SOCIAL LEARNING IN FRUIT FLIES

SOCIAL LEARNING ABOUT EGG-LAYING SITES IN *DROSOPHILA MELANOGASTER*

By

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Abstract

Social learning, defined as learning from other individuals has been well studied in vertebrates and social insect species. I used fruit fly egg laying site selecting as a paradigm to test for social learning in a non-social insect. Focal females that experienced novel food together with mated females (models), who had laid eggs on that food, subsequently exhibited a stronger preference for laying eggs on that food over another novel food than focal females that experienced the same food alone. This socially influenced learning was also observed when focal females experienced both foods, one with mated models and their eggs and one alone. In contrast to the strong effect of a food with mated models and their eggs, neither a food with virgin models nor the aggregation pheromone (cVA) alone generated socially influenced learning. These results provide the first evidence for social learning about egg laying substrate in fruit flies. Further research utilizing fruit flies as a model system may help us gain a better understanding of the evolution and neurogenetics of social learning.

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INTRODUCTION

Social learning

Social learning, or learning from others, is evolutionarily important because it can affect many decisions that an animal makes. These decisions include, but are not limited to, what, when, where and how to eat (Galef & Giraldeau, 2001), who to choose as a mate (Galef & White, 1998), and how to avoid predators (Griffin, 2004). Furthermore, social learning enables the spread of novel behaviors both within and between generations (Galef 1976; Dukas, 2008). Many animals exhibit a variety of socially influenced behaviours that could affect their learning (Galef 1976; Galef & Giraldeau 2001). Here, discussion of social learning is specifically focused on cases where an individual (observer) acquires new information through interaction with either another individual (model) or cues left by that individual.

A number of studies have examined the ecological settings that favour social over asocial learning (Boyd & Richerson, 1985; Laland, 2004; Kendal et al., 2009). One underlying theme of this research is that individuals must face the tradeoff of acquiring costly but accurate individual information versus the use of less costly but also less accurate social information (Boyd & Richerson, 1985). Although little is known about the evolution of social learning, Dukas (in press-a) suggests that it is most likely to evolve in species with parental care, to manifest to a lesser degree in species that exhibit frequent social interactions among individuals with overlapping generations, and to rarely occur in solitary, non-aggregating species with non-overlapping generations.

Social learning in insects

The majority of research that focuses on social learning has been conducted on mammals, birds and fish (Heyes & Galef, 1996). Among insects, social learning has been well studied only in social insects and more specifically in social hymenoptera (von Frisch, 1967; Franks & Richardson 2006; Leadbeater & Chittka 2007). Social insects are defined as insect species that share resources and reproduce cooperatively. The most heralded example of insect social learning is the waggle dance of honey bees in which successful foragers relay the precise location of floral food and other resources (von Frisch, 1967). Other demonstrations of social learning about food sources are known for stingless bees (Meliponini) (Nieh et al., 2004), and bumble bees (*Bombus* species) (Worden & Papaj, 2005; Leadbeater & Chitka, 2007). Research on social learning in non-social insects has been much more limited. Evidence suggests that wood crickets can use public information to learn about danger (Coolen et al., 2005); however, further experiments or replications have not been reported. There are also recent conflicting reports about mate choice copying in female fruit flies (Auld et al., 2009; Mery et al., 2009). Mery et al. (2009) reported that female fruit flies (*D. melanogaster*) exhibit mate choice copying. Females were observed to spend more time with males raised in a poor food medium but not males raised in a good food medium after observing those males with a model female. Further, females mated more with males of the color type (males were randomly dusted with either green or pink powder) they had previously observed copulating. Auld et al (2009) attempted to document mate choice in a different species of fruit fly, *D. serrata*, by measuring whether focal females mated more with males that had actual and apparent mating success compared to an unsuccessful male in a binary choice test. However, their experiments suggested that in these situations females do not copy the mate choice of others. Nonetheless, the prevalence of social learning among insects may actually be much higher than current research indicates (Chittka & Leadbeater, 2005; Dukas, 2008).

Although social learning by solitary insects may be limited due to lack of parental care (Dukas, in press-a), candidate model systems to examine social learning in insect species are those which exhibit overlapping generations and aggregate. Many non-social insects live in aggregations and some taxa even rely on pheromones for recruitment to such aggregations (Prokopy & Roitberg 2001; Wertheim et al. 2005). Under such circumstances, learning from other individuals could enhance fitness because it is often faster than individual learning, it circumvents costly errors associated with inexperience, and it could enable learning of otherwise inaccessible information (Galef 1976; Dukas & Simpson 2009). The co-occurrence in aggregations of animals with distinct experiences could allow for inexperienced individuals to gain important information from experienced conspecifics via social learning. Fruit flies (family Drosophilidae) are an attractive system because they are known to aggregate on food substrates when ovipositing (Werthheim, Van Baalen, Dicke & Vet, 2005) and they display individual learning in various contexts (Quinn, Harris & Benzer, 1974; Tempel, Bonini, Dawson & Quinn, 1983; Dukas 2005a; 2005b).

Learning in fruit flies

While in the past it has been suggested that insects should show little evidence of learning because of their relatively simple brains and short generation times, recent research suggests that a variety of insects rely heavily on learning for life activities including feeding, predator avoidance, aggression, social interactions, and sexual behavior (Dukas, 2008). Specifically, fruit flies have been an extremely useful system to improve our understanding of the cellular mechanisms underlying learning (Tully, 1996; Keene and Waddell 2007). Adult fruit flies are capable of avoiding odors associated with an electric shock (Quinn et al., 1974), as well as preferring odors associated with sugar water (Tempel et al., 1983). More ecologically relevant examples of learning have been demonstrated in the context of sexual behavior in both males and females. Males with experience courting unreceptive, recently mated females subsequently reduced courtship of mated but not virgin females, and males with experience courting unreceptive, immature females subsequently increased courtship of virgin but not mated females (Dukas, 2005a). Females who experienced courtship from only small-bodied, less attractive males are more likely to mate with small males in later encounters than females who experienced courtship by large males (Dukas, 2005b). Fruit flies demonstrate considerable genetic variation in learning ability. Using artificial selection to favour associative learning with regard to egg laying substrate choice, it is possible to artificially select for learning ability in flies (Mery & Kawecki, 2002).

Fruit flies: a model system for social behaviour?

Fruit flies are a non-social insect species; however, they do display pheromone mediated aggregations on food substrates where they mate, feed and oviposit (Werthheim et al., 2004). Fruit flies have been suggested as a potential system to study the genomics of social behavior because they exhibit these aggregations and powerful molecular techniques can analyze their neurogenetics (Robinson et al., 2005). There is also increasing evidence to suggest that social interactions have significantly impacted the evolution of fruit flies. Recent studies have indicated that the social group that a fly experiences can affect subsequent courtship signals and mating behaviours. Krupp et al. (2008) demonstrated that patterns of pheromone expression vary in relation to the social context a fly experiences, depending on whether the social group is composed of individuals that are genetically uniform or genetically different. Kent et al. (2008) quantified the influence of diurnal conditions, genotype, social environment and their interactions on variation in pheromone production. They found that phenotypic variation in important mating pheromones is largely influenced by the interaction of these parameters with social context. Therefore, a male fly's courtship signal is not just a product of his genotype, development and the time of day, but also of whom he is interacting with socially. Notably, aggregation pheromone is among those pheromones whose levels are known to respond to social context (Kent el al., 2008).

