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Authors for correspondence:
Zachary Durisko
e-mail: duriskzt@mcmaster.ca
Reuven Dukas
e-mail: dukas@mcmaster.ca

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Attraction to and learning from social cues in fruitfly larvae

Zachary Durisko and Reuven Dukas

Animal Behaviour Group, Department of Psychology, Neuroscience and Behaviour, McMaster University, 1280 Main St. West, Hamilton, Ontario, Canada L8S 4K1

We examined the use of social information in fruitfly larvae, which represent an ideal model system owing to their robust learning abilities, small number of neurons and well-studied neurogenetics. Focal larvae showed attraction to the distinct odour emanating from food occupied by other larvae. In controlled learning experiments, focal larvae preferred novel odours previously paired with food occupied by other larvae over novel odours previously paired with unoccupied food. When we gave groups of larvae a choice between food patches differing in quality, more larvae aggregated on the higher-quality food, suggesting that attraction to and learning about cues associated with other larvae can be beneficial. Furthermore, larvae were more likely to find the best available food patch in trials when that food patch was occupied by other larvae than in trials when that food patch was unoccupied. Our data suggest, however, that the benefits from joining others may be at least partially offset by the fitness costs of increased competition, because larvae reared in isolation did as well as or better than larvae reared in groups on three key fitness parameters: developmental rate, survival rate and adult dry body mass. Our work establishes fruitfly larvae as a highly tractable model species for further research on the mechanisms that modulate behaviour and learning in a social context.

1. Introduction

There has recently been increased interest in establishing simple, tractable model systems for research on the evolution of and neurogenetic mechanisms underlying social behaviour [1–3]. In addition to basic interest in social behaviour [4], such research may help us form the foundation for treatments of social disorders in humans [5–8]. A key feature of social animals is the ability to engage in social learning, defined as the acquisition of novel information from other individuals. We still do not know how prevalent social learning is among animal species. However, it has had remarkable effects on some species, most notably on humans, in which it has generated a rich culture [9]. While there has been intensive research on the evolution of social behaviour, empirical work on the evolution of social learning is rather limited. Furthermore, until recently, most research on social learning has focused on vertebrates and eusocial insects [10–12].

As a part of a series of experiments on the evolution of social learning in insects [13], we examined social behaviour and social information use in fruitfly (*Drosophila melanogaster*) larvae. Adult fruitflies are moderately social. Most notably, the pheromone *cis*-vaccenyl acetate (cVA), produced by males and transferred to females during copulation, serves as a long-distance attractant promoting adult aggregation [14–16]. Both cVA and an individual's cuticular hydrocarbons modulate aggression between males [17,18]. Social experience also influences fruitflies' circadian rhythms and the expression of cuticular hydrocarbons [19–21]. Finally, adult fruitflies show social learning in the contexts of egg laying and mate choice [22–25]. Because adult female fruitflies tend to aggregate and lay eggs at a single site, many larvae typically share a food substrate, and thus social behaviour may occur at the larval stage as well. Identifying social interactions among larvae opens opportunities for analysing social behaviour and the use of social information in a simple and tractable model

system with well-studied learning abilities [26–28] and neurobiology [29–31]. We began by examining social attraction in the larvae. We then tested whether larvae learn to prefer cues associated with other larvae. Finally, having found both social attraction to and learning from social cues, we assessed some of the benefits and costs larvae incur from joining other larvae.

2. Material and methods

(a) General

We maintained three population cages each containing several hundred *D. melanogaster* Canton-S on abundant standard food at 25°C and 60% relative humidity, and on a 12 L : 12 D cycle with lights on at 01.00. This irregular 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 on 85 mm (diameter) Petri dishes filled with 10 ml of standard food and covered with 0.7 ml of live-yeast suspension (30 g dry live yeast l^{-1} of warm water) to stimulate egg laying [22]. Immediately following egg laying, we transferred these dishes to an incubation chamber maintained at 25°C, high humidity and total darkness. We conducted all further manipulations and tests under far red light, which fruitflies cannot see [32], in order to minimize disturbance and phototaxis. Data are archived in Dryad (doi:10.5061/dryad.qq304).

(b) Food preparation

In several experiments, we created *social* and *non-social* food discs. We placed discs of food (ranging from 1.15 to 2.5 ml, depending on the experiment) in 85 mm Petri dishes containing a thin layer of agar. To social discs, we added groups of 20–30 randomly selected larvae, which fed on the discs for 18–42 h prior to testing, depending on the experiment. After such feeding, we considered food to be *used*, as opposed to unused *fresh* food, which was identical in quality and age, but had not been occupied by larvae. Used food has a notably different texture, smell and (presumably) taste than fresh food. Because the larvae on social stimuli may have provided social cues to the focal larvae, we refer to them throughout as 'models'.

3. Social attraction

We began our investigations by testing for simple social information use: attraction to a substrate frequented by others. We placed a social and a non-social food disc on opposite sides of a Petri dish containing a thin layer of agar (figure 1*a*). We tested each focal third-instar larva individually by placing it through a 1 cm hole in the lid at the centre of the Petri dish, equidistant to either disc, and recording its choice, defined as making contact with a disc within 5 min (see electronic supplementary material).

