

RESEARCH ARTICLE

Adult fruit fly attraction to larvae biases experience and mediates social learning

Zachary Durisko^{1,2,*}, Blake Anderson¹ and Reuven Dukas¹

ABSTRACT

We investigated whether adult fruit flies (*Drosophila melanogaster*) use cues of larvae as social information in their food patch choice decisions. Adult male and female fruit flies showed attraction to odours emanating from foraging larvae, and females preferred to lay eggs on food patches occupied by larvae over similar unoccupied patches. Females learned and subsequently preferred to lay eggs at patches with novel flavours previously associated with feeding larvae over patches with novel flavours previously associated with no larvae. However, when we controlled for the duration of exposure to each flavoured patch, females no longer preferred the flavour previously associated with feeding larvae. This suggests that social learning in this context is indirect, as a result of strong social attraction biasing experience.

KEY WORDS: Social behaviour, Social learning, Fruit flies, Larvae, *Drosophila melanogaster*

INTRODUCTION

There has been recent interest in establishing tractable model systems for examining the evolution and mechanisms of social behaviour and social learning (Dukas, 2010; Robinson et al., 2005; Sokolowski, 2010). One of the most suitable species for such research effort is the fruit fly (*Drosophila melanogaster*) owing to the availability of powerful tools for examining the links between its genes, neurons and behaviour (Chen et al., 2012; Dickson, 2008; Keleman et al., 2012; Vosshall and Stocker, 2007). Building on earlier knowledge (Prokopy and Roitberg, 2001; Wertheim et al., 2002; Wertheim et al., 2005), a few laboratories have developed new protocols for quantifying social interactions (Ardekani et al., 2013; Simon et al., 2012) and studying social information use in adult and larval fruit flies. Briefly, female fruit flies copy the egg-laying substrate choice of other females (Battesti et al., 2012; Sarin and Dukas, 2009) and the male phenotypes preferred by other females (Mery et al., 2009). There is significant genetic variation in social environment choice and social niche construction in male fruit flies (Saltz, 2011; Saltz and Foley, 2011), and mixed-sex groups of fruit flies rely on chemosensory cues to generate non-random social interaction networks, which vary between genetic lines (Schneider et al., 2012).

Social information is any cue or signal generated by another organism that reduces environmental uncertainty (Dall et al., 2005; Danchin et al., 2004), which animals typically use to improve

decisions. It has long been known that female fruit flies prefer to lay eggs at sites occupied by larvae (del Solar and Palomino, 1966), but this phenomenon has been dismissed as solely due to changes to the surface texture of the substrate and not to social cues provided by the eggs or larvae (Atkinson, 1983). However, our recent finding that individual fruit fly larvae show strong attraction to the distinctive odour of food consumed by other larvae and prefer cues associated with other larvae (Durisko and Dukas, 2013) led us to predict that the presence of larvae and food consumed by larvae would be a reliable indicator of high quality sites for adults. Adult flies, particularly egg-laying females, face uncertainty about where best to forage and lay eggs. The presence of larvae at a site indicates that both a previous egg-laying female and the larvae themselves, which are highly mobile and tend to aggregate at the best available local site (Durisko and Dukas, 2013; Gerber and Stocker, 2007; Gomez-Marin et al., 2011; Sokolowski, 1980), have found the site to be of sufficient quality. Adult males may utilize larval cues as social information to locate food and mates while females may use these cues to find suitable egg-laying sites. Additionally, by learning cues such as the specific fruit odour associated with sites commonly occupied by larvae, an individual adult could quickly identify similar sites without devoting the time and energy required for individual sampling. Such social learning would allow females to locate high quality sites efficiently without exposing their offspring to increased competition at sites already occupied.

We assessed whether adult flies use larval social cues in their patch choice decisions. First, we tested whether females prefer to lay eggs at patches occupied by larvae over unoccupied patches. Second, we assessed the attractiveness of the odours emanating from food occupied by larvae to both males and females. Third, we tested whether females would prefer to lay eggs at patches with flavours previously experienced with larvae over patches with flavours previously experienced without larvae.

