in *Drosophila melanogaster* (18-20, 23, 24, 41) we noticed a weather effect on social-learning that we tested in (20). As in the meantime our data set greatly increased, we re-tested that effect plus all potential confounding effects on the data set pooling all data from the present paper plus that previous paper (20). That data set contained 4,167 replicates for which the observer female was in a position to choose between the green and pink males, of which 2,280 concerned replicates in which the observer female was in a position to learn, thus excluding uninformed controls. We used that data set

to test 18 potential confounding effects, all of which but three proved to be non-significant (Table S1). Thus, in this paper, except for binomial tests that cannot be multivariate, all statistical analyses included the Block as a random effect, as well as the Experimenter-ID (when a data sets involved more than one experimenter) and Air-pressure as fixed effects.

The Experimenter-ID effect was probably due to the fact that most experimenters participated to part of the experiments. However, one experimenter always performed replicates of all treatments of a given experiment simultaneously, implying that this effect could neither have generated some of the experimental effect. Finally, this effect was not influential as the conclusions of all statistical analyses were not affected by the fact of including it or not (compare Fig. 1 to 4 and 6 with Fig. S7 to S11).

Analyses of each criterion

The departure of each treatment from random choice was tested with a binomial test. As binomial tests are univariate, these tests could not account for the Block, Experimenter-ID and Air-pressure effects. All other analyses used generalised linear mixed models with random effects (function glmer). They analysed mate-copying scores as a function of the experimental treatment, while accounting for the three significant confounding effects, Block as a random effect, and Experimenter-ID and Air-pressure as fix effects. Starting models included these effects plus interactions, and we reduced it by backward selection.

However, ignoring the three confounding effects resulted in very similar results. Fig. S7 to S11 replicate those of the paper replacing the Y axes by the mate-copying index (i.e. the proportions of flies choosing the male of the colour that was chosen during the demonstration) and provide the P values obtained without accounting for the Block, Air-pressure and Experimenter-ID effects. Results were only marginally changed (compare P values of Fig. S7 to S11 ignoring confounding effects with those of Fig. 1 to 5), revealing the robustness of our conclusions to the inclusion or exclusion of potential confounding effects.

Analyses of transmission chains

The probability P_i of one hexagon to keep the majority of the previous step under the null hypothesis that demonstrator females choose with probability p and the opposite with probability $1-p$ is:

$$P_i = C(6,4)*p^4*(1-p)^2 + C(6,5)*p^5*(1-p)^1 + C(6,6)*p^6*(1-p)^0 \text{ where } C(n,m) = n! / (m!(n-m)!)$$

In our case $p = 1-p = 0.5$ and $P_i = 0.3438$. P_i was used in two types of tests. The first test considers each step as an independent data point using a binomial test from one step to the next [Binomial test of parameters number of chains still alive after step $x+1$ (i.e. success) and step x respectively, tested against the null probability of $P_i = 0.3438$; these tests are reported in Fig. 6A and Table S2.1]. The second test considers each chain as a whole in Binomial tests with the first parameter being the number of chains still alive after step $x+1$ (i.e. success) and the initial number of chains (i.e. 36), and testing against the probability of $(0.3438)^x$ (reported in Fig. 6B and Table S2.2).

Using the same reasoning, the probability that at each step at least 3 (half) of 6 observer females choose the incorrect colour with a probability of choosing the colour chosen during the demonstration of 0.68 (the average mate-copying index calculated from 1737 informed replicates) is:

$$P_i(X \geq 3) = C(3,6)*p^3*(1-p)^3 + C(4,6)*p^4*(1-p)^2 + C(5,6)*p^5*(1-p)^1 + C(6,6)*p^6*(1-p)^0 = 0.2936$$

Thus, with a population of only 6 observer females the chance of ending the transmission chain at each step is fairly high (~29%). This probability drops to 0.1876, 0.0719, 0.0305, 0.0061, 0.0001 and <0.0000 with a population of 10, 20, 30, 50, 100 and 150 observer females respectively (a calculator can be found at <https://www.di-mgt.com.au/binomial-calculator.html>). This is why transmission chains durations in our experiment (Fig. 6) were short compared to those simulated with populations of 75, 100 or 150 observer females (Fig. 7B and C, and S4 to S6). Nonetheless, they lasted significantly longer than under the null hypothesis of observer female having not learned to prefer males of the colour that copulated during the demonstration (Fig. 6, significance in Table S2).

S1.6 Modelling the emergence of arbitrary local traditions in mating preference

We modelled a population as evenly composed of two kinds of females: demonstrator females, which are currently mating, and observer females. These females are engaged in a transmission chain in which observer females of a given step become the demonstrators of the next step, and so on. Demonstrators freely choose among pink or green males (in non-limiting amounts) with a given probability according to their already acquired preference, while the observers build a preference according to the mean behaviour observed in the demonstrator population. A key parameter of the model was the number n of observer females within the population. Assuming a balanced sex-ratio, the total adult population would be of at least $4n$ adult flies. The setting of their preference follows the response functions given in Fig. S3. For instance, for the white line (f is the frequency of choosing pink in demonstrator females, p is the probability of choosing pink in observer females): (a) if $0 < f < 0.4$ in the demonstration, then $p = 0.3$ in the observer population; (b) if $0.6 < f < 1$ in the demonstration, then $p = 0.7$ in the observer population; (c) if $0.4 < f < 0.6$ in the demonstration, then p in the observer population conservatively follows a straight line joining the two plateaus.