First, in experiment 1A, we gave focal larvae a choice between a social and a non-social disc. We conducted tests in 60 mm agar Petri dishes with the food discs placed on opposite sides. Focals were placed 7 mm from either disc. In this experiment, however, we reared focals with others for the first 2 days of life and so they may have learned to prefer the familiar cues associated with others. To eliminate this possibility, in experiment 1B, we reared each focal larva individually by placing each egg into its own 60 mm Petri dish containing 0.3 ml of standard food, which is abundant for a single larva. These isolated larvae experienced no other larvae prior to testing.

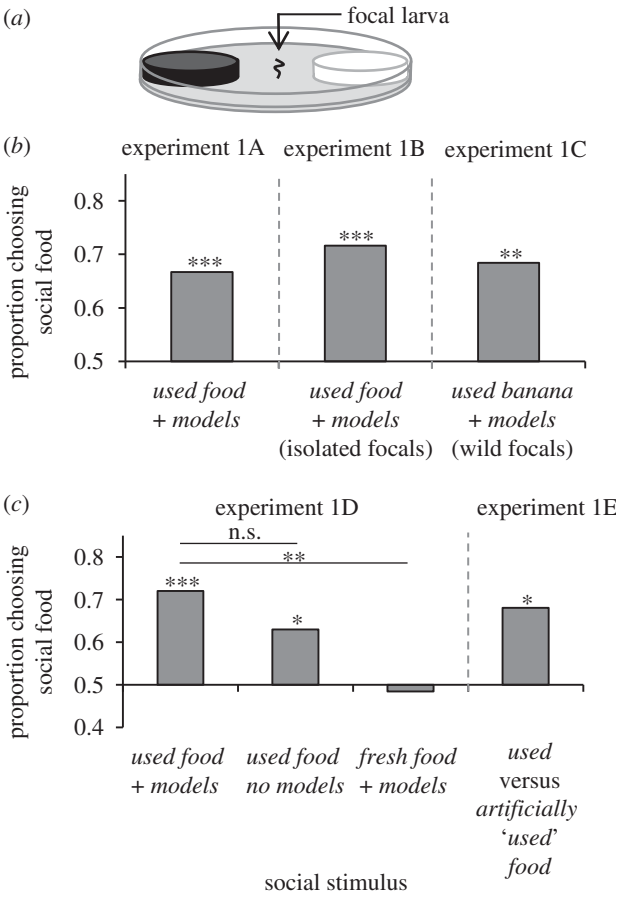

Figure 1. (*a*) In a series of experiments, we gave larvae a binary choice between a social food (black disc) and a non-social food (white disc). (*b*,*c*) Social foods and larval treatment varied between experiments, as noted on the *x*-axis legend. 'Focals' refers to the larvae being tested, and 'models' refers to the larvae providing social cues (see §3 for details). Dashed lines separate experiments. Asterisks indicate significant deviation from random (0.5): $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$; 'n.s.' indicates no significant difference.

Next, in experiment 1C, we tested whether the social attraction observed in the first two experiments was a general phenomenon by testing larvae from a wild-caught population feeding on and tested with ripe banana. In experiment 1D, we tested which cue served as the attractant for the focal larvae: the distinct odour emanating from food previously occupied by larvae for about a day or some cue directly originating from the model larvae. We compared larval attraction with social foods in two tests each involving a choice between foods: (i) *used food without models* versus *fresh food*, and (ii) *fresh food with models* versus *fresh food*. As a control, we also included our baseline test involving *used food with models* versus *fresh food*. Used food consisted of a food disc consumed by 20 early-third-instar model larvae for 24 h. Depending on the treatment, we left the models, removed models from used food discs or added models to fresh food discs immediately before testing.

Finally, in experiment 1E, we assessed whether the attractive cue associated with used food was due to the presence of larvae, and not due merely to an increased salience of the food (e.g. because of increased surface area). We tested larval attraction to *used food without models* versus *artificially 'used' fresh food*, which we had made to resemble used food by artificially simulating larval foraging with a needle, generating

small grooves and scratches in the surface and underside of the food disc.

(a) Results

Focal larvae reared both in groups and in isolation showed significant attraction to the social food discs of larvae and used food (groups: 66.7%, $n = 126$, generalized linear model (GLM) intercept: $\chi^2_1 = 13.8$, $p < 0.001$; figure 1*b*, experiment 1A; isolation: 71.6%, $n = 67$, $\chi^2_1 = 11.7$, $p = 0.001$; figure 1*b*, experiment 1B). Similarly, focal larvae from a wild population showed significant attraction to the banana slice that had been used by larvae overnight (68.4%, $n = 76$, $\chi^2_1 = 10.0$, $p = 0.002$; figure 1*b*, experiment 1C). In the test of the nature of the attractive cue, focal larvae showed significant attraction to the used food without model larvae but not to fresh food containing model larvae (respectively: 63.0%, $n = 81$, $\chi^2_1 = 5.5$, $p = 0.019$; and 48.4%, $n = 64$, $\chi^2_1 = 0.03$, $p = 0.874$; figure 1*c*, experiment 1D). As before, focal larvae showed significant attraction to used food occupied by models (72.0%, $n = 82$, $\chi^2_1 = 14.6$, $p < 0.001$; figure 1*c*, experiment 1D). Larval attraction to the social food was similar in the tests consisting of *used food with models* and *used food without models* ($p = 0.245$). Attraction to *fresh food with models* was significantly lower than attraction to *used food with models* ($p = 0.004$). Attraction to used food persisted even when the alternative food was similarly textured artificially 'used' food (68.0%, $n = 50$, $\chi^2_1 = 6.2$, $p = 0.013$; figure 1*c*, experiment 1E).