Aggregation pheromone

Female fruit flies emit an aggregation pheromone, identified as cis-vaccenyl acetate (cVA) (Bartelt, Schaner & Jackson, 1985), onto a food substrate when ovipositing. cVA is a lipid that is produced by males and transferred to females during copulation. At a long distance, cVA attracts both males and females to a food where they can feed and mate and where females can oviposit (Wertheim, 2002a). However, at a short distance cVA has different sex specific effects. For males, cVA acts as an anti-aphrodisiac inhibiting males from courting previously mated females (Ejima et al., 2007; Zawistowski & Richmond, 1986). Conversely, for females, cVA on a courting male, stimulates receptivity toward males (Kurtovic et al., 2007). cVA has been observed to activate a sexually dimorphic circuit within the brain, suggesting a mechanism by which the pheromone causes a differential behavioral response in males and females (Datta et al., 2008). The mechanism by which cVA is first transmitted and subsequently perceived by the brain from a neurogenetic perspective is currently a growing area of interest (see Benton, 2007 for a review).

Hundreds of non-social insect species exhibit pheromone mediated aggregations and Werthheim et al. (2005) suggest that a few functional explanations to help explain why aggregative pheromones have been selected for in many insect species. For fruit flies (*D. melanogaster*), an important hypothesized benefit of aggregation is for the larvae. Increased adult density on the substrate during egg-laying results in decreased fungal cover, decreased larval mortality, and larger emerging flies (Wertheim et al. 2002b). This benefit is primarily conferred to larvae because as adult flies feed on a food they also act as vectors for dispersal of yeast, on which larvae feed; thus, by having more flies feed on a food they inoculate the substrate with yeast and provide a better substrate on which larvae develop (Wertheim et al. 2002b). There are also costs associated with high densities of larvae, for example, increased larval competition (Wertheim et al., 2002a). Another cost associated with the use of aggregation pheromones is an increased conspicuousness to predators and parasites. A field study examining the behavioral response of a parasitoid, *Letopilna heterooma,* to cVA indicated that parasites are also attracted to this aggregation pheromone (Werthheim, Vet & Dicke, 2003).

Fruit fly egg laying site choice

An important decision that a female must make before reproduction is where to lay her eggs. However, aside from preferring fermenting fruit with aggregation pheromones, the manner in which females choose oviposition sites in nature is largely unknown (Reaume & Sokolowski, 2006). Females deposit their eggs one at a time after a set of behaviors. First, females exhibit a search behavior that takes varying lengths of time; next, they oviposit, and finally they clean and rest (Yang et al., 2008). Further, females avoided laying eggs on sweet media when they were in a chamber containing a sweet food and either a bitter, salty or plain medium. However, avoidance of sweet media decreased as the concentration of sucrose decreased and in the absence of other options females will lay eggs in sweet media (Yang et al. 2008). Habitat selection by fruit flies is also influenced by their natal habitat, a phenomenon referred to as natal habitat preference induction (NHPI) (Stamps & Blozis, 2006).

To test for and learn more about social learning in a non-social insect, I conducted a series of experiments with the fruit fly, *Drosophila melanogaster.* In the first set of experiments, I determined if females were able to learn to prefer an egg laying site. I then addressed the issue of social learning of egg laying site by testing whether inexperienced female fruit flies (focals) exhibit a preference for a food type previously encountered with experienced, mated conspecifics (models) over a food encountered alone. Furthermore, I tested how models' mating status would affect focal female's subsequent egg laying site choice. In the next set of experiments, I then examined the role of aggregation pheromone in influencing future egg laying site choice. Further experiments tested whether more ambiguous cues could influence focals' subsequent behaviour. I tested whether focals could learn about an egg laying site from models previously fed on and emitting odours from flavoured food and which food focals who experienced two foods, one socially and one alone, would prefer. My general prediction was that the food associated with the social experience would be preferred by females when ovipositing compared to a food associated with a solitary experience. Experiment specific predictions are given in the rationale of each experiment.

GENERAL METHODS

I obtained Canton-S flies from J. Levine (University of Toronto Mississauga Campus, Ontario, Canada). I kept the flies in 20 x 20 x 35 cm population cages, containing a total of a few thousand individuals, inside an environmental chamber at 25°C and 70% relative humidity (RH), on a 12:12 hour dark: light cycle, with lights on at 1000h. Each population cage contained two standard 240 ml food bottles each containing 50 ml of standard fly medium containing corn meal, glucose, yeast, sucrose, agar and methyl paraben. The flies used in the experiment were developed at a low density in food bottles containing about 200 larvae. I collected and sexed flies within 8 hours of eclosion and placed them in groups of 20 in single-sex vials containing water with sucrose (20g/l), agar (10g/l) and live yeast. All focal females that were tested were 4 days post-eclosion.

Flavoured food used in the experiments consisted of water, agar (10 g/l), sucrose (20 g/l) and either amyl acetate (AA, 0.9 ml/l) or benzaldehyde (BA, 0.09 ml/l). These chemical concentrations yielded equal food preference in previous experiments (A. Dunlap, unpublished data). In order to increase egg laying behaviour, I added 0.1 ml of a live yeast suspension (30g of yeast and 6 drops of red food colouring per 1 l of warm water) to the center of training vials and testing petri dishes. To increase egg visibility, I added 6 drops of green and blue commercial food colouring to the AA and BA foods respectively.

Training phase

Each experiment that tested for learning involved a training phase followed immediately by a test. For training, I introduced focal females individually into standard vials and provided them with distinct experiences as detailed below for each experiment. The vials were then placed inside the environmental chamber at full light. Models used in training phases were female flies that had fed and laid eggs on a flavoured food for 24 hours prior to the start of the training phase. I confirmed that models had laid at least 10 eggs per vial during that period. Unless otherwise stated, during the training phase models were presented with the same food they experienced, and on which they laid eggs. In order to differentiate between focals and models, I marked models by clipping the end of their right wings.

Test phase

I measured individual oviposition preference of focal females by gently aspirating each focal female into a 22.9 x 12.7 x 17.8 cm (L x W x H) transparent plastic cage. For all experiments in which oviposition preference was measured, the test phase began at 8pm and lasted for 12 hours. The test phase room was kept at dim lighting from 8pm-10pm and in darkness from 10pm until the end of the test. In experiments 1-4 and 7-9, each cage contained two 35 x 10 mm petri dishes, one with AA and the other with BA flavoured foods at the opposite sides of the cage close to the wall. I randomized the positions of the flavoured foods under the constraint that half the cages had AA on the right and half on the left side in each experiment. In all experiments, an observer blind to the experimental treatments then counted the eggs in each dish. I then examined the effect of experience on females' oviposition preference with ANOVAs conducted on the arcsine-square-root-transformed proportions of eggs. Analyses using non-parametric statistics revealed similar results. Only females that laid eggs during the testing phase were included in the analyses. In all three experiments, females belonging to distinct treatments laid similar average numbers of eggs.