At the following transmission step, the formerly observer females become demonstrator females, and now freely choose among pink or green males according to their learned preference, while a new batch of observer females can watch them and build their preference on the demonstrator choices. The process is repeated. All females belonging to the same category (demonstrator vs observer) at a given time step have the same probability to choose pink or green. The number of individuals is assumed to be constant through time. At the beginning of one simulation, the population of mating females is composed of demonstrator females with no preference, that is, with a probability f of choosing pink equal to 0.5, except for simulations of the transmission chains that initiated with $f = 1$.

We performed a graphical analysis to single out stable and unstable equilibria in the case where the dynamics is assumed to be deterministic (i.e. infinite population). To account for stochastic processes (finite populations) we simulated the dynamics in an individual-based model using the R software.

As observed in this study, the white line of Fig. S3 models the mate-copying response function of the probability that an observer female chooses pink in function of the frequency of demonstrator females choosing pink at the previous transmission step. We also assumed it to be symmetrical and continuous. As observed in this study (Fig. 5), for frequencies of choosing pink in the demonstrator population below 0.4 (and symmetrically above 0.6), the probability that an observing female chooses pink is constant (first plateau). Between 0.4 and 0.6, the response is conservatively assumed to increase linearly. Under these assumptions, the response function is defined by two parameters, the height of the first plateau π and the threshold ϕ beyond which the response quits the plateau. To match results of Fig. 5, in all of our simulation we fixed $\pi = 0.3$ and $\phi = 0.4$, except in simulations of Fig. 7 where we fixed $\pi = 0.32$, i.e. 1-0.68, the latter value being the average mate-copying index calculated from 1737 informed replicates to match the specific conditions of transmission chains. In Fig. S3 note that the threshold ϕ corresponds to the fraction closest to 0.5 that is attainable with our 6 compartment experimental device, i.e. $\phi = 2/5 = 0.4$.

In infinite populations, the dynamics is deterministic. A graphical analysis shows that with a mate-copying strategy corresponding to the white line of Fig. S3 (where $\pi < \phi$), the dynamics of the frequency of choice f has two stable equilibria ($f = \pi$ and $f = 1 - \pi$, the two white dots on Fig. S3) and one unstable equilibrium corresponding to no preference ($f = 0.5$, black dot of Fig. S3).

We also simulate a situation without conformity corresponding to the blue line of Fig. S3 where $\phi < \pi < 0.5$, which leads to a single stable equilibrium (no preference, $f = 0.5$, black dot of Fig. S3) as we found in the thin blue line of Fig. 7C.

Impact of population size

In finite populations, at every step the choice of demonstrator females represents a sample of the preference in the population. Simulations of an individual-based model using the R software (version 3.3.2 2016; packages used: lme4, car, binGroup, binom and DAKS) shows how the dynamics has periods of population mate-choice preference of different durations, with sampling effects leading preferences to sometimes randomly shift from pink to green preference and vice-versa. All other parameters being equal, the mean duration of such population

preferences greatly increased with population size, except in the absence of conformity (Fig. 7B). These periods of mate-choice traditions correspond to the stable equilibria of the infinite population size model. We also simulated a situation without conformity corresponding to the blue line of Fig. S3 where $\phi < \pi < 0.5$, which leads to a single stable equilibrium (no preference, $f = 0.5$, black dot of Fig. S3) as we document in the thin blue line of Fig 7C.

Impact of copying rate

The parameter π provides a measure of the copying rate. For a given population size the duration of episodes of population preference was positively related to π . For instance, with $\pi = 0.2$ we obtained situations similar to that of Fig. S6 (i.e. 150 observer females when $\pi = 0.32$ leading traditions to last for more than 100,000 transmission steps) with a population of only 50 observer females (pink and circle curve of Fig. 7B).

Examples of dynamics obtained in our simulations

Here are more results using different parameter settings to illustrate the importance of population size. While the ecology of *Drosophila melanogaster* in the field is poorly known, we performed a quick field estimation of the fruit fly population exploiting a single average size fig tree (Toulouse, South of France). The number of flies per fig varied from 0 to 14 at a time. As there were at least 500 figs in that tree this led to a minimum of 1,000 flies in that single tree, well above the population sizes used in these simulations.

Based on Criterion 3, we can consider that transmission steps can occur every day, one generation thus corresponding to 11 transmission steps in our simulation model. Note however, that all the extrapolations to estimate the durations of traditions in all the following simulations are purely theoretical and rest on the assumption that transmission steps occur in infinite and uninterrupted sequences. They are only meant to illustrate the potential of the documented social learning function to lead to the emergence of traditions.

