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Selection, Sex and Sun: Social transmission of a sexual preference in *Drosophila melanogaster*

Anne-Cécile Dagaëff

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THÈSE

En vue de l'obtention du

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Selection, Sex and Sun :

*Social transmission of a sexual preference in
Drosophila melanogaster*

'Although the phylogenetic distance between humans and insects is vast, the basic adaptive logic behind holding on to a mate shows striking parallels" (David Buss, 1994)

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- Lors de colloques scientifiques :
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Abstract

Mate choice is a major fitness-affecting decision in sexually reproducing organisms. A form of mate choice is mate copying, in which females choose potential mates by copying the mate choice of conspecifics. While many studies documented mate copying in vertebrates, little is known about this behaviour in invertebrates. In this thesis, I studied mate copying in *Drosophila melanogaster* females. I showed that female flies can build a sexual preference for one male characteristic after witnessing a single mate choice event and that the efficiency of mate copying correlates with air pressure and its variations. Then I studied the characteristics of mate copying to see whether a preference for one type of male can be transmitted into the population. Finally I tried to find some molecules that could be involved in this behaviour. These results indicate that fruit flies can express complex behaviour, which can potentially lead to cultural transmission, reproductive isolation and speciation.

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Introduction

1. Evolution and sexual selection

Charles Darwin transformed the field of biology in 1859 with his book: "On the origin of species by means of natural selection". In this book, Darwin proposed that species could gradually change over time (Darwin, 1859). He postulated that reproduction always goes hand in hand with variation, that leads to the disparate survival of individuals in a given environment. Individuals possessing characteristics that best fit an environment have more chances to reproduce and their offspring are expected to inherit their advantageous traits. If these traits remain beneficial, they should spread through the population over generations. This idea is the base of Darwin's theory of *natural selection* (Darwin, 1859). In brief, the mechanisms of natural selection emerge as soon as three conditions are met: there is some variation among individuals within a population, at least a part of it is heritable, and this heritable variation induces consistent fitness differences between the variants (Lewontin, 1970; Pocheville, 2010). For example a bird with a very long tail could be slower and more easily caught by predators than its conspecifics with shorter tail. Consequently, if this variation is heritable, shorter tails should become more prevalent in the next generation.

Counter intuitively some animals display characteristics that seem detrimental to their survival. For example the peacock has bright colours and a very long tail, that can be easily seen by predators. The presence of these characteristics could not be explained solely by the theory of natural selection. In the *Origin of Species*, Darwin actually wrote : "I am convinced that natural selection has been the main but not exclusive means of modification (Darwin, 1859)". Darwin considered that another kind of selection was possible: sexual selection (Darwin 1859, chap. IV, a view expanded in : *The Descent of Man and Selection in Relation to Sex*, Darwin, 1871). According to Darwin this process of sexual

selection "depends on the advantage which certain individuals have over other individuals of the same sex and species, in exclusive relation to reproduction" (Darwin, 1871). For instance if a bird has a very long tail that slows him down, it may be more easily caught by predators but such ornamentation could also be very attractive to potential mates. If the bird is able to survive until breeding, it will attract more sexual partners and thus will have more offspring than birds with shorter tails. This phenomenon has been studied in females of the long-tailed widowbird (*Euplectes progne*). They are actually so attracted to males with long tails, that the males with artificially elongated tails are the most successful (Andersson, 1982). Then, as explained by Darwin: "If the individuals of one sex were during a long series of generations to prefer pairing with certain individuals of the other sex, characterized in some peculiar manner, the offspring would slowly but surely become modified in the same manner." (Darwin, 1871). So this long tail character will be likely to survive to the next generation and become more prevalent in the population.

The theory of sexual selection, as defined by Darwin, gave a significant role to females in the process of evolution, a revolutionary assertion for the time (Vandermassen, 2004). This idea was rejected by many of Darwin's contemporaries who preferred the belief that females were passive entities in the mating process (Gowaty, 1992; Vandermassen, 2004). This rejection was so strong that after Darwin's death the role of females in sexual selection was almost completely forgotten (Vandermassen, 2004). It was only in the 1970's, once women's place in society had advanced, that scientists started to embrace this aspect of sexual selection and began to study female strategies to choose and compete for mates (Miller, 2000; Vandermassen, 2004).

Variation between individuals in their ability to attract more and/or better partners leads to a differential transmission of particular characteristics. Selection outcomes can be a result of competition between members of the same sex (intrasexual competition) or attraction of one sex to the other (intersexual mate choice) (Eva and Wood, 2006; Panhuis et al., 2001). For instance using

the example of our imaginary bird, males could fight between each others to gain access to females. Then traits that are favourable for fighting (for example increased size), will spread in the population. Now let's imagine that selection arises through female choice. Males with traits that are appealing to females or that reveal something about their health will be chosen more frequently by females. Such traits would then be more common in the next generation. If a group of females begin to prefer one type of ornamentation and another group of the same species prefers another type, then different populations may develop different preferences and thus start to diverge in terms of male characteristics. Reproductive isolation can subsequently be then generated independent of environmental differences and thus lead to a rapid divergence between populations. Sexual selection is therefore an important driver of speciation which is the split of one species into two or more species (Panhuis et al., 2001).

Sexual preferences have been mainly studied from an ecological and genetic point of view but recently the importance of social factors in the development of such preferences has been highlighted (Kirkpatrick and Dugatkin, 1994). In a changing environment, genetic transmission of useful information may occur too slowly to be beneficial, so acquiring information may be more effective to adapt in real time to changing conditions. Given that a poor mate choice can negatively impact an individual's reproductive output, could monitoring others behaviour be advantageous in the absence of other information?

2. Utilization of social information in the context of mate choice

2.1. Social information: clues given by others

To acquire information from their environment, individuals engage in a trial-and-error strategy. The information obtained is called *personal information* (Danchin et al., 2004; Valone, 1989). Alternatively, individuals can monitor others interactions with the environment and extract an

information called *social information* (Danchin et al., 2004; Danchin and Wagner, 2010; Valone, 1989).

Social information encompasses information intentionally communicated through signals as well as information extracted from cues inadvertently provided by others. Watching other individuals location can actually indicate where resources are (i.e. location cues) whereas watching the performance of others that share similar requirements provides information about the quality of a resource (Inadvertent Social Information as part of Public information, Danchin et al., 2004; Danchin and Wagner, 2010; Valone, 1989). Such public information can be used to separate resources that appear to be of similar quality, when discriminating using only personal information is not possible.

For example in a sexual context, young inexperienced females can monitor the choice of other females to gather information about male attractiveness or quality. This can provide the inexperienced females with additional information helping them to develop their own preferences for a given male phenotype.

2.2. Mate choice copying: obtaining public information by watching other mate

The mating performance of potential mates can provide rich public information on their attractiveness to members of the population (Nordell and Valone, 1998), and females of many species have been shown to develop mating preferences that are affected by the observation of other females' sexual preferences (i.e. public information, Westneat et al., 2000). This behaviour is called mate-choice copying or more simply *mate copying*. This can be defined as an increased probability of mating with another conspecifics if this individual has been previously observed to have successful sexual encounters (Pruett-Jones, 1992; Valone, 2007). Observing the mate choice of rivals can be a quick and easy way to acquire integrated information about potential mates particularly when learning about their quality requires previous experience (Dugatkin and Godin,

1993), is costly to obtain (Bowers et al., 2012) or when discrimination is difficult (i.e. males with similar quality) (Nordell and Valone, 1998; Valone and Templeton, 2002).

Empirical studies show that females use mate copying mostly in situations where discrimination is difficult and additional information is required to reduce this uncertainty (Valone and Templeton, 2002). Relying first on personal assessment and not only on the behavioural decisions of others could avoid to enter in an erroneous informational chain which appear when the individual initiating the behaviour makes a poor decision that gives the worst payoffs (Giraldeau et al., 2002). Nevertheless, it has also been shown that females of various species use public information on male attractiveness to develop sexual preferences to the extent that public information can fully reverse a pre-existing preference (Dugatkin and Godin, 1992; Witte and Noltemeier, 2002).

When copying the mate choice of others, females can adopt two strategies. They can be biased towards one of the observed specific males, resulting in “individual-based” mate choice copying. Alternatively they can generalize their bias towards other individuals with similar traits, resulting in “trait-based” copying (Bowers et al., 2012). The trait-based strategy enables individuals to avoid some of the suggested cost of mate copying, such as: time spent waiting until the end of the copulation, resistance to the new suitor or sperm depletion (Bowers et al., 2012; Valone and Templeton, 2002). Moreover the evolutionary impact of these two types of mate copying profoundly differs because individual-based copying only affects the mating success of a given male, while trait-based copying potentially affects the fitness of all males with the same phenotypes. Thus trait-based mate copying may amplify sexual selection on males leading them in different evolutionary pathways in different populations, and it incorporates the potential to transfer general mating preferences across individuals and generations. This latter characteristic implies that sexual preferences can be transmitted among population members from older to younger individuals potentially leading to their vertical social transmission across generations (Danchin et al., 2011).

2.3. A brief review about mate copying

Most studies about mate choice copying in animals focused on the choice of an observer female toward two specific males that it has previously observed with or without another female (Vakirtzis, 2011; White and Galef, 2000) thus corresponding to an “individual-based” copying strategy.

To our knowledge, only 6 studies have focused on “trait-based” copying in 6 different species (underlined in table 1): quails (White and Galef, 2000), sailfin mollies (Witte and Noltemeier, 2002) guppies (Godin et al., 2005), zebra finch (Swaddle et al., 2005), fruit flies (Mery et al 2009) and humans (Bowers et al., 2012)

Species	Evidence for mate copying	No evidence for mate copying	Uncertain
Whitebelly damselfish (<i>Amblyglyphidodon leucogaster</i>)	(Goulet and Goulet, 2006)		
Great snipe (<i>Callinago media</i>)		(Fiske et al., 1996)	
Sage grouse (<i>Centrocercus urophasianus</i>)	(Gibson et al., 1991)	(Spurrier et al., 1994)	
Japanese quail (<i>Coturnix coturnix japonica</i>)	Notably :Galef and White, 1998; <u>White and Galef, 2000</u> ; reviews : (Galef, 2008; White, 2004)		(WHITE and GALEF JR, 1999)
Fallow deer (<i>Dama dama</i>)		(Clutton-Brock and McComb, 1993; McComb and Clutton-Brock, 1994)	
Fruit fly (<i>Drosophila melanogaster</i>)	(<u>Mery et al., 2009</u>)		
Fruit fly (<i>Drosophila serrata</i>)		(Auld et al., 2009)	
Pied flycatcher (<i>Ficedula hypoleuca</i>)		(SLAGSVOLD and VILJUGREIN, 1999)	
Three-spined stickleback (<i>Gasterosteus aculeatus</i>)	(Frommen et al., 2008)	(Patriquin-Meldrum and Godin, 1998)	
Human (<i>Homo sapiens</i>)	Notably : (<u>Bowers et al., 2012</u> ; Eva and Wood, 2006; Place et al., 2010; Waynforth, 2007)	(Uller and Johansson, 2003)	
Humpback limia (<i>Limia nigrofasciata</i>)	(Munger et al., 2004)		
Perugia’s limia (<i>Limia perugiae</i>)		(Applebaum and Cruz, 2000)	
Brown-headed cowbird (<i>Molothrus ater</i>)	(Freed-Brown and White, 2009)		
Mouse (<i>Mus musculus</i>)	(Kavaliers et al., 2006)		
Japanese medaka (<i>Oryzias latipes</i>)	(Grant and Green, 1996)	(Howard et al., 1998)	
Amazon molly (<i>Poecilia formosa</i>)	(Heubel et al., 2008)		
Sailfin molly (<i>Poecilia latipinna</i>)	Notably : (Schlupp and Ryan, 1997; Witte and Massmann, 2003; <u>Witte and Noltemeier, 2002</u> ; Witte and Ryan, 2002, 1998; Witte and Ueding, 2003)		
Mexican molly (<i>Poecilia mexicana</i>)	(Heubel et al., 2008)		
Guppy (<i>Poecilia reticulata</i>)	(Amlacher and Dugatkin,	(Brooks, 1996; Lafleur et	

	2005; Dugatkin et al., al., 1997) 2003; Dugatkin and Godin, 1998, 1993, 1992; <u>Godin et al., 2005)</u>	
Sand goby (<i>Pomatoschistus minutus</i>)		(Forsgren et al., 1996)
Common goby (<i>Pomatoschistus microps</i>)		(Reynolds and Jones, 1999)
Norway rats (<i>Rattus norvegicus</i>)	(Galef et al., 2008)	
Ocellated wrasse (<i>Symphodus ocellatus</i>)	(Alonzo, 2008)	
Deep-snouted pipefish (<i>Syngnathus typhle</i>)	(Widemo, 2006) in males only	
Zebra finch (<i>Taeniopygia guttata</i>)	(Drullion and Dubois, 2008; <u>Swaddle et al., 2005)</u>	(Doucet et al., 2004)
Black grouse (<i>Tetrao tetrix</i>)	(Höglund et al., 1995, 1990)	

**Table 1: Review of studies on mate choice copying in animals (adapted from Vakirtzis, 2011),
underlined : trait based mate copying**

3. Why using *Drosophila* to study mate copying ?

Drosophila species have long been used in biological studies. With their short generation time, high reproductive rate and the possibility to do large-scale experiments, they are model organisms for the genetic studies. Furthermore, according to Andersson and Simmons (2006) *Drosophila* have "the properties desirable in a model system for analysis of sexual selection and mate choice". Indeed they have a conspicuous sexual behaviour, that is positively correlated to strong sexual selection (Andersson and Simmons, 2006). Their very short generation time permits experimental analysis of sexual selection and its consequences over many generations. Moreover, *Drosophila* are genetically well studied (Adams et al., 2000) which allows researchers to link their genetics to their physiology and behaviour (Leadbeater, 2009). Consequently genetic and phenotypic analyses of evolution by sexual and other forms of natural selection can be combined advancing the field of behavioural ecology.

Moreover, while sophisticated behaviours are well accepted in vertebrates, only a few studies studied complex behaviours in invertebrates, long considered as too simple. Nonetheless Darwin observed that the honeybees "merely imitated the humble bees" (Darwin, 1841) techniques of

nectar-robbing. This behaviour consists of removing flowers nectar by cutting holes into their corolla instead of entering inside them and carrying pollen. This observation was actually very unique: at that time, following Descartes thought, animal bodies were indeed considered as machines. Yet Darwin attributed them to have high cognitive abilities (Leadbeater and Chittka, 2007). Later studies supported that view: not only can insects learn but they are also capable of using social information from members of their own species or from other species (Chittka and Leadbeater, 2005; Leadbeater and Chittka, 2007).

Recent work studying *Drosophila* has revealed that they use social information in various contexts (Battesti et al., 2012; Schneider et al., 2012). Even though they are considered non social insects, fruit flies often spend their life aggregating on food sources (Reaume and Sokolowski, 2006), suggesting that they have many opportunities to dynamically interact. They can modulate their behaviour according to the social context through chemical/olfactory, visual or auditory communication (Krupp et al 2008; Schneider et al 2012). The social context has also been shown to impact learning performances (Chabaud et al, 2009), oviposition sites (Battesti et al., 2012; Sarin and Dukas, 2009) as well as mate choice (Mery et al., 2009). In fact, this last study is the cornerstone of all the research undertaken within this thesis.

4. Questions studied in the thesis

In their article, Mery et al (2009) demonstrated that female *Drosophila* mate choice could be influenced by the mate decisions of other females. The goal of this thesis is to study this mate copying phenomenon and to determine whether the preference for one male phenotype is likely to be transmitted across generations in a way that could resemble cultural transmission

We intended to further explore the central themes of Mery et al. by asking the following questions: can the observation of Mery et al. be reproduced? Are there ways to simplify the experimental protocol and make it closer to a natural situation? Are there external factors that could influence the female choice? These questions are addressed in Chapter 1. The second chapter addresses the question of the potential transmission of a mating preference through a population of flies, to test whether this preference could be transmitted across generation and so to persist through time. In order for a mate preference to spread into the population, females have to generalize their inclination to all the males sharing the same phenotype as the one of the male observed being chosen. Females also have to retain their newly acquired preference until they encounter potential mating partners. We thus tested whether *Drosophila* females were able to do some trait based copying and how long they could retain this generalized preference. However in a population of flies, not all females will copy the mate preference. We thus studied whether females will follow the preference of the majority. Then we built a theoretical model to test whether a phenotype preference could to spread across the population. Finally, in Chapter 3, we started to study some of the molecular mechanism underlying mate copying. This constitutes preliminary studies but revealed interesting results that could be used for future studies.

5. Experimental setup

5.1. Fly maintenance

We used the common laboratory Canton-S strain of *Drosophila melanogaster*. Individuals were raised in 8 ml (9.5 cm x 2.5 cm) vials containing a standard corn flour-agar-yeast medium. Each vial contained 6 males and 6 females that were removed after 3 days. Ambient conditions were fixed at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and $60\% \pm 5\%$ humidity, both during rearing of individuals and experimental phases. Light followed a 12:12 h light/dark cycle, starting at 7:30 am.

Vials were emptied daily to collect newly emerged individuals for experiments. These flies were sorted without anaesthesia within 6h after emergence and then kept in unisex groups of 6 individuals during 3 or 4 days. That is an age at which females are sexually mature (Manning, 1967) and able to learn easily. Fly maintenance and experiments took place in the same room and all manipulations were always performed by gentle aspiration.

5.2. Experimental devices

For the experiments, two experimental devices were used:

“Tube devices”

This device was made of two small plastic tubes (3 cm each) separated by a thin glass partition that could be either opaque (controls) or transparent (figure 1) according to the protocol. The tubes were closed by small cotton plugs.

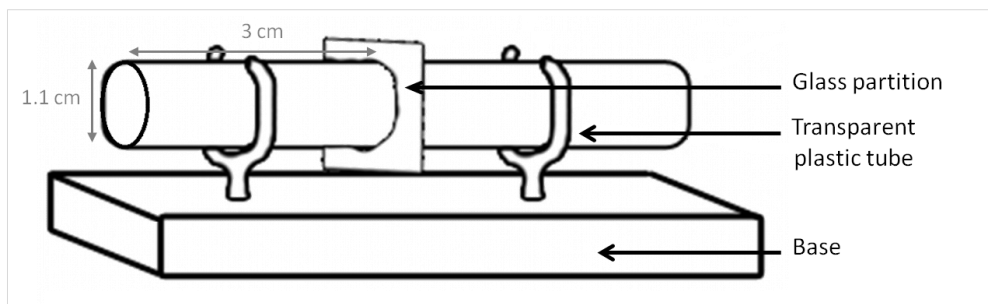


Figure 1: Tubes device. At the beginning of each experiment, one virgin observer female would be placed in one tube, while the demonstrator flies were put on the other side. The position of the flies were determined randomly before each experiment.

“Hexagonal devices”

For some experiments, a new device was necessary to allow demonstrations encompassing several females choosing between two males at a time. This scenario resembles more to what a fly could see in nature. This device was designed with the help of Laurent Polizzi (IRSAMC, Laboratoire Collisions Agrégats Réactivité - UMR5589). It encompassed a central compartment surrounded by 6 peripheral boxes each separated from the central compartment by a glass partition. The size of the boxes were designed to offer to flies the same space volume as in the tube experiments.

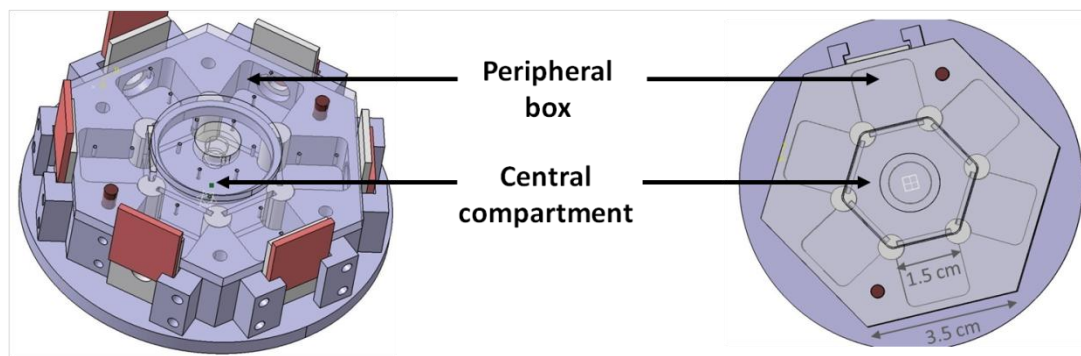


Figure 2: Hexagonal device. We put up to 6 observer females in the central compartment, while the demonstrator flies were placed in the peripheral boxes. This allows having up to six observer females looking at several demonstrations of one female choosing between two males of contrasted phenotypes.

We put up to 6 observer females in the central compartment, while the demonstrator flies were placed in the peripheral boxes. This allows having up to six observer females looking at several demonstrations of one female choosing between two males of contrasted phenotypes.

5.3. Mate copying experiments

For the experiments, the observer fly/flyes were placed on one side of the device while the demonstrations occurred on the other side. The device used, type of flies and number of demonstrations depend on the experimental protocol. After each demonstration phase, a test phase

occur. This was done in the same device or in another device (for example from the hexagon to the plastic tube) immediately after the test phase or with some delay (depending on the protocol).

The test phase occurred in the same way for all experiments. The observer female was placed in the device. Then one male of each colour was placed with it and the test began. *Drosophila* males have specific courtship behaviour (see figure 3). A male was considered as courting when it displayed the wing vibration behaviour. The time and the colour of the male was noted. We also documented the copulation time and the colour of the chosen male.