RESULTS**Experiment 1 – are adults attracted to cues of larvae?**

We began our investigations by testing for simple social information use: oviposition preference for and attraction to a site containing larvae. First, we placed females individually in cages containing two food dishes, one of which contained larvae (Fig. 1A). Females strongly preferred to lay eggs on the dish containing larvae over similar dishes without larvae, both under standard photoperiod (mean proportion of eggs on social dishes \pm s.e.m., 0.995 ± 0.004 ; $N=128$, one-sample Wilcoxon signed-ranks test: $W=8128$, $P<0.001$; Fig. 1B) and under continuous lighting (0.978 ± 0.022 ; $N=46$, one-sample Wilcoxon signed-ranks test: $W=1034$, $P<0.001$; Fig. 1B), which ensures that females were able to find both patches. In these two experiments, each female laid on average 35.2 ± 1.7 and 26.5 ± 2.5 eggs, respectively. This preference, however, could have been due to the altered texture of the food containing larvae and not due to attraction to social cues per se. Next, we directly tested

¹Animal Behaviour Group, Department of Psychology, Neuroscience & Behaviour, McMaster University, 1280 Main Street W, Hamilton, ON, Canada L8S 4K1. ²Social Aetiology of Mental Illness (SAMi) CIHR Training Program, Centre for Addiction and Mental Health, 33 Russell Street, Toronto, ON, Canada M5S 2S1.

*Author for correspondence (zachary.durisko@camh.ca)

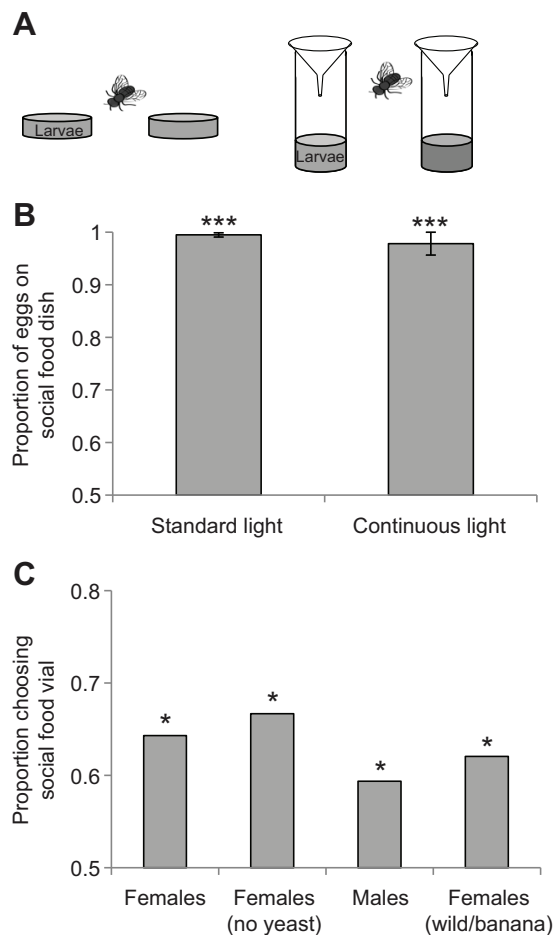


Fig. 1. Choices of flies between two food patches, one containing larvae and one unoccupied. (A) The experiments used either dishes (left) from which we calculated the proportion of eggs laid by each female on the social dish containing larvae, or vial-traps (right) from which we calculated the proportion of flies entering the social vial. (B) Females strongly preferred to lay their eggs (mean \pm s.e.m.) on dishes containing larvae in both standard (lights off from 22:00 h to 10:00 h; $N=128$) and continuous lighting conditions ($N=46$). (C) More females and males entered vials containing larvae and food than entered vials containing food alone. Females were tested first with vials containing standard lab diet, which contains live yeast (first bar on the left, $N=56$), then females (second bar, $N=53$) and males (third bar, $N=155$) were tested with vials containing a modified recipe that did not contain live yeast. Finally, females from a wild-caught population were tested with wild-caught larvae on banana (right bar, $N=108$). Asterisks indicate a significant difference from chance (0.5): * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

whether adult females and males are attracted to olfactory cues emanating from foraging larvae. We placed individual flies into cages containing two vials of food, one of which contained larvae, as before. We capped the vials with funnels, effectively forming 1-way traps (Fig. 1A), so that flies could not exit a vial after making a choice. Females significantly preferred vials containing larvae and used food over vials containing unused food (proportion choosing social vial: 0.643; binomial test: $N=56$, $P=0.044$; Fig. 1C). Females showed a similar preference when we controlled for the potential presence of live yeast (0.660; binomial test: $N=53$, $P=0.027$; Fig. 1C). Males also preferred vials containing larvae and used food over vials containing unused food alone (0.594; binomial test: $N=155$, $P=0.024$; Fig. 1C). Finally, we tested whether this attraction is a general phenomenon under more naturalistic conditions. Females from a wild-caught population chose vials containing

larvae and banana significantly more often than vials containing only banana (0.620; binomial test: $N=108$, $P=0.016$; Fig. 1C).