1.) Individual and social learning of egg laying substrate

1.1) Experiment 1: Individual learning of egg laying substrate

Rationale

The purpose of this experiment was to test whether focal females who experienced a flavoured food for a long period of time would learn to preferentially lay eggs on that food. I predicted that females who had experience with a food would lay a greater proportion of their eggs on that food.

Protocol

I mated 4 day-old females on the morning of the experiment and kept them in groups of 10 in vials containing sugar and agar food until 8pm. At 8pm, I transferred the females in the same groups of 10 into vials containing either AA or BA flavored food for a 24 hour training phase. After 24 hours, I immediately transferred the females individually into the test cages, where they remained for 12 hours. I tested 120 females of which 75 (36 AA trained and 39 BA trained) laid eggs. Only females that laid eggs during the testing phase were included in analyses.

Results

The females laid a significantly greater proportion of their eggs on the food experienced during training ($F_{1,72} = 7.3$, $p < 0.001$; Fig 1) and there was no significant effect of side ($F_{1,72} = 1.6$, $p = 0.207$).

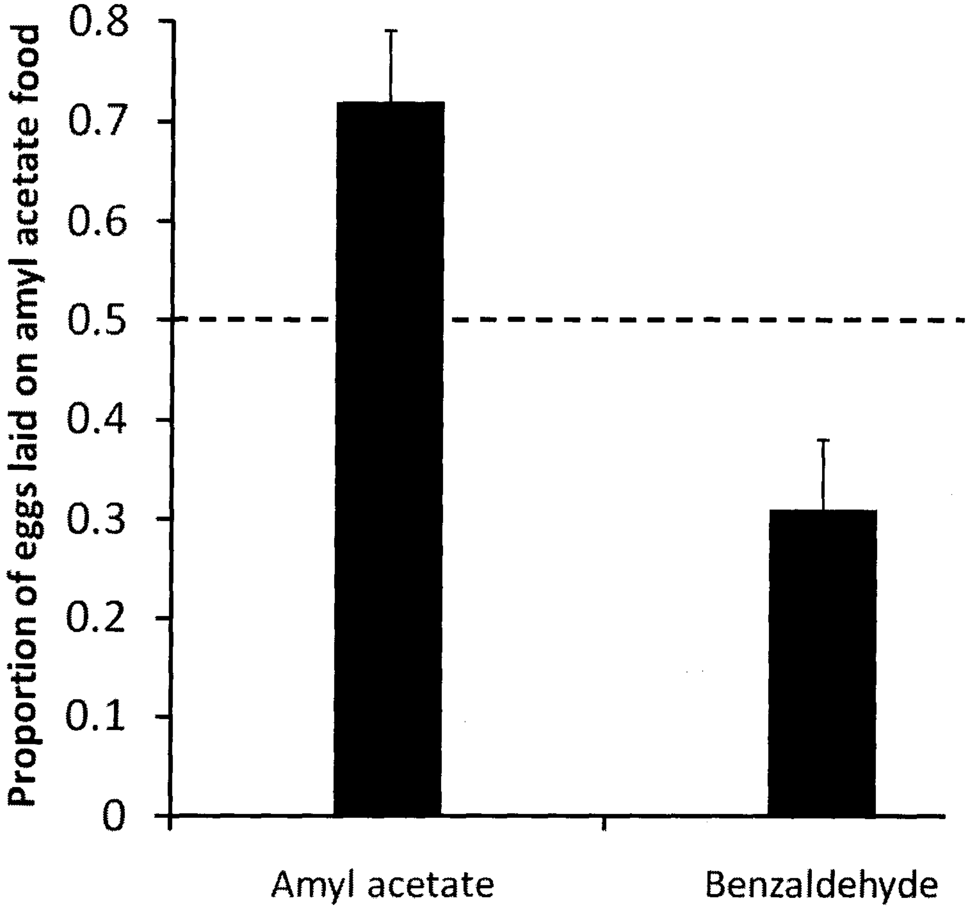

Fig 1: the mean (+1 SE) proportion of eggs laid on amyl acetate flavoured food out of the total numbers of eggs laid by females (n = 75) trained with 9 other females for 24 hours on either amyl acetate (AA) or benzaldehyde (BA) flavoured foods in Experiment 1.

Discussion

Experiment 1 suggested that females can individually learn to prefer to lay their eggs on a flavoured food substrate. After 24 hours of experience with other mated females on a food, females will significantly lay a greater proportion of their eggs on that food (Fig 1).

1.2) Experiment 2: Social learning with models

Rationale

The purpose of this experiment was to test whether focal females exhibit social learning of oviposition substrate. Focal females experienced a food alone or in the presence of two model females who had fed and laid eggs on the food for 24 hours prior to the introduction of the focal. I predicted that social experience with a food would result in females laying a greater proportion of their eggs on that food compared to females that experienced that same food alone.

Protocol

Experiment 2 consisted of 8 replicates during which I trained and tested 68 females per replicate. I mated and placed focal females in individual vials containing sugar water and agar until the training phase. For the training phase, which commenced at 7pm and lasted for 1 hour, I randomly selected focals and placed them in one of four treatments for 1 hour. Females experienced either: (i) AA flavoured food in the presence of models, (ii) BA flavoured food in the presence of models, (iii) AA flavoured food alone or (iv) BA flavoured food alone. Note that models had 24 of previous experience with the training food during which time they fed and laid eggs, before the introduction of the focal female. Immediately after the training phase, I tested the oviposition preference of each individual female tested for 12 hours. Of the 544 females tested, 356 laid eggs (89 AA trained alone, 91 AA trained with models, 87 BA trained alone and 89 BA trained with models).

Results

Focal females that had experienced food with models and their eggs laid a greater proportion of their eggs on that food than did focal females that had experienced food alone ($F_{1,350}$=6.0, p=0.015, Fig. 2). The effects of food type ($F_{1,350} = 0.34$, $p = 0.85$) and side ($F_{1,350}$=1.4, p=0.24) were not significant. Separate analyses for each of the two treatments revealed that both focal females with models ($F_{1,178}$ =41.6, p<0.001) and focal females alone ($F_{1,174}$=6.45, p=0.012) laid a greater proportion of their eggs on the food they had experienced. That is, all focal females preferred the food they had previously experienced, but the experience together with models had a significantly larger effect than the experience alone.