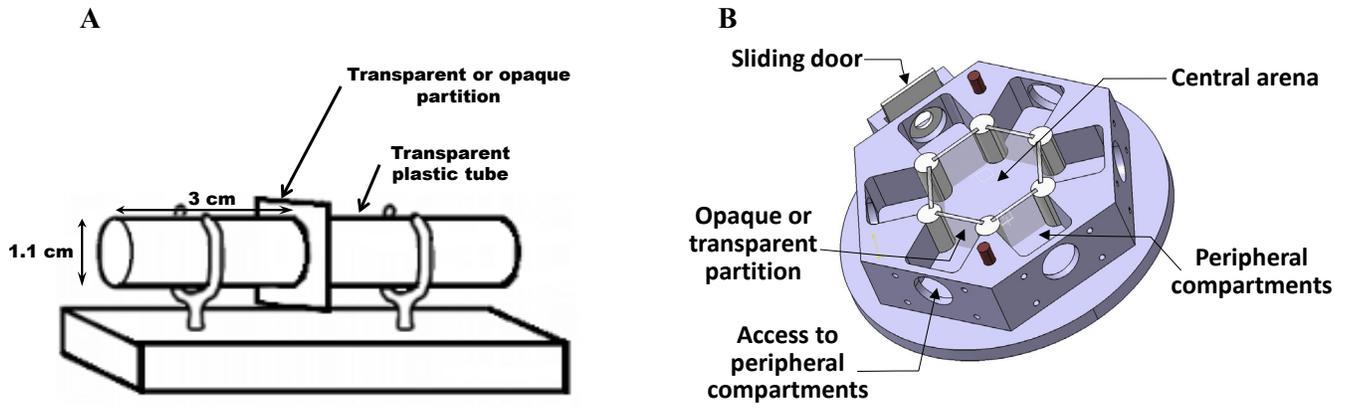


Figure S1: The experimental devices. **A)** The tube device used in most experiments. Informed females could watch through a transparent partition a demonstration in the other compartment. Uninformed females (controls) could not because the partition was opaque. **B)** The hexagon device used to test for conformity and transmission chains.

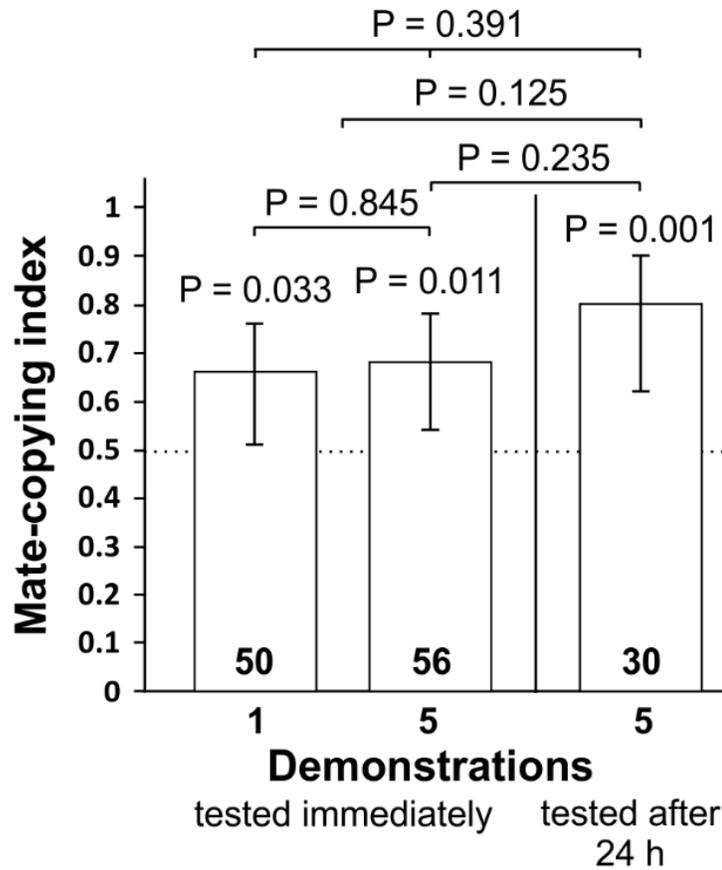


Fig. S2: Origin of the high mate-copying index observed in long-term memory. The left treatment replicates the usual experiment (except that in the 3 treatments here, flies did not receive sucrose before experiment) of a single demonstration immediately followed by a mate-choice. The two other treatment received 5 demonstrations spaced by 15-30 min resting periods. The middle treatment was spaced trained and mate-choice test followed immediately. The right bar duplicated the informed treatment of Fig3A and gave similar result. Thus, the high mate-copying index of Fig. 3 is observed again only in this condition, suggesting that the high index is observed only when spaced training is followed by a long delay during which de novo protein synthesis allows the establishment of long-term memory.

