The Hexagon - A New Tool for Multiple Simultaneous Demonstrations in Social Learning

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Introduction & Methods

The Hexagon is a new tool for multiple simultaneous demonstrations in behavioural experiments of social learning. We are studying mate-copying, a special form of social learning, in which individuals use the mate choice of conspecifics as source of information revealing potential partners' quality or compatibility. It leads females to either mate preferentially with the specific male they saw being chosen by another female (individual-based copying), or with a male showing similar characteristics as the male they saw being chosen by another female (trait-based copying) [1]. This behavioural pattern was documented in many vertebrate species, and in one invertebrate species: *Drosophila melanogaster* [2-4].

The experimental design to test mate-copying in *D. melanogaster* involves a demonstration during which one demonstrator female copulates with one of two males, one dusted in green, the other in pink powders, followed by a mate-choice test during which the observer female encounters two virgin males, one pink and one green, and can choose to mate with one of them. Several studies [3, 4] showed that females can perform trait-based mate-copying of artificially-coloured male phenotypes (green versus pink), after watching only a single live demonstration.

However, in the wild, drosophila often live in dense populations and aggregate on rotting pieces of fruits. Females can thus witness multiple and simultaneous mate choice demonstrations and can also be affected by the population sex-ratio [5], which determines the availability of a mating partner. We can expect females to adapt their choosiness to the population sex-ratio, and possibly, to mate-copy more or less often depending on the context. In order to address these questions and measure mate-copying in a more natural situation of multiple simultaneous demonstrations, we created a hexagonal device, called the Hexagon.

The Hexagon is composed of a central arena devoted to the observers, and six peripheral compartments devoted to the demonstrators. Each peripheral compartment can be seen from the central arena, as they are separated by a glass partition. Each peripheral chamber can host a copulating couple and a single male of the opposite colour, providing a demonstration to the observers placed on the central arena (see figure).

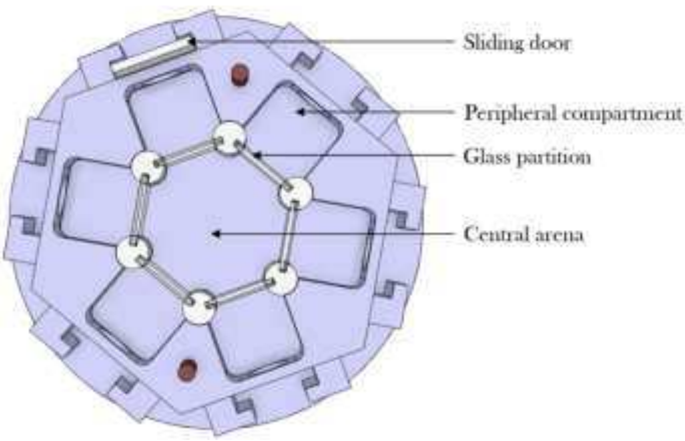

Figure: The Hexagon device allows observer females placed in the central arena to witness up to six simultaneous mate-choice demonstrations, occurring in the peripheral compartments [4]. The device “Hexagon” can be purchased from Toulouse Tech Transfer and Paul Sabatier University through a Material Transfer Agreement.

As the hexagon allows us to choose the number of observer females and demonstrator trios (and thus group size and sex-ratio), we created a gradient of sex-ratio during demonstrations of mate-copying experiments by changing the number of females observing from the central arena six simultaneous demonstrations unfolding in the six peripheral compartments of our hexagonal device.

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Results & Discussion

We found that experimental male:female sex-ratio during demonstrations did not affect mate-copying indices, but positively affected the proportion of both males courting the female during mate-choice tests, as well as male courtship duration. Thus, as predicted, the sex-ratio affected female choosiness positively, and drosophila females seem to have evolved a mate-copying ability independently of sex-ratio, and a capacity to adapt their choosiness to male availability.

Conclusion & Outlook

Addressing the effect of sex-ratio and group size on mate-copying is only one example for the range of application of the Hexagon. Our device can be used to study many questions related to mate-copying or more generally visual-based social learning in fruit flies. For instance, it has been used in a mate-copying context to demonstrate conformism, i.e., that flies show an exaggerated tendency to copy the majority [6]. We were able to show that flies can detect majorities as subtle as 60% and learned to prefer males of the most commonly chosen colour as efficiently whatever the majority during the demonstrations (range from 100% down to only 60%). The Hexagon can also be used to detect the ability to count (or to estimate numeric values), to study the behavioural response to the commonness of a phenotype (by using two male phenotypes which proportions differ from 50% each) or of a specific situation such as the presence of stressors, in a mate-copying context. Finally, this device can be used for a diversity of behavioural experiments involving observers and demonstrators (i.e. situations of social learning) as long as vision only is needed, like mate choice or laying site preference.

With its multiple usage options the Hexagon can greatly help in gaining insight into cognition and social learning.

Ethical statement

Our study involved a population of *Drosophila melanogaster* that have been maintained exclusively under laboratory conditions for hundreds of generations. The experiments contained behavioural observations of *D. melanogaster*, which required no ethical approval and complied with French laws regarding animal welfare. We kept the number of flies used in this study as small as possible. We handled flies by gentle aspiration without anaesthesia to minimize damage and discomfort. After the experiments individuals were euthanized in a freezer.

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