4. Learning from social cues

Next, we asked whether larvae learn to prefer novel cues associated with other larvae. All experiments consisted of pairing one novel odour with a social food and another novel odour with a non-social food, and then testing the subsequent odour preference (figure 2*a*). Training and preference test (see electronic supplementary material) were adapted from previous larval learning assays [27,28,33].

In experiment 2A, one odour was paired with a 1.25 ml social food disc occupied by 30 early-third-instar models, which had been feeding on that disc for 18 h, and the other odour was paired with a non-social food disc, consisting of fresh food without models. Next, in experiment 2B, we tested which component of the social experience was critical for the learned odour preference: used food or the model larvae *per se*. We had two treatments in which we trained larvae with one odour paired with non-social food (*fresh food without models*) and the other odour paired with either (i) *used food without models* or (ii) *fresh food with models*. As a control, we also included our baseline test, which paired one odour with *used food with models* and the other with *fresh food*. Additionally, we removed a circle of 1 cm diameter (0.1 ml) from the centre of each disc to ensure that focal larvae could more easily contact model larvae, which often crawl beneath the food discs. For *used food without models* and *fresh food with models*, we removed or added models, respectively, immediately prior to training.

The results from experiment 2B indicated that focal larvae learned to prefer novel odours associated with both used food with no larvae and models on fresh food (figure 2*b*). In experiment 2C, we directly tested which factor was more important to the larvae: used food or other larvae. We

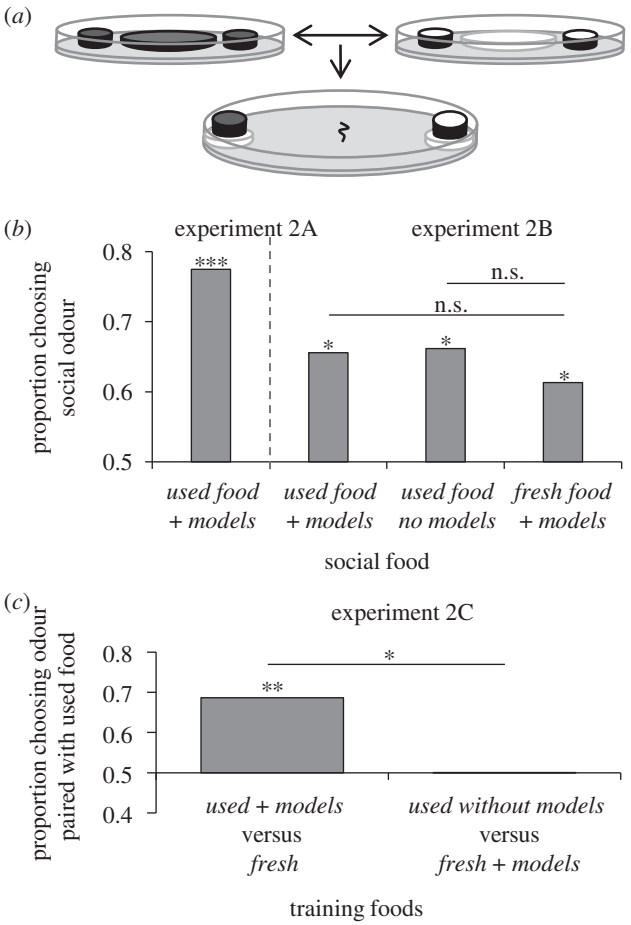

Figure 2. (*a*) We trained larvae with one odour paired with a social food (black odour cups and black disc), and another odour paired with non-social food (white odour cups and white disc), then gave them a choice between the two odours. (*b*) Social foods varied between experiments, as noted on the *x*-axis legend (see §4). The dashed line separates experiments. (*c*) We directly tested which factor was more important to the larvae: used food or other larvae. In control trials (left bar), we gave larvae a choice between an odour previously paired with *used food with models* and an odour paired with *unused food without models*. In test trials (right bar; at 0.5), we gave larvae a choice between an odour paired with *used food without models* and an odour paired with *unused food with models*. Asterisks indicate significance from random chance (0.5): $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$; 'n.s.' indicates no significant difference.

tested whether focal larvae preferred an odour previously paired with (i) *used food without models* or an odour previously paired with (ii) *fresh food with models*. As a control, we simultaneously replicated experiment 2A. If larvae do not learn from their direct interactions with others, then they should prefer an odour paired with used food over an odour paired with others on fresh food. If, however, direct interactions with others improve the perceived quality of a food, then they should prefer the odour paired with used food less strongly than controls. Additionally, we observed a subset (71%) of the *fresh food with larvae* training dishes to quantify social interactions between focals and models. We recorded the proportion of time (out of the total nine possible minutes) that each focal larva spent within 2 mm (approx. 1 body length) of a model larva. Typically, focal larvae crawled beside and remained in contact with other larvae. Once a focal was near models, it usually stayed close to them for the remainder of the training session.