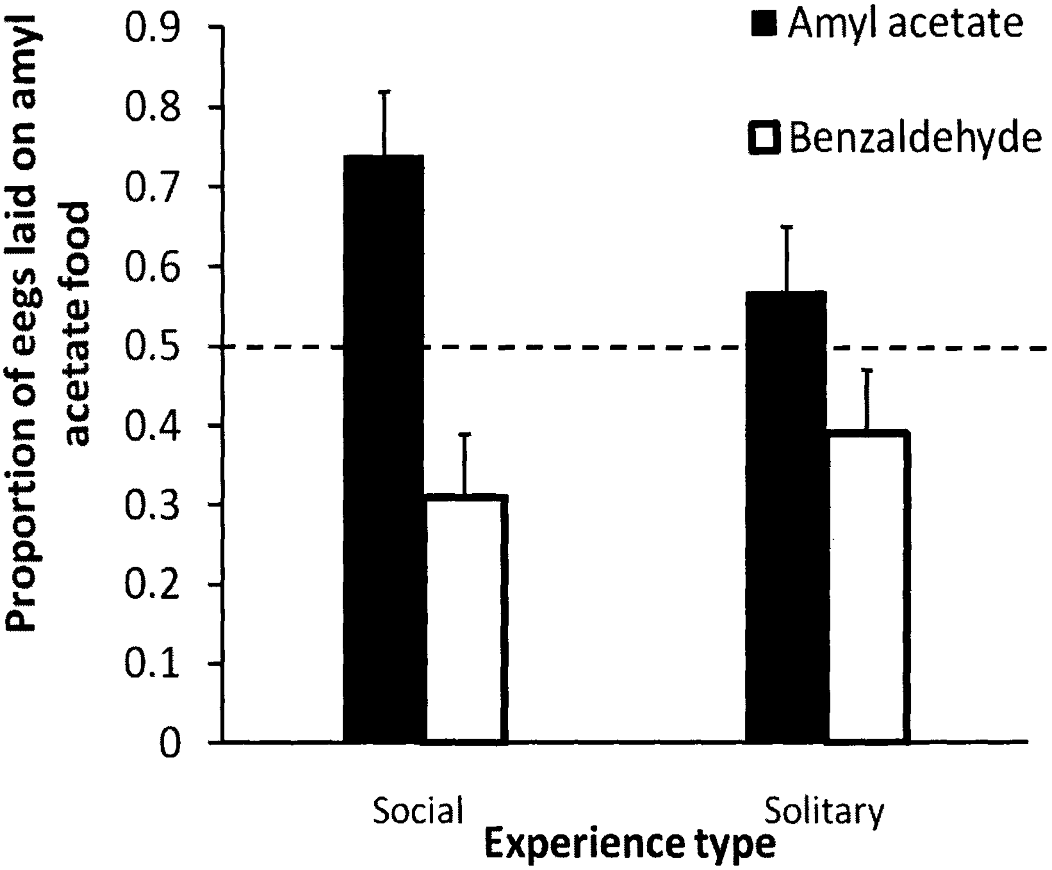

Fig 2 The mean (+1 SE) proportion of eggs laid on amyl acetate flavoured food out of the total numbers of eggs laid by females (n = 356) trained either with two models or alone on either amyl acetate (AA) or benzaldehyde (BA) flavoured foods in Experiment 2.

Discussion

In Experiment 2 focal females preferred a flavoured food more when they had a brief experience with it together with experienced, mated models and their eggs compared to focals who experienced the same food alone (Fig 2). Also, focals preferred food that they were trained with alone, though this effect was not as large after a social experience with a food. Thus, this experiment suggests that egg laying preference can be socially influenced by the presence of other mated females and their eggs.

1.3) Experiment 3: Social learning with virgin or mated models

Rationale

The purpose of this experiment was to test whether virgin females, who neither emit cVA nor lay fertilized eggs, would be as effective models as mated females. I measured egg laying preference for females who experienced a food i) alone, ii) with two mated models or iii) with two unmated models. I predicted that females who experienced foods with mated models would lay a greater proportion of their eggs on those foods than females who experienced the same food with virgin models. Further, I predicted that females that experienced food with virgin models would lay a greater proportion of their eggs on that food than females that experienced the same food alone. In order to test these predictions I conducted two planned comparisons in order to compare the proportion of eggs laid in the training phase food by focals that experienced i) a food with mated models versus a food alone, and ii) a food with virgin models versus a food alone.

Protocol

This experiment consisted of 10 replicates in which I trained and tested 66 females per replicate. I mated focal females on the morning of the experiment and then placed them in individual vials containing sugar water and agar with a bit of live yeast until the training phase. The training phase began when a randomly selected focal was placed in one of six treatments for 1 hour. Focal females experienced: (i) AA food and two mated models, (ii) BA food and two mated models, (iii) AA food and two virgin models, (iv) BA food and two virgin models, (v) AA food alone or (vi) BA food alone. Immediately after the training phase, I tested oviposition preference of each individual female. Of the 660 females tested, 420 laid eggs (68 AA trained alone, 62 AA trained with virgin models, 79 AA trained with mated models, 79 BA trained alone, 50 BA trained with virgin models and 82 BA trained with mated models). In order to test my predictions, I conducted two planned contrasts between females that experienced (1) food with virgin models and versus food with mated models and (2) food alone versus food with virgin models.

Results

Females that experienced food with mated models laid a greater proportion of their eggs on that food compared to females that experienced food with virgin models ($t_{417} = 4.18, p < 0.001$) while there was no difference between females that experienced a food with virgin models and females that experienced a food alone ($t_{417} = 0.01, p = 0.99$). Separate analyses for each treatment revealed that only focals that experienced mated models laid a significantly greater proportion of their eggs on the experienced food ($F_{1,161}$=54.12, p<0.001 for focal with mated models; $F_{1,110}$=0.081, p=.78 for focals with virgins models; $F_{1,145}$=.069, p=0.79 for focals alone). Further analysis using data from experiments 2 and 3 revealed that similar proportions of females laid eggs after training with mated models or alone ($F_{1,32}$=1.41, p=.244).

