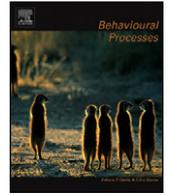




Contents lists available at ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc

Rapid reports

Do female fruit flies (*Drosophila serrata*) copy the mate choice of others?Heather L. Auld^a, David Punzalan^a, Jean-Guy J. Godin^b, Howard D. Rundle^{a,*}^a Department of Biology and Centre for Advanced Research in Environmental Genomics, University of Ottawa, 30 Marie-Curie, Ottawa, ON K1N 6N5, Canada^b Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, ON K1S 5B6, Canada

ARTICLE INFO

Article history:

Received 3 January 2009

Received in revised form 6 March 2009

Accepted 7 March 2009

Keywords:

Drosophila serrata

Mate-choice copying

Mate preferences

Sexual selection

Social learning

ABSTRACT

Female mate-choice copying is a social learning phenomenon whereby a female's observation of a successful sexual interaction between a male and another female increases her likelihood of subsequently preferring that male. Although mate-choice copying has been documented in several vertebrate species, to our knowledge it has not yet been investigated in insects. Here, we investigated whether female mate-choice copying occurs in the fruit fly *Drosophila serrata*, a model system for the study of mate preferences and the sexual selection they generate. We used two complementary experiments in which focal females were given a choice between two males that differed in either their apparent (as determined visually by the focal female) or actual recent mating success. Mate-choice copying was evaluated by testing whether focal females mated more frequently with the 'preferred' male as opposed to the other male. In both experiments, however, we found no evidence for mate-choice copying. We discuss possible reasons for the apparent absence of mate-choice copying in this species.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Female mate-choice copying is a phenomenon in which females bias mating towards males they have previously observed in successful sexual encounters with female conspecifics. Mate-choice copying can evolve under a range of conditions (e.g., Losey et al., 1986; Pruett-Jones, 1992; Servedio and Kirkpatrick, 1996; Stöhr, 1998) and can have potentially important implications for key evolutionary processes including the direction and intensity of sexual selection (Wade and Pruett-Jones, 1990; Laland, 1994) and the maintenance/erosion of genetic variation in secondary sexual traits and mating preferences (Kirkpatrick and Dugatkin, 1994; Agrawal, 2001; Witte, 2006). Because a number of *Drosophila* species are model organisms for the study of sexual selection and the evolution of mating systems (Powell, 1997), the existence of mate-choice copying in these taxa would be of particular interest.

Here, we present an empirical investigation of female mate-choice copying in the fruit fly *Drosophila serrata*. Although social learning has not been investigated in *D. serrata*, the existence of mate-choice copying in this species would have important implications because they are a model organism for the study of sexual selection arising from mate preferences (reviewed in Rundle and Boughman, in press). Mate preferences within *D. serrata* have been well characterized via a series of quantitative genetic, behavioural,

and evolutionary experiments, and are based in part on a suite of contact pheromones composed of cuticular hydrocarbons (CHCs). Both sexes express the same CHCs, although they are sexually dimorphic in relative concentrations, and both males and females discriminate among potential mates using variation in the relative concentrations of these CHCs (Chenoweth and Blows, 2005). To date, the study of mate preferences in this species has occurred exclusively in the absence of any opportunity for the transmission of social information.

Whether mate-choice copying occurs in *D. serrata* is also of interest in understanding the taxonomic distribution of this behaviour. Although mate-choice copying has been reported in a number of vertebrate species (Gibson and Höglund, 1992; Galef and White, 2000; Westneat et al., 2000; Witte, 2006), we are not aware of any previously published investigations in insects despite suggestions that the role of learning in insect sexual behaviour has likely been underestimated (Dukas, 2006). *Drosophila* species, for example, are promiscuous and mating occurs in aggregations on or near ephemeral food sources (Powell, 1997). *D. melanogaster* females have been shown to use chemical cues to discriminate among prospective mates based on age, and to avoid recently mated males (Scott et al., 1988; Ejima et al., 2005, 2007). Learning from prior mating experiences has also been shown to be an important component of mate choice (Dukas, 2005a, b, 2008a), suggesting a cognitive capacity for mate-choice copying.

We conducted two complementary experiments in which individual focal *D. serrata* females were presented with a binary choice between males that differed in their apparent (as determined visually) and actual recent mating success. We tested whether focal

* Corresponding author.

E-mail address: hrundle@uottawa.ca (H.D. Rundle).

females would copy the observed mate choice of the model female by preferentially mating with the apparently preferred male over the non-preferred male.