(a) Results

In experiment 2A, focal larvae chose the odour previously paired with social food (*used food with models*) more frequently than the odour paired with non-social food (77.5%, $n = 71$, GLM: $\chi^2_1 = 15.4$, $p < 0.001$; figure 2*b*, experiment 2A). In experiment 2B, focals chose the odour previously paired with the social food more frequently in all treatments: when the social food was *used food with models* (65.6%, $n = 61$; GLM: $\chi^2_1 = 5.5$, $p = 0.019$; figure 2*b*, experiment 2B), *used food without models* (66.2%, $n = 65$, $\chi^2_1 = 6.4$, $p = 0.012$) and *fresh food with models* (61.3%, $n = 62$; $\chi^2_1 = 3.8$, $p = 0.050$). There was no overall difference in the frequency of social choices between the three tests (GLM: $\chi^2_2 = 0.429$, $p = 0.807$), and pairwise comparisons revealed no significant differences between the three tests (all $p > 0.528$; figure 2*b*, experiment 2B). In experiment 2C, focals did not differ in preference for odours previously paired with *used food without models* or *fresh food with models* (50%, $n = 48$, GLM: $\chi^2_1 = 0.01$, $p = 0.937$; figure 2*c*), and the presence of model larvae on the fresh food significantly reduced preference for the odour paired with used food in test trials compared with controls (GLM: $\chi^2_1 = 4.1$, $p = 0.044$; figure 2*c*). We replicated our previous results from experiment 2A, with larvae choosing an odour previously paired with *used food with models* significantly more often than an odour previously paired with fresh food alone (68.6%, $n = 51$, GLM: $\chi^2_1 = 7.1$, $p = 0.008$). Our quantification of social interactions revealed that focal larvae spent 52.4 ± 3.8% ($n = 41$) of their time within 2 mm of model larvae.

5. Benefits and costs of joining others

In our final three experiments, we addressed the ultimate evolutionary question of *why* focal larvae prefer to join others. First, we asked whether an aggregation of larvae can be a valuable source of foraging information to other larvae. If groups of larvae tend to aggregate at the best sites in their environment, then individuals can rely on the cues of foraging conspecifics to quickly locate high-quality sites. In experiment 3A, we tested whether groups of larvae are more likely to aggregate on the best available food in their environment. We randomly selected 30 larvae and placed them at the edge of an 85 mm agar dish, 3 cm from two 2.5 ml discs of food (2.3 cm diameter, 6 mm thick). Dishes contained either (i) one disc of standard food (100%) and one disc of 50% food, or (ii) one disc of 50% food and one disc of 25% food. Additionally, the food discs were presented in one of two possible configurations: touching or separated by 1 cm.

In experiment 3B, we tested whether individual larvae were better at locating the best locally available food patch when that patch was occupied by other larvae than when it was unoccupied. We allowed focal larvae to choose between a low- and high-quality food in one of two conditions. In the models-absent condition, individual focal larvae could choose between the two food patches based on food-derived cues only. In the models-present condition, we placed 30 larvae on the higher-quality food disc 18 h prior to testing. In short, we gave larvae a choice between (i) low-quality food and (ii) either social or non-social high-quality food. We analysed the frequency of choices with a generalized linear model with a binomial distribution and logit link function,

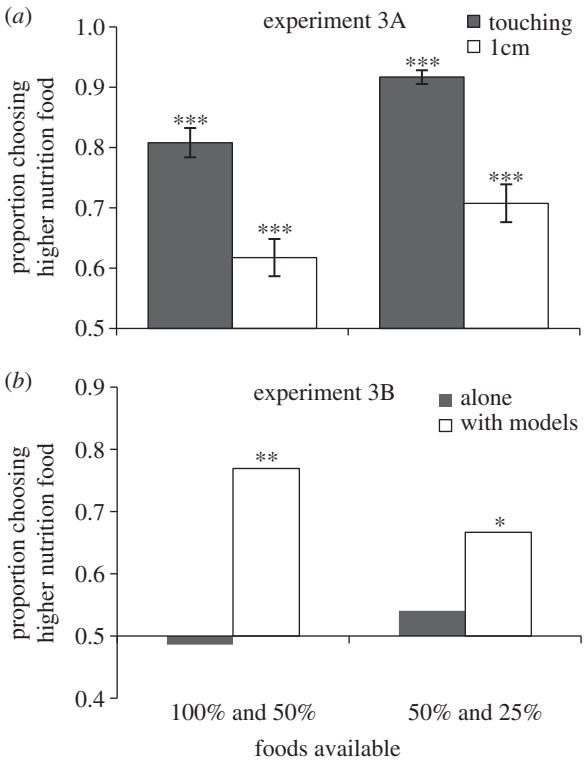

Figure 3. (*a*) We gave groups of larvae a choice between two food discs of different quality placed together (black bars) or 1 cm apart (white bars), and recorded the proportion feeding from the higher-quality food after 18 h. (*b*) We gave individuals a choice between two food discs of different quality, either alone or with a group of larvae on the higher-quality food. Asterisks indicate significant difference from chance (0.5) or significant differences between treatments: *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

including factors for the presence/absence of model larvae, foods available, side of food discs and relevant interactions.

Finally, in experiment 3C, we assessed the developmental effects of group foraging. We measured key parameters related to fitness as a function of larval group size. We transferred 1, 3, 10 or 50 eggs to dishes with 2.5 ml of standard food immediately after egg laying. As a reference, fruitfly laboratories typically rear a few dozen flies per vial containing 5 ml of standard food [34,35]. We recorded larval developmental rate, egg-to-adult survival and adult body mass. See electronic supplementary material for further details. If foraging aggregations improve fitness in this context, then we would expect moderately sized groups of larvae to develop faster, larger and with lower mortality rates than either larvae reared alone or in large groups with increased competition.