Experiment 2 – does adult attraction to larvae result in social learning?

We asked whether attraction to foods occupied by larvae could result in female learning, which would bias future oviposition decisions in the absence of larvae. We exposed individual females simultaneously to one novel flavour of food that contained larvae and another novel flavour of food without larvae, and then gave them a choice to lay eggs on either flavour without larvae. Females significantly preferred to lay eggs on the flavour that had been paired with larvae and used food during training ($N=254$; permutation test: $P=0.004$; Fig. 2A). Side of flavour presentation, whether females laid eggs during training and their interaction had no significant effect (all $P>0.255$). This experiment simulated realistic settings in which females are free to sample, experience and lay eggs on both social and non-social patches. The learned preference for the social flavour, however, may have been due exclusively to the strong attraction to social cues, biasing subsequent individual experience. That is, it is possible that many females were attracted to the social food and did not experience the non-social alternative at all and subsequently showed preference for the familiar cues. When we exposed individual females to the flavours with and without larvae separately for an equal duration, females did not prefer the flavour previously experienced with larvae over the flavour previously experienced without larvae ($N=114$; permutation test: $P=1.000$; Fig. 2A). Again, there was no significant effect of the side of flavour presentation, the order of training, whether females laid eggs during training, or their interaction (all $P>0.141$). Having found no evidence of social learning when we controlled for exposure to both flavours, we finally tested whether a female experiencing only a single flavour with larvae would show a stronger preference for this cue than another female experiencing the cue without larvae. The presence of larvae on a flavour during training did not increase female preference for this flavour during the test ($N=67$; effect of larvae \times flavour experienced, permutation test: $P=0.157$; Fig. 2B). We did, however, observe a main effect of the flavour experienced during training on later preference (permutation test: $P=0.027$), indicating a preference for familiar flavours. There was no significant effect of the side of flavour presentation, whether females laid eggs during training, or their interaction (all $P>0.170$). Overall, females showed socially influenced learning when we simulated natural settings, but no social learning when we controlled for exposure duration to social and non-social flavours.

DISCUSSION

In this study we showed, first, that female fruit flies strongly prefer to lay eggs on a food substrate already occupied and consumed by larvae (Fig. 1B). Second, both female and male adult fruit flies are attracted to cues emanating from food that is occupied by larvae (Fig. 1C). Third, females learn to prefer novel cues associated with food consumed by larvae over novel cues associated with unused food of similar quality (Fig. 2A). Fourth, if females experience a flavour associated with food used by larvae for the same duration as another flavour associated with food without larvae, they do not subsequently prefer the flavour associated with larvae (Fig. 2A). Finally, females that experience only a single flavoured food used by larvae do not subsequently show a stronger preference for that flavour compared with females that experience a single flavoured food without larvae (Fig. 2B). Altogether, this suggests that female

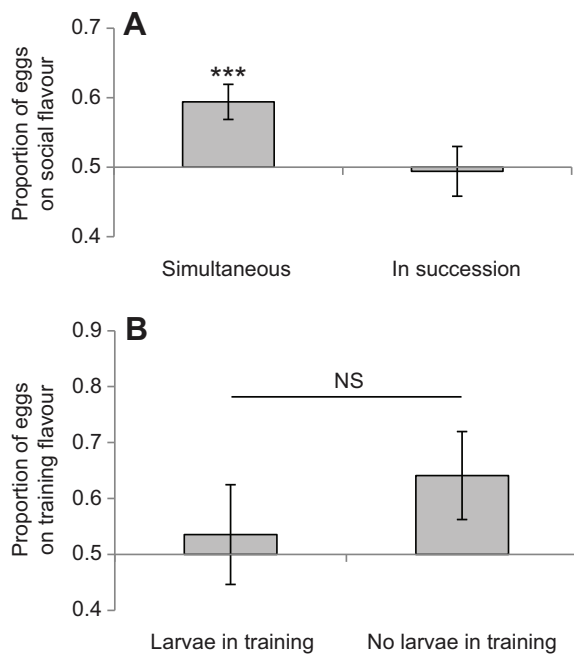


Fig. 2. Egg-laying patch flavour preference after experience. (A) We trained females with a dish of each of two flavours, only one of which contained larvae, either simultaneously ($N=254$) or successively ($N=114$), with the latter controlling for duration of exposure to each flavour. During a subsequent test, females significantly preferred to lay eggs on the flavour paired with larvae only if they had been trained with the two flavours simultaneously. When we controlled for exposure duration, females no longer preferred the social flavour. (B) We trained females with a single flavoured dish, which either did or did not contain larvae. Females trained with a flavour containing larvae did not prefer that flavour more than females experiencing the flavour without larvae ($N=67$). NS, not significant. Error bars indicate ± 1 s.e.m.