2. Materials and methods

Our experiments utilized a stock population of *D. serrata* (Rundle et al., 2006) maintained with non-overlapping generations at large population size (16 half-litre bottles) on a yeast-based medium at 25 °C, 12L: 12D, and at approximately 60% relative humidity. *D. birchii* females used in conjunction with the non-preferred males (see below) came from a stock originally established in Brisbane, Australia, from which a large sample of flies was moved to Ottawa in 2007 and maintained under the same conditions as above in eight half-litre bottles.

Flies for use in the experiments were collected as virgins within 24 h of emergence from low density vials. These vials were created in the previous generation by allowing a single, randomly chosen virgin female and male from the stock population to mate and oviposit for 48 h. After collection, offspring were held separately by sex at low density (six males/vial; eight females/vial) for 4 days prior to use in the experiments.

In both experiments, replicate trials consisted of placing a single focal virgin *D. serrata* female in the middle compartment of a three-chambered arena (see supplementary online material) partitioned by transparent dividers. A single *D. serrata* 'stimulus' male was present in either end chamber of the arena. These males had been differentially marked prior to the experiment by feeding them live yeast saturated with either red or blue food colouring in a balanced design (the coloured yeast is visible through the abdomen under a dissecting microscope for up to 24 h). To one end chamber two virgin *D. serrata* 'model' females were then added, designating the male in that chamber as 'preferred'. To the other end of the chamber, two *D. birchii* virgin females of a similar age were added, designating that male as 'non-preferred'. *D. serrata* males will court *D. birchii* females, but these females consistently reject *D. serrata* males, preventing copulation (Hoikkala et al., 2000). The use of *D. birchii* females ensured that while both males actively courted in the arena, only the preferred male could successfully mate.

During each mating trial, the focal female was permitted to observe the males in both end chambers until one of the model *D. serrata* females mated with the preferred male. Immediately following this copulation, the two model females and the two *D. birchii* females were removed and the dividers were lifted, permitting the focal female to interact directly with both stimulus males. Arenas were then checked at approximately 5 min intervals and once mating occurred between the focal female and one of the two stimulus males, all flies were removed and the identity of the chosen and rejected males was recorded.

In Experiment 1, stimulus males were full-sibling brothers, thereby minimizing phenotypic differences between them. This was done to reduce the possibility that mate-choice copying could be obscured by female preferences for particular phenotypic traits in males, independent of any social information (e.g., Dugatkin, 1996). All stimulus males were also given the opportunity to practice courting (and to potentially mate) via the addition of six random virgin females to each holding vial of six males approximately 36 h prior to their use in the mating trials. After 24 h, these females were removed and discarded. Finally, to control for the recent mating history of the two stimulus males, males that were to be designated as non-preferred were individually mated, out of sight of the focal female, to a virgin *D. serrata* female immediately prior to their use in the mating trials. This 'pre-mating' of the non-preferred male meant that, once a focal female was released

from her center chamber during a mating trial, she had a choice between two brothers, both of whom had just mated, but only one of these matings (involving the preferred male) had she potentially witnessed. Fifty-two replicate mating trials were performed using unique individuals and families (for the brothers) in each.

Experiment 2 differed slightly and was designed to address three possible causes of a failure to observe mate-choice copying in Experiment 1. First, the phenotypic differences between the two stimulus males (brothers) may have been so small as to hamper the focal female's ability to discriminate between them. To address this possibility, stimulus males in Experiment 2 were randomly chosen from the stock population. Second, as a control of their recent mating history, non-preferred males were pre-mated immediately prior to the mating trials in Experiment 1. Although this occurred out of sight of the focal female, females may utilize chemosensory as opposed to visual cues to infer the mating history of potential mates. Focal females may therefore have perceived both stimulus males as being equally attractive to other females. To address this possibility, non-preferred males in Experiment 2 were not pre-mated, permitting females to utilize both visual and non-visual cues. Third, if females do use non-visual cues to infer male mating history, the period of courtship practice provided to the stimulus males in Experiment 1 may have generated variance among males in their longer term (36 h) mating history that may have affected female mate choice. To address this possibility, stimulus males in Experiment 2 were not given an opportunity to practice courting, thereby standardizing their lifetime mating history. Sixty-two replicate mating trials were performed using unique individuals in each.