(a) Results

In experiment 3A (group choice), larvae showed significant preference for aggregating on the higher-quality food for all food combinations and configurations (all $t_{49} > 3.7$, all $p < 0.001$; figure 3*a*). For the 100% versus 50% nutrition food tests, the proportion of larvae choosing the 100% food was 0.808 ± 0.024 when the foods were touching and 0.617 ± 0.031 when 1 cm apart. For the 50% versus 25% food tests, the proportion choosing the 50% food was 0.917 ± 0.011 when touching and 0.708 ± 0.031 when 1 cm apart (figure 3*a*). When the discs were touching, a significantly greater proportion of larvae chose the higher-quality food than when the discs were 1 cm apart ($F_{1,192} = 56.3$, $p < 0.001$).

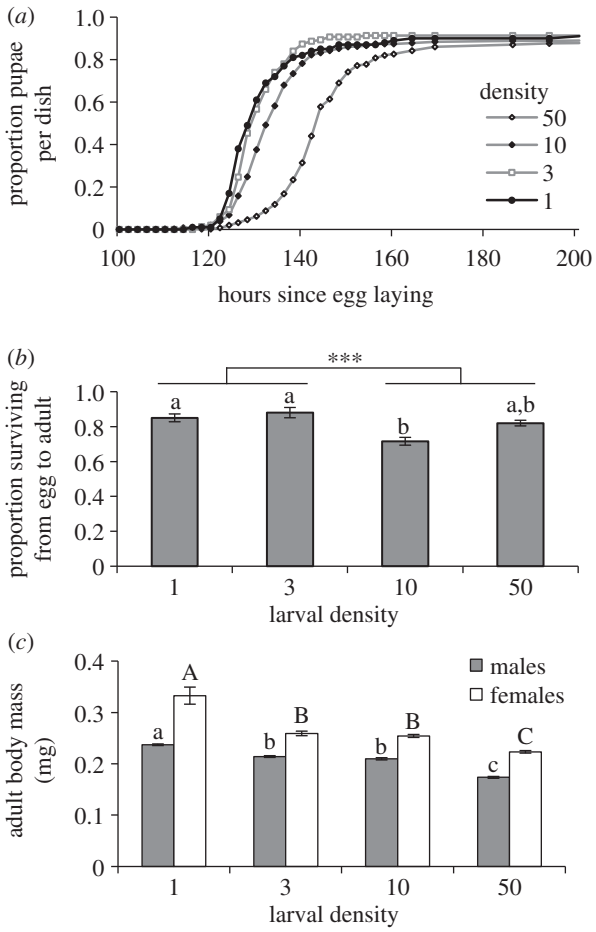

Figure 4. We monitored (*a*) larval developmental rates, (*b*) egg-to-adult survival (mean ± s.e.) and (*c*) adult body mass of flies reared at different larval densities. Letters above bars indicate significant differences in *post hoc* tests, with upper and lower case in panel (*c*) reflecting independent comparisons within females and males, respectively.

When the available foods were 50% and 25%, a greater proportion of larvae chose the higher-quality food than when the two foods were 100% and 50% ($F_{1,192}$= 18.6, $p < 0.001$). There was no significant effect of side ($F_{1,192} = 1.0$, $p = 0.331$), and no significant interactions (all $p > 0.365$).

In experiment 3B (individual choice), focal larvae chose the higher-quality food more often in the presence than the absence of model larvae (GLM: $\chi^2_1 = 6.7$, $p = 0.009$). In the presence of models, focal larvae chose the higher-nutrition food significantly more frequently in both the 100% versus 50% and the 50% versus 25% food conditions (respectively, 76.9%, $n = 39$, GLM: $\chi^2_1 = 10.0$, $p = 0.002$; and 66.7%, $n = 39$, $\chi^2_1 = 4.1$, $p = 0.042$; figure 3*b*). Without model larvae on the higher-quality food, focals did not differ from chance (respectively, 48.6%, $n = 37$, $\chi^2_1 = 0.4$, $p = 0.842$; and 54.1%, $n = 37$, $\chi^2_1 = 0.2$, $p = 0.619$; figure 3*b*). There was no significant effect of food types available, side of food disc presentation or the interaction between available foods and the presence of model larvae (all $p \geq 0.288$). The presence of model larvae did not affect choice latency (58.7 ± 6.3 versus 61.0 ± 8.0 s, with and without larvae, respectively; $t_{150} = 0.2$, $p = 0.815$).

In experiment 3C, larval density negatively affected developmental rate, survival and adult body mass (figure 4). Larval density decreased developmental rate (Kaplan–Meier survival analysis with Mantel–Cox log rank chi-square: $\chi^2_3 = 29.6$, $p < 0.001$; figure 4*a*). *Post hoc* comparisons revealed that larval development was significantly slower in the density of 50 larvae than all others (all $p < 0.001$), and that 1 and 3 versus 10 approached significance (respectively, $p = 0.090$ and $p = 0.059$). Density negatively affected egg-to-adult survivorship ($F_{3,36} = 6.4$, $p = 0.001$; figure 4*b*). *Post hoc* comparisons showed that the density of 10 larvae had the lowest survivorship, significantly lower than densities of 1 and 3 larvae (Tukey HSD, respectively, $p = 0.025$ and $p = 0.001$). A planned contrast of *low density* (1 and 3) versus *high density* (10 and 50) revealed a significantly lower survivorship in the higher-density than the low-density treatments ($t_{36} = 3.7$, $p <$ 0.001). Increasing density also significantly reduced adult body mass in both males ($F_{3,98} = 118.5$, $p < 0.001$) and females ($F_{3,79} = 69.3$, $p < 0.001$; figure 4*c*). See electronic supplementary material for further details.