experience with a flavour regardless of the presence of larvae can explain the later preference for that flavour, but that larval presence is attractive and can bias female experience. We should note that our previous work on social attraction in larval fruit flies critically established that such social attraction is mediated by odours because we conducted all our experiments either in total darkness or under far-red light not perceived by fruit flies (Durisko and Dukas, 2013). Furthermore, work in our laboratory (Venu et al., 2014) has recently identified larval gut bacteria as the source of the odour cues that are attractive to larval and adult fruit flies. Because we have critical evidence for odours being the social cues, we preferred to conduct the experiments described in this study under the regular photoperiod to help flies orient in the cages. While the experiments reported in this study do not rule out a possible role for visual cues, experiments to be reported elsewhere show no social attraction by larval and adult fruit flies when only visual cues are available (Venu et al., 2014).

Our easiest result to explain is that males are attracted to food that has been used by larvae. The odour emanating from such food is probably a cue that can lead them to a food source and to sexually receptive females. Females' attraction to such food patches with larvae is more complex, as choosing an oviposition site that already contains larvae will have both costs and benefits. Like with males, the cues associated with foraging larvae will guide a female to a good site that has already been chosen by both other females and the larvae themselves. Given a choice between food patches of varying quality, larvae will settle on the better alternative (Durisko and

Dukas, 2013), suggesting that the presence of larvae may be a particularly informative cue. Additionally, larvae may actually improve the quality of the substrate for subsequent larvae owing to changes in texture, suppression of mould and facilitation of favourable microbes, including beneficial yeast species (Rohlf and Hoffmeister, 2003; Stamps et al., 2012; Wertheim et al., 2002).

While the informational value of odours emanating from feeding larvae is probably substantial, there are obvious costs as well. Because it takes about a day for eggs to hatch, a female laying eggs on substrates already occupied by larvae guarantees that her larvae will encounter a substrate containing harmful waste products (Borash et al., 1998) and likely competition for food. Increased larval density can slow development rate, increase mortality and, even with one versus three larvae on abundant lab food, decrease adult body mass (Durisko and Dukas, 2013). Similar effects of density on larval success under different experimental settings have been previously reported (Sang, 1949; Wertheim et al., 2002).

Given the cost–benefit tradeoffs associated with laying eggs on substrates containing other larvae, we expect that females would be attracted to low larval densities and repelled by very high densities. Indeed, some reports have indicated that oviposition is inhibited by extremely used food (Chess and Ringo, 1985; Chiang and Hodson, 1950), but this effect has yet to be investigated directly. Interestingly, interactions between different larval species (Budnik and Brncic, 1974; Budnik and Brncic, 1975; Hodge et al., 1999; Miller, 1964) and genotypes (Dawood and Strickberger, 1969; Lewontin, 1955; Saltz et al., 2012) can affect larval development and survival differently, and it would be interesting to see whether females can attend to and modulate their attraction to cues associated with different species and genotypes accordingly. We expect females to be more strongly attracted to cues associated with beneficial larval species, genotypes and densities.

Given that larval presence at a food patch is perhaps the best indication that it is highly suitable for larval development, it is clear why females that were attracted to substrates occupied by larvae learned and subsequently sought out similar substrates (Fig. 2A). However, females did not show direct social learning under strictly controlled conditions that equalized the duration of fly exposure to social and non-social flavoured food patches (Fig. 2B). Further analyses indeed agree with our interpretation that this difference is due to females spending relatively more time on the social food during training when they experienced the flavours simultaneously than when they experienced the flavours in succession. Females laid $95.0 \pm 1.5\%$ versus $72.2 \pm 4.1\%$ of their eggs on the social food during training in the simultaneous and succession experiments, respectively (Mann–Whitney $U=5050.5$, $Z=4.8$, $P<0.001$). Thus it appears that social learning in this context is due to strong social attraction coupled with individual experience. While we must be careful not to over-interpret null results, adult females have been shown across labs with similar protocols to engage in robust direct social learning from other adults (Battesti et al., 2012; Sarin and Dukas, 2009), and so we may speculate on why females do not learn directly from interactions with larvae. Given that females possess the necessary learning abilities and can perceive both the cues of larvae and the food flavours, presumably they would have evolved to learn directly from larvae if it were beneficial. One possible explanation for the absence of more direct social learning is that, in this context, strong social attraction combined with individual learning may be mechanistically simpler yet will result in a similar outcome: attraction to similar patches in subsequent decisions. Another possible explanation is that a female's individual experience is the most relevant indicator of site quality. Thus, while larval cues