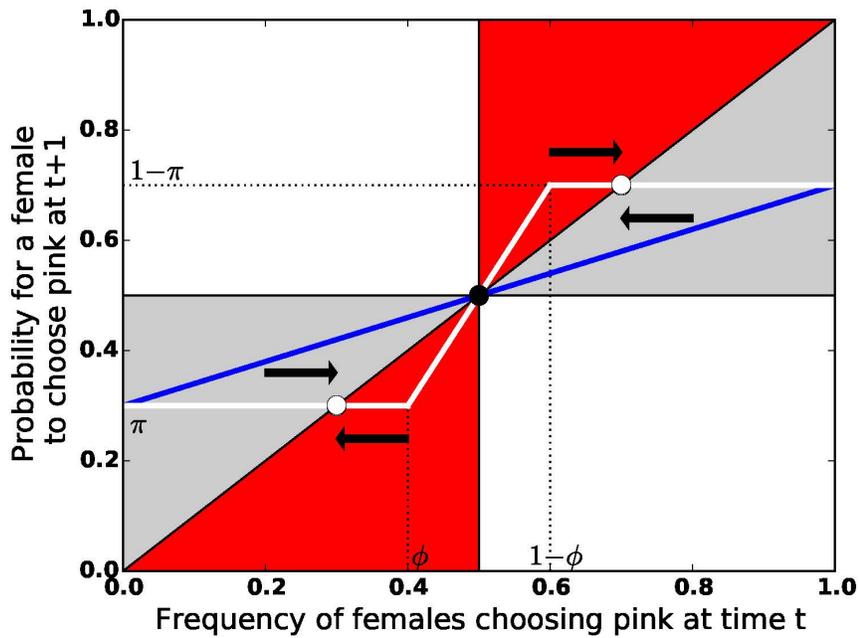


Fig. S3: Areas representing the set of possible response functions. X-axis: the frequency of choosing pink as shown by the population at transmission step t . Y-axis: mating preference acquired by observer females after watching demonstrator females; this preference will translate into a probability that an observer female chooses pink at the next transmission step. The line $y = x$ corresponds to situations where the probability p that an individual chooses pink is equal to the frequency f of choosing pink in the demonstrator female population. In grey areas, ($p > f$), observer females copy the population choice with attenuation (their preference is closer to the mean), thus the preference in the population tends to regress towards the mean (black dot). In red areas ($p < f$), individuals copy the population choice with exaggeration (conformity), thus the preference in the population tends to go further away from the mean. In white areas, individuals do the reverse of the majority (ignored here). Blue line: without conformity.

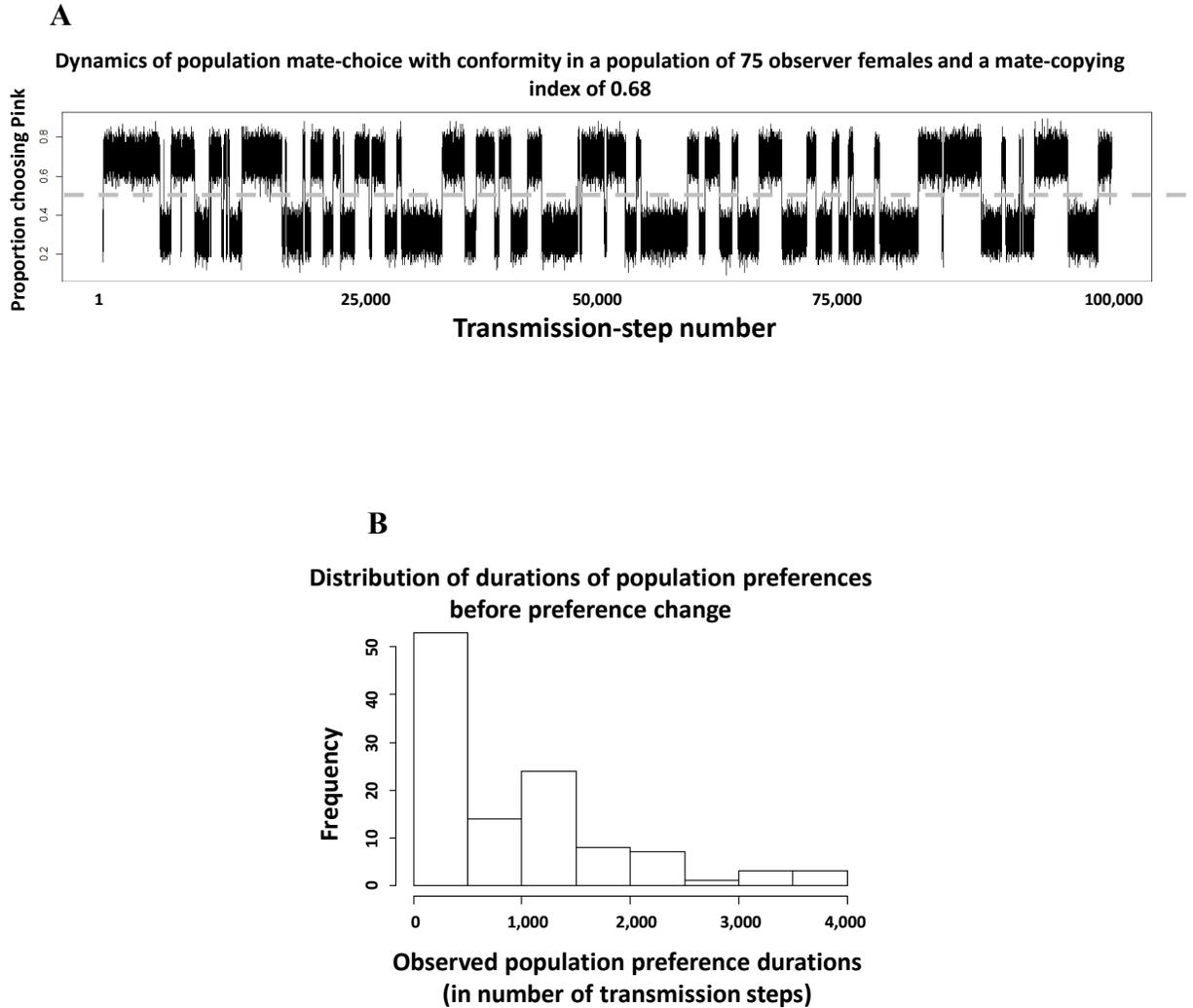


Fig. S4: Simulations with conformity. Simulations as in the white line of Fig. 7A and thick dark-red line of Fig. 7C with a population of 75 observer females. **A)** Typical dynamics. Horizontal dotted line: mean expected value under random choice. **B)** Distribution of population preference durations obtained over one sequence of 100,000 transmission steps. Periods of tradition readily emerge (top: preference for pink males, bottom: preference for green males). Accepting that transmission steps occur every day (Fig. 3), 4,000 transmission steps would potentially represent almost 11 years, corresponding to about 350 generations.