6. Discussion

Our main findings were that (i) fruitfly larvae are attracted to odours emanating from food used by other larvae; (ii) larvae prefer novel odours previously associated with other larvae over novel odours previously associated with non-social alternatives; (iii) for a foraging larva, other larvae can be a useful source of social information about high-quality food; and (iv) when larvae join others, they may incur costs owing to competition. We discuss each of these results in turn.

(a) Social attraction

In our first series of experiments, we found that focal larvae showed significant attraction to food patches occupied by other larvae, and this was consistent whether or not we reared focal larvae in a group or in isolation (figure 1*b*). This indicates that focal larvae did not merely show attraction to an already-familiar group setting. Furthermore, we replicated the social attraction results using larvae from a recently collected wild population reared on natural fruit (figure 1*b*). Larvae far away from food rely on cues that lead them back to food, and cues of other feeding larvae are especially relevant because they indicate that others have found a site with sufficiently high-quality food. Moreover, food patches that have been occupied by larvae for several hours develop a distinct odour. Experiment 1E suggests that larvae are attracted to this odour (figure 1*c*) and not to the direct presence of larvae at a food site. Finally, experiment 1E indicates that the attractive odour is associated with feeding larvae rather than with mere mechanical disturbance of the food. The tendency of animals to join others and form aggregations has been studied for a long time [36–38]. Our experimental work on fruitfly larvae allows us to link work on social attraction to simple cases of social information use in a leading model system highly amenable to experimental manipulation in both evolutionary ecological and neurogenetic arenas.

One could argue that the larvae in our experiments (figure 1) did not actually show social attraction in the strict sense because they were not attracted directly to others, but instead to the volatiles in food consumed by others. However, social attraction should always be based on the most relevant and salient cues available, and the ultimate cause of all social attraction is some fitness benefit such as the opportunity to locate and feed on higher-quality food [37,38].

(b) Learning from social cues

To assess the magnitude of social information use by larvae, we asked whether larvae assigned higher values to novel odours associated with relevant social settings. In agreement with the data for social attraction, we found that the larvae preferred novel odours previously associated with either used food occupied by larvae or used food from which we had removed the larvae (figure 2*b*). Interestingly, larvae also preferred odours paired with fresh food occupied by larvae over odours paired with fresh, unoccupied food (figure 2*b*), and larvae did not prefer odours paired with used food over odours paired with fresh food containing models (figure 2*c*), which suggests that experiencing direct interactions with other larvae on a food increases the perceived quality of that food.

(c) Benefits and costs of joining others

Our model system is somewhat unique because it allows us to quantify potential benefits and costs of social information use. We found that, given a choice between foods of different quality, groups of larvae were more likely to settle on the better option (figure 3*a*). Importantly, the distance between the high- and low-quality food patches had strong effects on larval choice, with fewer larvae settling on the high-quality food when the inter-patch distance was greater (figure 3*a*, white versus black bars), suggesting that limited mobility and perception may prevent larvae from readily locating the best available food patches. Given such limitations, it may be highly beneficial for larvae to be attracted to odour cues associated with others and to learn about novel cues associated with others. Indeed, we found that focal larvae were significantly better at locating a higher-quality food when that food was occupied by larvae than when it was unoccupied (figure 3*b*).

While the information gleaned by seeking others has obvious benefits, we also documented some costs. Isolated larvae had the heaviest adult dry body mass (figure 4*c*). This can translate into higher fitness, because males prefer larger females, which are more fecund [39,40], and larger males have a mating advantage owing to both superior fighting ability and female preference for larger males [41–43]. Moreover, isolated larvae did as well as or better than a modest group of 10 larvae in terms of developmental rate and survival from egg to adult (figure 4*a*,*b*). Costs associated with aggregation are well known from a large variety of species [36,38], and our results are consistent with those showing such costs among *D. melanogaster* in both laboratory and natural settings [44,45].

One can imagine some benefits from being in a small group, including suppressing mould, enhancing the growth of preferred species of yeast and bacteria, and improved ability to dig into the substrate [45–49]. Such benefits, however, may not be important in our laboratory settings, where we provide larvae with a diet containing yeast and a mould inhibitor. We cannot yet provide an estimate of the net benefit larvae may gain from joining others in natural settings. Overall, though, our results are in agreement with previous work highlighting the trade-offs involved in joining others: individuals searching for the best available site may rely on the inadvertent social information of others who have already found such a site; by joining others, however, an individual increases the level of competition at that site [36,38].

