\section{Introduction}

Sexual selection is at the origin of an intense struggle between conspecifics. Some individuals win by harm and it is a matter of weight, strength or weapons \*{anderson\_grey\_1985, clutton-brock\_functions\_1982}. While others win by charms and the opposite sex chooses the breeders, in a much more peaceful contest. Mate-choice (i.e intersexual selection) can lead to captivating and highly complex traits to attract the opposite sex, such as courtships or songs in birds \*{danchin\_ecologie\_2005}. Nevertheless, many species do not exhibit such traits and the choice is based on much more discreet signs. Yet, as stated a century ago by A.R Fisher: “The most difficult and important act of choice is the choice of a mate” \*{fisher\_evolution\_1915}, any mistake can be very expensive since it directly impacts individual’s offspring. To avoid mistakes, many species have acquired the capacity to learn from the observation of others and can therefore use social learning in numerous decision-making processes.

Social learning can take many forms as the transmission of information can be

intentional (teaching), or not. The latter is simpler since it does not require an active and intentional participation of the demonstrator. Copying is likely to be more common, and even exists in non-social invertebrates\*{coolen\_social\_2005, laidre\_mark\_e.\_how\_2010}. Many behaviors can be copied, whether trivial \*{van\_leeuwen\_group-specific\_2014}or decisive for the individual’s fitness \*{mery\_public\_2009}. It’s particularly beneficial when individual learning is costly (time consuming or dangerous; see also\*{webster\_m.m\_social\_2008}) as in mate-choice. Therefore, copying the mate-choice of potentially more experienced conspecifics can be a good solution for naive individuals to avoid the extra costs of individual learning

Mate-copying is a form of social learning in which the observation of a sexual interaction in conspecifics biases the subsequent mate-choice decision of the observer \*{brown\_fish\_2011}. It has been first demonstrated in fishes \*{dugatkin\_lee\_alan\_reversal\_1992}, followed by observations in many vertebrates \*{galef\_mate-choice\_1998, yorzinski\_same-sex\_2010} and recently in invertebrates \*{mery\_public\_2009, fowler-finn\_complexities\_2015}. Its benefits are double-sided as it facilitates the decision of the mate-choice of naïve individuals and make sure that their descendants will be preferred by conspecifics. By reproducing with a partner of the most preferred phenotype, their descendants will have chances to possess in their turn the preferred trait.

Interestingly, in population with genetic preferences, mate-copying can override them \*{dugatkin\_interface\_1996, witte\_male\_1998}. At a larger scale, mate-copying can even shape preferences of entire populations: a trait-based preference, transmitted vertically and horizontally and possibly for a long time can lead to long-lasting local tradition that can be considered as a form of animal culture\*{brooks\_importance\_1998, danchin\_cultural\_2018}.

The existence of culture in non-human species has long been disputed \*{laland\_animals\_2003} but is increasingly accepted among scientists \*{aplin\_experimentally\_2015, whitehead\_geneculture\_2017}. The list of animals for which form of culture was documented is growing constantly \*{van\_schaik\_orangutan\_2003, thornton\_alex\_multi-generational\_2010, whiten\_culture\_2017} , and one of the most recent may surprise many, ^{*Drosophila melanogaster*} \*{danchin\_cultural\_2018}. Very few, if none, species have been studied as deeply, with as extensive knowledge in every scientific field (genetics, development, neuroscience…) as ^{*Drosophila melanogaster}*. as ^{D.melanogaster} is a species of flies that have been intensively studied for more than a century as a model study in many different biological sciences. Its genetic and neuronal homology with humans and the relative simplicity of its brain allow the manipulation and relatively easy study of common mechanisms that have led to numerous discoveries \*{bellen\_100\_2010, ugur\_drosophila\_2016, hewitt\_mechanisms\_2017}.Thus, existence of mate-copying in this species represents a wonderful opportunity to understand the obscure neuronal roots of an evolutionary process widely shared among animals.

Since a century, the works of Pavlov and Skinner have led to considerable advances in the study of associative learning mechanisms \*{pavlov\_conditioned\_1927, skinner\_behavior\_1938). It exists two main methods to study associative learning: pavlovian conditioning and operant conditioning. In pavlovian conditioning experiment, it is possible to teach them to react to a previously neutral stimulus, by associating for example a sound with the presence of sugar. In operant conditioning, it is possible to teach them to change their behavior in response to a stimulus (operant conditioning). For example, by exposing them to an electric shock as they move in a specific direction. Contrary to Pavlovian, operant conditioning requires a specific action of the animal which is active.With the help of genetic and neuronal tools, it is possible to know the neuronal groups involved in these behavioral associations In drosophila, depending on the valence of the stimulus (either appetitive or aversive), different groups of neurons are involved in the learning process \*{vogt\_shared\_2014, busto\_olfactory\_2010}. Therefore, our first step was to test whether mate-copying implies aversive or appetitive memory. During a classic mate-copying experiment, the demonstration contains several types of information, the acceptance of one male and the rejection of another. We considered that a rejection represents a negative stimulus and acceptance of copulation a positive stimulus for an observer female. So, we created two treatments by presenting to an observer, a male rejected by a female ("Rejection" treatment) or a male accepted by a female ("Acceptance" treatment), and we measured the observer’s inclination to copy.