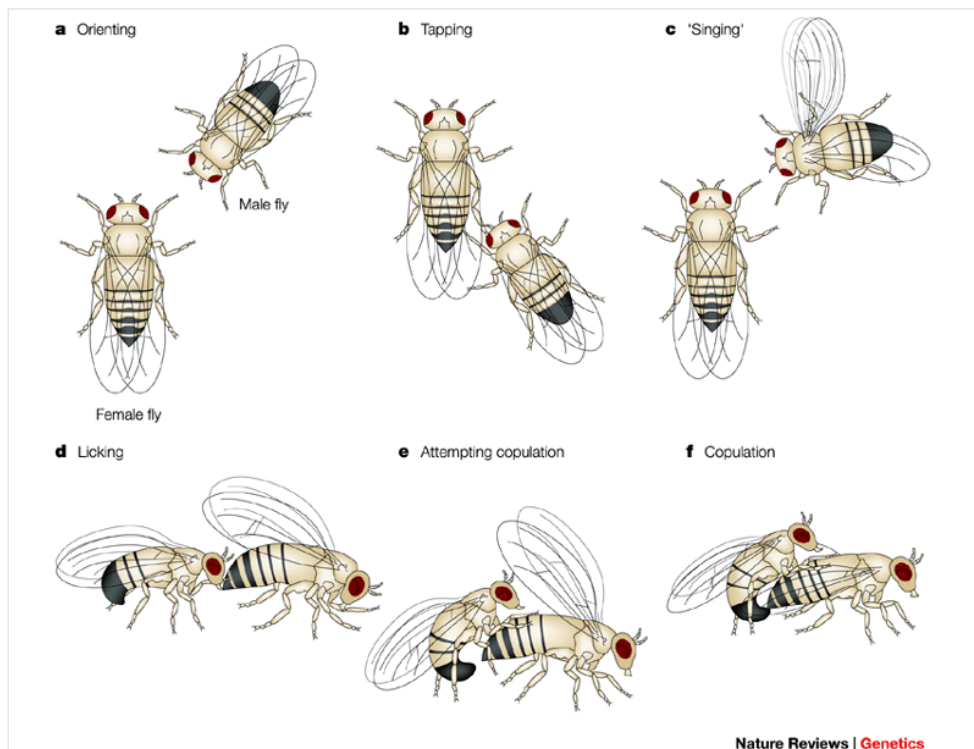


Figure 3: "Sequence of courtship behaviours shown by *Drosophila melanogaster* males towards females. a) The male fruit fly orientates towards the female, then follows her, b) taps her, and c) sings a species-specific courtship song by vibrating one wing. d) Finally, he licks the genitalia of the female, and e) curls his abdomen in an attempt to copulate with her".(Sokolowski, 2001)

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5.4. Statistical analyses

All statistical analyses were carried out with the R software version 3.1.2 (*R Core Team. R: A language and environment for statistical computing*, 2014).

For a given replicate, a Mate Copying Score was defined as 1 when the observer female copulated with the male of the phenotype that was 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 these conditions, and indicated female learning. Values around 0.5 indicate random choice by observer females while values above 0.5 reveal mate copying. The Mate Copying Score was analyzed through generalized linear mixed model with binary logistic regression with the package *lme4* (Bates D et al., 2014). A Wald chi-square post hoc analysis then tested whether the observed proportions differed from random choice (package *binGroup*, Zhang et al., 2012). All models included the colour of the chosen male during the demonstration (pink or green) as well as the date as random effects to control for potential colour preferences and day effect. In experiments with the hexagonal device, we further added a block effect to account for the fact that the 6 observer females belonged to the same hexagon. Courtships and copulation latencies were analyzed through generalized linear mixed model (package *lme4*) with the colour of the chosen male during the demonstration (pink or green) as well as the date as random effects. A Shapiro test was then performed to check for the residuals normality.

This experimental protocol was used in all experiments that will be described in this manuscript.

**Chapter 1: Mate copying in
Drosophila melanogaster and air
pressure influence**

Before studying the mate preference transmission, we wanted to study whether we could easily reproduce Mery et al's results and if there were ways to improve it. Their mate copying protocol is actually very long and heavy going, not very suitable to use in a study of mate preference transmission between generations. Thus the questions tackle in this chapter are:

- Can we induce a mate preference in a female *Drosophila*?
- Is there a way to have a really simple experimental protocol?
- Is there external factors influencing the female choice?

Papers awaiting decision after submission in *Animal Behaviour*:

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

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Abstract

Mate choice is a decision having major effects on 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 same sex individuals. While many studies have documented mate copying, little is known about the effect of environmental conditions on this behaviour. Here we report (1) the first evidence that *Drosophila melanogaster* females can build a sexual preference for one male characteristic after witnessing a single mate choice event (i.e. speed learning), and (2) that mate copying correlates with air pressure and its variation so that females copy far more when air pressure is increasing, i.e. in improving weather conditions. These results highlight the potential importance of climatic conditions for mate copying, a behaviour potentially driving reproductive isolation.

Keywords:

Mate choice, mate copying, social information, social learning, air pressure, *Drosophila melanogaster*

Introduction

“If bees stay at home, rain will soon come; if they fly away, fine will be the day” (English Proverb)

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 trials-and-errors tactics (i.e. using private information) or by monitoring other individuals with similar requirements (i.e. 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 phase followed by a test phase. During the demonstration phase, 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 test phase, the observer female preference is assessed either by the relative amount of time the female spends by the two males or through actual copulation with one of them. By copying the mate choices 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 Jr. & White, 2000 for a review) and in only one invertebrate, the fruit fly *Drosophila melanogaster* (Mery et al., 2009). In the latter study, the experimental design differed from those used in vertebrates in that the observer female did not witness an actual choice between two males by the demonstrator female, but witnessed instead the behaviour of six females alternatively mating with one male phenotype, or rejecting the other phenotype (Figure 1.1).

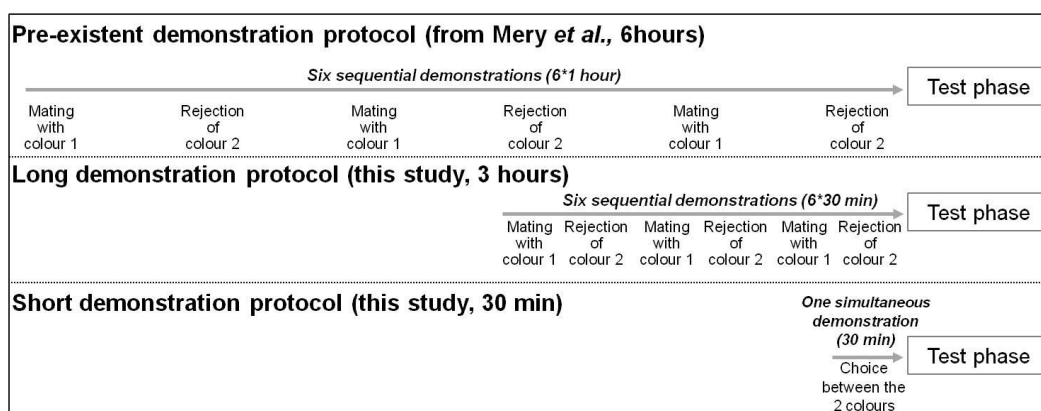


Figure 1. 1: *Drosophila* mate copying protocols. The first protocol was design by Mery et al. (Mery et al., 2009). The two following ones are those of this study. Our long demonstration protocol follows

the same design as Mery's one, which consists of a sequence of demonstrations involving one female mating with a male of one colour, followed by another demonstration with a female rejecting the male of the other colour. This was repeated 3 times and lasted 3 hours for our long demonstration protocol. The short demonstration protocol only involved one live demonstration of a female choosing between two differently coloured males. This shorter demonstration phase lasted only 30 min. In both protocols, a test phase is run just after the demonstration and the observer female preference is recorded.

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). A change in weather, in particular the arrival of heavy rains or storms, can have serious fitness consequences on 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, 2006). Air pressure variation needs also to be considered: a rapid drop indicates the upcoming of a storm or heavy winds (Ahrens, 2006). Even though the influence of weather on animal behaviour has been observed by humans for centuries, it has been investigated only in 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). Mating behaviours have been shown to be affected by air pressure changes in the cucurbit beetle, the true armyworm moth and the potato aphid (Pellegrino et al., 2013). In *D. melanogaster*, only two studies focused on the influence of air pressure on the prevalence of sexual behaviour (Ankney, 1984; Austin et al., 2014). First, Ankney (1984) found that *Drosophila* mating frequency decreases in low air pressure conditions. Second, Austin et al (2014) found an effect of pressure variation on *D. melanogaster* courtship and mating frequency: in decreasing air pressure some flies reduced their mating activity, others in contrast increased it. But the effects of air pressure on other aspects of sexual behaviour such as mate choice or, more generally, cognitive abilities, have never been investigated.

Here, we first investigated whether *Drosophila* females can perform mate copying in a protocol similar to those traditionally used in studies of vertebrates mate copying. For that goal, we realised a first experimental study comparing results from two designs. The first one (adapted from Mery et al's study, Mery et al., 2009) involved 6 apparent female choices in a sequence (long demonstration protocol, Figure 1.1). The second design involved a single live demonstration of a female choosing between two males of contrasting phenotypes (short demonstration protocol, Figure 1.1). Very little is known about *Drosophila* ecology in the wild (Reaume & Sokolowski, 2006), thus it is unsure whether female *Drosophila* would have the possibility to experience sequential demonstrations of mate choice in nature as in the long demonstration protocol. In addition to bridging the gap with vertebrate studies, the rationale for our short demonstration protocol is 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. After this first experiment, we realised a second experimental study to see whether a young virgin female fly could be influenced in its mate choice by the observation of just the copulation of a single other female. Finally, for all of these experiments, we analysed the effect of natural variation in air pressure across experimental days to explore the impact of weather conditions on mate copying performance.

Methods

Fly maintenance and general procedures

We used the common laboratory Canton-S strain of *D. melanogaster*. Flies were raised in 8 ml vials containing a standard wheat flour-agar-yeast medium 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 6h after emergence and kept in unisex groups of 6 individuals before experiments. All *Drosophila* used for the experiments were 3 or 4 days old. Fly manipulations were performed by gentle aspiration. All experiments were

conducted under controlled photoperiod (12 hours daylight), temperature ($25^{\circ} \pm 1^{\circ}\text{C}$), and humidity ($60\% \pm 5\%$). The two artificial male phenotypes were created by randomly 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 (see figure 1). In all test phases, as observer females courted by only one male were not in a position to choose mates, we only kept replicates in which both males courted the female and discarded all the others.

Experiment 1: preliminary experiment

One virgin observer female was placed in the tube with a male of each colour for 30 minutes during which we recorded the identity and number of males courting the female (i.e. if the males were displaying a wing vibration or “singing”, Sokolowski, 2001), as well as the copulation time and colour chosen.

Experiments 2 and 3: Mate copying protocols

Assays were conducted over many days. 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*1h in Mery et al.,2009), and another one with a unique live demonstration that better mimicked natural conditions (Figure 1.1).

The long demonstration protocol consisted in one demonstration of a virgin female mating with a

male of one colour for 30 minutes (Figure 1.1). As virgin females readily accept copulation, this provided to observer females positive information about this male phenotype. For the next 30 minutes, the demonstration involved another male of the other colour together with a recently mated female. As mated females reject every males 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 (Figure 1.1). For the control group, the same protocol was performed with an opaque partition separating the tubes, so that the observer female could not see the demonstration.

The short demonstration protocol consisted in a single demonstration of one female placed with two males, one of each colour for 30 minutes (Figure 1.1). The copulation of the demonstrator female with one of the males provided positive information for that male phenotype and negative information for the other male phenotype. In *Drosophila melanogaster*, copulations last 20 minutes on average (Pavković-Lučić, S, Lučić, L, Miličić, D, Tomić, V, & Savić, T, 2014). So a thirty-minute demonstration ensures that copulation had the time to start and last for long enough to inform the observer female. In the control group, the demonstration consisted in a presentation of a male 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 to control whether observer females had an innate preference for one of the phenotypes.

The test phase immediately followed every demonstration. The test males were previously coloured using the same protocol as the ones used for the demonstration males, consequently males used in all test phases were new ones (they differed from those used in demonstrations), came from different vials and were not powdered at the same time as the demonstrator males. Then, a new pair of males of each colour was placed with the observer females for 30 minutes during which we recorded the time, identity and number of males courting the female (i.e. displaying a wing vibration or “singing”(Sokolowski, 2001)). The courtship latency was defined as the time between the insertion of the two males and the first wing vibration by one of the males. We also recorded the copulation

time and thus were able to have the time between the first courtship and the beginning of copulation. We only kept replicates in which both males courted the female and discarded all the others (for the long demonstration protocol, we kept 125 replicates out of 543 trials; for the short demonstration protocol, we kept 159 replicates out of 472 trials). However, 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.

For demonstrations of the experiment 3, we preliminary put virgin females in tubes with males of the desired colour. Once one mating occurred, the couple was placed on one side of the experimental plastic tube (Figure 1), and a male of the opposite colour was inserted next to the couple. This triad mimicked a situation in which the demonstrator female had chosen one male phenotype over the other. This demonstration lasted 20 minutes. Then the test phase was performed as in experiment 2. As in the previous experiments, we only kept replicates in which both males courted the female (161 replicates on 399 trials).

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 or atmospheric condition was the mean of Mate Copying Scores for these conditions, and indicated female learning. Values around 0.5 indicate random choice by observer females while values above 0.5 reveal mate copying.

Effect of external parameters

We used records of barometric conditions taken every thirty minutes. We examined the effects of 1) absolute air pressure at the onset of the experiment and 2) its variation during the 6 hours

preceding the start of each replicate. The variation in air pressure was calculated as the difference between the absolute air pressure at the moment of the onset of the replicate and the absolute air pressure 6 hours before, divided by 6. This gave the average rate of air pressure change per hour during the preceding 6 hours, 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 supplementary information for more material about the pressure distribution during the experimental days.

Statistical analyses

All statistical analyses were performed with the R software, version 3.1.2 (*R Core Team. R: A language and environment for statistical computing*, 2014). The Mate Copying Score was analyzed through generalized linear mixed model with binary logistic regression with the package *lme4* (Bates D, Maechler M, Bolker B, & Walker S, 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 random effect to control for potential day effect. For the control of the short demonstration protocol, as no phenotype was preferred during the demonstration, the number of pink chosen during the test was set as the Mate Copying Index for the pink demonstration and the number of green chosen during the test for the Mate Copying Index for the green demonstration. We used the same generalized linear mixed model to test the effects of air pressure on the Mate Copying Index, except that we pooled together green and pink demonstrations and included the colour of the chosen male during the demonstration (pink or green) as a random effect.

A 3D graph with Mate Copying Index predicted values according to air pressure at the beginning of the experiment and air pressure variation during the 6 preceding hours illustrates the correlation between climatic conditions and Mate Copying Index. We used a generalized linear model with

binary logistic regression (package *lme4* (Bates D et al., 2014)) with the Mate Copying Score as a function of air pressure value and variation. We used the R packages *plot3D* (Soetaert, 2014) and *rgl*(Adler et al., 2014) to create the 3D graph.

Results

Experiment 1: preliminary control

We first ran a preliminary control to test whether the observer female could have an innate preference for one coloured phenotype. When put in the presence of the two male phenotypes, without any prior information about them, the observer females chose randomly between green and pink males: in 63 trials, we got 30 copulations with pink males and 33 with green males. No statistically significant pattern was detected in this control (Chi-square test: green *versus* pink, $\chi^2_{1} = 0.127$, $p = 0.722$, $n = 63$). We thus concluded that the observer females do not show any innate preference for one or the other coloured phenotype, a result consistent with those of other experiments using this type of powders (Pavković-Lučić, S et al., 2014).

Experiment 2: mate copying under two experimental protocols

When analysing results of the whole data set including the two types of protocols, observer females mate choice depended on the type of demonstration prior to the test phase (logistic regression, Wald test: uninformed i.e. control *versus* informed females, $\chi^2_{1} = 10.261$, $p = 0.0014$, $n = 284$) but not on the type of protocol (logistic regression, Wald test: short *versus* long demonstration protocol for informed females, $\chi^2_{1} = 0.320$, $p = 0.572$, $n = 199$, Figure 1.2). 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 no matter the colour preferred during the demonstration phase (long demonstration protocol: demonstration with pink, Wald chi-square test: $\chi^2_{41} = 2.917$, $p = 0.0057$, $n = 42$; 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: $\chi^2_1 = 0.002$, $p = 0.965$, $n = 115$; Figure 1.2)

For the long demonstration protocol, in controls with an opaque glass partition (preventing the observer female from gathering visual information about the two male phenotypes' mating success, uninformed females 1, Figure 1.2), 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 is slightly different: observer females were shown one green and one pink male without demonstrator female during the demonstration phase (uninformed females 2). We chose this control to allow the observer females to get used to the new phenotypes during the same amount of time as the other observer females from the non-control replicates. Moreover the opaque partition prevented from seeing but not necessarily from hearing or smelling. Similarly as in the control (using an opaque partition) of the long demonstration protocol, no pattern 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 effect of the type of protocol suggests that observer females built equivalent mating preferences under the two protocols implying that females *D. melanogaster* can copy a mate preference even after witnessing a single live demonstration, a situation we call "speed learning".

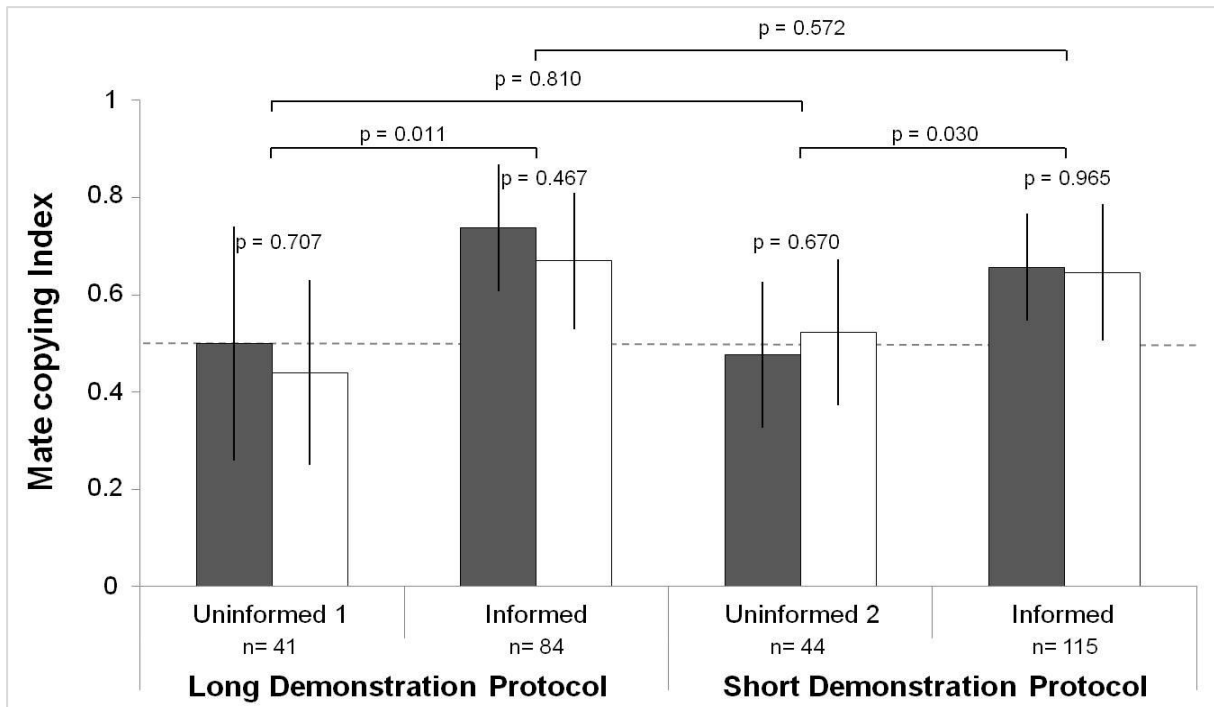


Figure 1. 2: Mate Copying Index according to demonstration protocols (see Figure 1.1). The Mate Copying Index was the proportion of observer females that copied the choice of demonstrator females. The two protocols differed in their demonstrations duration: 3 hours versus 30 minutes and in their demonstration type: sequential versus simultaneous for the long and short demonstration protocol respectively. For each protocol, grey bars: Mate Copying Index of flies that saw a demonstration during which a pink male was preferred; white bars: Mate Copying Index of flies that saw a 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 females 1, $n = 41$) or because there were only two males without any female in the demonstrator side (uninformed females 2, $n = 44$). P values: comparison between two conditions. Vertical bars: confidence intervals; horizontal dash line: expected value if males were chosen randomly.

Experiment 3: influence of the observation of one copulation

In the short demonstration protocol, observer females could gather information from two kinds of mating behaviours: the male courtship performance and/or the female mating choice. To distinguish these two sources of information we carried out a supplementary experiment. The protocol was the same as the short demonstration protocol, except that the observer female was

shown a demonstrator female already mated with one male phenotype, and a male of the other phenotype standing next to the couple. Consequently, the observer female could not gather information about males other than their copulating success. In this design, again, observer females mated preferentially with the male of the colour phenotype they saw mounting the demonstrator females during the demonstration phase (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: $\chi^2_1 = 0.005$, $p = 0.943$, $n = 80$; Figure 1.3). In the control group with an opaque partition (uninformed females), no pattern 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: $\chi^2_1 = 0.245$, $p = 0.621$, $n = 80$; Figure 1.3), and controls and treatments differed significantly (logistic regression, Wald test: uninformed control *versus* informed flies, $\chi^2_1 = 10.566$, $p = 0.001$, $n=160$). The observer female preference thus was influenced by the sole vision of a single other copulating female.

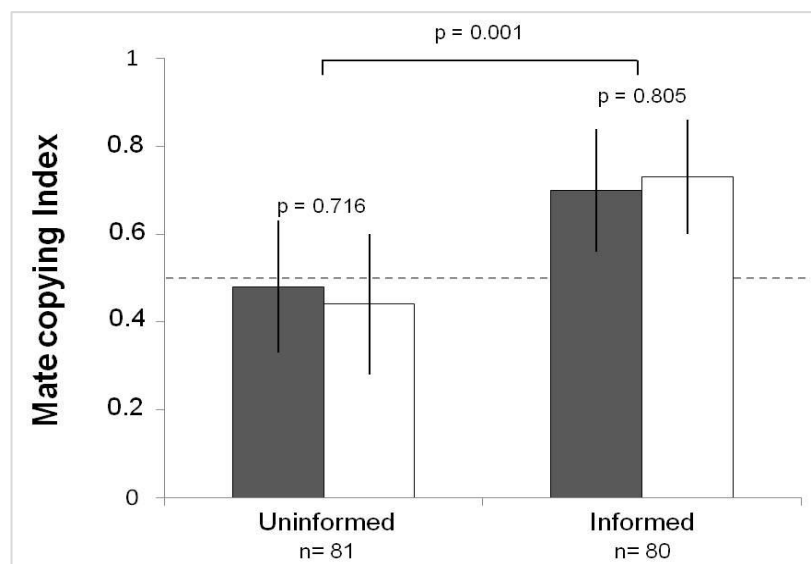


Figure 1. 3 : Mate copying Index in experiment 3. In this experiment, demonstrations consisted in an already formed couple plus a male of the other phenotype so that the observer female only saw a mating and no prior courtship during 20 minutes. Grey: Mate Copying Index of flies that saw a demonstration during which a pink male was preferred; white bars: Mate Copying Index of flies that

saw a green male being chosen. In controls, the observer flies did not see any demonstrator female choice because the partition was opaque (uninformed 1, $n = 81$). P values: comparison between two conditions. Vertical bars: confidence intervals; horizontal dash line: expected value if males were chosen randomly.