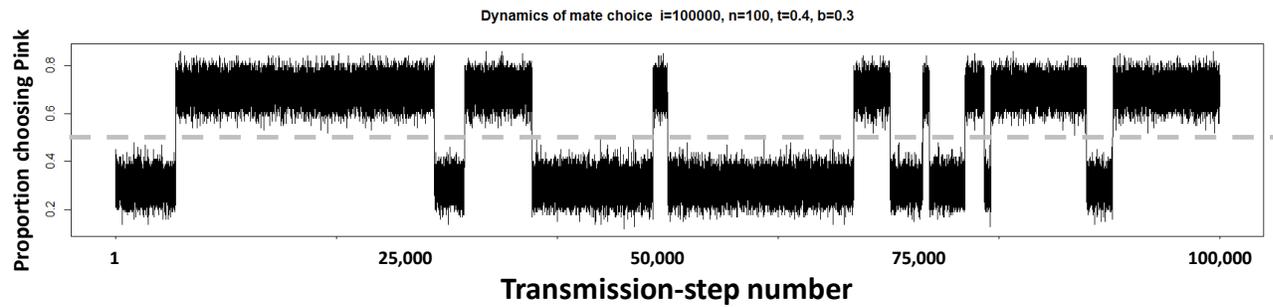
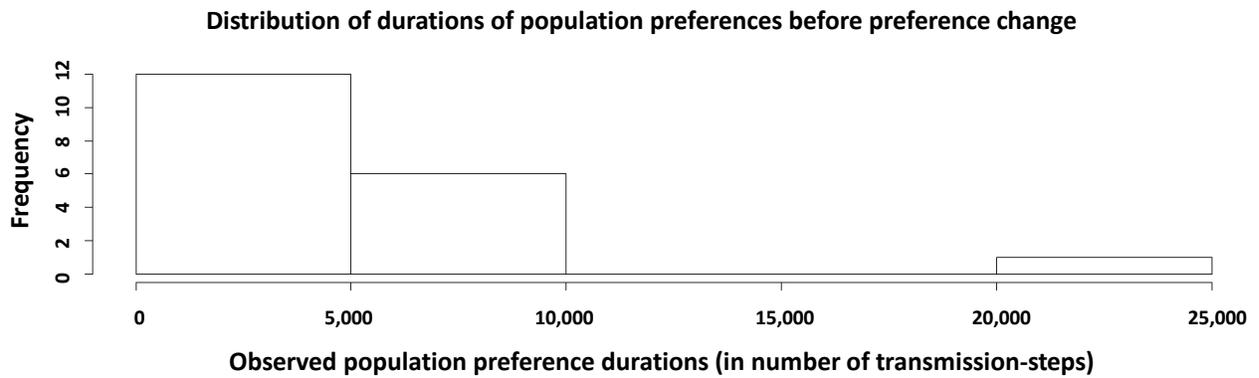
A**B**

Fig. S5: Simulations with conformity. Simulations as in the white line of Fig. 7A with a population of 100 observer females. **A)** Typical dynamics. Horizontal dash line: mean expected value under random choice. **B)** Distribution of population preference durations obtained over one sequence of 100,000 transmission steps. Under these conditions, traditions lasted for very long periods, and mean maximum traditions duration over 5 simulations was of over 16,000 transmission steps. Accepting that transmission steps occur every day (Fig. 3), 25,000 transmission steps would potentially represent some 68 years, corresponding to about 2,200 generations.