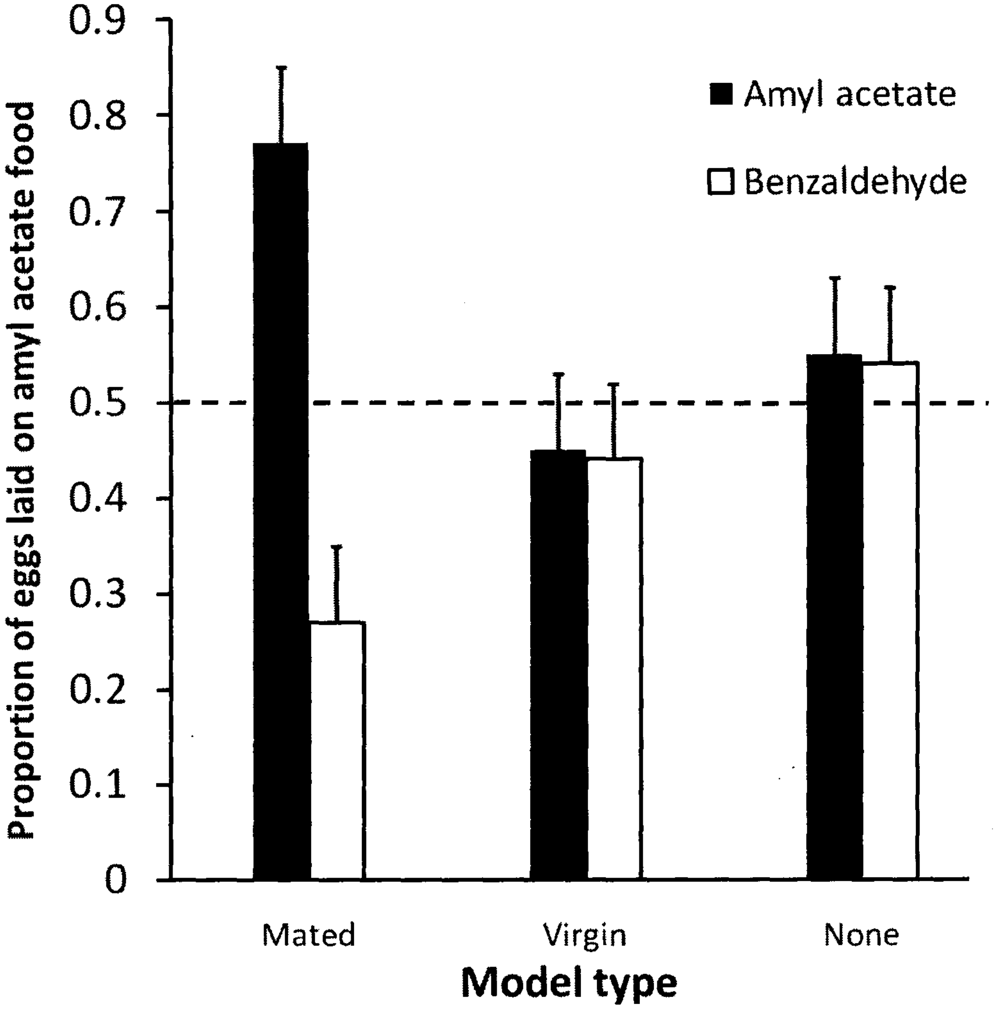

Fig 3 The mean (+1 SE) proportion of eggs laid on amyl acetate flavoured food out of the total numbers of eggs laid by females (n = 420) trained with mated models, virgin models or alone on either amyl acetate (AA) or benzaldehyde (BA) flavoured foods in Experiment 3.

Discussion

Experiment 3 replicated the finding in experiment 2 since focal females exhibited a greater preference for a flavoured food when they experienced it together with mated models and their eggs compared to females who experienced the same food alone. However, females who experienced a food and virgin models and their unfertilized eggs laid similar proportions of their eggs on that food compared to females who experienced the same food alone. Also, data using experiments 2 and 3 suggests that focals are as likely to lay eggs after a training with mated models or alone. Further, in contrast to experiment 2, those focal females that experienced a food alone did not show a subsequent preference for that food. Thus, experiment 3 suggested that females would exhibit socially biased learning of egg laying site only after an experience with a food and mated but not virgin models.

1.4) Experiment 4: Behavioral observations during the training phase

Rationale

Experiments 2 and 3 suggested that after an hour long training in a vial with a food, females who have a social experience with a food will preferentially lay their eggs in that food whereas, females that had a solitary experience with a food exhibited a lesser preference or no preference at all. However, what females were actually doing during this training was unknown. The purpose of this experiment was to examine the behavior of focal females when they encounter a novel flavoured food either alone or with two model females. I predicted that females that experience a food with models would spend more time on that food than females who experience a food alone.

Protocol

In order to differentiate between focals and models, focals were marked with a white dot on the abdomen. Focal females were placed in a vial that contained flavoured food either alone or with two models. Two observers used a computer program to record time spent with the food and the number of interactions with the models. Interactions were defined as focal females being in physical contact with models for at least 2 seconds. Each observer recorded data for 4 focal females simultaneously. In total, observations were recorded for 64 focal females.

Results

The focal females spent short but highly variable durations on the food. Focal females with or without models present did not spend significantly different amounts of time on the food ($U = 1.50$, $N_1 = N_2 = 32$, $p = 0.13$). The median durations on food were 0 and 6.5 s for the solitary and social treatments and the overall median duration on food was 4 s. Focal females had an average of 1.97 ±0 .37 SE interactions with models.

Discussion

The observations from experiment 4 suggest that, during a typical training phase, focal females generally interact directly with the food for a short time. The results also suggest that focals in the social treatment were at least aware of the presence of models as focals were observed to interact with models about 2 times during training. Also, while in the training vial focal females are constantly being exposed to the flavoured food odours and in the social treatment the odour of other mated females.

2.) Influence of aggregation pheromone on egg laying preference

2.1) Experiment 5: Innate preference for aggregation pheromone

Rationale

The purpose of this experiment was to test whether the presence of synthetic cis-Vaccenyl Acetate (Z-11-octadecenyl acetate), abbreviated as cVA (Bartelt et al. 1985), causes females to preferentially lay eggs on a food substrate. I placed mated females in an environment where they were exposed to two identical flavoured foods differing only in the presence of synthetic cVA diluted in hexane. I predicted that females would lay a greater proportion of their eggs on the substrate with cVA added.

Protocol

The experiment consisted of 2 replicates during which I tested 68 females per replicate. There was no training phase as this experiment was designed to determine females' innate tendency to lay eggs on a substrate with cVA added. I mated females on the morning of the experiment and kept them in individual vials with sugar water and agar food until testing. The testing phase was identical to the testing phase in the other experiments except that each cage contained two identical flavored foods (either AA or BA) where one dish had 15 μl of synthetic cVA added and the other dish had 15 μl of hexane added. I diluted the cVA (99% pure, Pherobank, Wageningen) in hexane and applied it in a standard dose of 4.5μg in 15 μl hexane, approximately the equivalent of the deposition by 15 recently mated females (Bartelt et al., 1985). I randomly placed females in cages containing either BA or AA flavoured foods. To control for possible side bias, I counter-balanced petri dish side such that the cVA treated food was placed on the left hand side of the cages for half of the trials. Of the136 females tested, 85 laid eggs (47 AA tested and 38 BA tested).

Results

Focal females laid a significantly greater proportion of their eggs in the medium treated with cVA than hexane ($T_{,84}= 6.58$, $p =<0.001$; Fig. 3). Further, there was no main effect of food type ($F_{1,82}= 0.065$, $p =0.80$) or side ($F_{1,82}= 0.016$, $p =0.90$).