Effect of external parameters

We explored with a correlative approach the climatic parameters that could be involved on the pooled data set (we pooled the data because there was no difference between the demonstration with a green or a pink male on the copying rate of the observer female's preference).

To this aim, we defined the Mate Copying Index, which is the fraction of flies copying the mate choice observed in the demonstration (see the Methods section). Given the insight of former studies (Ankney, 1984; Austin et al., 2014), and the fact that temperature and humidity were controlled in the experimental room, we hypothesised that an influential weather parameter, if any, should be linked to air pressure. We nevertheless explored possible relationships of Mate Copying Index with small residual variations in temperature and humidity. In experiment 2, for both protocols, we did not find any significant covariation of the Mate Copying Index neither with temperature (long demonstration protocol, logistic regression, Wald test: $\chi^2_1 = 0.047$, $p = 0.829$, $n = 84$; short demonstration protocol, logistic regression, Wald test: $\chi^2_1 = 0.161$, $p = 0.688$, $n = 115$) nor humidity (long demonstration protocol, logistic regression, Wald test: $\chi^2_1 = 0.851$, $p = 0.356$, $n = 84$; short demonstration protocol, logistic regression, Wald test: $\chi^2_1 = 1.979$, $p = 0.160$, $n = 115$). The same result was found in experiment 3 (logistic regression, Wald test: temperature: $\chi^2_1 = 3.571$, $p = 0.059$, $n = 80$; humidity: $\chi^2_1 = 1.588$, $p = 0.208$, $n = 80$).

For a relationship between the Mate Copying Index and air pressure, in the full data set of experiment 2, the model selected included both absolute air pressure and change in air pressure through time. For the short demonstration protocol, the interaction term between absolute air pressure and its variation covaried significantly with the Mate Copying Index (logistic regression,

Wald test: $\chi^2_1 = 6.793$, $p = 0.009$, $n = 115$). Replicates performed in high and increasing air pressure led to significantly higher Mate Copying Indexes than those in other conditions (Figure 1.4). For the long demonstration protocol, there was no significant effect of absolute or variation in air pressure on the Mate Copying Index (logistic regression, Wald test: $\chi^2_1 = 2.043$, $p = 0.153$; $\chi^2_1 = 0.636$, $p = 0.425$, respectively, $n = 84$). We also found a significant correlation between the interaction term between air pressure and its variation and the Mate Copying Index in experiment 3 (logistic regression, Wald test: $\chi^2_1 = 11.439$, $p < 0.001$, $n = 80$).

Furthermore, in experiment 2, absolute air pressure and air pressure variation covaried significantly with courtship latency, i.e. the time until first wing vibration (Wald test: $\chi^2_1 = 14.141$, $p < 0.001$; $\chi^2_1 = 5.424$, $p = 0.020$, respectively; protocol effect: $\chi^2_1 = 0.741$, $p = 0.389$, demonstration effect: $\chi^2_1 = 2.245$, $p = 0.134$; $n = 249$). This latency was significantly shorter in high and increasing pressures (for example, mean latency = $157.9 \text{ s} \pm 19.8 \text{ s}$, $n = 51$ when the air pressure was $> 1013 \text{ hPa}$ and increasing, *versus* $256.03 \text{ s} \pm 29.2 \text{ s}$, $n = 36$ when the air pressure was $< 1013 \text{ hPa}$ and decreasing). We did not detect any significant relationship between air pressure and the time between the first wing vibration and copulation (Wald test: absolute air pressure: $\chi^2_1 = 0.004$, $p = 0.951$; air pressure variation: $\chi^2_1 = 0.228$, $p = 0.633$; $n = 249$), which is mainly under female control (for example, mean time = $219.5 \text{ s} \pm 25.5 \text{ s}$, $n = 51$ when the air pressure was $> 1013 \text{ hPa}$ and increasing, *versus* $210.4 \text{ s} \pm 36 \text{ s}$, $n = 36$ when the air pressure was $< 1013 \text{ hPa}$ and decreasing). The female can indeed accept a courting male by slowing down its walk and allowing the male to copulate (Kimura, Sato, Koganezawa, & Yamamoto, 2015) or reject it using various ways such as decamping, kicking the male or extruding its ovipositor (Connolly & Cook, 1973).

In experiment 3, there was a significant correlation of absolute air pressure but not air pressure variation with courtship latency (Wald test: $\chi^2_1 = 4.678$, $p = 0.030$; $\chi^2_1 = 0.004$, $p = 0.948$, respectively; $n = 160$). As in the other experiment, we did not detect any significant correlation between air pressure and the time between the first wing vibration and copulation (Wald test: absolute air pressure: $\chi^2_1 = 0.224$, $p = 0.636$; air pressure variation: $\chi^2_1 = 0.940$, $p = 0.332$, $n = 160$).

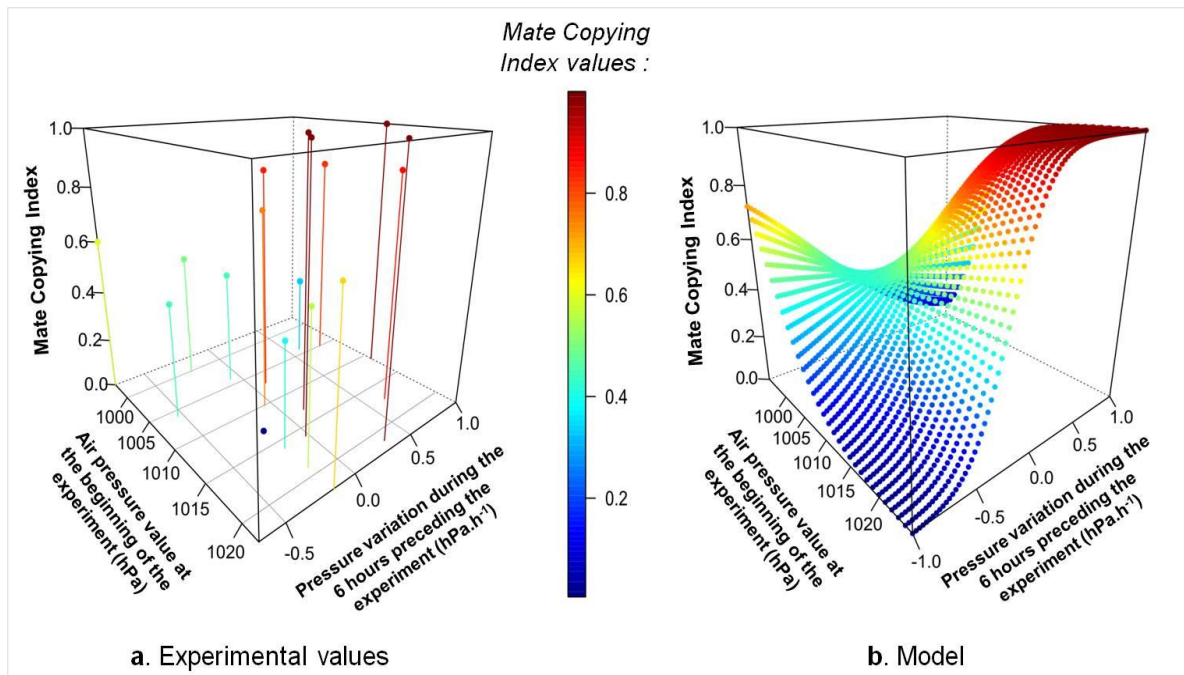


Figure 1. 4: Mate Copying Index according to absolute air pressure and its variation during the 6 hours preceding the experiment for the short demonstration protocol (experiment 2). The short demonstration protocol consisted in only one demonstration of a female choosing between two males with different phenotypes. The mate copying Index is the proportion of observer females that copied the choice of demonstrator females. Part a) shows the experimental Mate Copying Index calculated for each couple of pressure (in hPa) and pressure variation (in hPa.h⁻¹). Part b) shows the statistical model obtained with the experimental points. Its surface represents predicted values of Mate Copying Index (n = 115).

Discussion

Our study confirms that *D. melanogaster* can perform social learning in the form of 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 such social learning is similar under the short and long protocols, in spite of the fact that the quantity of available information differed drastically between them (Figure 1.1). This reveals an unsuspected capacity for social learning and mate copying in this species.

In the short demonstration protocol, demonstrator females chose freely between two males, 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. We found a slight but non-significant preference for pink males in demonstrator females (in the short demonstration protocol, on 115 trials, demonstrator females mated 67 times with the pink male versus 48 times with the green male, Wald chi-square: $\chi^2_{114} = 1.756$, $p = 0.082$) but no such tendency in control groups, nor in the preliminary control (experiment 1). Moreover the copying rate was similar when green or pink males were chosen during the demonstration phase. Last, the results of experiment 3, where the choice of the demonstrator female was entirely controlled, corroborated those of experiment 2. Altogether, these considerations suggest that observer female preference was mainly driven by the final choice of the demonstrator fly, i.e. by the copulation itself. The fact that observing a single copulation event seems enough to induce a preference in the observer females invites us to speculate that this kind of observational learning may also occur in nature.

Temperature, humidity and photoperiod were controlled in our experimental room. We did not find any significant correlation of the residual variations of temperature and humidity with the Mate Copying Index (of course, these results do not rule out a potential impact of temperature and humidity in experiments where they would be controlled as varying explanatory variables). As regards air pressure, in the short demonstration protocol, we found a strong correlation between the Mate Copying Index and air pressure (absolute air pressure and recent variation). Female social learning was more efficient in high and increasing air pressure, i.e. according to meteorological studies (Ahrens, 2006), when climatic conditions were improving.

The correlation between air pressure and mate copying rate was only significant in the short demonstration 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 hyped for 3 hours.

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). Such weather effects also exist in children that 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 focussed on overall activity which decreased 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; Rouse, 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) and thus after an increase in air pressure. Here we show that mate copying in a speed learning design is higher in good or improving climatic conditions. This suggests that accounting for air pressure and more generally external conditions might be important in insect behavioural studies. The effect on cognition may be either direct or indirect, for instance through an increase in activity, potentially improving information gathering. The latter interpretation seems supported by the fact that in both protocols males started courting more rapidly in improving climatic conditions and by the fact that the long demonstration protocol seemed to “overcome” the effect of external conditions.

In conclusion, we showed that *D. melanogaster* can perform mate copying even in a speed learning context, and that this behaviour seems more frequent under improving climatic conditions. Though little is known on the ecology of *Drosophila* in the wild, we can speculate that mating occurs under such good or improving climatic conditions, giving occasions for females to copy mate choices by others. The importance of these mate copying abilities in the field, and their potential impact on *Drosophila*'s evolution, need to be further evaluated.

Acknowledgements

We thank Thomas Achkar, Mélanie Allain and Amelia Landre 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 French funding agency ANR (project ANR-13-BSV7-0007-01 to ED) that also provided part of ACD's salary.

**Chapter 2: Testing criteria of
culture in *Drosophila
melanogaster***

Introduction

In chapter 1, we showed that *Drosophila melanogaster* females copy the mate choice of other females. This acquisition of a new behaviour through the observation of other animals is called social learning (Brown and Laland, 2003). The idea that animals could acquire new behaviours through social learning dates back to Antiquity. Aristotle indeed described some social learning of song in birds (Aristotle, *Histoire des animaux*, livre 4, chapitre IX ; Hoppitt and Laland, 2013). The bird's behaviour (among other animal behaviours) was also noticed by the evolutionists of the 19th century, such as Alfred Wallace, George Romanes and Conwy Lloyd Morgan. For them, the transmission of behaviours in animals (and thus their traditions) was the origin of adaptative behaviour (Laland and Galef, 2009).

Nowadays, the two most well known examples of a social transmission in animals are the ones that have been described in the second half of the 20th century. The first one is the opening of milk bottles by British tits (great tits, *Parus major*, and blue tits *Cyanistes caeruleus* ; Fisher and Hinde, 1949). The second one is the spread of potato washing techniques in a group of Japanese macaques (*Macaca fuscata*) on the island of Koshima (Kawai, 1965). These observations started the modern debate over the existence of animal traditions or animal culture (Laland and Galef, 2009).

One of the major problems that came across the development of the field of research on animal culture was to find a clear and universally-recognized definition of the term culture. Using the existing definitions employed in human culture studies would have been too narrow because these definitions had been specifically created to describe human behaviour, and thus could not generally be applied to all animal species (Laland and Hoppitt, 2003). On the other hand, too broad definitions do not truly capture the cultural phenomenon because some criteria are lacking (for example the definition of John Tyler Bonner who describes culture as "the transfer of information by behavioral means" (Bonner and Farge, 1989) lacks the transmission of the information between generations). Here we will use the definition of Danchin et al. (2004) who describe culture as the "sum of

traditions and information that vary among groups; the transmission of these differences across generations rests on social interactions (imprinting, imitation, learning, or teaching) that change the phenotype lastingly. Culture therefore consists of nongenetic, heritable differences among populations and requires overlapping generations that allow intergenerational transmission of phenotypic traits" (Danchin et al., 2004).

Cultural transmission has been accepted and reported in a restricted set of non-human mammals and few bird species, known for their high cognitive abilities such as primates (chimpanzees (Whiten, 2005; Whiten et al., 2009), orangutans (van Schaik et al., 2003), vervet monkeys (van de Waal et al., 2013), cetaceans (whales Allen et al., 2013; Whitehead, 1998), dolphins (Krützen et al., 2005), see also: Rendell and Whitehead, 2001) and in zebra finches (Fehér et al., 2009) and cowbirds (Freeberg, 1998). This is a non exhaustive list and the field is still growing. All these studies show the continuity between animal and human culture (Whiten et al., 2011). But why not having this continuity spanning across other taxa as well? Intuitively it seems likely that other taxa might demonstrate social learning and traditions, thus having the predispositions for cultural evolution. The current lack of studies of culture in other taxa might results from the absence of clear methods to determine whether a trait is culturally inherited or not (Danchin et al., 2010).

For this purpose, four testable criteria were introduced by Danchin and collaborators. They proposed that if a trait is shown to fulfil these four criteria simultaneously, it can be accepted as at least partly culturally transmitted (Danchin et al., 2011; Danchin and Wagner, 2010):

1. The first criterion is that the trait must be socially learned *i.e.* learned from others (Danchin et al., 2004; Whiten and van Schaik, 2007), this is the touchstone of all the definitions of culture.

2. Second, individuals have to be able to generalize the newly acquired information and use it in new contexts. Only general rules can indeed be transmitted across generations, as specific situations might change or disappear over time (Bowers et al., 2012a; White and Galef, 2000).

3. Third, the behaviour of the individual has to be modified for a sufficient time to allow others to observe and learn the new trait (Brooks, 1998).

4. Finally, the socially learned information has to be transmitted across generations, from older to younger individuals (Avital and Jablonka, 2000). Thus, individuals of different generations have to be in interaction.

Despite the fact that the first and last criteria could be considered as sufficient to demonstrate that a trait is culturally transmitted, the other ones have been included because they create favourable conditions for the realisation of the fourth criterion (transmission across generations). Altogether, these criteria provide conceptual and practical tools to identify cultural traits (Danchin et al., 2011). To our knowledge, they have never been tested simultaneously on a single system.

The goal of this thesis is to study whether *Drosophila melanogaster* has the potential to transmit mate choice partly culturally, and thus to test the four previous criteria on the transmission of a mate preference in *Drosophila*. The following experiments have been realised with the help of Eva Gazagne and Guillaume Gomez. The theoretical model and analyses have been made in collaboration with Arnaud Pocheville (University of Sydney).

1. Generalization in mate copying in *D. melanogaster* (criterion 2)

In chapter 1, we showed that *Drosophila* females could socially acquire a preference for a male phenotype. We thus verified the **criterion 1**. In the experiment with the short demonstration protocol, we provided two new males to the observer female during the test phase (see figure 2.1.)

Short demonstration protocol (*One simultaneous demonstration of 30 min*)

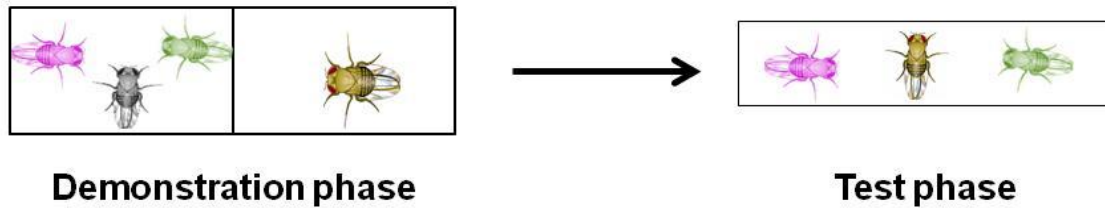


Figure 2. 1: *Drosophila* mate copying protocols. The short demonstration protocol as been described in chapter 1 and is used in all experiments of this thesis. It involves one observer female observing another female choosing between a pink or a green male. This demonstration phase lasted 30 min. Then a test phase is run after the demonstration: two new males dusted in pink and green are put with the observer female and its preference is recorded.

Even though the test males are not the same as the one used during the demonstration phase, we could not rule out the possibility that the female believed they were the same males as the demonstration. We thus realized a new experiment to eliminate this uncertainty. This time, during the test phase, we used some phenotypic mutant males dusted in green or pink. They thus had one physical characteristic that differed from the demonstrator males but presented the same colours (green or pink). This allowed to clearly test the second criterion and see whether females were able to generalize the newly acquired information and thus performing trait-based mate copying.

1.1. Methods

In this experiment the demonstration and test phases take place into plastic tubes separated in the middle by a thin glass partition (see figure 1). The naive observer female is placed at one side of the tube device and the demonstrator flies in the other side with the two coloured males.

During the demonstration phase, a wild type observer female was shown another wild type female choosing between two wild type males, following the short demonstration protocol described in chapter 1 (see also figure 2.1). For the test phase, we provided the observer females two males with the same mutant phenotype (with curly instead of straight wings or white instead of red eyes).

As a control we ran in parallel the same experiment with wild type males for the demonstration and test phase. Thus during the test phase the observer female had to choose between two males dusted with green or pink powders, that could look like the one seen during the demonstration phase (wild type males, control situation), or have different wings (*curly* phenotype), or a different eye colour (*white* phenotype); (figure 2.2).

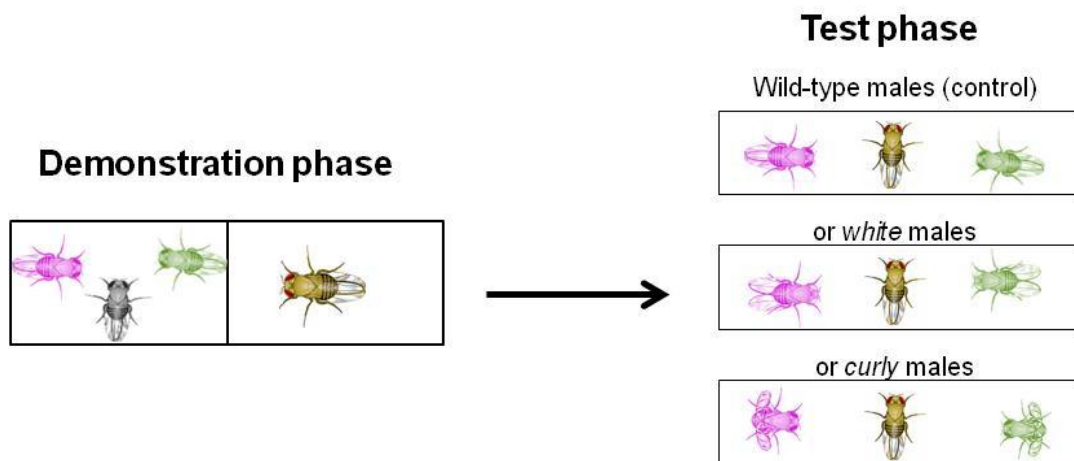


Figure 2. 2: Experimental protocol. This protocol is based on the short demonstration protocol except that during the test phase, the observer female has to choose between two wild-type, *curly* or *white* males.

As in the experiments in chapter 1, we recorded both the courtship and copulation latencies and the colour of the male(s) which had been involved during the test phase. If no copulation happened during 30 min, a replicate was recorded as a failure, and as in the previous experiments we only kept the situations in which both males did a courtship during the test phase (so 142 replicates over 612).

1.2. Results

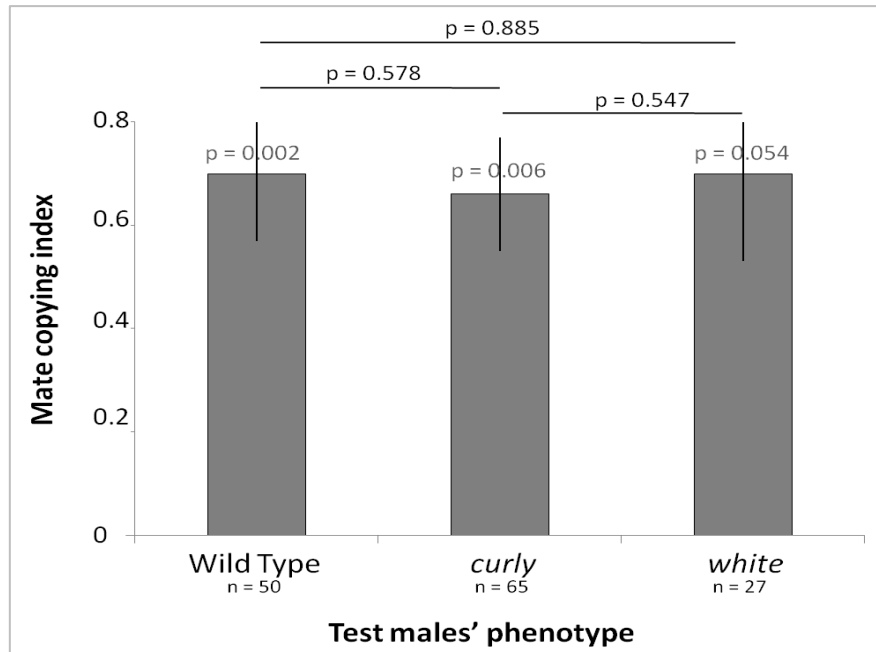


Figure 2. 3: Mate copying index according to the test males' phenotype. The mate copying protocol used is the short demonstration one except that the test males were from wild type, *curly*, or *white* phenotypes. The Mate Copying Index is the proportion of observer females that copied the choice of demonstrator females. Vertical bars are confidence intervals; p values are comparisons between two groups or , for the ones above the bars (in grey) , correspond to Wald chi-square tests between the experimental Mate Copying Index and a random choice.