may be attractive at a distance, experiencing a high quality food regardless of larvae overrules social information and results in a learned preference for this flavour. Fruit flies, which are a growing model for the study of social information use and social learning, can help shed light on the mechanisms and evolution of social attraction and social information use.

MATERIALS AND METHODS

General

We maintained three population cages each containing several hundred *D. melanogaster* Canton-S on abundant standard food at 25°C, 60% relative humidity and a 12 h:12 h light:dark cycle with lights on at 01:00 h. This light cycle placed peak egg laying at midday so that we could collect experimental eggs within a very short time window of about 1 h. We collected eggs for experimental larvae on 85 mm diameter Petri dishes filled with 10 ml of standard food, 11 of which contained: 60 g dextrose, 30 g sucrose, 32 g yeast, 75 g commmeal, 20 g agar and 2 g methyl paraben dissolved in 20 ml ethanol. Immediately following egg laying, we transferred these dishes to an incubation chamber maintained at 25°C and high humidity. For experimental adults, we collected and sexed flies with light CO₂ anaesthesia within 8 h of eclosion. We stored the males in small cages (24×11×11 cm) and females in standard vials at a density no greater than 20 per vial. We provided flies with abundant 2 mol l⁻¹ sucrose solution diets hardened with agar (20 g l⁻¹), and gave females an additional sprinkle of live yeast as a protein supplement to encourage egg development. In all female oviposition or choice experiments, we added females to the cage of males for mating about 16 h prior to experiments so that they did not have experience with larvae before testing as larvae hatch after about 22 h. All flies were 3–4 days old during testing.

Experiment 1: oviposition preference and social attraction

We placed one social and one non-social 35 mm diameter Petri dish containing 4 ml of standard food each into small cages (20×12×13 cm). Social dishes each contained 30 early 2nd instar larvae, which had been feeding at the dishes for 24 h prior to the test so that the food ('used food') had a noticeably different surface texture and smell. As a control, we treated the non-social dishes identically, including a sham addition of larvae using the same paintbrushes used to transfer larvae. Food contained a few drops of blue colouring to increase the visibility of eggs. We placed dishes 5 cm apart in opposite corners, furthest from a lamp scattering diffuse light to the ceiling timed to turn off at 22:00 h. We alternated the locations of social and non-social dishes between tests to control for side bias. Testing began at 18:00 h with each focal female introduced into the cage through a hole in the centre of the side opposite the food, 17 cm from each dish, to minimize any bias (Fig. 1A). We left females to lay eggs overnight for 16 h. At 10:00 h the following morning, we removed females, randomized the dishes and counted eggs while blind to female identity. For each female we calculated the proportion of eggs laid on the social dish. Because of violations of normality, we compared these proportions to random chance (0.5) with a one-sample Wilcoxon signed-ranks test. Next, we repeated this protocol but with continuous lighting throughout the night in order to ensure that females could perceive both food dishes. We analysed only those females that laid eggs during testing.

We tested whether females were attracted to olfactory cues emanating from larvae. We used vials of food capped with opaque funnels (Fig. 1A). Flies had to enter the vials via the funnels and, once inside, could not exit (R.D., unpublished data). Vials contained 5 ml of standard food, either with or without larvae, as before. Each female therefore made a single choice of either the social or non-social vial, and we analysed the frequency of choices with a binomial test. We analysed only those flies that entered a vial. Larvae typically burrow into the surface of the food, making the use of visual cues unlikely. By far the most salient feature of larval foraging is the odour. Females, however, may have been attracted not to a smell generated directly by the larvae but to metabolites produced by live yeast previously consumed and transferred within the guts of larvae (Coluccio et al., 2008; Stamps et al., 2012). We therefore repeated this protocol with a modified larval diet containing no live yeast. We used this new diet recipe for all subsequent

experiments. We repeated this protocol separately with virgin male fruit flies.