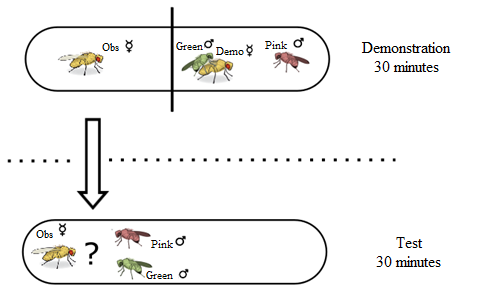
In a second part, we went deeper into the neuronal mechanisms of mate-copying by searching which group of dopaminergic neurons is required for mate-copying. The neuronal mechanisms underlying non-social visual and olfactory learning are very well known in drosophila (reviewed in #{ guo\_vision\_2017} and #{cognigni\_right\_2018}). Regarding their roles in non-social learning, two brain structures are particularly prone to be involved in mate-copying, the central complex and the mushroom bodies. The central complex localized in the center of the insect brain plays a major role in decoding visual information. It receives visual inputs from the rest of the brain and controls vision-related behaviors, memory and learning. The mushroom bodies (MBs) are formed by two symmetrical groups of approximately 2500 neurons in the center of the insect brain. Several studies studying its structure showed that it can be divided in different sub-area: calyx, pedunculus, medial lobes and vertical lobes \*{aso\_neuronal\_2016}. MBs are an integrative center involved in learning, memory and decision-making. It has been compared to the hippocampus of mammals due to the similar role they play in learning \*{strausfeld\_evolution\_1998}.

Notably, a recent study found that γ-lobes are required for visual associative memory \*{ vogt\_shared\_2014} it's consistent with previous study showing that neurons involved in appetitive and aversive memories are heavily connected to γ-lobes \*{cladrige-chang\_writing\_2009, burke\_ layered\_2012}.

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On the contrary, despite a rich repertoire of well-studied social processes \*{pasquaretta\_how\_2016, teseo\_fighting\_2016, dawson\_social\_2018}, neuronal mechanisms of social learning are still poorly understood. However, a recent study found that dopamine is required in mate-copying \*{monier\_dopamine\_2018}. Dopamine is a neurotransmitter, that drives a variety of brain functions among which the formation of appetitive and aversive memory \*{riemensperger\_punishment\_2005, sitaraman\_serotonin\_2008, alekseyenko\_targeted\_2010, berry\_dopamine\_2012, yamamoto\_dopamine\_2014}. Dopamine is produced in dopaminergic neurons, but if they are involved in the formation of appetitive and aversive memory, we do not know which are for mate-copying. However, we do know some of the neurons required for non-social visual learning, Ddc and TH-labbeled neurons. Ddc neurons are involved in the acquisition of olfactive and visual appetitive memory \*{liu\_subset\_2012, vogt\_shared\_2014}. In pavlovian conditioning, the impairment of those neurons prevents flies from learning to associate a neutral stimulus (light or odor) with a reward (sugar). Meanwhile TH neurons are involved in the acquisition of olfactive and visual aversive memory. If TH neurons are silenced, flies can no longer associate an odor or color to a punishment (electric shock). Since these 2 groups of neurons are essential for visual learning, we expected these neurons to be required in mate-copying.

Thanks to #{kitamoto\_conditional\_2001}, we know that UAS-GAL4 technology coupled with the thermosensitive Shibire protein can be used to block specific sets of neurons (see also #{kasuya\_neuronal\_2009}). Precisely, mutant flies containing both transgenes UAS-shits and GAL4(expressed in a specific group of neurons) are exposed to a restrictive temperature (33°C) that silences neurons where GAL4 is expressed. Our goal was to use this technique to study mate-copying. We used mutant flies with the transgene UAS-shits and TH-GAL4 or Ddc-GAL4. This allowed us to have a temporal control on TH and Ddc-labelled neurons activity and to silence them during the demonstration in order to see if they are required in mate-copying (for more details, see "Fly strains and crossings" section). We thus created two treatments, depending on the group of neurons silenced, and measured mate-copying scores in each of them. If one group is involved in mate-copying, the corresponding treatment will not display mate-copying (score similar to random choice), due to the incapacity of mutants to learn.



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Figure 1: classic mate-copying protocol

All experimental flies were virgin. During demonstration, observer female observes the free choice of demonstrator female. Observer females are tested immediately after the end of the demonstration. After all copulations ended, demonstrators were removed.