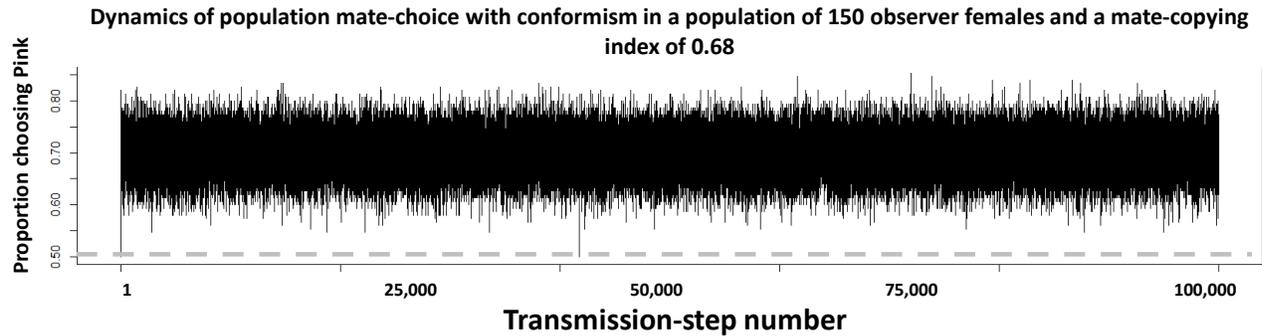


Fig. S6: Simulations with conformity. Simulations as in the white line of Fig. 7A with a population of 150 observer females. Typical dynamics obtained over a sequence of 100,000 transmission steps. Note that the horizontal grey dash line that represents the mean expected value under random choice is situated at the bottom of the figure, meaning that in this specific simulation a tradition for pink emerged at the beginning of the simulation and remained unchanged over the 100,000 transmission steps of the simulation. This kind of dynamics was obtained in 4 of the 5 performed simulations with these parameters. In the fifth one we observed only one tradition change. Accepting that transmission steps occur every day (Fig. 3), 100,000 transmission steps would theoretically represent almost 275 years, corresponding to about 9,000 generations.

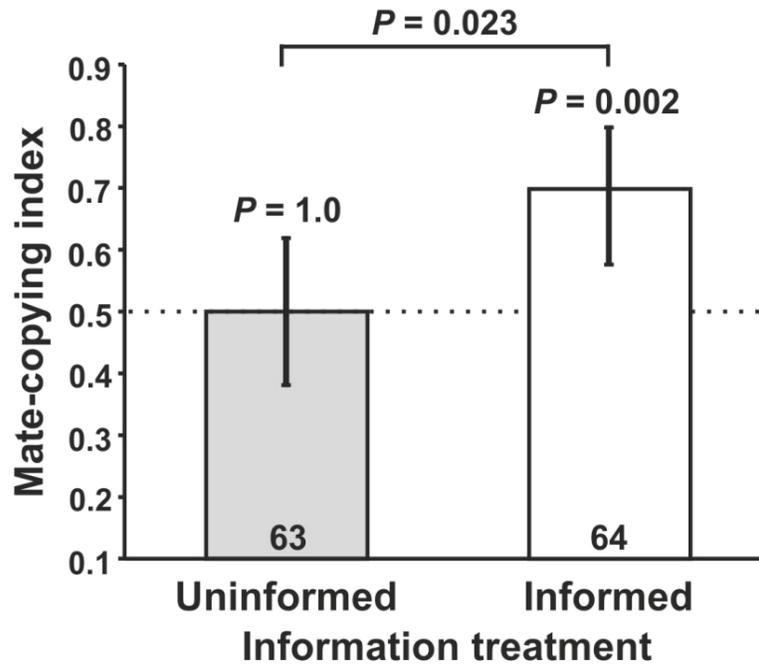


Fig. S7: Criterion 1 of social learning. Figure equivalent to Fig. 1C but replacing the Y axis by the mate-copying index. Vertical bars: Agresti-Coull-intervals.

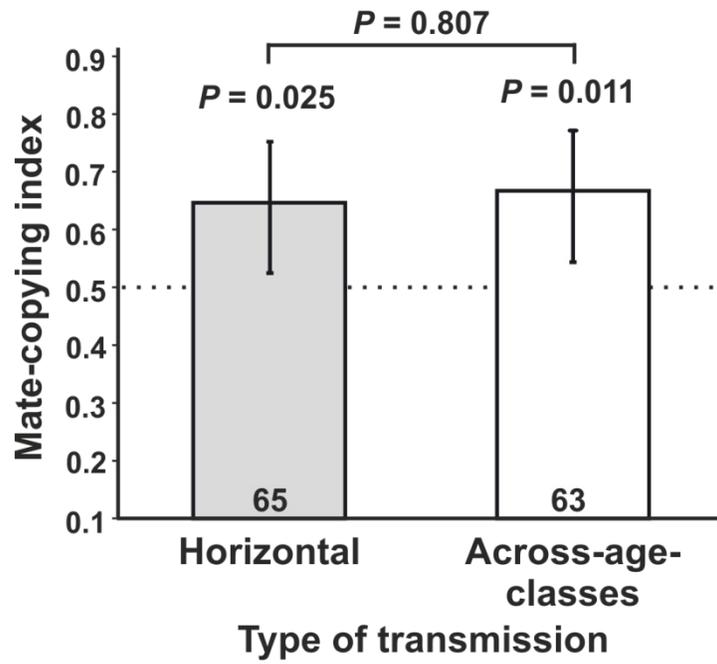


Fig. S8: Criterion 2 of across age-class social transmission. Figure equivalent to Fig. 2 but replacing the Y axis by the mate-copying index. Vertical bars: Agresti-Coull-intervals.