(d) Conclusions and prospects

We have established fruitfly larvae as a simple, highly tractable model system for studying social behaviour and socially influenced learning. This is especially exciting given that larvae have only about 3000 functional neurons and that there are powerful tools available for studying their neurogenetics [30,50,51]. The most logically consistent explanation for our results is that focal larvae use cues of others as a guide to superior feeding sites. Learning about novel cues associated with others and then preferring such cues over alternatives constitutes social learning, defined as the acquisition of new information by an individual (observer) through interaction with either another individual (model) or cues left by that individual [22]. While one can question whether such simple social learning can inform us about elaborate cases of social learning among vertebrates, experience clearly indicates that simple, tractable behaviours and brain functions identified in fruitflies have been instrumental for furthering our understanding of behaviour and cognition in more complex animals, including humans [52,53]. Further work on fruitfly larvae can elucidate the social cues or signals they rely on, and the neurobiological pathways that modulate behaviour and learning in a social context.

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Supplementary Material for:

Attraction to and learning from social cues in fruit fly larvae

Zachary Durisko & Reuven Dukas

2. SOCIAL ATTRACTION

Supplemental Methods

In Experiment 1A, disks consisted of 2.5 mL of standard food, 2.3 cm in diameter and 6 mm thick. Social disks contained 30 model larvae that had been feeding on that substrate for 42 hours. Social and nonsocial food disks in Experiment 1B and subsequent experiments were 1.25 mL of standard food, 3.4 cm diameter and 1.4 mm thick, which were thinner and made it easier to locate and observe larvae. Social disks contained 30 model larvae reared on these disks for 18 hours prior to testing. We conducted tests similar to the previous experiment, but in 85 mm agar petri dishes with the social and nonsocial food disks 10 mm apart, and placed larvae 5 mm from either disk.

For Experiment 1C, we captured a few hundred *Drosophila melanogaster* from several locations in southern Ontario and maintained them in the laboratory. We collected the eggs of first and second generation offspring on 85 mm petri dishes filled with 30 g of mashed ripe banana. The social and nonsocial food disks consisted of fresh, 2-mm thick slices of ripe banana. Each social disk contained 30 randomly selected model larvae that had fed on the banana slice for 18 hours prior to testing. Model larvae remained on the banana slice during testing. We conducted tests in 85 mm agar petri dishes with the slices placed on opposite sides, 1 cm apart, placing larvae 5 mm from either banana slice.

We placed larvae parallel to the midline, facing perpendicular to either disk so that they could not make a choice by simply crawling straight ahead. Typically, larvae crawled along the midline before turning and contacting a food disk, with the mean (±SEM) latency to make a choice ranging from 37.4 ± 4.9 seconds in Experiment 1C to 88.8 ± 7.1 seconds in Experiment 1A. We alternated the side of social and nonsocial disks between trials to control for side bias. We tested each focal once and always on a fresh dish of agar to prevent larvae from following a trail established by others. All larvae were in the feeding stage of their third-instar at the time of testing, approximately 90 hours after egg laying. In all experiments we analyzed only those larvae that made a choice during the test phase, comparing social and nonsocial choices with generalized linear models (GzLM) with a binomial distribution and logit link function, including side of social disk as a factor. Wald χ^2 statistics are reported.

3. LEARNING FROM SOCIAL CUES

Supplemental Methods

Prior to training, focal larvae fed on standard food dyed with blue food colouring so that they were easily distinguishable from model larvae. We gave focals six 3-minute training sessions alternating between odour/food pairings with 1-minute breaks between sessions, during which we rinsed larvae in a droplet of fresh water and placed them on an empty petri dish. For each training session, we placed larvae directly on top or in the center (depending on experiment) of the food disk between two small cups (polypropylene NMR tube caps, Sigma) each containing 10 uL of chemical odourant, either 1-butanol (BUT; Fisher) or propyl acetate (PA; Sigma), the latter diluted in paraffin oil prior to each experiment to a concentration that naïve larvae prefer equally (ranging from 1:300 to 1:1000; data not shown). The vapours of both odours are strongly attractive to larvae (Kaun et al. 2007; Kreher et al. 2008). We alternated the odours paired with each food type between tests to control for odour preference. The odour cups had lids made of mosquito-net mesh, which allowed ample evaporation of the odours but prevented larvae from making contact with the chemicals. The petri-dish lids remained on the dishes during training so that odour vapours could collect, but each lid had a series of small holes along the midline of the dish to improve aeration (Neuser et al. 2005). In all cases, we trained and tested focal larvae individually and used training and test dishes only once. We tested larval odour preference immediately following training. We placed each focal larva on the midline of an 85 mm petri dish, between two fresh odour cups filled with 10 uL of the respective odours on opposite sides, each atop a 1 cm diameter disk of fresh food. We placed focals 3 cm from each odour, parallel to the midline, perpendicular to both odours, so that they could not make a choice by simply crawling straight ahead. We gave focals up to 10 minutes to choose an odour, defined as contacting the corresponding food disk underneath an odour cup. We shuffled odour cups before testing to randomize sides of chemicals and to ensure that the observer was blind to odour identity. As in training, we perforated the lids of the petri dishes along the midline to improve aeration, draw odours to the center and minimize odour mixing. In all experiments we analyzed only those larvae that made a choice during the test phase. We assessed odour choice (BUT or PA) using GzLMs with a binomial distribution and logit link function. As factors, we included the identity of the social odour (BUT or PA), the order of training (social or nonsocial first), the side of odour presentation, and relevant interactions. We compared learning between treatments with a GzLM on the frequency of choices (social or nonsocial) including the identity of the social stimulus as a factor.