In all treatments there was a significant effect of the demonstration on the observer female's choice (GLMM: $p < 0.0001$, $n = 142$). We found no significant effect of the test male's phenotype on the choice of the observer female (GLMM: $p = 0.779$, $n = 142$) and no significant difference when we compared two phenotypes at a time (GLMM: Wild-type and *curly*: $p = 0.578$, $n = 115$, Wild-type and *white*: $p = 0.885$, $n = 77$, *curly* and *white*: $p = 0.547$, $n = 92$, see figure 2.3).

However, we found a significant effect of the male's phenotype on the courtship latency ($p = 0.0022$, $n = 142$), with white eye males taking more time to start courting (298 seconds in

average) than *curly* (139 seconds in average) and wild-type flies (152 seconds in average). The male's phenotype had also a significant effect on the time between the courtship and the copulation ($p < 0.0001$, $n = 142$). A wild type male needed an average of 195 seconds before the copulation while the duration is 318 seconds in average for a *curly* male and 534 seconds in average for a *white* male.

The small sample size of the white group is due to a high number of failed copulations during the test phase: on 168 trials, only 57 had a copulation and in 27 both males courted the female. The behaviour of white eyes flies can explain this high proportion of failure during the copulation (66%). The failures obtained can be explained by the *white* mutation: male mating success has been shown to be correlated with the amount of eye pigmentation (Connolly et al., 1969; Geer and Green, 1962). Moreover, *white* mutants have an attenuated visual acuity (Kalmus, 1943) which gives males difficulties to find females and maintain contact with them (Connolly et al., 1969). This phenomenon is consistent with our data: *white* males were significantly slower to begin the first courtship than wild type or *curly* males.

The duration between the first courtship and the copulation is under female control. In *Drosophila melanogaster*, the female can indeed accept a courting male by slowing down its walk and allowing the male to copulate (Kimura et al., 2015) or reject it using various ways such as decamping, kicking the male or extruding its ovipositor (Connolly and Cook, 1973). Thus the higher duration found for *white* and *curly* mutants seems to show that wild-type females are more reluctant to mate with them. However, even though wild type females were less motivated to mate with mutants, they chose mainly the same colour as the one seen preferred during the demonstrations, thus showing some trait-based copying.

In conclusion the female flies are able to learn socially to prefer one type of male and to generalize this preference. However to transfer this preference to other females, this information needs to be kept a sufficient time to allow the finding of new males. So we studied how long the

females were able to retain this information and if this time period could be sufficient to encounter new males.

2. Durability of the acquired preference (criterion 3)

The goal of the following experiment was to study how long a *Drosophila* female could retain the preference for a male phenotype. In our experimental protocol, in order to see the female's preference we observed which male it would mate with. This protocol did not allow to test the same female multiple times as *Drosophila melanogaster* females do not remate during 7 days in average after a first copulation (Singh et al., 2002). We thus used the hexagonal device in order to have a group of females watching the same demonstration and then to be able to test their preference at different times.

As *Drosophila* is a model system for the study of learning and memory, numerous studies exist on this topic. Olfactory learning with a single training session had been shown to last up to one day (Margulies et al., 2005; Tempel et al., 1983; Tully et al., 1994). In visual learning paradigm, Folkers, (1982) tested her flies 2 hours after the training session, but the learning score dropped after the first 30 minutes. Ofstad et al. (2011) tested the flies up to 8 hours after the training session and had significant learning scores until 2 hours after the training phase. Based on these studies we decided to first test our flies 1 hour and 3 hours after the demonstration phase. We then ran another series of experiments, testing the flies 24h and 48h after the demonstration.

2.1. Methods

To study the durability of the learnt preference, we put six observer females in the central compartment of the hexagonal device and showed them 6 other females mating with males of the same colour simultaneously. These pairs were firstly created in plastic tubes in order to control the number and colour of demonstration. With the demonstrator mating couples, we also placed a male

of the complementary colour (pink if the demonstrator female fly was mating with a green male or green if the demonstrator fly was with a pink male) in each peripheral compartment. These triads provided positive public information for the phenotype of the male who is copulating with the demonstrator female (see figure 2.4).

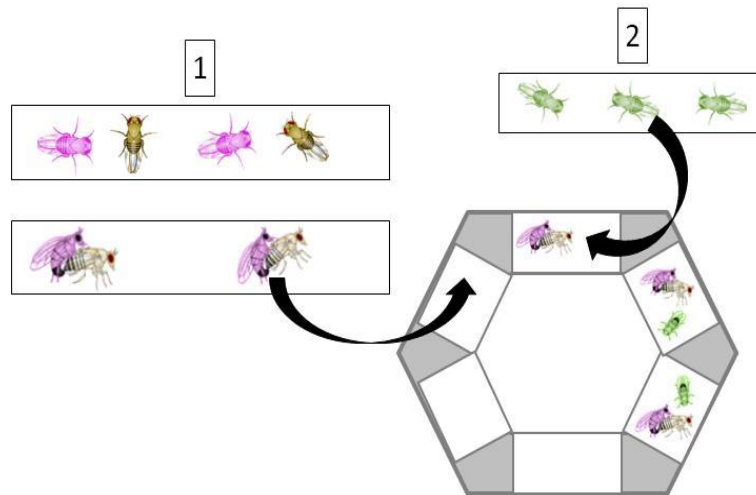


Figure 2. 4: Schematic representation of the method used to make a constraint demonstration.

Step 1: virgin females were placed in tubes with males of the desired color (here is pink as an example). Once they started mating, the couples were placed in the peripheral chambers of the hexagonal device. Then, in step 2, a male of the opposite colour (here green) was inserted next to the couple. These two steps mimicked a situation in which a female had chosen a pink male over a green male and allowed to easily have 6 copulations with the same male colour.

At the end of the demonstration, the observer females were shifted to vials containing medium. For the test phase, we followed the same protocol as in the previous experiment, and tested one group of two females right after the demonstration, another group 1 hour after the demonstration and the last group 3 hours after the demonstration.

We conducted another series of experiments and repeated this protocol but this time, we tested one group right after the demonstration, the other one 24h after and the last one 48 hours after the demonstration (see figure 2.5). As before, we only kept the replicates in which both males did a

courtship during the test phase (that is 144 replicates among 306 for the first experiment and 171 replicates among 363 for the second experiment).

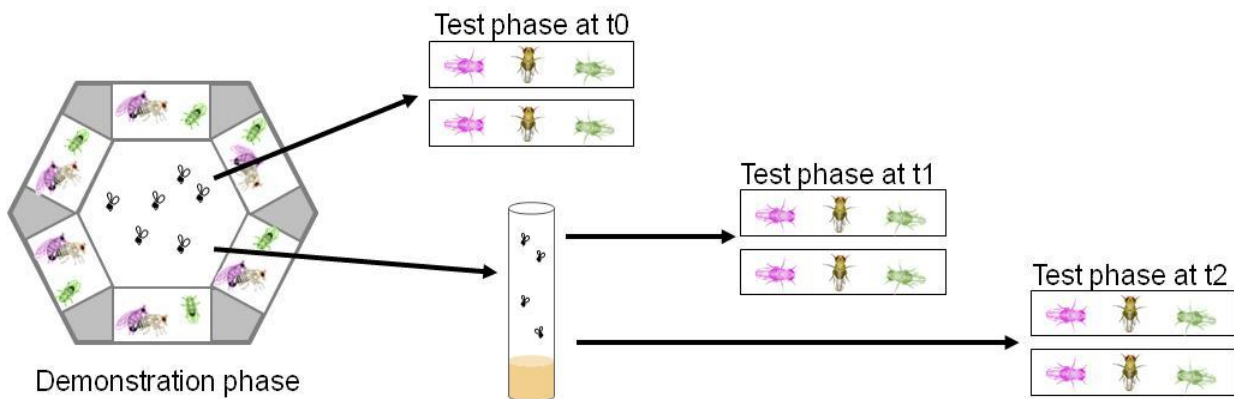


Figure 2. 5: Schematic representation of the protocol used for the experiment of durability. After the constraint demonstration, 2 of the observer females are taken for the test phase at t0. The other observer females are placed in a vial with medium. Then 2 of the observer females are tested for their male preference at t1 (that is 1h or 24h after the demonstration, depending on the experiment) and the 2 remaining ones are tested at time t2 (that is 3h or 48h after the demonstration, depending on the experiment)

2.2. Results

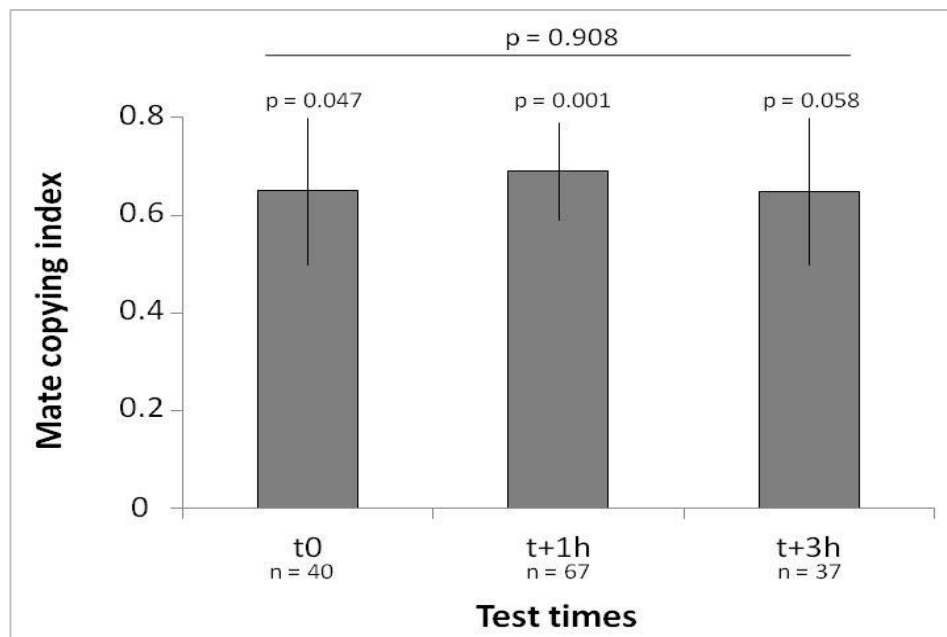


Figure 2. 6: Mate copying index according to the test times for the first set. The mate copying protocol used is the short demonstration one. The Mate Copying Index is the proportion of observer

females that copied the choice of demonstrator females. Females were tested right after the demonstration (t_0), one hour ($t + 1h$) or three hours ($t + 3h$) after. Vertical bars are confidence intervals, p values above the bars correspond to Wald chi-square tests between the experimental Mate Copying Index and a random choice or the comparison between the groups.

In all treatments there was a significant effect of the demonstration on the observer female's choice (see figure 2.6) for the test at t_0 (Chi-square: $p = 0.047$, $n = 40$) and at $t+1h$ (Chi-square: $p = 0.001$, $n = 67$) and a strong trend for the test at $t+3h$ (Chi-square: $p = 0.058$, $n = 37$). Moreover we found no significant difference between the three test times (GLMM: $p = 0.908$, $n = 144$).

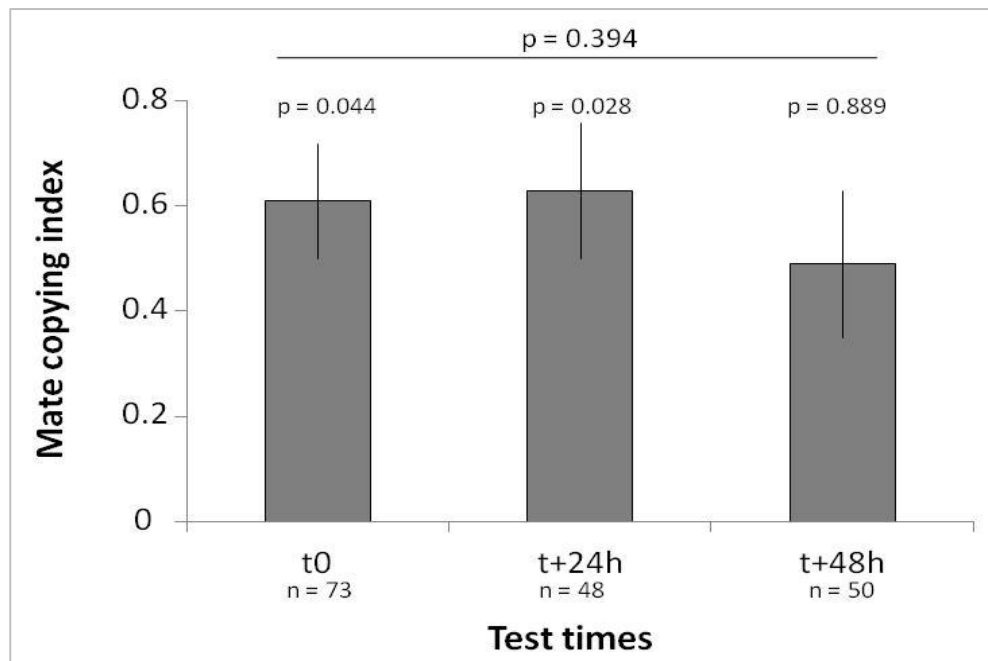


Figure 2. 7: Mate copying index according to the test times for the second set. The mate copying protocol used is the short demonstration one. The Mate Copying Index is the proportion of observer females that copied the choice of demonstrator females. Females were tested right after the demonstration (t_0), one day ($t + 24h$) or two days ($t + 48h$) after. Vertical bars are confidence intervals, p values above the bars correspond to Wald chi-square tests between the experimental Mate Copying Index and a random choice or the comparison between the groups.

We found no significant difference between the 3 different test times (GLMM: $p = 0.394$, $n = 171$). However there was a significant effect of the demonstration on the observer female's choice only for the test at t_0 (Chi-square: $p = 0.044$, $n = 73$) and $t+24h$ (Chi-square: $p = 0.028$, $n = 48$) but no significant effect for the test 48h after the demonstration (Chi-square: $p = 0.889$, $n = 50$).

According to the results, the flies seemed to retain an acquired preference at least 3h after seeing another female's copulation. They might also retain the information up to 24h after the demonstration but there is only a tendency and more replicates are needed to draw clearer conclusions.

Studies on odour-avoidance response in *Drosophila* have shown that "massed training" (multiple consecutive sessions of training) was improving the memory duration up to 3 days (Margulies et al., 2005). If these sessions are spaced with a rest interval of 15 minutes, the flies retained the information for a week (Tully et al., 1994). Thus as a future study with our observational learning paradigm, it would be very interesting to see the impact of the number of demonstrations (spaced or not with a rest interval) on the durability of the acquired preference.

3. Transmission across generations (Criterion 4)

The previous experiments have shown that *Drosophila* females were able to socially learn to prefer one type of male (criterion 1), to generalize this preference (criterion 2) and that they could keep this preference up to 24 hours (criterion 3). As the last two criteria are fulfilled, this creates favourable conditions for the realization of the transmission of the preference across generations (Danchin and Wagner, 2010).

Drosophila melanogaster is not a group living species strictly speaking. They aggregate and lay eggs on food sources (Reaume and Sokolowski, 2006), but there is no parental care and the offspring

needs an average of 10 days at 25°C to reach the adult stage (Ashburner, 1989). So it is unlikely that one female can act as a demonstrator for its own progeny. However new flies are emerging every day, so flies at different ages and mating status are interacting. Thus in order to have a transmission of the preference through time, the preference has to spread across the population. We used the hexagonal device to investigate whether we could create a transmission chain of a preference for one phenotype.

3.1. Transmission chain

The goal of the following experiment was to study if the observer females could in their turn become demonstrator flies for a new set of observer females (see figure 2.8). In this way, a transmission chain of the preference could appear and this new behavior could spread across the population.

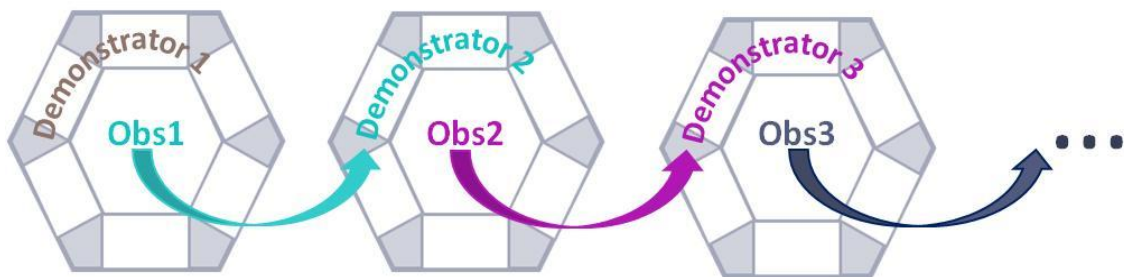


Figure 2. 8: Theoretical design of the transmission chain.

According to the results of our previous experiments, the copying rate is less than 100%. In the transmission chain, we only control the demonstration 1. All the other demonstrations are made by the former observer flies. Thus the new set of observer flies won't have a homogeneous demonstration because of some flies not manifesting the copying behaviour. Thus our first question was: do *Drosophila* females copy the preference of the majority? In other words is there a conformist bias?

3.2. Conformism

Conformism is the phenomenon in which individuals preferentially adopt the behaviours that are the most frequent in the local population despite the presence of other options (Henrich and Boyd, 1998; Waal, 2013). In terms of probabilities, there is a conformist bias when the rate of copying the most common behaviour is higher than the frequency of that behaviour in the population (Wakano and Aoki, 2007).

To test whether there was conformism in *D. melanogaster*, we decided to conduct an experiment with a controlled demonstration composed only of 6 matings but not all with the same male phenotype, to see whether the observer females could acquire the preference of the majority.

We realised the demonstration phase in the same way as the previous experiment with the hexagonal device: we put already formed couples with the males of the desired colour, plus a single male of the other colour (see figure 2.4). This experiment was composed of seven different demonstration phases. The first ones were either an homogeneous preference with 6 demonstrator females mating with the same coloured males and conversely (6P/0G : 6 pink males chosen or 0P/6G: 6 green males chosen); or with no preference for one male phenotype with 3 couples of each colour (control situation 3P/3G: 3 demonstrator females mating with a pink male and 3 with a green male, see figure 2.9). We then introduced contradiction in the demonstration to see whether flies could acquire the preference of the majority. To do that we put 5 females mating with one colour and 1 mating with the other colour (1 contradiction: 5P/1G or 1P/5G) or 4 females mating with one colour and 2 mating with the other colour (2 contradictions: 4P/2G or 2P/4G), see figure 2.9.

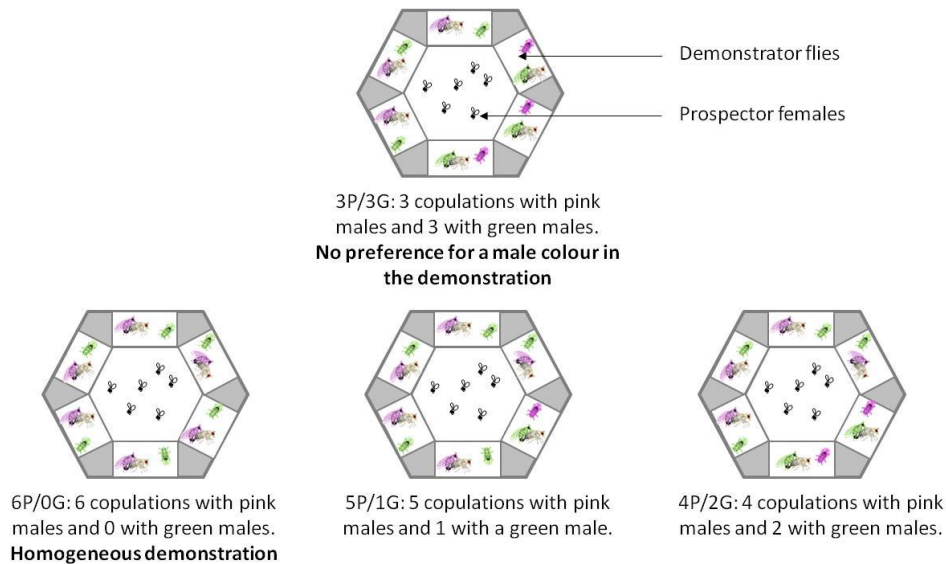


Figure 2. 9: Representation of the experimental protocol. Here is presented an example with the majority of the demonstrator flies choosing the pink male phenotype. We also ran the symmetrical demonstration phases with the majority of flies choosing the green male phenotype. Just after the demonstration phase, we performed a test phase in the same way as in previous experiments. As in the other protocols, we kept only the cases in which two males did the courtship (that is 223 among 879 replicats).

Results

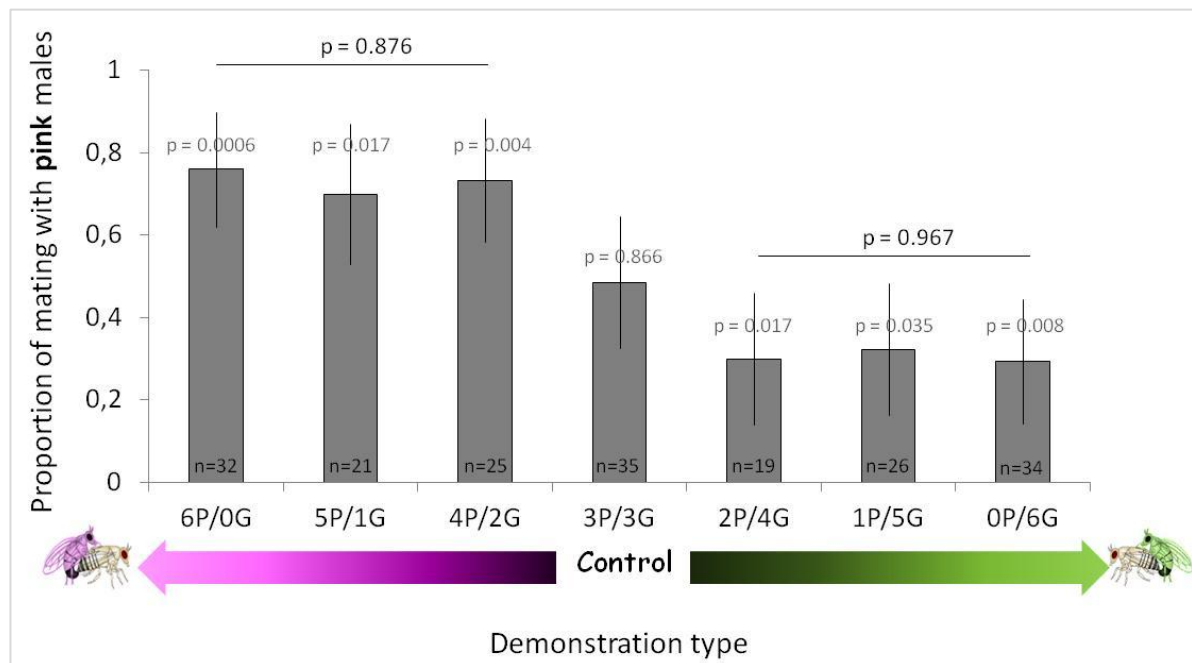


Figure 2. 10: Proportion of matings with pink males according to the number of contradictions during the demonstration phase. The mate copying protocol used is the short demonstration one.