Finally, having shown that gravid females are attracted to and prefer to lay eggs on sites containing conspecific larvae on standard lab food, we tested whether this phenomenon exists among wild populations of fruit flies and fruit. We caught a few hundred wild *D. melanogaster* from several locations in Southern Ontario using plastic bottle traps with slices of banana seeded with a sprinkle of live yeast. We maintained these flies on our standard lab diet and conducted our experiment within 12 generations of collection. Similar to the previous experiments, we gave females a choice between vial-traps but this time containing similarly sized 1 cm slices of ripe banana (~2.5 g), either with or without larvae.

Experiment 2: socially influenced learning

We tested whether attraction to foods occupied by larvae could result in female learning, which would bias future oviposition decisions in the absence of larvae. We collected and stored males, females and larvae as before. We transferred mated females individually into training cages each containing a social and a non-social dish of standard food, one flavoured with cherry and one with orange (sugar-free Kool-Aid drink mixes, 3 and 4.3 g l⁻¹, respectively, which flies preferred approximately equally in preliminary tests), placed in opposite corners at the rear of the cage. Each female received a 4 h training session (14:00 h–18:00 h) with the two flavours, one of which was associated with larvae and used food. We counted eggs laid on each dish during training as an indication of each female's experience. We alternated the side of flavour presentation and the flavour paired with larvae across females to control for bias. For testing, we replaced training dishes with one new dish of each flavour without larvae. The location of each flavour was the same during training and testing. We also spread 0.8 ml of yeast suspension (3 g live yeast l⁻¹ warm water) on the surface of each test dish and allowed it to dry for at least 1 h before testing. This amount of yeast is sufficient to stimulate egg laying but does not overpower the orange and cherry flavours. Females laid eggs overnight (18:00 h–10:00 h, with lights off at 22:00 h). We randomized the dishes to ensure that observers were blind to female identity, and then counted the eggs laid on each flavour. We analysed only those females that laid eggs during the test phase. Many females (52%) exclusively preferred one flavour during testing, resulting in highly non-normal data, so we analysed oviposition flavour preference (proportion of eggs laid on cherry flavoured food) with a non-parametric permutation ANOVA (using the R package 'ImPerm', version 1.2) (Wheeler, 2010). We included factors for the side of cherry presentation, identity of the social flavour, whether females laid eggs during training, and the interaction between social flavour and whether females laid eggs during training. A parametric ANOVA revealed similar results.

Training females with the two flavours simultaneously simulated realistic settings in which females are free to sample, experience and lay eggs on both social and non-social patches as they prefer. Any learned preference for the social flavour, however, may be due exclusively to the strong attraction to social cues biasing subsequent individual experience. That is, it is possible that many females were attracted to the social food and did not experience the non-social alternative at all and subsequently showed preference for the familiar cues. Therefore, we tested whether a female would prefer the flavour paired with larvae during training if we exposed the female to the social and non-social flavours for equal durations. All collection, storing, training and testing of the females was identical, except that we trained females with the two distinct patches and flavours in succession. We gave each female a 4 h training session (10:00 h–14:00 h) with a food dish of the first flavour, and then a second training session (14:00 h–18:00 h) with a dish of the other flavour, only one of which contained larvae, followed by a test phase (18:00 h–10:00 h) with both flavours without larvae. During training, we alternated the order and side of flavour presentation, as well as which flavour contained larvae, between females. The location of each flavour was the same during training and testing. As before, results were non-normal with 40.4% of females laying eggs exclusively on one flavour. We again analysed oviposition flavour preference (proportion of eggs on cherry flavoured food) with non-parametric permutation ANOVA, here including factors for the flavour paired with larvae, the order of training flavours, the

side of flavour presentation, whether females laid eggs during training, and relevant interactions.

Having found no evidence of social learning when we controlled for exposure, next we tested whether a female experiencing a novel cue with larvae would show a stronger preference for this cue than another female experiencing the cue without larvae [a test of social learning similar to Sarin and Dukas (Sarin and Dukas, 2009)]. All collection, storing, training and testing of the females was identical to the previous two experiments, except that we trained each female with one flavour only. Each female received a single 8 h (10:00 h–18:00 h) training session with one flavoured food dish, either with or without larvae, followed by a test phase (18:00 h–10:00 h) with both flavours without larvae. Again, results were non-normal with 94.0% of females exclusively preferring one flavour during the test, and so we analysed oviposition flavour preference (proportion of eggs laid on cherry flavoured food) with non-parametric permutation ANOVA, here including factors for the flavour experienced, the presence or absence of larvae during training, the side of flavour presentation, whether females laid eggs during training, and relevant interactions.