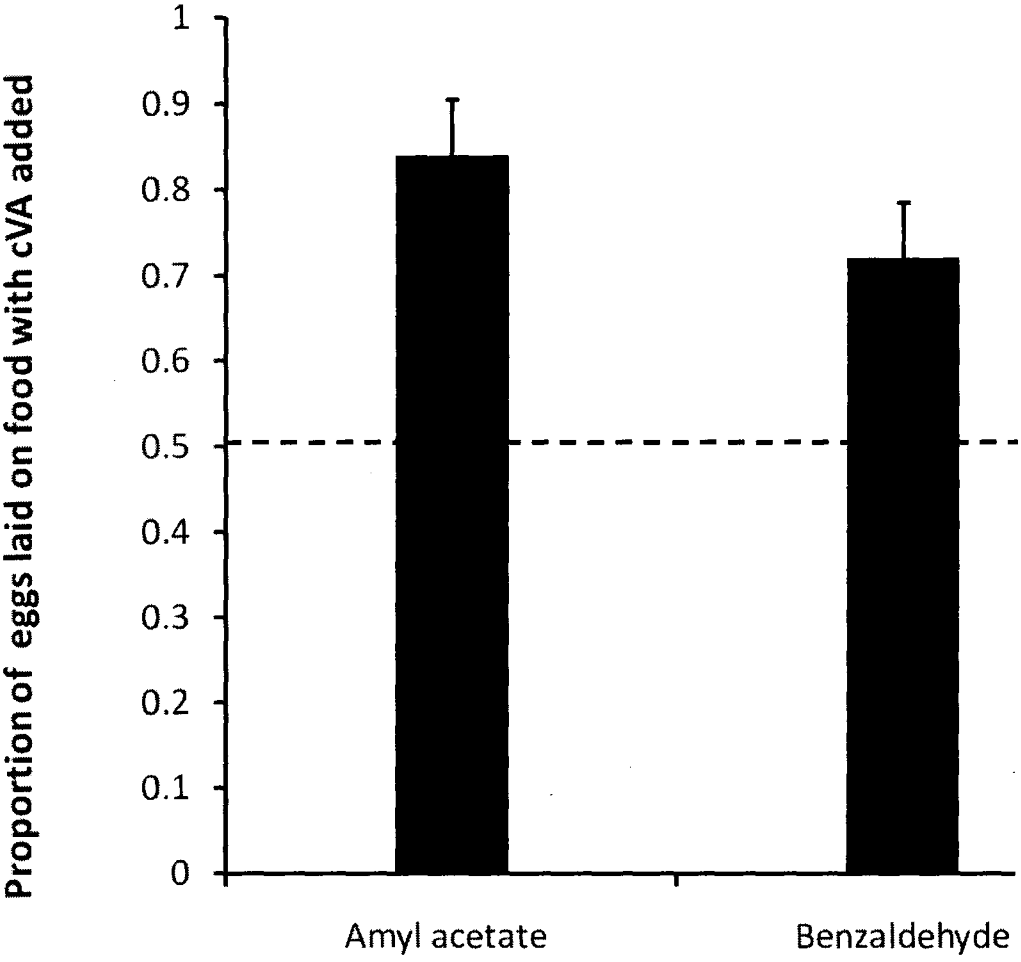

Fig 4: The mean proportion (+ 1 S.E.) of eggs laid by females on the food with cVA added. The females (n = 85) were given either two AA or two BA flavoured foods on which they could lay eggs.

Discussion

Experiment 5 demonstrated that females have an innate preference for egg laying sites with aggregation pheromone (cVA) (Fig 5). This is consistent with previous findings that greater numbers of flies visit pheromone treated substrates compared to control substrates in indoor flight cages and outdoor population cages (Werthheim et. al, 2002a).

2.2) Experiment 6: Social learning with aggregation pheromone

Rationale

The purpose of this experiment was to test whether cVA is used as a social cue by females to help evaluate egg laying site quality. I placed individual females in a vial containing a flavoured food with either hexane or synthetic cVA added. I predicted that females who experienced a flavoured food treated with cVA would lay a greater proportion of their eggs on that flavour of food than females who experienced a flavoured food treated with hexane.

Protocol

This experiment consisted of 8 replicates during which I trained and tested 68 females per replicate. I mated focal females on the morning of the experiment and then placed them in individual vials containing sugar water, agar and live yeast until the training phase. During the training phase, I randomly selected focals and placed individually them in one of four treatments for 1 hour. Females experienced either: (i) cVA and the AA food, (ii) cVA and the BA food, (iii) hexane and the AA food and (iv) hexane and the BA food. Immediately after the training phase, I tested oviposition preference of each individual female. Of the 544 females tested, 335 laid eggs (86 AA with hexane added, 91 AA with cVA added, 73 BA with hexane added, 85 BA with cVA added).

Results

Focal females that experienced food with cVA did not lay a larger proportion of eggs on that food than focal females that experienced food with hexane ($F_{1,331}=0.003$, $p=0.96$, Fig. 2). The effects of food type ($F_{1,331}=0.034$, $p=0.81$) and side ($F_{1,331}=0.27$, $p=0.49$) were not significant. Separate analyses for each treatment revealed that focal females laid a greater proportion of their eggs on the food they had experienced whether it contained cVA ($F_{1,174}=13.4$, $p<0.001$) or hexane ($F_{1,157}=12.8$, $p<0.001$)

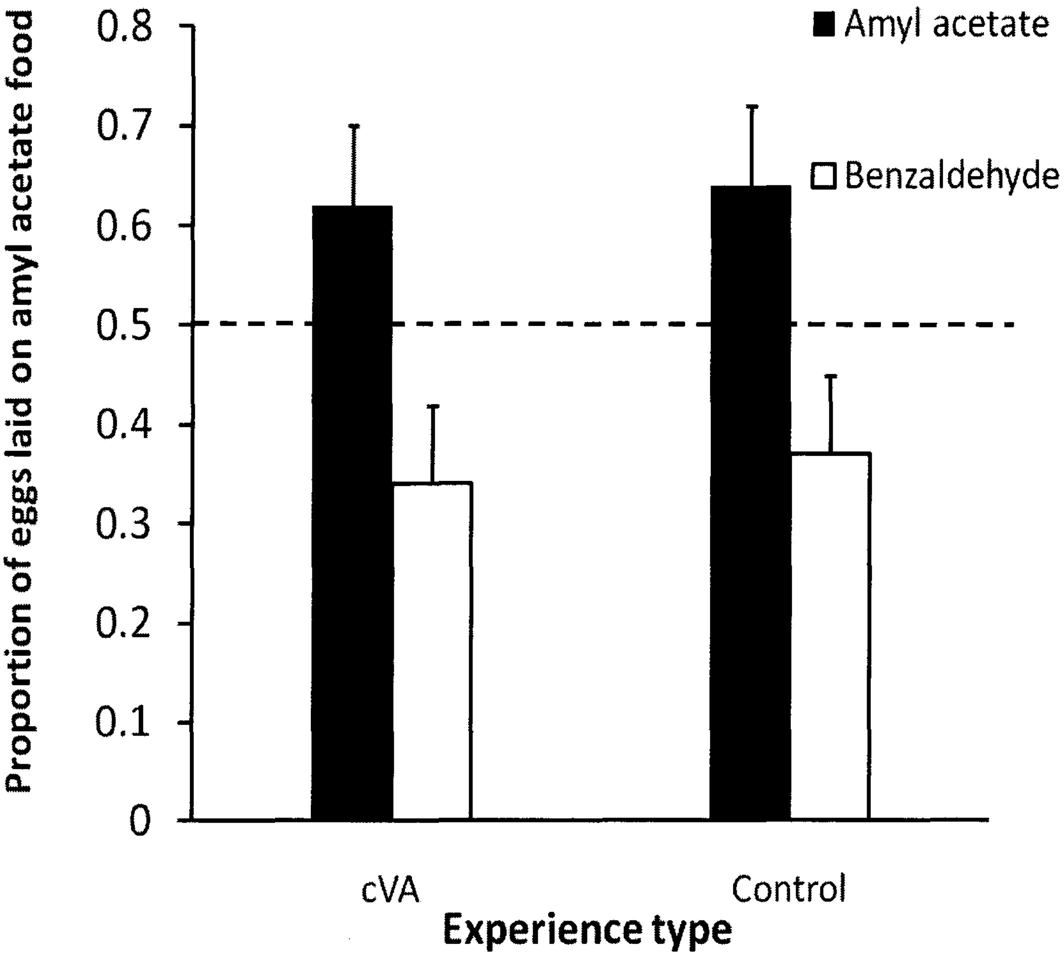

Fig 5 The mean (+1 SE) proportion of eggs laid on amyl acetate flavoured food out of the total numbers of eggs laid by females (n = 335) trained with either cVA or hexane added to either amyl acetate (AA) or benzaldehyde (BA) flavoured foods.