Contrary to the previous graphs, where the Mate copying Index was given, here is presented the proportion of females mating with pink males during the test phase (thus the proportion of green males chosen is the exact reciprocal). The demonstration type corresponds to the proportion of matings with pink and/or green males during the demonstration phase (as in figure 2.9). 6P/0G and 0P/6G are demonstrations in which only pink or green males respectively mated with the demonstrator females. p values above the bars correspond to Wald chi-square tests between the experimental Mate Copying Index and a random choice or the comparison between the groups.

There is a significant difference between the demonstrations with a majority of pink and the ones with a majority of green (GLMM: $p < 0.0001$, $n = 188$, figure 2.10). There is no significant difference between the different demonstrations in which pink males were preferred by the majority in the proportion of pink males chosen during the test (GLMM: $p = 0.876$, $n = 93$). This result is also found in the demonstrations in which green males were preferred by the majority (GLMM: $p = 0.967$, $n = 95$, figure 2.10). However, there is a significant difference between these groups and the control with 3P/3G (GLMM pink vs control group: $p = 0.010$, $n = 93$; green vs control group: $p = 0.048$, $n = 95$).

These results suggest that females *Drosophila* copy the choice of the demonstrators females even when there is some contradictory information in the demonstration. They thus copy the majority. This behaviour is part of the social-learning strategies (Laland, 2004). These strategies determine the optimal conditions of usage of social information (Grüter and Leadbeater, 2014). If the behaviour of most individuals is indeed the most successful one, then copying the majority is a good strategy. Sometimes the majority behaviour is suboptimal and thus this strategy is not adaptive (Giraldeau et al., 2002; Grüter and Leadbeater, 2014). However, in a mate choice situation, even if the preferred phenotype does not correlate with the best male quality, copying the majority might be still adaptive. According to Fisher's "sexy son hypothesis" (Fisher, 1930), choosing the preferred male will tend to produce offspring with this attractive phenotype and thus with the highest chance of reproductive success. Mating with the preferred phenotype is thus an advantageous strategy if the

preference remains constant in the population. Nonetheless, if the preference disappear, the offspring quality won't be worse than the one of the majority (Vakirtzis, 2011). In this case following the majority's preference is thus a strategy enabling to reduce one's variance in fitness.

The results of this experiment indicate that observer females are copying the preference of the majority. However, all the demonstrations were constrained (that is, observer females did not see a real choice). We thus decided to investigate whether we could obtain the same phenomenon with a demonstration composed of 6 demonstrator females choosing freely between a green and a pink male.

We followed the same experimental protocol as in the previous experiment, but without constraining the demonstrator female. We put six virgin observer females into the centre of the experimental device and six virgin females with a male of each colour (green and pink) into the peripheral chambers. As in other experiments, this demonstration phase lasted 30 minutes.

As we did not control which male the demonstrator flies would chose, 3 different scenarios could be obtained:

- (i) The demonstrator females would mostly mate with pink males. So the phenotype preferred by the majority would be pink.
- (ii) The demonstrator females would mostly mate with green males. So the phenotype preferred by the majority would be green.
- (iii) The demonstrator females would mate with as many green males as pink males (control): no phenotype would be predominantly preferred

Moreover in each peripheral chamber of the hexagon, 3 scenarios are possible: either the demonstrator female mates with the green male or with the pink male or no copulation occurs (so

none of them is chosen). Thus 28 different demonstrations are possible in this new device (see table 2)

	Number of demonstrator females mated with	
	Pink males	Green males
Preference of the majority for pink males	6	0
	5	0
	5	1
	4	0
	4	1
	4	2
	3	0
	3	1
	3	2
	2	0
	2	1
	1	0
Preference of the majority for green males	0	6
	0	5
	1	5
	0	4
	1	4
	2	4
	0	3
	1	3
	2	3
	0	2
	1	2
0	1	
No difference in the preference	3	3
	2	2
	1	1
	0	0

Table 2: The 28 different demonstrations possible in the hexagonal device. The control situations are: 3 flies choose a pink male and 3 choose a green male (3P/3G); 2 flies mate with a pink male, 2 mate with a green male and 2 do not mate (2P/2G); only two flies mate one with a pink male and the other one with a green male (1P/1G); or no copulations occur at all.

Then a test phase followed the demonstration phase. The protocol used is the same as in previous experiments. We kept only the replicates with 2 courtships during the test phase that is 109 replicates on a total of 572.

Results

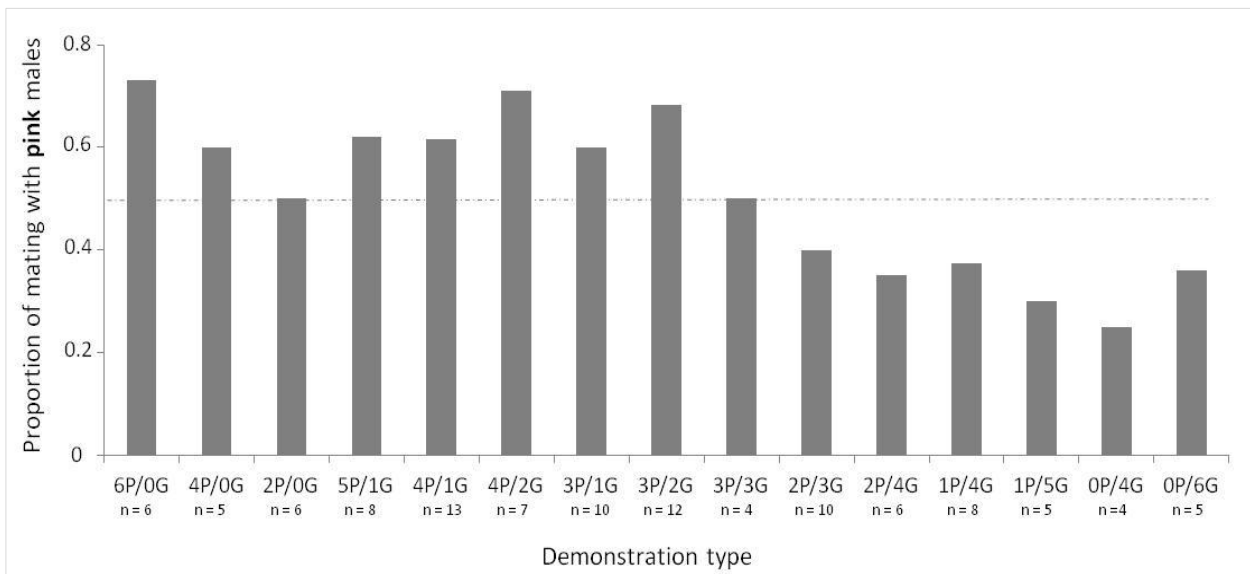


Figure 2. 11: Proportion of pink males chosen during the test phase according to the demonstration type. The mate copying protocol used is the short demonstration one and here is presented the proportion of females mating with pink males during the test phase. The demonstration type corresponds to the proportion of matings with pink and/or green males during the demonstration phase, for example 4P/2G stands for 4 demonstrator females mating with pink males and 2 mating with green males. The horizontal dash line corresponds to the expected value if the males were chosen randomly. P values are not shown because the statistical test were not meaningful due to the small number of replicates in each situations.

Due to the variability of the demonstrations, this experiment was inconclusive. After 572 replicates (in which only 109 had two courtships during the final test and thus were kept), we got 15 different demonstrations (see figure 2.11) with only a few replicates in each and we stopped the experiment. The presence of failed matings raised a statistical issue, as the same ratio of green vs pink matings could be obtained in different situations, with unknown effects on the copying

behaviours. For instance, a ratio of 2 pink for 1 green could be obtained with 4 pink males and 2 green males being chosen by females, or with 2 pink males and 1 green male (and 3 females that did not chose any of the males). It is unclear whether observer females would be affected by the first situation in the same way as the second. Thus, would the dynamics of conformism be the same when many females fail to copulate or not? For instance, observer females could interpret the presence of non-mating females as a signal that neither green nor pink males are suitable for mating.

Due to these concerns, we decided to create a theoretical model to test whether a preference for a phenotype could spread across the population. The questions this model is aimed at answering are in particular:

- (i). Is the copying response observed in our hexagonal device sufficient to obtain a spread of the preference in the population?
- (ii). We expect that due to random cultural accidents, small populations be more prone to loose cultural traditions (that is, at first sight small populations should have more short lived cultural traditions). What is the precise effect of population size on the potential conformist traditions in our set-up?

3.3. Theoretical model

This model was created in collaboration with Arnaud Pocheville (University of Sydney). The population is conceived as being evenly composed of two kinds of females: those that are currently mating, and the observer ones (somehow, the younger generation). The mating females freely choose among pink or green males according to their own preference, with some probability, while the observer females “observe” and update their own preference according to the mean behaviour observed in the population (the setting of their preference follows the function given in figure 2.12). At the following time step, the previously observer females become demonstrator females, and now

freely choose among pink or green males, with some probability of choosing pink or green (again, according to their updated preference). For instance, a female may have a preference for green males and choose green with probability 0.7 and pink with probability 0.3. For simplicity, all females belonging to the same category (mating vs observer) at a given time step are assumed to behave in the same way and the number of individuals stays fixed through time. The first population of mating females is composed of females with no preference, that is, with a probability of choosing a pink or green male equal to 0.5.

If most of the demonstrator females (above a given threshold value of frequency) mate with pink (respectively green) males, then during the following mating event, the majority of observer females (but not all of them) will mate with pink (respectively green). In a perfect conformist situation, the threshold value to obtain majority would be 0.5. Thus, if more than 50% of the demonstrator flies are choosing one phenotype, then the observer females will choose the same phenotype. Experimentally we saw that there are always some observer females that would not copy the choice of the demonstrator flies. We called "dissimilarity rate" this proportion of non-copying females (around 30% in our experiments). Thus the proportion of the pink male chosen after the demonstration is never equal to 100% (around 70% in our experiments). For the threshold value, due to our hexagonal device, the closest proportion to a 50 % situation is 66 % (2G/4P or 2P/4G). Thus we cannot test if flies follow the majority when the most frequent behaviour is expressed between more than 50 % and less than 66 % of the flies. In contrast, we can test if the observer females show no biased preference after having experienced a demonstration with 50 % females choosing pink and 50 % choosing green. Thus, we used this experimental value in our model and fixed the threshold value at 1/3 (or, symmetrically, 2/3). Between these points, we considered that the response was linear for simplicity. The linear response is also a less "charitable" constraint than other non-linear functions with a steeper slope, which would correspond to a stronger conformist bias and thus lead more easily to mate choice copying traditions (the R code of this model is presented in Annexe 3).

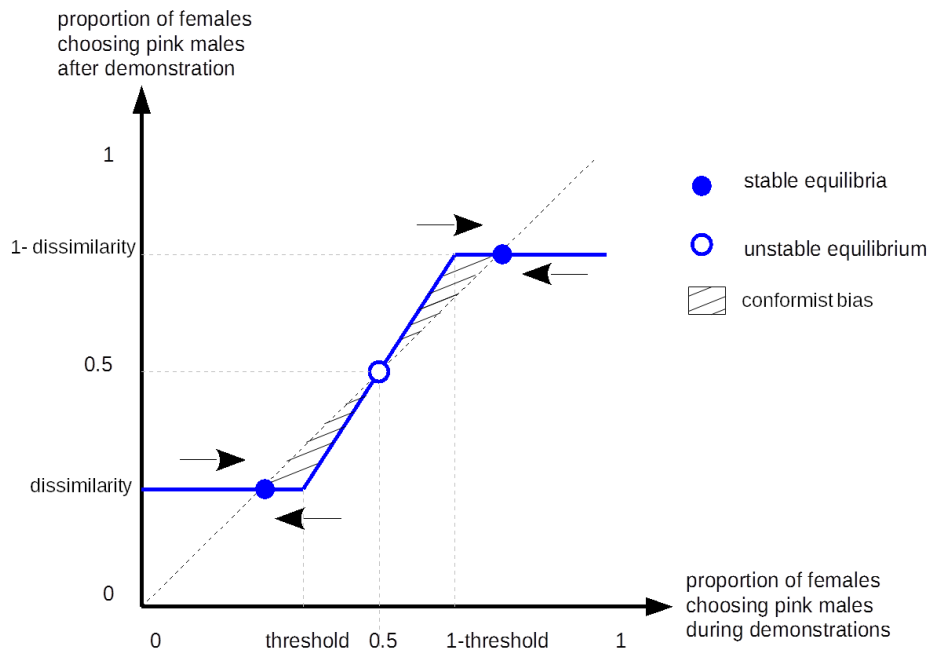


Figure 2. 12: Theoretical model 1: Mate choice copying response. This function models the conformist response of females when they set their preferences according to the behaviour they observe in the population. When mating females show a marked preference for green males (up to a certain threshold), observing females set their preference to a given constant (here named as dissimilarity). The situation where mating females show a marked preference for pink males is assumed to be symmetrical. Between the two plateaux, the response of observing females is assumed to be continuous and linear. Arrows represent the tendency of the dynamics of the frequency of choices in the population.

Depending on the value used for dissimilarity, the model exhibits either one stable equilibrium (absence of preference), or two stable equilibria (located at the points where the choice copying response crosses the line $x=y$) and one unstable equilibrium (absence of preference) (fig. 2.12).

One can see on fig. 2.12 (and 2.13 b) that when the copying response (blue curve) is *below* the line $x=y$ on the left (resp. above on the right) of the 0.5 point, the choice of the majority will tend to be exaggerated by the following generation, that is, the frequency of the most common behaviour will tend to increase in the population. (For instance, if the frequency of choosing pink is 0.3 at time t

and the copying response is such that observer flies will tend to choose pink with probability 0.1 at $t+1$, then choosing pink will tend to disappear in the population.)

By contrast, when the choice copying response (blue curve) is *above* the line $x=y$ on the left (resp. below on the right) (see fig 2.13 a), the choice of the majority will tend to be attenuated by the following generation, that is, the frequency of the most common behaviour will tend to decrease in the population. (For instance, if the frequency of choosing pink is 0.3 at time t and the copying response is such that observer flies will tend to choose pink with probability 0.5 at $t+1$, then the population will tend to choosing pink and green with even probabilities.)

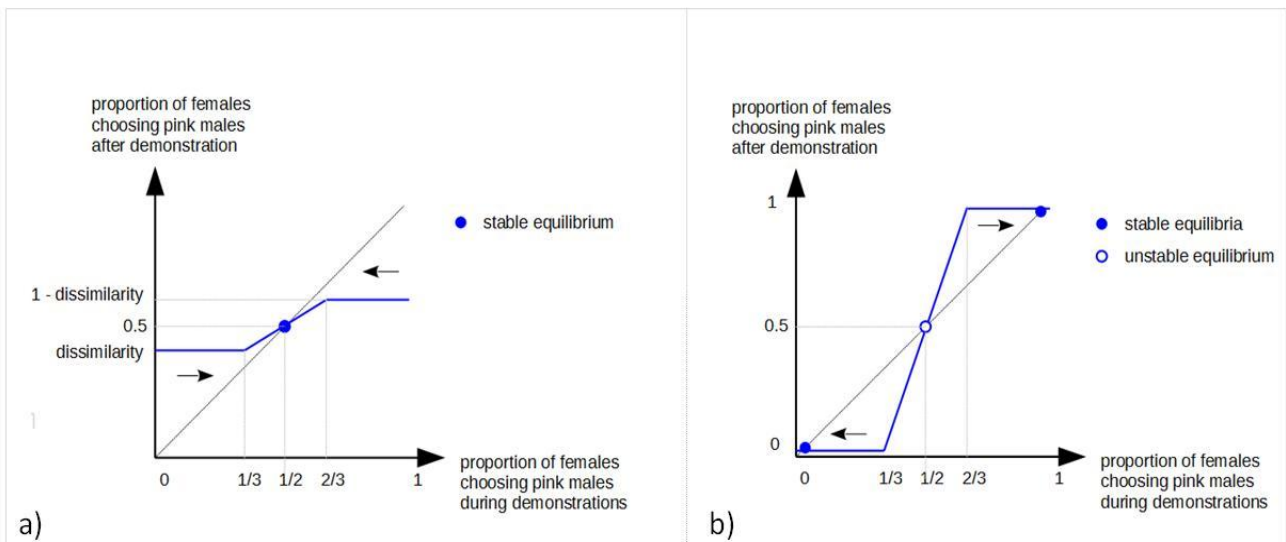


Figure 2. 13: Two other possible conformist responses illustrating the behaviour of the equilibria.

In the first example, flies do tend to copy the choice of the majority at a rate which is above even randomness (0.5), but which is lower than the frequency of the behaviour in the population. The population tends to evolve toward a stable equilibrium where flies evenly choose among pink and green males. In the second example by contrast, the copying rate is as strong as possible, and the population tends to evolve towards a situation where all flies choose the same colour. Due to the fact that probabilities 0 and 1 correspond to events which occur (or not) with certainty, once the population gets stuck on one stable equilibrium, it can't escape to shift to the other stable equilibrium in this particular situation (by contrast with the situation in figure 2.12).

As we explained previously, the hexagonal device constrains the resolution of the copying response we can model (our resolution on the x -axis is $1/6, 2/6, 3/6, 4/6, 5/6, 6/6$). This has consequences on the conditions of possibility to obtain a conformist response. Indeed, as we can see on the graphical representation of the copying response (fig. 2.12), if the dissimilarity is greater than $1/3$, there is almost no conformist bias. This would be true even in situations where flies would significantly copy the majority, that is, in situations where observing flies would show choices which significantly depart from 0.5 at $t+1$.

Behavioural events in the wild probably occur with a random character of some sort. That is, even if a population tends to evolve towards a stable equilibrium, stochastic events can happen and prevent it from reaching this equilibrium, or move it out of this equilibrium if the population has already reached it. For instance, if the population is in a stable equilibrium where flies choose pink with probability 0.8, randomness still makes possible that the flies choose green with a frequency of 100% (this randomness comes from the probability of choice by the females in our model, and can also come from other factors in the wild, such as males' availability). Of course the probability that, for example, 100 % of flies choose "green" when their preference is to choose pink, will depend on their level of preference (that is, in the model, their choice probability) and on the population size. The higher their preference, and the greater the population size, the more stable we expect the preference transmission.

In particular we expect that small populations show more random shifts from a majority to another, whereas big populations should get more easily stuck in one "tradition". To repeat, as the preference of the females is represented by a given probability to choose pink or green, the higher the number of females, the most "faithful" the choice exerted by demonstrator females will represent their preference. As observer females set their own preference according to the demonstration they observe, larger populations will have demonstrations which tend to better represent this preference, and which will be faithfully copied by the next generation.

In the limit case, an infinite population cannot move away from a stable equilibrium. However, for finite populations, the precise impact of population size (and of the strength of the preference) on the duration of traditions is less clear. How, precisely, does the population size affect the shifting from one tradition (if any) to another? This is the aim of the next section.

3.3.1. Impact of population size

To provide a first grasp on the behaviour of the model depending on population size, we ran 500 simulations of this model with each time the same parameters (number of flies, of iterations, dissimilarity rate) to see how the preference for one type of male could spread in the population and be maintained through generations. To do so, for each simulation, when a preference was first met (for a type of male), we counted the number of iterations before the preference changes. We then plotted the number of simulations in function of the number of iterations during which the choice of the majority remained the same – that is, the “transmission” of the preference. We repeated this procedure for 3 different population sizes: 10, 100 and 1000 demonstrator flies (figures 2.14, 2.15, 2.16).

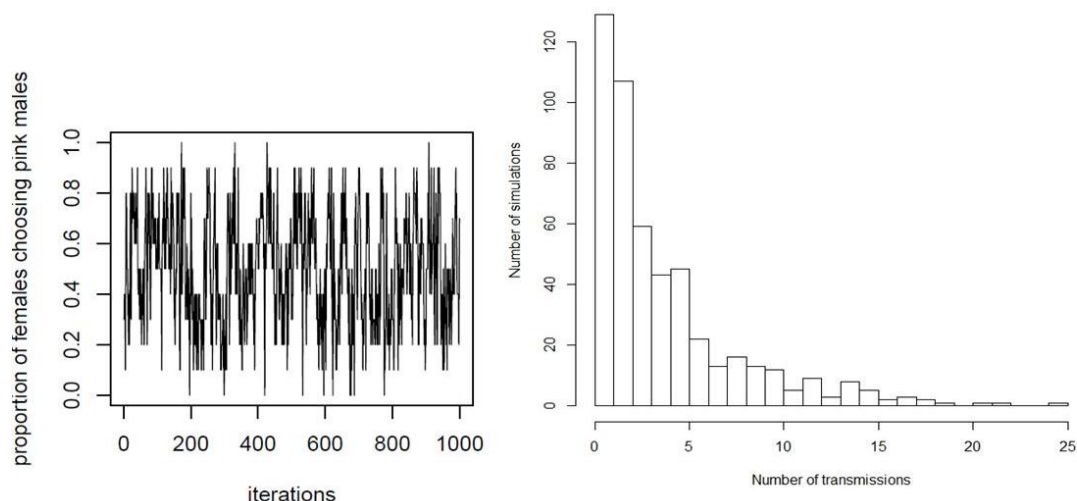


Figure 2. 14: Output of the model for a population of 10 demonstrator females, dissimilarity rate of 30%, and threshold at $1/3$, 500 simulations. On the left is presented an example of the dynamics of

mate choice for one simulation. On the right is shown the distribution of "transmission events" before the preference changes.