Acknowledgements

We thank M. Belyk, A. Luby, I. Venu and A. Scott for assistance, and S. Balshine and L. Dukas for comments on the manuscript.

Competing interests

The authors declare no competing financial interests.

Author contributions

This work was developed by Z.D., B.A. and R.D., conducted by Z.D. and B.A., analysed by Z.D., and the manuscript written by Z.D. and R.D.

Funding

This work was supported by the Natural Sciences and Engineering Research Council of Canada (R.D.); Canada Foundation for Innovation (R.D.); Ontario Innovation Trust (R.D.); and McMaster School of Graduate Studies (Z.D.).

References

- Ardekani, R., Biyani, A., Dalton, J. E., Saltz, J. B., Arbeitman, M. N., Tower, J., Nuzhdin, S. and Tavaré, S. (2013). Three-dimensional tracking and behaviour monitoring of multiple fruit flies. *J. R. Soc. Interface* **10**, 20120547.
- Atkinson, W. (1983). Gregarious oviposition in *Drosophila melanogaster* is explained by surface texture. *Aust. J. Zool.* **31**, 925–929.
- Battesti, M., Moreno, C., Joly, D. and Mery, F. (2012). Spread of social information and dynamics of social transmission within *Drosophila* groups. *Curr. Biol.* **22**, 309–313.
- Borash, D. J., Gibbs, A. G., Joshi, A. and Mueller, L. D. (1998). A genetic polymorphism maintained by natural selection in a temporally varying environment. *Am. Nat.* **151**, 148–156.
- Budnik, M. and Brncic, D. (1974). Preadult competition between *Drosophila pavani* and *Drosophila melanogaster*, *Drosophila simulans*, and *Drosophila willistoni*. *Ecology* **55**, 657–661.
- Budnik, M. and Brncic, D. (1975). Effects of larval biotic residues on viability in four species of *Drosophila*. *Evolution* **29**, 777–780.
- Chen, C. C., Wu, J. K., Lin, H. W., Pai, T. P., Fu, T. F., Wu, C. L., Tully, T. and Chiang, A. S. (2012). Visualizing long-term memory formation in two neurons of the *Drosophila* brain. *Science* **335**, 678–685.
- Chess, K. and Ringo, J. (1985). Oviposition site selection by *Drosophila melanogaster* and *Drosophila simulans*. *Evolution* **39**, 869–877.
- Chiang, H. and Hodson, A. (1950). An analytical study of population growth in *Drosophila melanogaster*. *Ecol. Monogr.* **20**, 173–206.
- Coluccio, A. E., Rodriguez, R. K., Kernan, M. J. and Neiman, A. M. (2008). The yeast spore wall enables spores to survive passage through the digestive tract of *Drosophila*. *PLoS ONE* **3**, e2873.
- Dall, S. R. X., Giraldeau, L.-A., Olsson, O., McNamara, J. M. and Stephens, D. W. (2005). Information and its use by animals in evolutionary ecology. *Trends Ecol. Evol.* **20**, 187–193.
- Danchin, E., Giraldeau, L.-A., Valone, T. J. and Wagner, R. H. (2004). Public information: from nosy neighbors to cultural evolution. *Science* **305**, 487–491.
- Dawood, M. M. and Strickberger, M. W. (1969). The effect of larval interaction on viability in *Drosophila melanogaster*. 3. Effects of biotic residues. *Genetics* **63**, 213–220.
- del Solar, E. and Palomino, H. (1966). Choice of oviposition in *Drosophila melanogaster*. *Am. Nat.* **100**, 127–133.
- Dickson, B. J. (2008). Wired for sex: the neurobiology of *Drosophila* mating decisions. *Science* **322**, 904–909.
- Dukas, R. (2010). Social learning in insects. In *Encyclopedia of Animal Behavior* (ed. M. Breed and J. Moore), pp. 176–179. Oxford: Academic Press.
- Durisko, Z. and Dukas, R. (2013). Attraction to and learning from social cues in fruitfly larvae. *Proc. Biol. Sci.* **280**, 20131398.
- Gerber, B. and Stocker, R. F. (2007). The *Drosophila* larva as a model for studying chemosensation and chemosensory learning: a review. *Chem. Senses* **32**, 65–89.
- Gomez-Marin, A., Stephens, G. J. and Louis, M. (2011). Active sampling and decision making in *Drosophila* chemotaxis. *Nat. Commun.* **2**, 441.