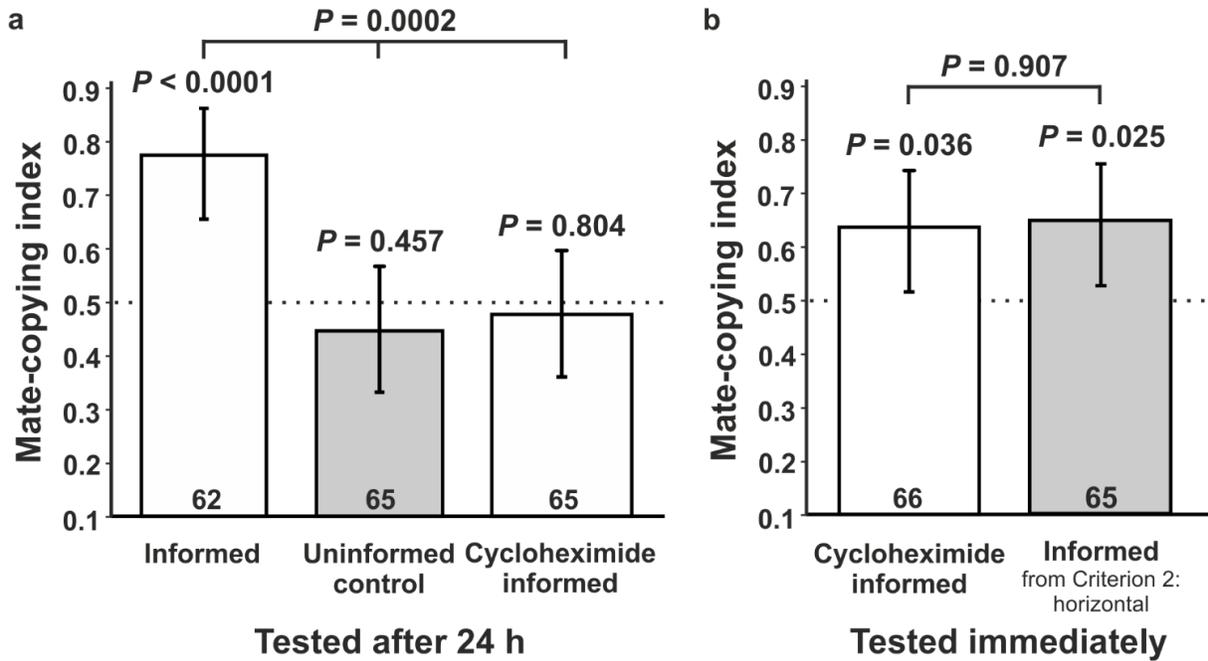


Fig. S9: Criterion 3 of durability of socially acquired mating-preferences. Figure equivalent to Fig. 3 but replacing the Y axis by the mate-copying index. Vertical bars: Agresti-Coull-intervals.

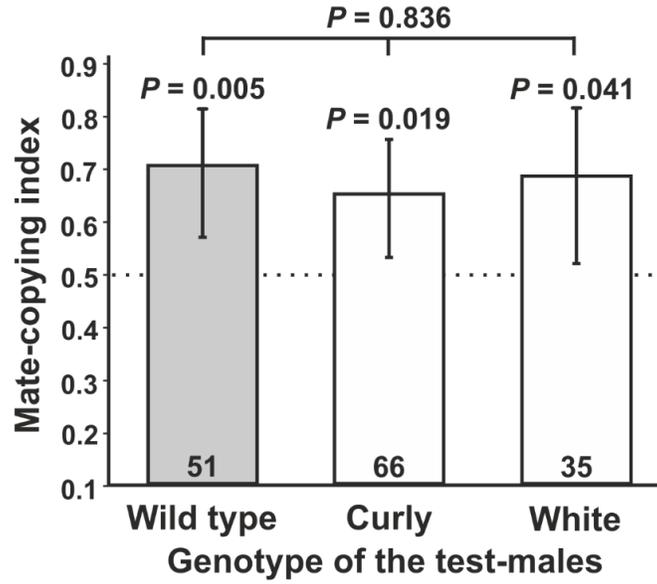


Fig. S10: Criterion 4 of trait-based copying. Figure equivalent to Fig. 4 but replacing the Y axis by the mate-copying index. Vertical bars: Agresti-Coull-intervals