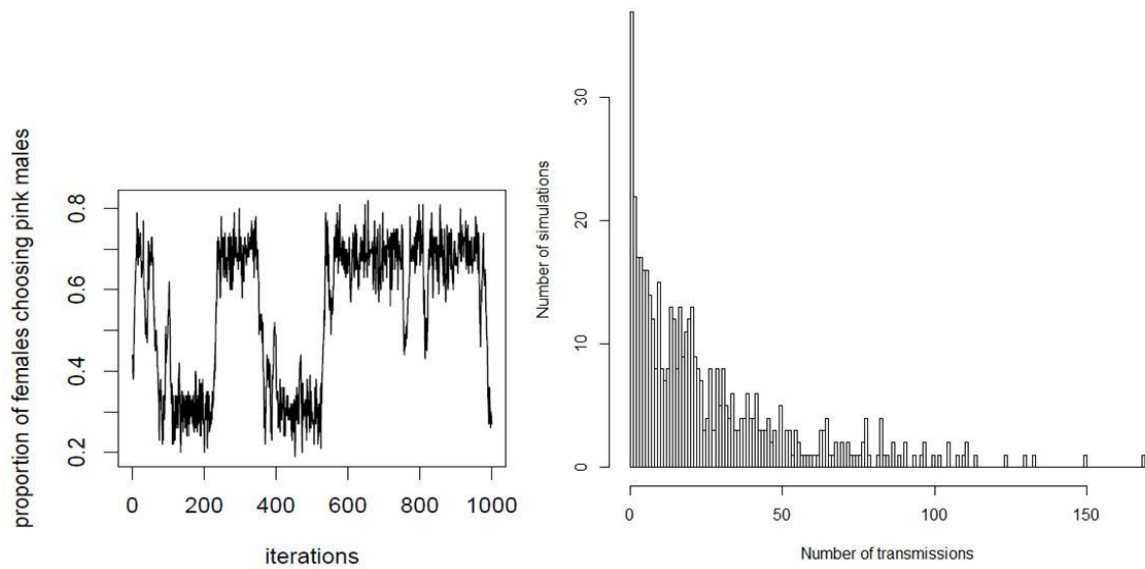


Figure 2. 15: Output of the model for a population of 100 demonstrator females, dissimilarity rate of 30%, and threshold at $1/3$, 500 simulations. On the left is presented an example of the dynamics of mate choice for one simulation. On the right is shown the distribution of "transmission events" before the preference changes.

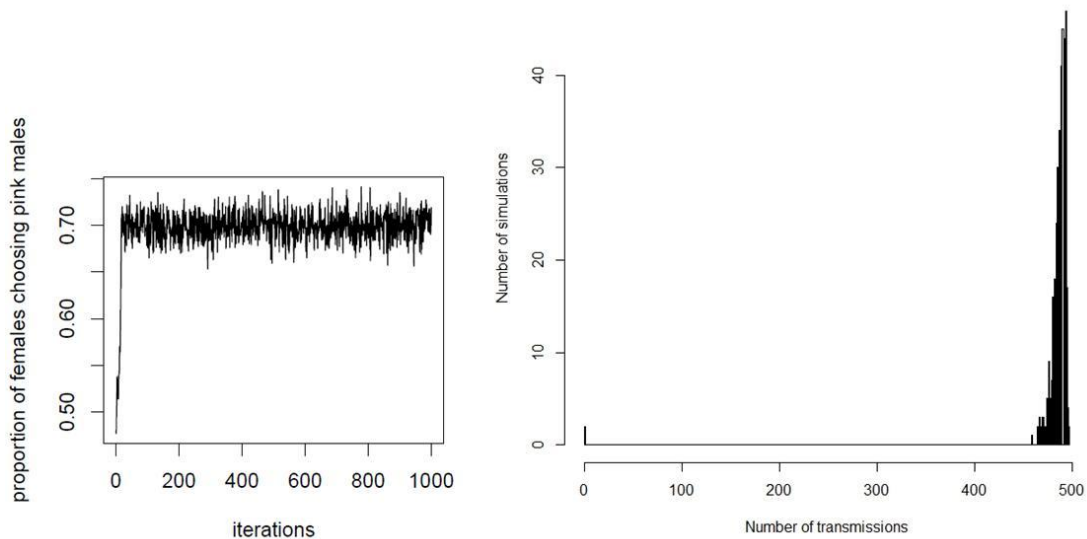


Figure 2. 16: Output of the model for a population of 1000 demonstrator females, dissimilarity rate of 30%, and threshold at $1/3$, 500 simulations. On the left is presented an example of the dynamics of mate choice for one simulation. At the considered time-scale, the population appears to be stuck in one tradition. On the right is shown the distribution of "transmission events" before the

preference changes. Notice the distribution of time spans before majority changes is truncated as we set the number of iterations to 500.

As shown by these outputs, the time spans of conformism depends on population size. As expected, small populations show more random shifts from a majority to another whereas big populations get stuck in one “tradition”.

To have a better grasp on the behaviour of the model depending on population size, we first ran models with the same parameters as the previous ones (dissimilarity rate of 30%) with a population size varying between 10 and 100 female flies. For each population size, we ran 500 models and we calculated the mean number of “transmission events” of the majority's choice (see figure 2.17).

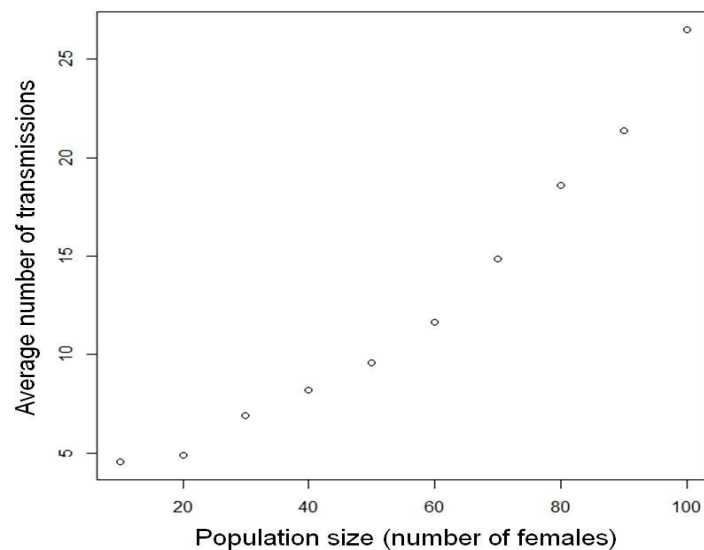


Figure 2. 17: Average number of "transmissions events" according to the population size (from 10 to 100 demonstrator females), dissimilarity rate of 30%, and threshold at $1/3$, 500 simulations.

With this model, the average number of "transmissions events" increases with the size of the population. No maximum time span is expected to happen if increasing the population size. However, it would be interesting to develop a version of the model to take into account the fact that in a big group, individuals are not likely equally impacted by all members of the group. As the mate

choice copying requires observation of others, neighbours might have a greater weight than remote individuals. Thus it could be interesting to take into account the spatial distribution of flies.

3.3.2 Impact of copying rate

As we saw during the experimental protocols, the Mate Copying Index varies between days, these variations being correlated with air pressure value and air pressure changes (see chapter 1). In our model, the proportion of flies that do not copy the preferred phenotype is modelled by the dissimilarity rate. We set this rate at 0.3 because in most experiments the Mate Copying Index is around 0.7 (figure 2.17). As we saw in chapter 1, in a condition of high (> 1015 hPa) and increasing barometric pressure, the copying rate is higher than 0.8. i.e. dissimilarity lower than 0.2). We thus took advantage of the model to study the impact of the copying rate and ran another set of simulations with a dissimilarity rate of 0.2 (figure 2.18).

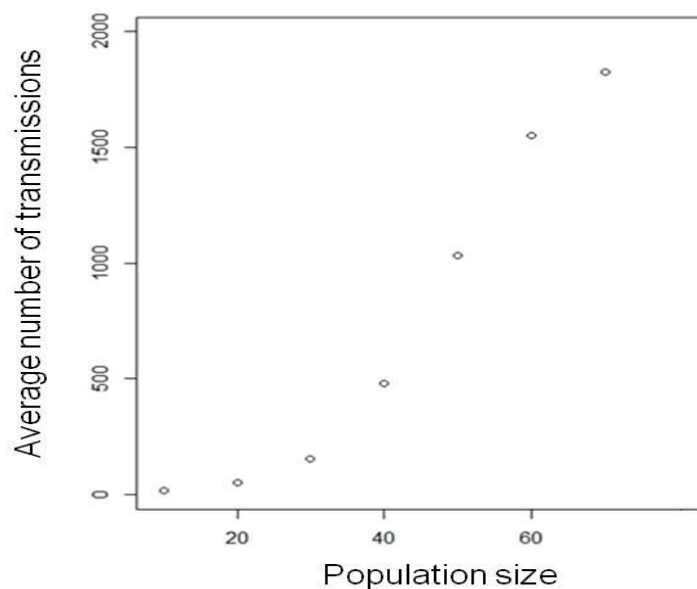


Figure 2. 18: Average number of transmissions according to the population size (from 10 to 100 demonstrator females), dissimilarity rate of 20%, and threshold at 1/3, 500 simulations of 2000 iterations.

Results indicates that the average time-span of a "tradition" was indeed impacted by this factor and rose very quickly with population size until reaching the number of iterations run.

By contrast, when the climatic conditions are unfavourable, we saw in chap. 1 that the Mate Copying Index dropped to 0.6 or below. As an index of 0.6 corresponds to a dissimilarity of 0.4 which is greater than $1/3$, the mate preference is not expected to last in the model with this set of parameters (figure 2.19)

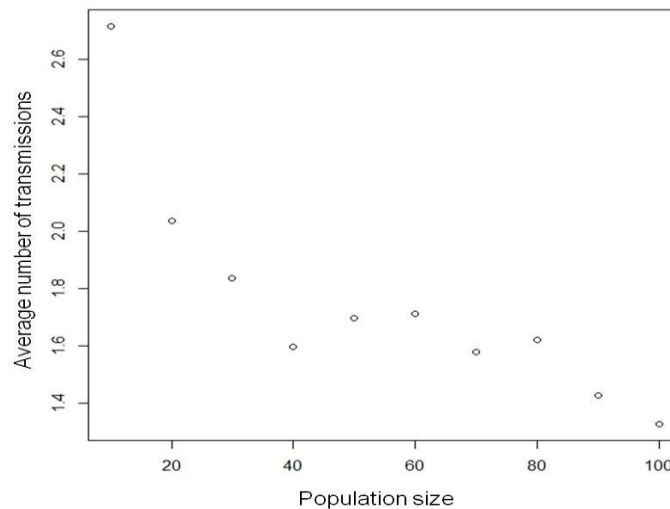


Figure 2. 19: Average number of transmissions according to the population size (from 10 to 100 demonstrator females), dissimilarity rate of 40%, and threshold at $1/3$, 500 simulations.

3.3.3. Discussion

This model was created to test whether a preference for a phenotype could spread across the population and be transmitted across generations. We saw that, with a sufficient number of individuals, the preference might be durably transmitted across the population and last for a sufficient time to allow new females to observe and learn this preference. While the copying rate is set to a constant in our model (with the parameter “dissimilarity”), it is possible that several consecutive observations enhance the learning response of flies (Margulies et al., 2005; Tully et al., 1994). In parallel, the longer a preference lasts for a given male phenotype, the more important it

might be for a female to follow the majority's choice. Indeed, if the male phenotype is heritable, then a fly choosing a phenotype which is not preferred by the majority might jeopardize the reproductive success of its offspring (we dwell on this in the Discussion chapter). Thus if the population is big enough, there might be both more occasions for a female to assess the most common behaviour in the population, and more fitness incentives of following the choice of the majority.

By contrast, in small populations, there might be little occasions for a consistent cultural tradition to form and little fitness advantages of durably remembering an acquired preference.

As studies on *Drosophila* natural history are very scarce, it is difficult to have a precise idea of how many flies could be present on one food source. In addition, other factors would be interesting to take into account, such as the mortality and migration rates, the rate of copulation failures, the spatial segregation of social interactions, and the production of offspring. Without proper ecological knowledge of these parameters, the model can, of course, at best point to their potential importance. An immediately interesting study will be to take into account the variability of certain factors through time, such as that of the copying rate with climatic conditions. For instance, can conditions remain favourable for a long enough period of time to lead to the virtual disappearance of a male phenotype in the population?

Chapter 3: Molecular insights on biological processes supporting mate copying

In this last part we worked toward identifying some of the molecular mechanisms underlying the cognitive capacity revealed by mate copying. The work presented here was partly realized with the help of Guillaume Gomez and Thomas Crouchet. It constituted a preliminary study and revealed interesting results that could be used for future studies. Very little is known about the genetic and molecular nature of heritable variation in learning performances (Mery et al., 2007). The detailed genetic knowledge in *D. melanogaster* (Adams et al., 2000) and the numerous studies on the genetics of learning (such as Dubnau and Tully, 1998; Liu et al., 2006 for observational learning), is making fruit flies a perfect model to study the molecular nature of variation in learning performances. The available genetic tools and the small and highly structured *Drosophila* brain (100 000 cells) allow the manipulation of gene expression while recording the behaviour (Isabel and Preat, 2008).

Historically, the way to identify mutants in learning ability was through conditioning protocols during which the fly would learn to associate an odour with electric shocks (Quinn et al., 1974). Thanks to genetic tools (generation of random single-gene mutations, P-element insertion in different lines, interference RNA to disrupt gene expression, etc), *Drosophila* mutants have been produced and some neural structures supporting learning and memory have been identified (see Isabel and Preat, 2008 for a review). Natural *Drosophila* variants are also helpful for behavioural genetics analyses. They indeed show behaviour-specific alterations but without the pleiotropic effects often caused by the generation of null-alleles (Sokolowski, 2001).

Using some of *Drosophila* learning and memory mutants, our goal was to answer the following questions: What are the neural structures involved in mate copying? And what are the molecular pathways required? To do this, we used the natural variants on the foraging gene (*for*), known to have an effect on learning and memory in larval and adult stages (Reaume et al., 2010). We also used the *Drosophila dunce* (*dnc*) and *rutabaga* (*rut*) mutants which historically were among the first learning and/or memory mutants characterized during the seventies (Aceves-Piña et al., 1983; Dudai et al., 1976; Livingstone et al., 1984).

1. Influence of *foraging*

Two foraging strategies in *D. melanogaster* were identified in the 80's: rover and sitter. They coexist in natural population with approximate phenotypic frequencies of 70% *rovers* and 30% *sitters* (Sokolowski, 1980). These variants differ in the foraging gene (*for*) which encodes a cGMP-dependent protein kinase (PKG). Flies with a rover allele (*for^R*) have higher PKG activities in their head than the flies homozygous for the sitter allele (*for^S*) (Reaume et al., 2010; Sokolowski, 2001).

Rover and *sitter* flies are known to differ in a suite of behavioural and metabolic traits. At a larval stage, *rovers* show longer foraging trails on food than *sitters*. They also have a greater tendency to travel and leave a food patch more readily than *sitters* (Reaume et al., 2010; Sokolowski, 2001). *Rovers* also visit more and farther patches than *sitters* and tend to avoid revisiting previous patches (Stamps et al., 2005).

The cGMP-dependent protein kinase (PKG) encoded by *for*, is required in associative olfactory learning and memory in a social environment (Kohn et al., 2013). Indeed, in an associative olfactory learning and memory paradigm, *rover* flies retain better the information in a short-term than *sitters*, but they seem to remember less in the long term (Mery et al., 2007; Reaume et al., 2010). PKG is also involved in the operant visual learning tasks. Visual pattern memory is normal in *rover* variants but impaired in *sitter* variants (Wang et al., 2008).

In addition, Foucaud et al. (2013) showed that *sitters* are more likely to use or display social information than *rovers*. We could thus predict that *sitters* would be more prone to perform mate copying.

Before starting the mate copying experiment, we conducted a preliminary study to test whether there was a natural preference for one of the variant. For example would female *sitter* prefer to mate with *sitter* flies? In other words do they show a natural biased preference?

1.1. Experiment 1: is there a natural preference for one variant?

1.1.1 Methods

In order to test the existence or absence of a natural biased preference, we used *rover* and *sitter* lines kindly provided by Frederic Mery. We placed during 30 minutes a *rover* or *sitter* female with a *rover* or *sitter* male, dusted with green or pink powder (see table 3). This coloration allowed to recognize the male variant and to have experimental conditions similar to the one of the mate copying experiment.

Females	Males	
	Treatment 1	Treatment 2
Rover	pink <i>rover</i> + green <i>sitter</i>	pink <i>sitter</i> + green <i>rover</i>
sitter	pink <i>rover</i> + green <i>sitter</i>	pink <i>sitter</i> + green <i>rover</i>

Table 3: Experimental design

During the test, we registered time, identity and number of males courting the female, as well as the copulation time and the variant chosen. As in all experiments, we only kept replicates in which both males courted the female and discarded all the others (so 113 among 404 replicates).

1.1.2. Results

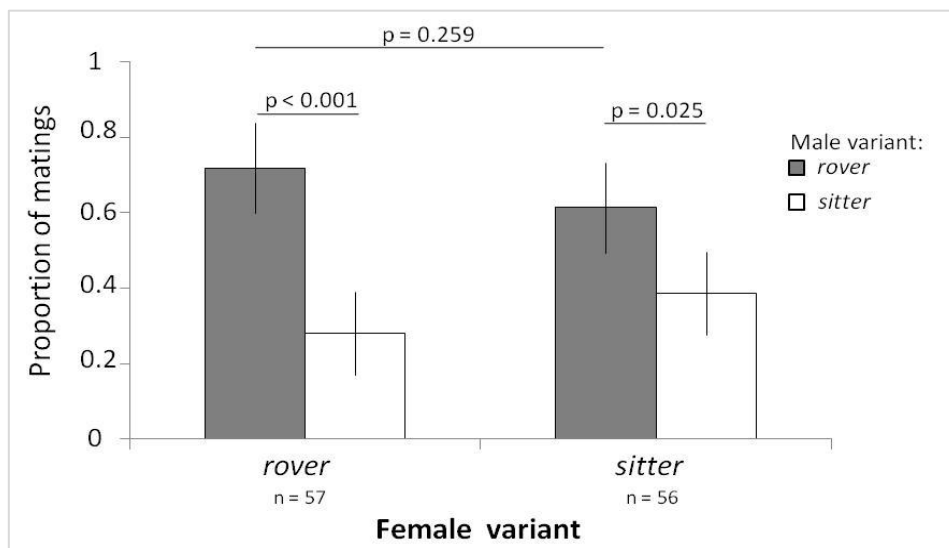


Figure 3. 1: Female mating preference according to its natural variant. The females were either *rover* or *sitter* and were provided with one male of each variant. The bars represent their choice: the

grey bars correspond to the proportion of matings with *rover* male while the white bars show the proportion of matings with *sitter* males. Vertical bars are confidence intervals, p values are comparisons between two groups.

Rover males are significantly preferred over *sitter* males by *rover* females (Chi-square: $p < 0.001$, $n = 57$) as well as *sitter* females (Chi-square: $p = 0.025$, $n = 56$). There is no significant difference between the females variant regarding their male's preferences (GLMM: $p = 0.259$, $n = 113$).

There is no significant effect of the male variant on the courtship latency (GLMM: $p = 0.305$, $n = 113$), but there is a trend to a shorter courtship latency toward *sitter* female (GLMM: $p = 0.063$, $n = 113$). There is a significant effect of the male variant on the time spent mating with the female: *sitter* males spent significantly more time on the female than *rover* males (GLMM: $p < 0.001$, $n = 113$) no matter the female's phenotype (GLMM: $p = 0.742$, $n = 113$).

Overall these results show that *rover* males are preferred by females while, when chosen, *sitter* males spend more time mating with females. Yet the copulation duration and fertility are genetically correlated (Gromko, 1987). Two strategies seem to appear : *rover* males being successful and mating with a lot of females, *sitter* males having less success but when chosen spending more time with the female thus maybe siring as many offspring as *rover* males. These results open interesting ways for future studies.

Our wild type flies used for the mate copying experiments are composed of *rover* and *sitter* variants (70% of *rovers* vs 30% of *sitters* , Sokolowski et al., 1997). This experiment could give further indications about the observer female's choice during the final test of mate copying experiment. The results could actually explain some of the non-copying situations: it might happen that the observer female is provided with one *rover* and one *sitter* male. The colour of the *rover* may or may not match the one chosen during the demonstration. Thus a *rover* preference can sometimes strengthen and other times lower the copying behaviour in experiments using wild-type flies.

This experiment shows that the variant could have an influence on the result of the final test during mate copying experiment. Could this variant also have an impact on the information transfer and copying behaviour?

1.2. Experiment 2: Is there a difference in mate copying according to the variant?

The aim of the following experiment was to study whether the *for* gene could have an influence on the use of social information. Foucaud et al. (2013) showed that *sitters* are more likely to use or display social information than *rovers*. Thus we wanted to test the influence of the natural variant on the propensity to copy. For example would *sitter* females copy more the preference of other *sitter* females? Are *sitter* females the best demonstrator flies?

1.2.1 Methods

For this experiment we followed the short demonstration protocol (as described in chapter 1). The observer female could be either from the *rover* or *sitter* variant. This female had a demonstration made by *rover* (treatment 1) or *sitter* (treatment 2) flies (table 4). During the final test, we gave to the female two males from the same variant as the demonstrator flies (*rover* for treatment 1 or *sitter* for treatment two).

	Demonstrator and test males flies	
Females	Treatment 1	Treatment 2
<i>rover</i>	<i>rover</i>	<i>sitter</i>
<i>sitter</i>	<i>rover</i>	<i>sitter</i>

Table 4: Experimental design

We recorded the time, identity and number of males courting the female. For the mate copying index, we only kept replicates in which both males courted the female and discarded the others (that is 99 among 304 replicates).