- Hodge, S., Mitchell, P. and Arthur, W. (1999). Factors affecting the occurrence of facilitative effects in interspecific interactions: an experiment using two species of *Drosophila* and *Aspergillus niger*. *Oikos* **87**, 166–174.
- Keleman, K., Vrontou, E., Krüttner, S., Yu, J. Y., Kurtovic-Kozaric, A. and Dickson, B. J. (2012). Dopamine neurons modulate pheromone responses in *Drosophila* courtship learning. *Nature* **489**, 145–149.
- Lewontin, R. (1955). The effects of population density and composition on viability in *Drosophila melanogaster*. *Evolution* **9**, 27–41.
- Mery, F., Varela, S. A. M., Danchin, E., Blanchet, S., Parejo, D., Coolen, I. and Wagner, R. H. (2009). Public versus personal information for mate copying in an invertebrate. *Curr. Biol.* **19**, 730–734.
- Miller, R. (1964). Larval competition in *Drosophila melanogaster* and *D. simulans*. *Ecology* **45**, 132–148.
- Prokopy, R. J. and Roitberg, B. D. (2001). Joining and avoidance behavior in nonsocial insects. *Annu. Rev. Entomol.* **46**, 631–665.
- Robinson, G. E., Grozinger, C. M. and Whitfield, C. W. (2005). Sociogenomics: social life in molecular terms. *Nat. Rev. Genet.* **6**, 257–270.
- Rohlf, M. and Hoffmeister, T. S. (2003). An evolutionary explanation of the aggregation model of species coexistence. *Proc. Biol. Sci.* **270** Suppl. **1**, S33–S35.
- Saltz, J. B. (2011). Natural genetic variation in social environment choice: context-dependent gene-environment correlation in *Drosophila melanogaster*. *Evolution* **65**, 2325–2334.
- Saltz, J. B. and Foley, B. R. (2011). Natural genetic variation in social niche construction: social effects of aggression drive disruptive sexual selection in *Drosophila melanogaster*. *Am. Nat.* **177**, 645–654.
- Saltz, J. B., Alicuben, E. T., Grubman, J., Harkenrider, M., Megowan, N. and Nuzhdin, S. V. (2012). Nonadditive indirect effects of group genetic diversity on larval viability in *Drosophila melanogaster* imply key role of maternal decision-making. *Mol. Ecol.* **21**, 2270–2281.
- Sang, J. H. (1949). The ecological determinants of population growth in a *Drosophila* culture; larval and pupal survival. *Physiol. Zool.* **22**, 183–202.
- Sarin, S. and Dukas, R. (2009). Social learning about egg-laying substrates in fruitflies. *Proc. Biol. Sci.* **276**, 4323–4328.
- Schneider, J., Dickinson, M. H. and Levine, J. D. (2012). Social structures depend on innate determinants and chemosensory processing in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **109** Suppl. **2**, 17174–17179.
- Simon, A. F., Chou, M.-T., Salazar, E. D., Nicholson, T., Saini, N., Metchev, S. and Krantz, D. E. (2012). A simple assay to study social behavior in *Drosophila*: measurement of social space within a group. *Genes Brain Behav.* **11**, 243–252.
- Sokolowski, M. B. (1980). Foraging strategies of *Drosophila melanogaster*: a chromosomal analysis. *Behav. Genet.* **10**, 291–302.
- Sokolowski, M. B. (2010). Social interactions in “simple” model systems. *Neuron* **65**, 780–794.
- Stamps, J. A., Yang, L. H., Morales, V. M. and Boundy-Mills, K. L. (2012). *Drosophila* regulate yeast density and increase yeast community similarity in a natural substrate. *PLoS ONE* **7**, e42238.
- Vosshall, L. B. and Stocker, R. F. (2007). Molecular architecture of smell and taste in *Drosophila*. *Annu. Rev. Neurosci.* **30**, 505–533.
- Wertheim, B., Marchais, J., Vet, L. E. M. and Dicke, M. (2002). Allee effect in larval resource exploitation in *Drosophila*: an interaction among density of adults, larvae, and micro-organisms. *Ecol. Entomol.* **27**, 608–617.
- Wertheim, B., van Baalen, E.-J. A., Dicke, M. and Vet, L. E. M. (2005). Pheromone-mediated aggregation in nonsocial arthropods: an evolutionary ecological perspective. *Annu. Rev. Entomol.* **50**, 321–346.
- Wheeler, R. (2010). *lmpPerm: Permutation Tests for Linear Models*. R package version 1.1-2.