Discussion

Experiment 6 suggested that cVA alone was not the social cue utilized by females to influence their evaluation of substrate quality. Focal females who experienced a food with cVA did not lay more of their eggs in that food than females who experienced a food with hexane alone (Fig 5).

3) Examining other social cues

3.1) Experiment 7: Perfumed models

Rationale

The purpose of this experiment was to test whether residual odour of food on models could influence egg laying behaviour of focal females. Each focal female experienced either 6 models or 6 cotton balls that smelled of a flavoured food. This is in contrast to previously described experiments as focal females did not actually experience either of the flavoured foods prior to testing. I predicted that females who experience models as opposed to cotton balls will subsequently lay a greater proportion of their eggs on that food.

Protocol

Experiment 7 consisted of 8 replicates during which I trained and tested 68 four day-old focal females per replicate. I mated focal females and placed focals in individual vials containing sugar water, agar and live yeast until the training phase. Immediately prior to the training phase model females were transferred from flavoured vials into vials containing sugar and agar. I randomly selected focal females and placed them in one of four training treatments for 1 hour. Training consisted of focal females in a vial containing sugar water and agar and either: (i) six models that fed on AA flavoured food, (ii) six models that fed on BA flavoured food, (iii) cotton that smelled of AA flavoured food or (iv) cotton that smelled of BA flavoured food. For control groups, pieces of cotton that have been on flavoured food were pinned to the vial plug. Immediately after the training phase I individually tested focal females' egg laying preference for 12 hours. Of the 544 females tested, 292 laid eggs (71 trained with cotton that smelled of AA food, 70 trained with models that fed on AA food, 76 trained with cotton that smelled of BA food and 75 trained with models that fed on BA food).

Results

Focal females that experienced models or cotton balls that smelled of a flavoured food laid similar proportions of their eggs on that food ($F_{1,284} = 0.051$, $p = 0.81$; Fig 6). The effects of food type ($F_{1,284} = 0.41$, $p = 0.52$) and side ($F_{1,284} = 0.154$, $p = 0.69$) were also not significant.

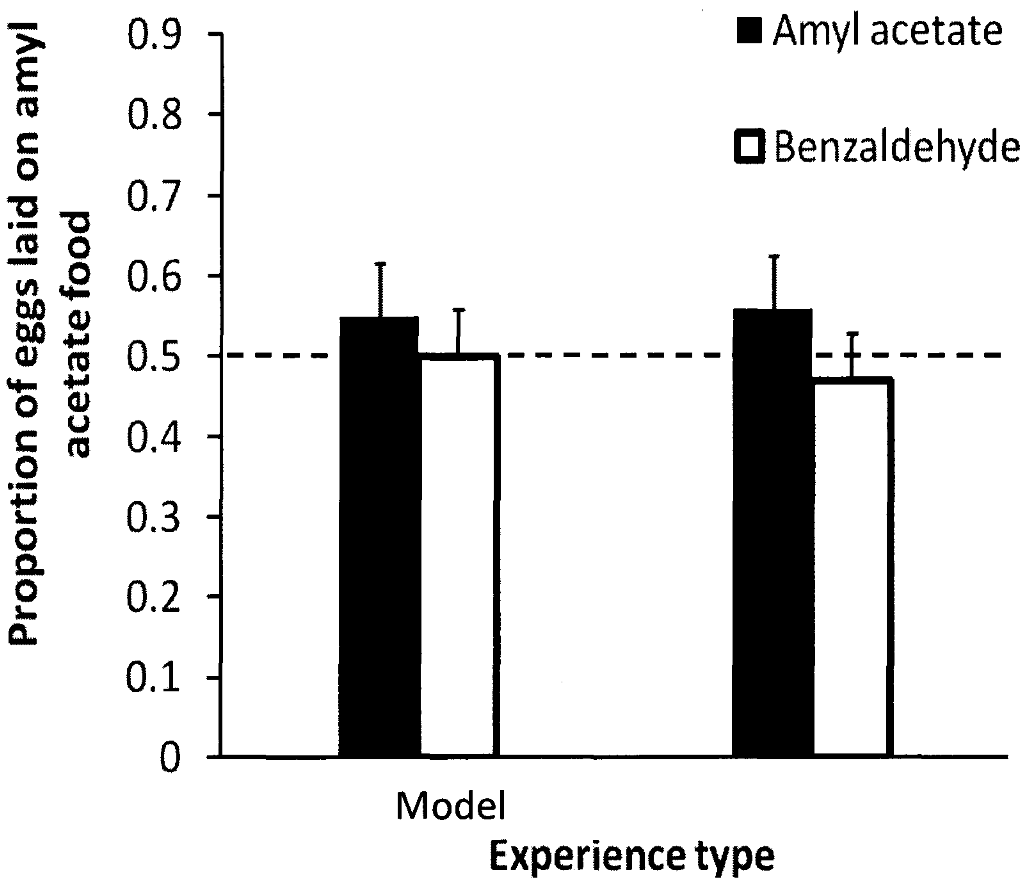

Fig 6: The mean (+1 SE) proportion of eggs laid on amyl acetate flavoured food out of the total numbers of eggs laid by females (n = 292) trained with either models or cotton balls that smelled of either amyl acetate (AA) or benzaldehyde (BA) flavoured foods in Experiment 7.

Discussion

Focal females that experienced mated models that smelled of a food did not exhibit socially influenced learning (Fig 6). After a brief experience with mated models and the food they laid eggs on, focals showed a significant alteration in their egg laying preference (Fig 2, 3). However, when focals experienced just mated models that had the residual odour of food, this did not result in focals laying more of their eggs on that food. It may be that the odour was not sufficiently conspicuous for focals to attend to the cue, or alternatively focals may not attend to food cues while away from food.

3.2) Experiment 8: Females experience both foods

Rationale

In all the previous tests for learning females were trained with one of the two flavoured foods that they would eventually be tested with. The purpose of this experiment was to test whether focal females who were trained with two foods, one alone and one in the presence of mated models (socially), would lay a greater proportion of their eggs on the socially experienced food. I predicted that females would lay a greater proportion of their eggs on the flavored food they experienced with models than on the food they experienced alone.

Protocol

The experiment consisted of 8 replicates during which I trained and tested 68 four day-old focal females per replicate. I mated focal females and placed them in individual vials containing sugar water, agar and live yeast until the training phase. I randomly selected focal females and placed them in one of four treatments. Focals experienced two flavoured foods for one hour each and separated by a 15 minute break during which females were placed back in individual sugar water and agar vials. Thus, focals experienced one of the following four training treatments (i) two models with AA flavoured food followed by BA flavored food alone, (ii) two models with BA flavoured food followed by AA flavored food alone, (iii) AA flavoured food alone followed by BA flavored food with two models (iv) or BA flavoured food alone followed by AA flavored food with two models. Of the 544 females tested, 288 laid eggs (85 trained on AA with models followed by BA food alone, 62 trained on BA food alone followed by AA food with models, 66 trained on BA food with mated models followed by AA food alone and 75 trained on AA food alone followed by BA food with models). Immediately following the training phase, I individually tested focal females' egg laying preference.