1.2.2. Results

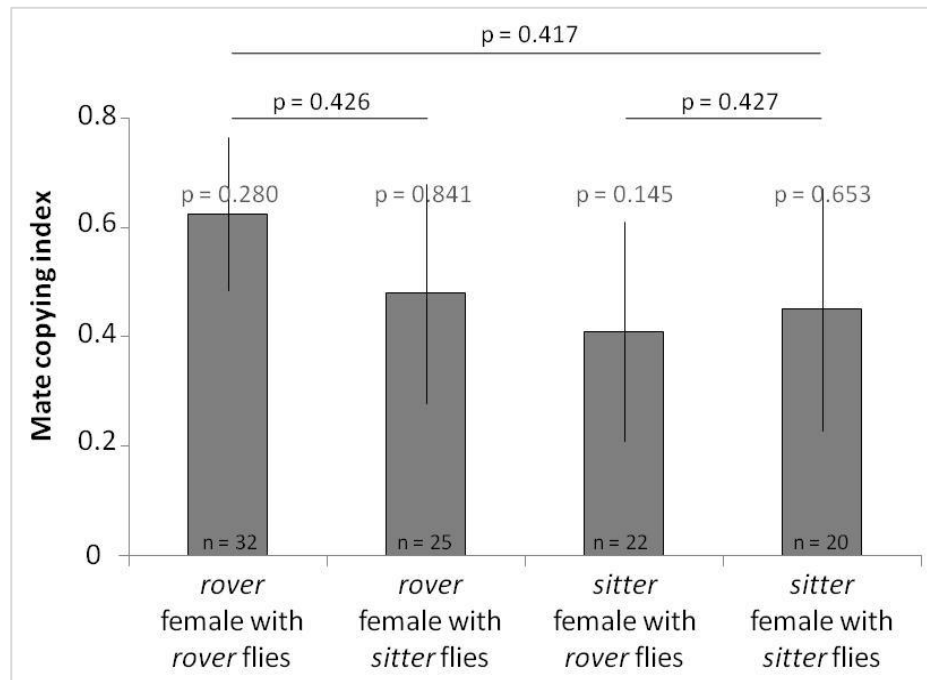


Figure 3. 2: Mate coping index according to the observer female natural variant and the type of demonstrator and test male flies. The mate copying protocol used is the short demonstration one except that the observer females were either *rover* or *sitter*. The demonstrator and test flies could be either from the *rover* or *sitter* variants. The Mate Copying Index is the proportion of observer females that copied the choice of demonstrator females. Vertical bars are confidence intervals, p values above the bars correspond to Wald chi-square tests between the experimental Mate Copying Index and a random choice or the comparison between the groups.

Even though the Mate Copying Index is higher for the group of *rover* observer females with *rover* flies, we found no significant difference between the treatments (GLMM: $p = 0.417$, $n = 99$). Moreover there was no significant effect of the demonstration on the observer female's choice (Chi-square: *rover* observer female with *rover* flies: $p = 0.280$, $n = 32$; *rover* observer female with *sitter* flies: $p = 0.841$, $n = 25$; *sitter* observer female with *rover* flies: $p = 0.145$, $n = 22$; *sitter* observer female with *sitter* flies: $p = 0.653$, $n = 20$). This result has to be moderated due to the very small sample size we obtained. This study has been stopped at the end of Thomas Crouchet's internship and would need to be continued in order to draw conclusions. For the moment with these

preliminary results, no difference appears between *rover* and *sitter* flies. This situation is opposite to our hypothesis that was that *sitter* flies would copy more or be better demonstrators than *rover* flies. This hypothesis was based on the results of Foucaud et al. (2013) study. In their experiment, they showed that *sitter* flies learnt better to find the cooler zone in their 'heat maze' apparatus when they were trained in group comparing to when they were alone. One of their conclusions was that *sitter* flies may use more social information than *rover* do. This pattern does not appear yet in our experiment. But the lack of difference might also be due to the fact that our observer females are trained and tested alone. Another possible study, would be to test whether a observer female learns better in a group or alone depending on its natural variant. The *for^S* allele indeed promote group living (Sokolowski, 2001) so it is possible that *sitter* female would learn better if they were trained in a group. This experiment could easily be done with the hexagonal device and could give potential interesting results.

1.3. Pressure influence on Mate copying Index according to the variant

As described in chapter 1, changes in air pressure correlates with changes in the propensity to copy by observer females. Using the same model as in chapter 1, we studied how observer females with a different allele of the *for* gene would be impacted by air pressure in their propensity to copy. Due to the low number of replicates, we pooled together demonstrations made by *sitter* and *rover* demonstrator flies and showed the Mate Copying Index of *rover* or *sitter* observer females according to the changes (Pressure Index) and actual values (Test pressure) of air pressure (figure 3.3).

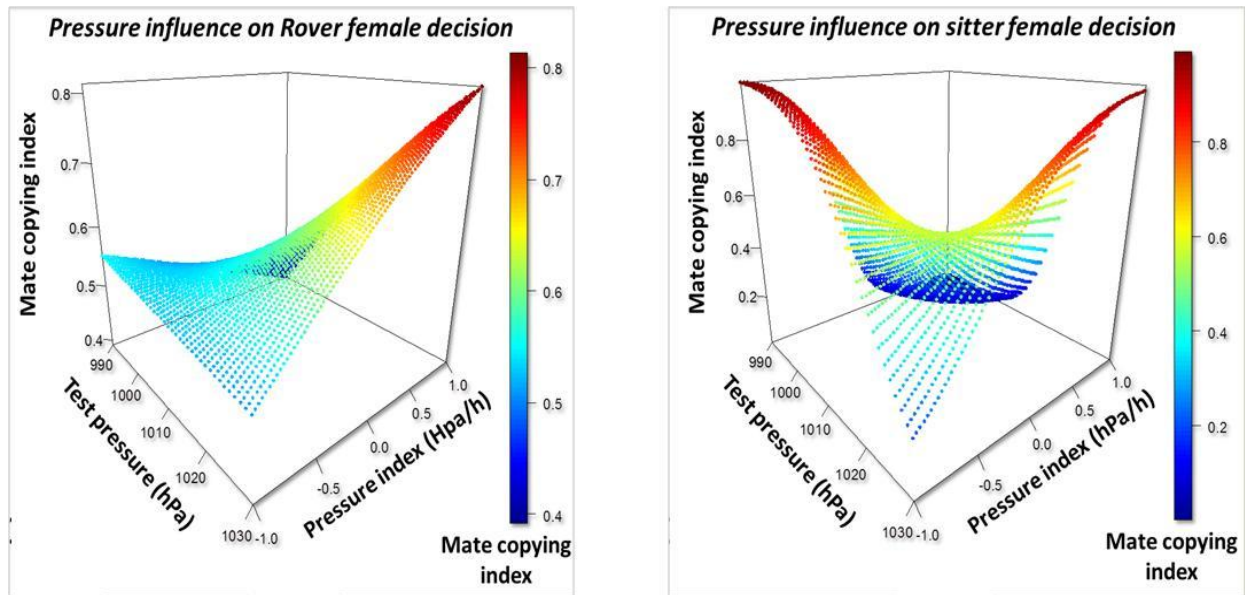


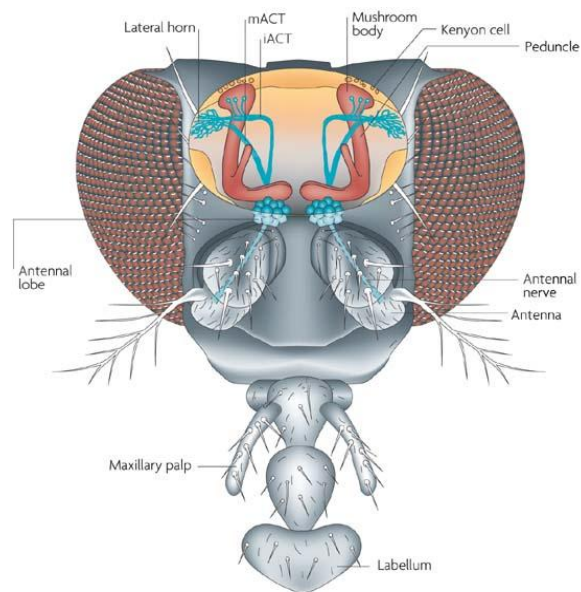
Figure 3. 3: Mate Copying Index according to absolute air pressure (Test pressure) and its variation during the 6 hours preceding the experiment (Pressure Index). The protocol used is the short demonstration protocol. The mate copying Index is the proportion of observer females that copied the choice of demonstrator females. The statistical model has been obtained with the experimental points and its surface represents predicted values of Mate Copying Index. The graph on the left represents the Mate Copying Index of a *rover* observer female tested with *rover* or *sitter* flies (n = 57). The graph on the right represents the Mate Copying Index of a *sitter* observer female tested with *rover* or *sitter* flies (n = 42).

The results show a difference in the response to change in air pressure according to the natural variant of the observer females. The *sitter* females seem actually to copy more when the air pressure is high and increasing and when the air pressure is low and decreasing. More studies need to be carried out to understand this phenomena. But we can relate it with the augmentation of copying observed in the third figure of chapter 1 (figure 1.3) for the lowest values of air pressure. Maybe this phenomenon is due to the *sitter* variants of our Wild Type population.

To conclude, the foraging gene (*for*) might have an influence on the observer females' propensity to copy but further studies need to be undertaken to draw clear conclusions. In the mean time, we also studied the influence of *dunce* and *rutabaga* proteins on the mate copying behaviour.

2. Roles of Rutabaga and Dunce proteins in mate copying

Many learning/memory mutants were isolated in *Drosophila*. The first of them were *dunce* (*dnc*) and *rutabaga* (*rut*) (Dubnau and Tully 1998). These two mutants are deficient in enzymes located into the Kenyon cells constituting the mushroom bodies (Figure 3.4), a cerebral structure involved in olfactory memory (Keene and Waddell, 2007).



Nature Reviews | Neuroscience

Figure 3. 4: (Keene and Waddell, 2007): "*Drosophila melanogaster* head. Dorsal view of a cutaway fly head showing the main elements of the olfactory pathway"

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Biochemical approaches showed that *rut* flies are deficient in the activity of a Ca²⁺/calmodulin-sensitive adenylyl cyclase which converts ATP into cAMP, and that *dnc* flies are deficient in one form of cAMP phosphodiesterase activity which degrades cAMP. Thus *rut* mutants have low levels of cAMP and *dnc* mutants show elevated levels of cAMP, (Davis and Dauwalder, 1991). cAMP is an important signal transducer and its synthesis is involved in the cell response to environmental changes (figure 3.5).

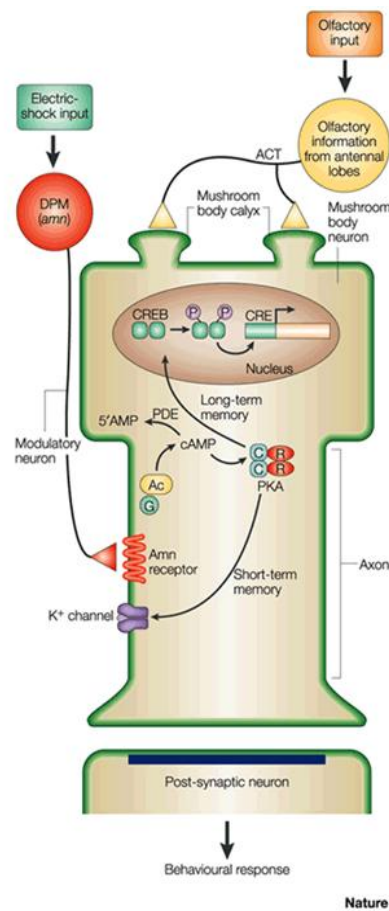


Figure 3. 5: (Sokolowski, 2001): "A model for olfactory-based shock-avoidance learning in *Drosophila* mushroom body neurons. A mushroom body neuron gets olfactory information from: the antennal lobes and from DPM (dorsally paired medial neurons), which release the amnesic (Amn) neuropeptide after the delivery of an electric shock to the fly. The axons of the DPM neurons, in which *amn* is expressed, are thought to synapse onto mushroom body axons to cause the release of putative modulatory neuropeptides. The simultaneous activity of these two pathways causes the stimulation of adenylate cyclase (Ac), encoded by *rutabaga* (*rut*). The stimulated Ac then activates a G-protein-coupled receptor (G), which causes elevated cAMP levels. The increase in cAMP gives rise

to either a short-lived change in the excitability of the mushroom body neuron (short-term memory) or a long-lasting change (long-term memory). The *dunce*-encoded cAMP phosphodiesterase (PDE) then degrades cAMP".

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Rut adenylyl cyclase is also located in both the fan-shaped body and the ellipsoid body, which are parts of the central complex (Figure 3.6). This last structure has been shown to be involved, among other functions, in visual memory (Liu et al., 2006; Wang et al., 2008). Furthermore, *Rut* adenylyl cyclase acts during learning as a coincidence detector (for a review see Isabel and Preat, 2008).

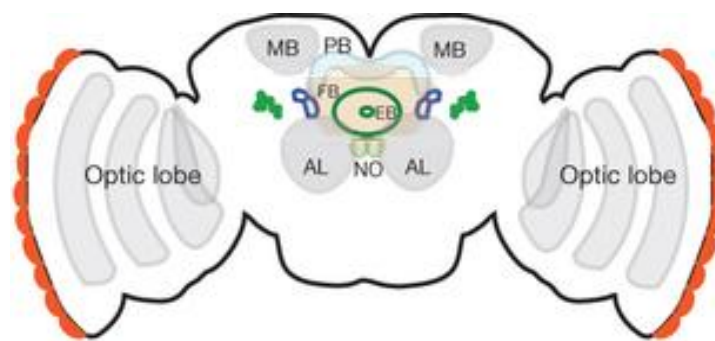


Figure 3. 6: (Seelig and Jayaraman, 2013) : "Schematic of fly central brain showing antennal lobe (AL), mushroom bodies calyces (MB) and optic lobes along with sub-structures of the central complex: ellipsoid body (EB), fan-shaped body (FB), protocerebral bridge (PB) and noduli (NO)".

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To evaluate the involvement of *rutabaga* and *dunce* in observational social learning, we have tested *rut* and *dnc* mutant flies, in our mate copying protocol. We predicted that both *dnc* and *rut* mutants should show much lower mate copying indices than wild-type flies, because of the disruptions of key-molecules involved in learning.

2.1 Methods

For the following experiment we used the memory mutants *rutabaga* and *dunce*. We followed the short demonstration protocol (as described in chapter 1) except that the observer female was from the wild type Canton-S, *dunce* or *rutabaga* type. We recorded the time, identity and number of males courting the female. For the mate copying index, we only kept replicates in which both males courted the female and discarded the others (that is we kept 116 among 406 replicates).

2.2. Results

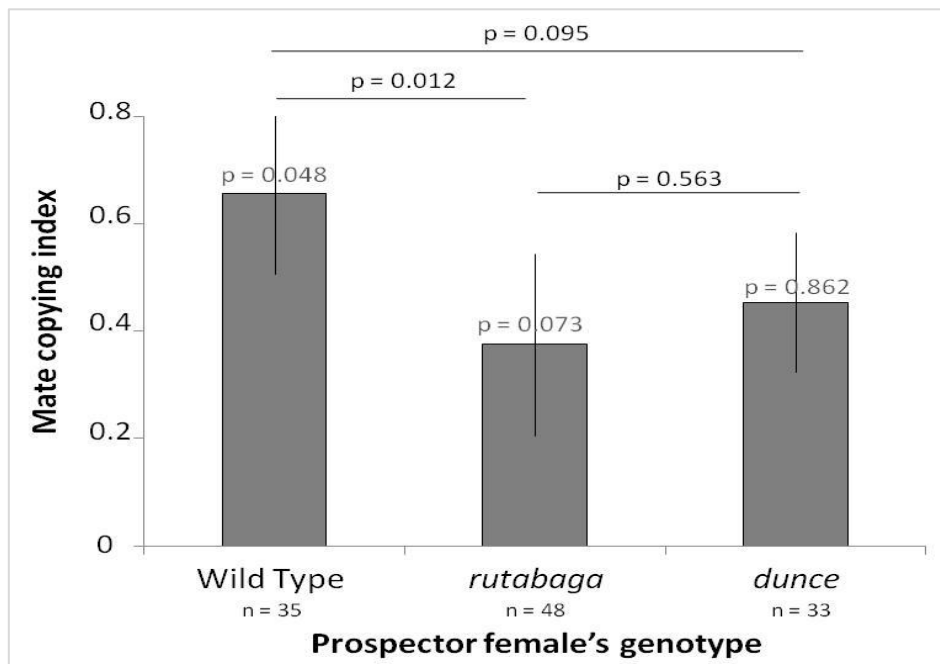


Figure 3. 7: Mate coping index according to the observer female's genotype. The mate copying protocol used is the short demonstration one except that the observer females were either from Wild type, *rutabaga* or *dunce* genotypes. The Mate Copying Index is the proportion of observer females that copied the choice of demonstrator females. Vertical bars are confidence intervals, p values above the bars correspond to Wald chi-square tests between the experimental Mate Copying Index and a random choice or the comparison between the groups.

These results show a significant effect of the observer female's phenotype on the Mate Copying Index (GLMM: $p = 0.041$, $n = 116$). There is actually a significant difference between Wild Type and *rut* flies (GLMM: $p = 0.012$, $n = 83$) and a trend between Wild Type and *dnc* flies (GLMM: $p = 0.095$, $n = 68$). We found no significant difference between the *rut* and *dnc* groups (GLMM: $p = 0.563$, $n = 81$).

This series of experiment highlights the effect of *rut* and *dnc* genes in the mate copying process. This preliminary work will be the basis of a new study carried out by Sabine Nöbel and described below.

2.3.Further studies

On the basis of these results, two approaches will be envisaged to discover the neural structure(s) required in Mate-Copying. The first study will focus on the different neural circuits composing the ellipsoid body (EB) and the fan-shape body (FSB) (see figure 3.6), given their crucial importance for visual learning and memory (Wang et al., 2008) and the mushroom bodies (MBs) recently shown as involved in another associative task of visual memory (Vogt et al., 2014) . To unravel the neural structures depending on Rutabaga and Dunce proteins involved in the Mate-Copying, these two key proteins will be disrupted independently in different cerebral regions with the UAS/GAL4 system, as previously reported in a different paradigm (Wang et al., 2008 for example).

Second, populations of *Drosophila* will be artificially selected for improved mate copying according to a protocol inspired by Lagasse et al. (2012). This protocol will be repeated until selection of flies displaying a better performance than control lines. In the aim of revealing the gene putatively required in Mate-Choice-Copying, the genomes of the selected will be compared to the ones of control lines.

In the future, the detected putative difference between the two genomes will be tested to see if they could give rise to molecules (proteins, different RNA...) required in mate copying. The goal will be to confirm a causal link between the candidate genes revealed by genomics comparisons of the two lines and mate copying.

Studying the cognitive mechanisms in an ecological context provides a unique opportunity to bridge infra-individual approaches with supra-individual processes, which constitutes one of the major challenges of current biology (Danchin and Pocheville, 2014). This transdisciplinary project, based on the preliminary study with *rut* and *dnc* flies has the potential to lead to interesting discoveries.

Discussion

In chapter 1, we showed that *Drosophila melanogaster* females could socially acquire a mate preference. We replicated the results of Mery *et al.* (2009) and we greatly improved the experimental protocol. The new experimental protocol was closer to a natural situation in terms of duration and allowed to undertake many new experiments. Moreover, in the initial protocol of Mery *et al.*, a series of copulation and rejection are shown to the observer female. This sequence might have an effect on the acquisition of the preference. With only one simultaneous demonstration, our new protocol controlled for that point. This protocol revealed that the observer female's choice could be impacted by the observation of only one another female. Additionally, we showed that the copying rate was correlated with changes in air pressure; mate copying being more efficient under improving climatic conditions.

Through the experiments of chapter 2, we controlled that *Drosophila* females could generalize the socially acquired information, thus showing a trait-based copying of mate preference. This preference for a certain male phenotypes lasts at least 3 hours and might continue up to 24 hours. Then we tested the impact of contradictory information on the acquisition of a mate preference. This experiment revealed the existence of a "copy the majority" strategy and thus a conformist bias in the transmission. The creation of a theoretical model allowed to study the preference transmission in the population. This transmission is favoured in big populations of flies and/or when the copying rate is high, that is in good climatic conditions.

Finally, in Chapter 3, we tackled the question of the influence of behavioural variants on mate copying. *Rover* and *sitter* flies showed differences in their mate preferences, propensity to copy and reaction to changes in air pressures. These differences can explain some of the results obtained with our Wild-type flies. The experiments with *dunce* and *rutabaga* mutants underlined the role of cAMP in mate copying. These experiments constitute only preliminary studies but revealed interesting results that constituted a first step for further research that would be carried out by the team. The future work can also give clues to better understand the molecular basis of *Drosophila* behaviour, as well as the one of other taxa. Many genes found in *Drosophila* have actually functional structural or functional homologues in vertebrates, including humans (Sokolowski, 2001).

1. Mate copying in *Drosophila melanogaster*

Finding the new short demonstration protocol greatly improved the efficiency of the experiments on mate copying and allowed us to test many new aspects. The creation of the hexagonal device went one step further and allowed to make experiments that were not possible to undertake in the initial tube device. The new settings resemble more to natural situations, with several observer females looking at several demonstrations, which allowed to test whether observer females would conform to the mate preference of the majority. One problematic aspect of the hexagonal device, is that we cannot control what the females see inside the central compartment. There is a possibility that some females do not move from their spot and just see one couple or few couples instead of the 6 couples presented. According to personal observations, observer females are always moving in the central compartment, but we never ran an experiment to verify this point. An ideal control would be to record with a video camera the observer female activity during the mate copying experiment and then track their path. This would allow to be ascertain that females are moving during the experiments and thus have the potential to see all the couples presented.

The fact that constrained demonstrations were required for mate copying protocol used in most experiments in the hexagonal device gave additional interesting information. In this protocol, we only showed to the observer females already formed couples with one male of the other colour next to them. This amounted to demonstrate only the result of the choice, and not the actual choice, but was enough to induce a preference in our observer females. This may be compared to observations made in the transmission of the behaviour of nectar robbing in bumblebees or milk bottle opening in tits. In these two animals, only seeing changes to the environment caused by the novel behaviour favours as much the social transmission as the observation of this new behaviour itself (Fisher and Hinde, 1949; Sherry, 2008). One interesting thing would be to study if seeing an actual copulation is a necessary condition to acquire a mate preference. Could the observation of only a courtship behaviour and thus only another female interest for a phenotype (and not its actual choice) be sufficient to induce a preference, as raised by Valone and Templeton (2002)? Stopping a courtship sequence before the copulation would be easy to do in *Drosophila*; having one male courting the female but not the other would be, on the other hand, more difficult to obtain.