3. Results

There was no evidence of mate-choice copying in Experiment 1: focal females mated with the preferred male in 27 of 52 trials (sign test for deviation from random mating, $p=0.89$). Similarly, females mated randomly with respect to stimulus males in Experiment 2, although there was a non-significant trend towards focal female avoidance of the preferred males (the preferred male was chosen in 25 of 63 trials; sign test, $p=0.13$). There was no effect of male colour marking on female mate choice in either experiment (sign test; Experiment 1: 28 red vs. 24 blue matings, $p=0.678$; Experiment 2: 34 red vs. 29 blue matings, $p=0.615$).

4. Discussion

Female mate-choice copying occurs when females bias their choice of mates towards males they assess as having been preferred by other females. Using 115 independent mate choice trials, we found no evidence consistent with mate-choice copying in female *D. serrata*. This was true when females could visually assess males as differing in their apparent recent mating success (Experiment 1), and when females had the opportunity to utilize multiple cues (including chemosensory) to discriminate between males that differed in their actual mating success (Experiment 2). Although non-significant, in the latter case our results suggested the opposite trend in which females mated preferentially with recently un-mated males. This is consistent with previous work showing female aversion of recently mated males in *D. melanogaster* (Scott et al., 1988) and could indicate that, rather than copying each others' mating decisions, *D. serrata* females may use this social information to avoid recently mated males. Alternatively, males may experience a behavioural refractory period immediately following copulation that reduces their mating success. Insight into these alternatives could be gained by tracking changes in male courtship following mating.

Our failure to detect mate-choice copying in *D. serrata* suggests that it may not occur in this species. An absence of mate-choice copying may reflect weak selection for such a behaviour. Mate-choice copying is expected to evolve when copying increases the reliability with which females can assess mate quality, or when the costs of locating and assessing mates are high (Gibson and Höglund, 1992; Stöhr, 1998; but also see Servedio and Kirkpatrick, 1996). The strong phenotypic covariance between male sexual display traits (CHCs) and an index of male quality previously demonstrated in *D. serrata* (Hine et al., 2004) may mean that direct assessment of males is efficient in this species, and that mate-choice copying may therefore confer little benefit. Although choosiness is thought to be energetically costly in many taxa (Andersson, 1994), direct estimates of these costs are limited. Costs of mate choice in *D. serrata* may be low if females expend little energy locating or sampling multiple males (i.e., because males congregate at their food source). Such conditions will not favour the evolution of mate-choice copying.

Nevertheless, it remains possible that mate-choice copying could occur in *D. serrata* under conditions not considered here. The design of our experiment, including the configuration of our mating area, may not have been conducive to the expression of mate-choice copying for some unknown reason. Detecting visual learning in *D. melanogaster*, for example, appears to be highly dependent on the experimental design (e.g., Tang et al., 2004). It is also known that the expression of mate-choice copying in animals depends on the properties of both the observer and the model, the relative costs of independent choice versus copying, and certain environmental factors (e.g., Gibson and Höglund, 1992; Dugatkin, 1996; Galef and White, 2000; Westneat et al., 2000; Witte, 2006). Evaluating the potential influence of such factors will require additional studies using alternative experimental designs. A comprehensive understanding of the prevalence of mate-choice copying in insects will also require experiments employing a variety of species. Of particular interest will be those in which social learning has previously been documented (see Leadbeater and Chittka, 2007), although this remains largely unknown (Dukas, 2008b).

Acknowledgements

We thank M. Delcourt, S. Khair, L. Kwan, K. MacLellan, V. Rotondo, and P. Wu for assistance in the lab. This work was funded by a grant from the Natural Sciences and Engineering Research Council of Canada to HDR.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.beproc.2009.03.004.

References

Agrawal, A.F., 2001. The evolutionary consequences of mate copying on male traits. *Behav. Ecol. Sociobiol.* 51, 33–40.