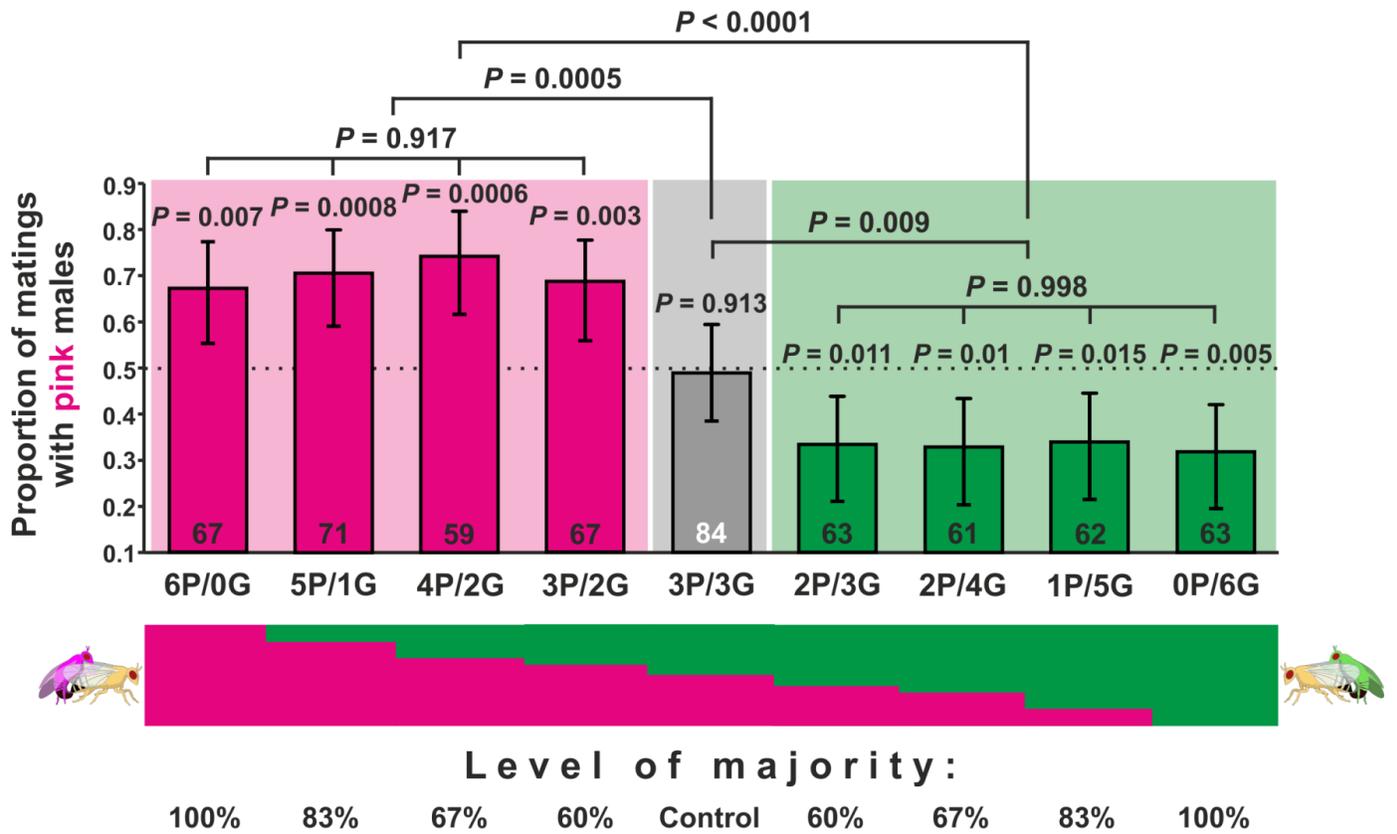


Fig. S11: Criterion 5 of conformity is mate-copying. Figure equivalent to Fig. 5 but replacing the Y axis by the mate-copying index. Vertical bars: Agresti-Coull-intervals.

Potential confounding effect	P value
Time of demonstration	0.116
Quadratic regression: Time of demonstration + (time of demonstration) ²	0.886 0.778
Room temperature at the onset of experiment	0.484
Room humidity at the onset of experiment	0.361
Type of medium used to raise flies	0.071
Year	0.404
Tube position in a batch of 6 simultaneously run tube devices	0.569
Actual tube device used (each device had an ID)	0.19
Fly strain (Rovers and sitter versus wild type Canton-S)	0.857
Experimental question: the five criteria of this paper, and those of previous papers	0.611
Demonstration device: tubes versus hexagons	0.761
Type of demonstration (free choice versus already formed copulating pairs)	0.579
Colour shown during demonstration	0.151
Day (may capture part of the Air-pressure effect)	0.31
Demonstration duration	0.823
Experimenter-ID effect	0.00049
Block	0.0001
Air pressure	0.0053

Table S1: Significance of potential confounding effects. As sample size is rather big ($n = 2,280$), for model selection we used a strict 0.05 threshold or the BIC information criterion, which is more suitable than AIC when using big data sets. These methods always led to the same conclusion. Bold: significant effects. The experimenter effect was largely due to the fact that different experimenters participated to different experiments. In all statistical analyses the three significant confounding effects were introduced either as random effects (Block) or as a fixed effect (Experimenter ID and Air pressure). However, note that accounting or ignoring them did not change any of our conclusions.

Step x	1. Test (Fig. 7.A)				2. Test (Fig. 7.B)				
	Observed number of Chains	Success over N	Threshold under random	Binomial test: P-value	Success over N	Threshold under random $(0.3438)^x$	Expected Number	Observed over Expected	Binomial test: P-value
0	36	36/36			36/36		36	1.00	
1	25	25/36	0.3438	2.41E-05	25/36	0.34380	12.377	2.02	2.41E-05
2	16	16/25	0.3438	0.002758	16/36	0.11820	4.255	3.76	1.01E-06
3	11	11/16	0.3438	0.006484	11/36	0.04064	1.463	7.52	1.17E-07
4	8	8/11	0.3438	0.01086	8/36	0.01397	0.503	15.91	3.10E-08
5	5	5/8	0.3438	0.1335	5/36	0.00480	0.173	28.92	8.49E-07
6	5	5/5	0.3438	0.004803	5/36	0.00165	0.059	84.11	4.42E-09
7	2	2/5	0.3438	1.0000	2/36	0.00057	0.020	97.86	0.0002
8	1	1/2	0.3438	1.0000	1/36	0.00020	0.007	142.31	0.00718

Table S2: Description of the binomial tests comparing observed transmission-chain durations to the ones that would be expected if all females selected randomly during the successive mate-choice tests. The purple left part compares for each step the observed proportion of chains that kept the initial population preference from one step to the next. This is thus a pairwise comparison that just tests the departure from random at each step independently from the previous transmission steps. In the blue right part, binomial tests take into account the fact that each step is part of a chain by comparing the observed frequency of surviving transmission chains since the beginning until step x to the theoretical one of $(0.3438)^x$.

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