While doing experiments in parallel in the hexagonal and in the tube devices we got a better Mate Copying Index with flies that had been trained in the hexagonal device. This is a personal observation so far and more studies are needed. However, such an observation, if confirmed, would raise the question whether *Drosophila* females show better mate copying when trained in a group. Social facilitation has already been shown in *Drosophila* in experiments involving olfactory memory (Chabaud et al., 2009). We could test if we find the same phenomenon in our observational learning situation by doing the mate copying experiment, but instead of testing 6 females in the central cavity we would test 1, 3 or 6 females. This experiment would allow to see whether social facilitation influences the females' propensity to copy.

In all our mate copying experiments, the number of courtships during the final test was an issue. As explained previously, a *Drosophila* female can mate with one male, only if it has previously courted her. To be meticulous in our experiments we only kept the situations in which both males had courted the female, so that the female could truly have the choice between the two males. Unfortunately, in most cases, only one of the male was willing to mate with the female. Thus in all of our datasets, we only could use one third of the data on average. Besides the fact that this phenomenon is costly in terms of flies used and time spent, it also causes problems for the transmission chain. The mate preference can be easily lost if the female cannot mate with the male she had a preference for, which adds stochasticity in the transmission. Thus, increasing the number of male courtships is the point that needs to be improved in order to be able to carry on a transmission chain. Some studies are made on that subject in the team in order to find some fruit flies "aphrodisiacs" for the males. One student started to study the effect of yeast. As flies mate on their food sources (Reaume and Sokolowski, 2006), the smell of a good meal could increase the males' willingness to mate. The data with only one courtship are nonetheless potentially interesting. We used them to study the correlation between air pressure and courtship latency and we are currently studying the results of the one courtship situations in the mate copying experiments.

Mate copying is a fascinating behaviour that have only been described in few species so far (see the brief review in the introduction) and many questions remain. An important one is the fitness advantage to copy the preferences of other females (Leadbeater, 2009). There is a lack of empirical studies on this topic. In our experiments, we did not detect a higher offspring production when the female had copied the choice of another one (Sabine Nöbel, unpublished data). One hypothesis is that as if many females are choosing the same type of male, it is likely to be a good male and the choice would be equal to or better than random choice. Moreover copying the choice of the majority will give offspring with a quality no worse than the average quality of the new generation (Vakirtzis, 2011). Finally if the preference spreads in the population, choosing the preferred male will produce

offspring with this attractive phenotype, thus with the highest chance of reproductive success ("sexy son hypothesis" , Fisher, 1930). However, as we saw in the first experiment of chapter 2 with the *white* mutants, if the male possesses the preferred trait but another non appealing characteristic the female will be more reluctant to mate. They thus rely primarily on their personal information, a phenomenon that has also been observed in other animals (Rieucau and Giraldeau, 2011).

Another interesting study to undertake would be to test whether females could be sensitive to the fact that one male has been rejected and avoid that phenotype in the future, that is, to test whether females can generalize mate *aversion* in addition to mate preference. This is an unexplored area in studies of mate copying (Vakirtzis, 2011), and could be done easily with *Drosophila* as already mated females refuse new copulations during many days. Such an experiment could give new insights on the acquisition of information and on the impact of this information on the individual's strategies. For instance, would demonstrations of mate aversion affect the preference of females in the same way as demonstrations of mate preference? Would contradiction (of, say, innate or acquired preferences) have the same effect whether females are shown demonstrations of preference, or aversion? One can suppose, for instance, that a female might be more inclined to follow the choice of the majority (whatever its potential dispositions before the experiment) in case of a demonstration of aversion than of preference, as going against the choice of the majority could be even more disastrous for the reproductive success of its offspring in case of aversion. To be run, this new experiment would involve several male phenotypes, and demonstrations for preference would single out one phenotype for mating, while demonstrations for aversion would single out one phenotype for *not* mating. As is, our current protocol involves only two phenotypes: males are either of the preferred phenotype, or of the avoided phenotype (an exception is in the first experiment of chapter 2 where we tested generalization with mutant phenotypes, but still males were either pink or green, or, more generally, of the preferred or avoided phenotype). It is possible that our experiments reveal copying of mate aversion, rather than mate preference. We could label this question of the generalization of aversion, rather than preference, as the "unsexy son hypothesis".

2. The climactic effect of climatic conditions

For a long time neglected, the influence of barometric pressure on insects behaviour has caught scientific interest very recently (Austin et al., 2014; McFarlane et al., 2015; Pellegrino et al., 2013). Our study about pressure is only correlative, to provide clear conclusion about the causal effect of air pressure effects on mate copying in *Drosophila* it would be necessary to artificially manipulate the changes in air pressure in a controlled experiment. However, as air pressure is the only climatic condition that we do not control in our experimental room and seems to be the only varying variable our *Drosophila* might be sensitive to.

How would *Drosophila* feel the air pressure changes? Unfortunately the organ sensitive to air pressure is not known yet in *Drosophila*. One study is undertaken by our team using mutants on the Johnston's organ. This organ is involved in audition and is used to detect air vibrations (Boekhoff-Falk, 2005; Eberl et al., 2000). As sound waves in air results in change in air pressure, the Johnston's organ could be involved in air pressure sensitivity.

One important question is why the females copy less when climatic conditions are deteriorating. One proximate hypothesis could be that deteriorating climatic conditions increases their stress level. This stress could either directly constraint their capacity to learn, for instance by preventing necessary resources to be allocated to learning, or just favouring the use of personal information. On an experiment on guppy, Dugatkin and Godin showed that only the most well-fed females copied the mate choice of others. Females deprived of food and thus stressed, chose randomly between the presented males (Dugatkin and Godin, 1998). More studies would be needed to see at which level air pressure has influence in *Drosophila*. Does it have an action on cognitive processes or memory? Or on the general behaviour diminishing the fly's attention? Or in contrast, are the flies focused on specific parameters and not on others' behaviour?

The reverse question is also valuable: why do females copy a lot when climatic conditions are improving? Are they more oriented toward reproduction and pay more attention to males and

mating events? An interesting point would be to know the behaviour of wild flies at the approach of rains and during period of good and sunny weather. Even though fruit flies are genetically well known, there is almost no studies about their behaviour in the wild (Leadbeater, 2009). Additional information about *Drosophila*'s natural history and behaviour in the wild, could improve lab studies and help orienting new researches in the laboratory and in the field (Reaume and Sokolowski, 2006).

3. Overall conclusion

Our results suggest that females fruit flies evolved the capacity to visually discriminate between two categories of males and to copy the majority (conformism strategy) for their mate choice. Lots of variables are influencing the probability that observer females will copy the mate choice of demonstrators females, the most important being the climatic conditions. Thus the social influence on *Drosophila* selection of a sexual partner is greater when the sun is shining. As we conducted our study in a laboratory, with a laboratory adapted population, we can only conclude that they have all the capacities to acquire a mate choice from other individuals and transmit it thorough the population. It would be interesting to investigate whether *Drosophila* use public information and if they exhibit mate choice copying in the wild.

We verified 3 of the 4 criteria defined by Danchin et al (2011) to test if a trait can be accepted as at least partly culturally transmitted. However, we showed that another criteria might need to be added to obtain cultural transmission: conformism. Too much conformism might prevent the apparition of new or improved behavioural variants, but individuals might copy with a little bit of conformism in order for new behaviours to spread in a population and across generations. Copying the majority, especially when arriving in a new environment, allows to exploit the knowledge of local experts (van de Waal et al., 2013) and to conform to the local social rules. As the saying goes "when in Rome, do what the Romans do".

Moreover, this work brings contribution to the increasing evidence that the use of public information in decision-making exists in very different taxa, suggesting that cultural evolution may be ancestral and perhaps more widespread than what was currently thought (Danchin *et al.*, 2004). This would considerably broaden the taxonomic range of cultural process, and plea for the inclusion of cultural inheritance into the general theory of evolution (Danchin *et al.*, 2010).

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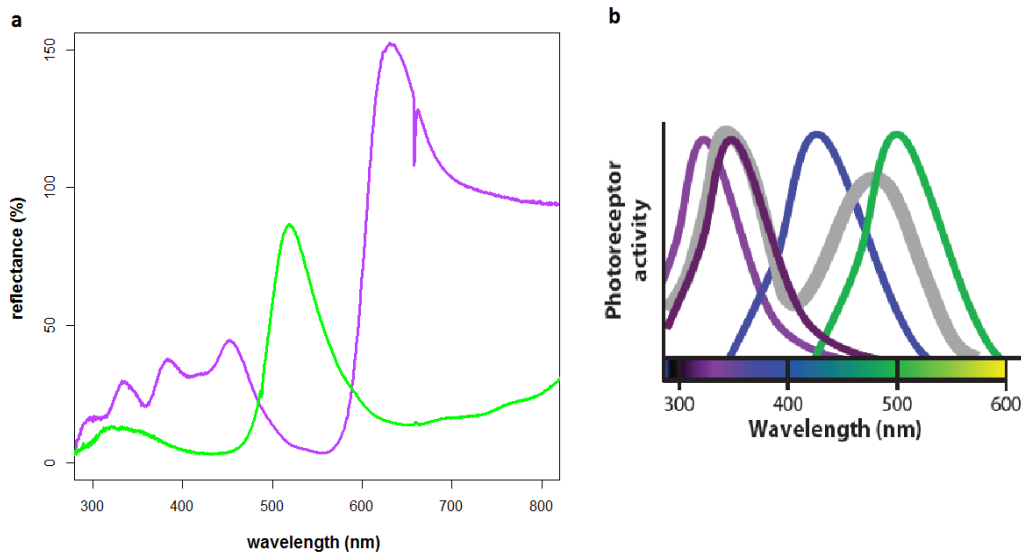
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Annexes

1. Characteristics of the powders used to create the males artificial phenotypes:



Annexe 1: a. Spectral reflectance curves of the green and pink powders. b. wavelengths of light detected by *D. melanogaster* (from the article of Paulk *et al.* 2012). The fly cannot see wavelength after 600 nm, but our pink powder also emits between 300 and 500 nm so making it visible for the fly's eyes. The fact that the two powders emits with two very distinct picks is best for the flies to distinguish the phenotypes.

2. Aspect of the dusted males



Annexe 2: The two artificial phenotypes with males dusted with green and pink powders. On this picture the males were just dusted. Then the 30 min cleaning period allows them to take out the excess of dust.

3. R code for the theoretical model

```
#Note: all lines until the #--- line are functions to be launched once only.
#Then the parameters and simulations can be set or launched by launching only
#the lines after the #--- line

update0 <- function (pop, n, thres, bias)
#This function sets the preference (green or pink) of a population of females
#according to the behavior of a same sized population observed at the
#previous iteration.
#A probability p is calculated according to the previous iteration mean choice.
#Then, a random value between 0 and 1 is calculated for each individual of
#the population.
#If this value is above p, the individual is considered to choose green. Else
#it is considered to choose pink.

#pop = table with choices of each individual at the previous
#iteration. 0 = green, 1 = pink
#n = number of individuals in the population
#thres = threshold value (under thres or higher than 1-thres the
#probability p is constant due to dissimilarity)
#bias = dissimilarity (limit value of the probability p) = proportion minimal
#of individuals making different choice than the majority

{
  mean_pop = mean(pop); #mean choice of the population at previous iteration
  pop = 1:n*0; #reinitialisation of the population for the new iteration

  a = (1 - 2*bias) / (1 - 2*thres); #slope of the the linear part of the
                                     #conformism function defining p, i.e. when
                                     #the proportion of females choosing pink at
                                     #the previous iteration is between thres and
                                     #1-thres

  #p = probability to choose pink
  p = (mean_pop - thres)*a + bias; #value of p in the linear part

  if (mean_pop < thres) #limit of p when proportion of pink at
    p = bias; #previous iteration is under threshold

  if (mean_pop > 1 - thres) #limit of p when proportion of pink at
    p = 1 - bias; #previous iteration is above threshold

  for (i in 1:n) #update of pop. choice for current iteration
  {
    if (runif(1,0,1) > p) #random choice compared to probability p
      pop[i]=0 #individual i choose green
    else
      pop[i]=1 #individual i choose pink
  }

  return(pop); #return the table with updated population
                                     #choice
}

simu <- function (Niter, nn, thres, bias, trac_simu)
#This function allows updating the population over Niter iterations.
#It returns a vector with the mean choice of the population at each iteration

#Niter = number of iterations = number of successive mating cycle, according
```

```

#that all females of a population mate at the same time, once per iteration.
#n = number of individuals in the population
#thres = threshold value (under thres or higher than 1-thres the
#probability p is constant due to dissimilarity)
#bias = dissimilarity (limit value of the probability p ) = proportion minimal
#of individuals making different choice than the majority
#trac_simu = indicates if choice in function of the iteration has to be plotted

{
  res=c();                                     #initialisation of the vector containing mean
                                              #choice of the population at each iteration

  pop=1:nn *0+0.5;                             #initiate the population to have a mean
                                              #choice of 0.5

  for (i in 1:Niter)                            #update the population choice during Niter
                                              #iterations
  {
    pop=update0(pop,nn,thres,bias); #use of update function to update the choices
    res=c(res,mean(pop));           #addition of the mean choice for iteration i
    #to the result vector
  }
  if (trac_simu == 1)                          #plot only if requested by trac_simu
  {
    iter = 1:Niter
    x11()                                       #command to ensure the
                                              #plot open in an new window
    plot(iter,res,                             #plot the choice in function of the
          xlab="Iteration",                    #iteration
          ylab="Proportion of females choosing pink male")
  }
  return(res);                                 #return the vector with mean choices for the
                                              #population at each of the Niter iterations
}

```

```

func_tableau<- function (nbSimu, nbIter,dissim, nbFemales,lim_downRandom,
lim_upRandom,thres,trac_simu)
#This function determines for each of initially identical nbSimu populations
#if the population has preferentially chosen pink (choice = 1),
#green (choice = -1) or no clear choice (choice = 0) for each of the nbIter
#iterations
#It returns a table with nbSimu columns and nbIter rows.
#Row i gives the preference for each population at the iteration i
#Columns j gives the preference at each iteration for population j

#nbSimu = number of similar simulations run = number of populations
#nbIter = number of iterations for 1 simulation = number of successive mating
#cycle for i simulation, according that all females of a population of a
#simulation mate at the same time, once per iteration.
#dissim = dissimilarity (limit value of the probability p ) = proportion minimal
#of individuals making different choice than the majority
#nbFemales = number of females in the population
#lim_downRandom = lower limit of the non-defined choice interval. When the mean
#choice of pink is in this interval, the population is considered not to have a
#significant preference for one color
#lim_upRandom = upper limit of the non-defined choice interval. When the mean
#choice of pink is in this interval, the population is considered not to have a
#significant preference for one color
#thres = threshold value (under thres or higher than 1-thres the
#probability p is constant due to dissimilarity)
#trac_simu = indicates if choice in function of the iteration has to be plotted

```



```

{
  if ((tableau[i,V]* tableau[i+1,V])==1) #test if a choice is transmitted
                                        #from iteration i to i+1 for
                                        #population V
  {
    j=1 #j = j-th iteration after the
        #current i-th iteration
    compteur=1 #start of the counter of
               #transmissions (as there is
               #transmission from iter i and to
               #iter i+1

    #the next loop compares all iterations after the (i+1)th iterations with
    #the i-th iter until the transmission is lost or until the end of
    #calculated iterations
    while (((tableau[i+1+j,V]*tableau[i,V])==1) & (j<(nbIter-(i))))
    {
      compteur=compteur+1 #increment the counter
      j = j+1 #next iteration to be compared
    }
    perdu = 1 #indicate that the transmission
              #is lost (this line is reached
              #if the transmission has been
              #started)
  }
  i=i+1 #next iteration if no
        #transmission has been detected
}
tableau_compteur[1,V]=compteur #Save the nb of transmissions
                               #calculated for population V
}
return (tableau_compteur) #return the number of
                           #transmissions for each
                           #populations
}

```

```

func_moyenne_compteur<-function (tableau_compteur)
#This function calculates the average number of transmissions in the nbSimu
#populations

#tableau_compteur = table with the number of transmissions for each nbSimu
#populations
{
  moyenne_compteur = matrix(0,nc=1,nr=1) #init of the average number
  colnames(moyenne_compteur) = c("prop moyenne")
  moyenne_compteur[,1] = rowMeans(tableau_compteur) #average number of
                                                    #transmissions for the nbSimu
                                                    #populations

  return (moyenne_compteur) #return the average number of
                             #transmissions
}

```

```

func_histo<- function (nbSimu,tableau_compteur)
#This function plots an histogram indicating, for each number of transmissions,
#how many populations among the nbSimu populations transmitted its preference
#exactly this number of times.
#The x-axis maximal value is the maximal number of transmissions reached in all

```



```

moy_compt = c() #init of the counter for each set of
#simulation
pop_siz = c() #init of the number of the females

for (i in 1:nb_pop) #loop on each set of simulations
{
  nbFemales1 = pas*i #nb of females for this set
  simu_one_set1 = simu_one_set(nbSimu, #run the simulation for 1 number of
  nbIter,nbFemales1,lim_upRandom, #females

  lim_downRandom,dissim,thres,trac,trac_simu)
  moy_compt = c(moy_compt,simu_one_set1)#fill the vector with the average number
#of transmission for this number of
#females
  pop_siz = c(pop_siz,nbFemales1) #fill the vector of the number of
#females
}

x11() #command to ensure the
#plot open in an new window
plot(pop_siz,moy_compt, #plot the average transmission time
xlab="Population size", #in a population in function of the
ylab="Average transmission time") #size of population

#Uncomment the following lines to write on the plot the parameters used
#for the simulation
#texte = paste("nbIter=", toString(nbIter))
#mtext(texte, 3, line = 0, las = 1,adj=0)
#texte = paste("nbSimu=" , toString(nbSimu))
#mtext(texte, 3, line = 1, las = 1,adj=0)
#texte = paste("lim_upRandom=" , toString(lim_upRandom))
#mtext(texte, 3, line = 0, las = 1,adj=0.5)
#texte = paste("lim_downRandom=" , toString(lim_downRandom))
#mtext(texte, 3, line = 1, las = 1,adj=0.5)
#texte = paste("Dissim=" , toString(dissim))
#mtext(texte, 3, line = 0, las = 1,adj=1)
#texte = paste("Thres=" , toString(round(thres,2)))
#mtext(texte, 3, line = 1, las = 1,adj=1)
#Stop unccommenting at the previous line

return (1)
}

#Functions defined above this line have to be executed once only

#-----

#Parameter of the simulations

#nbSimu = number of similar simulations run = number of populations
#nbIter = number of iterations for 1 simulation = number of successive mating
#cycle for i simulation, according that all females of a population of a
#simulation mate at the same time, once per iteration.
#lim_upRandom = upper limit of the non-defined choice interval. When the mean
#choice of pink is in this interval, the population is considered not to have a
#significant preference for one color
#lim_downRandom = lower limit of the non-defined choice interval. When the mean
#choice of pink is in this interval, the population is considered not to have a
#significant preference for one color
#dissim = dissimilarity (limit value of the probability p ) = proportion minimal

```

```

#of individuals making different choice than the majority
#thres = threshold value (under thres or higher than 1-thres the
#probability p is constant due to dissimilarity)

nbSimu= 500
nbIter=100
nbFemales=20
lim_upRandom=0.60
lim_downRandom=0.40
dissim = 0.3
thres = 1/3

x=simu_one_set (nbSimu,nbIter,nbFemales,           #execute the simulations for
lim_upRandom,lim_downRandom,dissim,             #one set of populations
thres,trac=1,trac_simu=0)                       #x = average transmission time

reel=c()
for (i in 1:10)                                  #loop to execute the same set
  {x=simu_one_set (nbSimu,nbIter,nbFemales,      #of populations ten times
lim_upRandom,lim_downRandom,
dissim,thres,trac=0,trac_simu=0)
reel=c(reel,x)
}
cbind("mean=",mean(reel))                       #return the mean value of
                                                #transmission number

#the following lines allows running nb_pop set of simulations with a variable
#population
pas = 10    #step used to increment the number of females in each set
nb_pop = 10 #number of set
x_pop=simu_param_pop (nbSimu,nbIter,lim_upRandom,
lim_downRandom,dissim,thres,pas,nb_pop,trac=0,trac_simu=0)

```

AUTEUR : Anne-Cecile DAGAEFF

TITRE : Selection, Sex and Sun : transmission sociale d'une préférence sexuelle chez la drosophile (*Drosophila melanogaster*)

DIRECTEUR(S) DE THESE : Etienne Danchin (directeur) et Guillaume Isabel (co-directeur)

LIEU ET DATE DE SOUTENANCE : Université Paul Sabatier – Toulouse III, le 20 Octobre 2015

RÉSUMÉ : Choisir un partenaire est une décision importante pour les organismes à reproduction sexuée. Une possibilité est de copier le choix d'autres individus, c'est ce que l'on appelle l'imitation du choix du partenaire, phénomène bien étudié chez les vertébrés, mais peu connu chez les invertébrés. Cette thèse porte sur l'imitation du choix du partenaire chez la drosophile (*Drosophila melanogaster*). J'ai tout d'abord montré que les femelles pouvaient être influencées dans leur choix par l'observation d'une seule autre femelle et que ceci était corrélé avec la pression atmosphérique. J'ai ensuite étudié si la préférence pour un type de mâle pouvait être transmise au sein de la population. Enfin j'ai commencé un travail exploratoire pour identifier les molécules impliquées dans ce processus d'imitation. Ces résultats montrent que les drosophiles peuvent exprimer des comportements complexes conduisant potentiellement à la transmission culturelle, l'isolement reproductif et la spéciation.

MOTS-CLES : imitation du choix du partenaire, *Drosophila melanogaster*, choix du partenaire, apprentissage social, transmission culturelle, sélection sexuelle

TITLE: Selection, Sex and Sun : social transmission of a sexual preference in *Drosophila melanogaster*

ABSTRACT: Mate choice is a major fitness-affecting decision in sexually reproducing organisms. A form of mate choice is mate copying, in which females choose potential mates by copying the mate choice of conspecifics. While many studies documented mate copying in vertebrates, little is known about this behaviour in invertebrates. In this thesis, I studied mate copying in *Drosophila melanogaster* females. I showed that female flies can build a sexual preference for one male characteristic after witnessing a single mate choice event and that the efficiency of mate copying correlates with air pressure and its variations. Then I studied the characteristics of mate copying to see whether a preference for one type of male can be transmitted into the population. Finally I tried to find some molecules that could be involved in this behaviour. These results indicate that fruit flies can express complex behaviour, which can potentially lead to cultural transmission, reproductive isolation and speciation.

KEYWORDS: Mate copying, *Drosophila melanogaster*, mate choice, social learning, cultural transmission, sexual selection

DISCIPLINE ADMINISTRATIVE : Ecologie, biodiversité et évolution

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