- Andersson, M., 1994. *Sexual Selection*. Princeton University Press, Princeton, New Jersey.
- Chenoweth, S.F., Blows, M.W., 2005. Contrasting mutual selection on homologous signal traits in *Drosophila serrata*. *Am. Nat.* 165, 281–289.
- Dugatkin, L.A., 1996. Interface between culturally based preferences and genetic preferences: female mate choice in *Poecilia reticulata*. *Proc. Natl. Acad. Sci. U.S.A.* 93, 2770–2773.
- Dukas, R., 2005a. Experience improves courtship in male fruit flies. *Anim. Behav.* 69, 1203–1209.
- Dukas, R., 2005b. Learning affects mate choice in female fruit flies. *Behav. Ecol.* 16, 800–804.
- Dukas, R., 2006. Learning in the context of sexual behavior in insects. *Anim. Biol.* 56, 125–141.
- Dukas, R., 2008a. Learning decreases heterospecific courtship and mating in fruit flies. *Biol. Lett.* 4, 645–647.
- Dukas, R., 2008b. Evolutionary biology of insect learning. *Annu. Rev. Entomol.* 53, 145–160.
- Ejima, A., Smith, B.P.C., Lucas, C., Levine, J.D., Griffith, L.C., 2005. Sequential learning of pheromonal cues modulates memory consolidation in trainer-specific associative courtship conditioning. *Curr. Biol.* 15, 194–206.
- Ejima, A., Smith, B.P.C., Lucas, C., van der Goes van Naters, W., Miller, C.J., Carlson, J.R., Levine, J.D., Griffith, L.C., 2007. Generalization of courtship learning in *Drosophila* is mediated by cis-vaccenyl acetate. *Curr. Biol.* 17, 599–605.
- Galef Jr., B.G., White, D.J., 2000. Evidence for social effects on mate choice in vertebrates. *Behav. Process.* 51, 167–175.
- Gibson, R.M., Höglund, J., 1992. Copying and sexual selection. *Trends Ecol. Evol.* 7, 229–232.
- Hine, E., Chenoweth, S., Blows, M.W., 2004. Multivariate quantitative genetics and the lek paradox: genetic variance in male sexually-selected traits of *Drosophila serrata* under field conditions. *Evolution* 58, 2754–2762.
- Hoikkala, A., Crossley, S., Castillo-Melendez, C., 2000. Copulatory courtship in *Drosophila birchii* and *D. serrata*, species recognition and sexual selection. *J. Insect Behav.* 13, 361–373.
- Kirkpatrick, M., Dugatkin, L.A., 1994. Sexual selection and the evolutionary effects of copying mate choice. *Behav. Ecol. Sociobiol.* 34, 443–449.
- Laland, K.N., 1994. Sexual selection with a culturally transmitted mating preference. *Theor. Popul. Biol.* 45, 1–15.
- Leadbeater, E., Chittka, L., 2007. Social learning in insects – from miniature brains to consensus building. *Curr. Biol.* 17, R703–R713.
- Losey, G., Stanton, F., Telecky, T., Tyler, W., the Zoology 691 Graduate Seminar class, 1986. Copying others, an evolutionarily stable strategy for mate choice: a model. *Am. Nat.* 128, 653–664.
- Powell, J.R., 1997. *Progress and Prospects in Evolutionary Biology: The Drosophila Model*. Oxford University Press Inc., New York.
- Pruett-Jones, S., 1992. Independent versus nonindependent mate choice—do females copy each other. *Am. Nat.* 140, 1000–1009.
- Rundle, H.D., Chenoweth, S.F., Blows, M.W., 2006. The roles of natural and sexual selection during adaptation to a novel environment. *Evolution* 60, 2218–2225.
- Rundle, H.D., Boughman, J.W. Behavioral ecology and speciation. In: Westneat, D.F., Fox, C.W. (Eds.), *Evolutionary Behavioral Ecology*. Oxford University Press, Oxford, in press.
- Scott, D., Richmond, R.C., Carlson, D.A., 1988. Pheromones exchanged during mating—a mechanism for mate assessment in *Drosophila*. *Anim. Behav.* 36, 1164–1173.
- Servedio, M.R., Kirkpatrick, M., 1996. The evolution of mate choice copying by indirect selection. *Am. Nat.* 148, 848–867.
- Stöhr, S., 1998. Evolution of mate-choice copying: a dynamic model. *Anim. Behav.* 55, 893–903.
- Tang, S., Wolf, R., Xu, S., Heisenberg, M., 2004. Visual pattern recognition in *Drosophila* is invariant for retinal position. *Science* 305, 1020–1022.
- Wade, M.J., Pruet-Jones, S.G., 1990. Female copying increases the variance in male mating success. *Proc. Natl. Acad. Sci. U.S.A.* 87, 5749–5753.
- Westneat, D.F., Walters, A., McCarthy, T.M., Hatch, M.I., Hein, W.K., 2000. Alternatively mechanisms of nonindependent mate choice. *Anim. Behav.* 59, 467–476.
- Witte, K., 2006. Learning and mate choice. In: Brown, C., Laland, K., Krause, J. (Eds.), *Fish Cognition and Behavior*. Blackwell Publishing Ltd., Oxford, pp. 70–95.