4. BENEFITS AND COSTS OF JOINING OTHERS

Exp 3: Larval Aggregation and Food Quality, Supplemental Methods

We collected eggs for experimental larvae on 85 mm petri dishes containing 10 mL of food with 50% of the sugar and yeast of our standard recipe (henceforth, “50% food”). We left larvae to develop normally on these dishes until late second instar. We tested

two different combinations of food quality, with one food always containing twice the nutrients as the other.

We alternated the side of food disks between replicates in order to control for any side bias. We left larvae for 18 h to forage freely, after which, we separated the disks, placed them in the freezer for 15 minutes to immobilize the larvae and counted the number of larvae on each disk. All proportions were arcsine square root transformed prior to statistical analyses to meet assumptions of normality. We compared whether the proportion of larvae feeding from the higher quality food differed from chance levels with one-sample t-tests and tested for differences due to treatment, side of presentation and distance apart with an ANOVA. We tested 200 dishes of larvae, 50 from each combination of foods and configuration.

Additionally, we confirmed the relative quality of the foods by monitoring pupation rates and adult body mass of individuals reared on 100, 50 or 25%. We made 20 vials of 5 mL of each food type, added 20 eggs to each immediately after egg-laying and left the larvae to develop normally. We counted the number of larvae reaching pupation twice per day (11am and 5 pm) starting 120 hours after egg laying. Upon eclosion, adults were collected in vials and stored in the freezer. We compared the rates of larvae reaching pupation in the three food types with Kaplan-Meier survival analysis with Mantel-Cox log rank chi-square tests, which allows comparison of the rate of reaching a well-defined endpoint. In our case, this endpoint was defined as when a vial reached 80% pupation (16 pupae out of the 20 possible), which we arbitrarily chose to indicate "successful" pupation while accounting for some mortality. Furthermore, we monitored vials for newly eclosed adults until there were no new adults for two consecutive days. Adults were stored in the freezer and then sexed and dried in an oven at 70°C for 3 days. Due to their small size, we compared the dry body mass of the adults by weighing 5 flies at a time on a microbalance. We transformed this value back to the weights of individual flies but counted each group as one data point for statistical analyses. We analyzed dry adult body mass with an ANOVA including factors for nutrition and sex as well as their interaction.

Exp 3: Larval Aggregation and Food Quality, Supplemental Results

Nutrition had a significant effect on rate of pupation ($\chi^2_2 = 9.0$, $p = 0.011$). Pairwise comparisons showed that larvae given 25% food reached pupation significantly later than the other two (25% versus 50%, $\chi^2_1 = 8.9$, $p = 0.003$; 25% versus 100%, $\chi^2_1 = 6.7$, $p = 0.009$), and that the 50% and 100% foods did not differ ($\chi^2_1 = 0.03$, $p = 0.856$). There was a significant effect of both nutrition and sex, as well as their interaction, on adult body mass (nutrition, $F_{2,86} = 37.2$, $p < 0.001$; sex, $F_{1,86} = 1169.0$, $p < 0.001$; nutrition X sex, $F_{2,86} = 47.5$, $p < 0.001$). Analysis of the males and females separately revealed that nutrition significantly affected the body mass of females ($F_{2,45} = 67.1$, $p < 0.001$) but not males ($F_{2,44} = 2.4$, $p = 0.106$). Among females, planned comparisons between 25-50% and 50%-100% were both significant (respectively, $t_{27} = 3.4$, $p = 0.002$; $t_{29} = 7.9$, $p < 0.001$). Among males, the 50% nutrition adults were slightly smaller than those from 25% ($t_{30} = 2.1$, $p = 0.045$), and there was no difference between 50% and 100% ($t_{30} = 0.8$, $p = 0.417$).

Exp 4: Developmental effects of foraging density, Supplemental Methods

For the analyses of larval developmental rate and egg-to-adult survival, dishes were analyzed in groups assigned *a priori* to give N = 10 for each density: dishes of 50 larvae were counted singly, dishes of 10 and 3 larvae were counted in groups of 5, and dishes of single larvae were counted in groups of 10. This categorization enabled us to analyze proportions of larvae in each group either reaching pupation or surviving.

Larval developmental rate: We counted the number of larvae reaching the pupal stage in each dish beginning 90 hours after egg-laying, before the expected start of pupation, and in 2 hour increments over the following 3 days. After 3 days, we counted pupae intermittently until 379 hours (16 days) post egg-laying. Upon eclosion, adults were collected in vials and stored in the freezer. The rates of reaching the pupal stage were analyzed with Kaplan-Meier survival analysis with Mantel-Cox log rank chi-square tests, similar to Experiment 3 (above).

Egg-to-Adult Survival: For our measure of *egg-to-adult survival*, eclosion success for each dish was monitored closely up to 16 days post egg-laying (3 days beyond our last recorded pupation event, and 9.5 days beyond the median time of pupation for the slowest developing group), at which time pupae that had not eclosed were considered dead. We attempted to count additional adults 11 days later, but many dishes contained substantial mould growth. We conducted an ANOVA on the arcsine square root transformed proportions surviving to adulthood for each group.

Adult Body Mass: We sexed and dried adult flies for 3 days in an oven at 70°C and weighed them on a microbalance in groups of 5. Groups of 5 flies were weighed together and counted as a single data point, although reported means and standard errors have been divided by 5 in order to show the mass of single flies. Data from males and females were analyzed separately with one-way ANOVAs.

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