Results

Focal females that experienced a flavoured food with models laid a greater proportion of their eggs on that food than on the food that they experienced alone ($F_{1,284} = 5.24$ $p = 0.023$; Fig 2). The effect of the order in which foods were experienced ($F_{1,284} = .13$ $p = 0.71$) and side ($F_{1,284} = 1.17$ $p = 0.28$) were not significant.

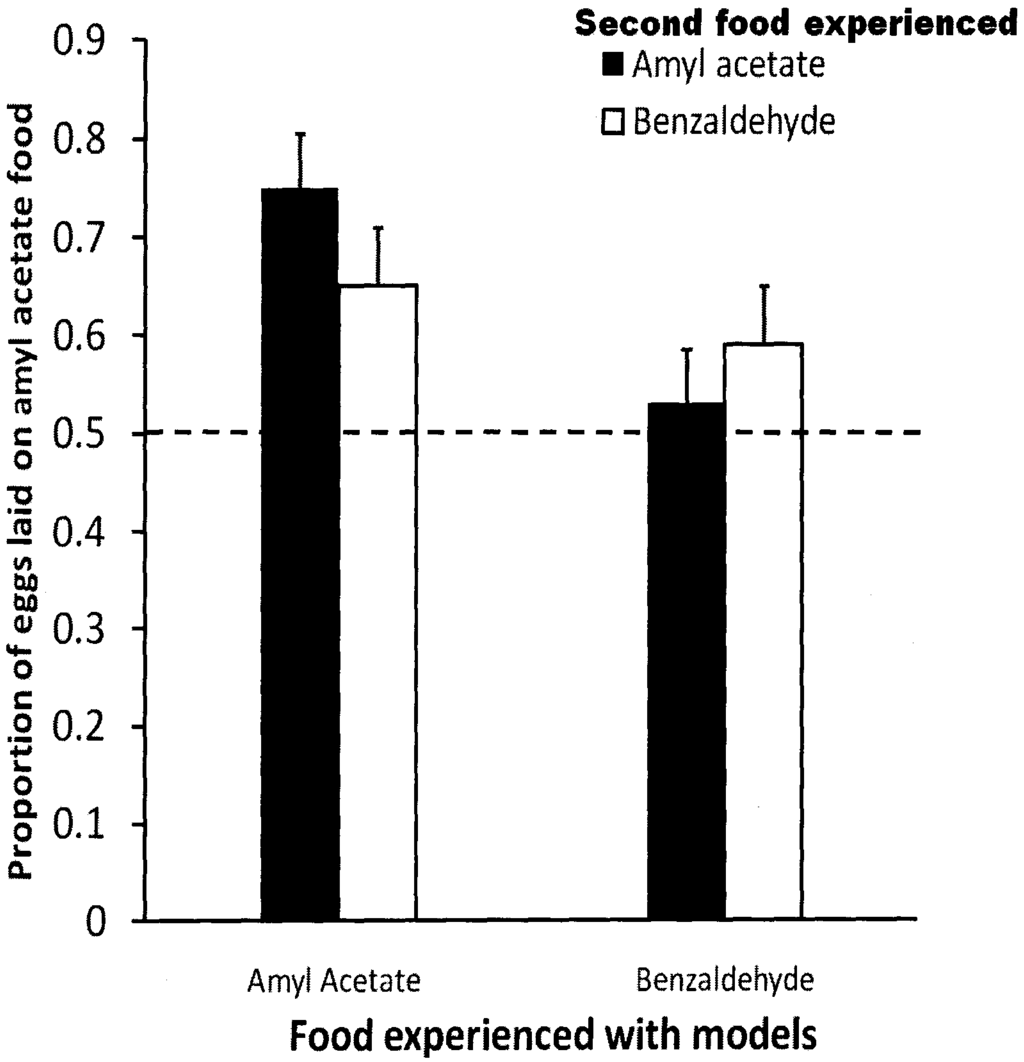

Fig 7: The mean (+1 SE) proportion of eggs laid on amyl acetate flavoured food out of the total numbers of eggs laid by females (n = 288) trained with mated models on either AA or BA flavoured foods in Experiment 8.

Discussion

Experiment 8 demonstrated that when females experience two foods, one alone and one in the presence of experienced models, they will prefer to lay their eggs in the food experienced with models. These findings are consistent with the previous experiments in which experience with mated models and their eggs on a food, affected subsequent egg laying behaviour. Furthermore, the order in which females were trained did not influence learning. However, the effect observed from this experiment appears to be weaker than the socially influenced learning described after females experienced only one of the flavoured foods. The reason for this may be that in experiments 2 and 3 females had some information about one of the foods and no information about the other whereas in this experiment females had information about both foods.

3.3) Experiment 9: Food with eggs vs. food with mated models and no eggs

Rationale

The purpose of this experiment was to determine if experience with a food and mated models or an experience with a food with eggs left from mated models can affect subsequent egg laying behavior in female fruit flies. Importantly, in order to increase the exposure to the food, vials were positioned such that the food was at the top of the vial rather than the bottom. My observations indicated that flies spent more time near the top of vials in general. Focal females experienced either i) a food alone, ii) a food that two other females had laid eggs on without those females being present or iii) an unused food with two model females that had been on and laid eggs on flavoured food. I predicted that females who experienced a food with mated models or a food that contained eggs as opposed to a food alone would subsequently lay a greater proportion of their eggs on that food. In order to test these predictions I conducted two planned comparisons comparing the proportion of eggs laid in the training phase food by focals that experienced i) a food with mated models versus a food alone, ii) food that contained eggs versus a food alone.

Protocol

Experiment 9 consisted of 12 replicates during which I trained and tested 66 four day-old focal females per replicate. I mated focal females and then placed them in individual vials containing sugar water and agar with live yeast until the training phase. Immediately prior to the training phase model females were transferred into fresh vials containing the same flavoured food that they had been on for 24 hours. The vials that models had fed and laid eggs on were subsequently used in the training phase described below. The training phase began when I randomly selected focal females and place them in one of six treatments for 1 hour. Focal females were placed in a vial containing either: (i) AA flavoured food alone, (ii) BA flavoured food alone, (iii) AA flavoured food containing eggs, (iv) BA flavoured food containing eggs, v) AA flavoured food with mated models or vi) BA flavoured food with mated models. Of the 792 females tested, 406 laid eggs (50 AA trained alone, 73 AA trained with eggs on a food, 70 AA trained with models on an unused food, 68 BA trained alone, 76 BA trained with eggs on a food, 69 BA trained with models on an unused food). Immediately after the experience phase, I individually tested focal females' egg laying preference.

Results

Focal females that experienced food with eggs ($t_{403} = 0.02$ $p = 0.77$; Fig 8) and food with models ($t_{403} = 0.03$ $p = 0.60$; Fig 8) laid similar proportions of their eggs on that food compared to females who experienced a food alone.

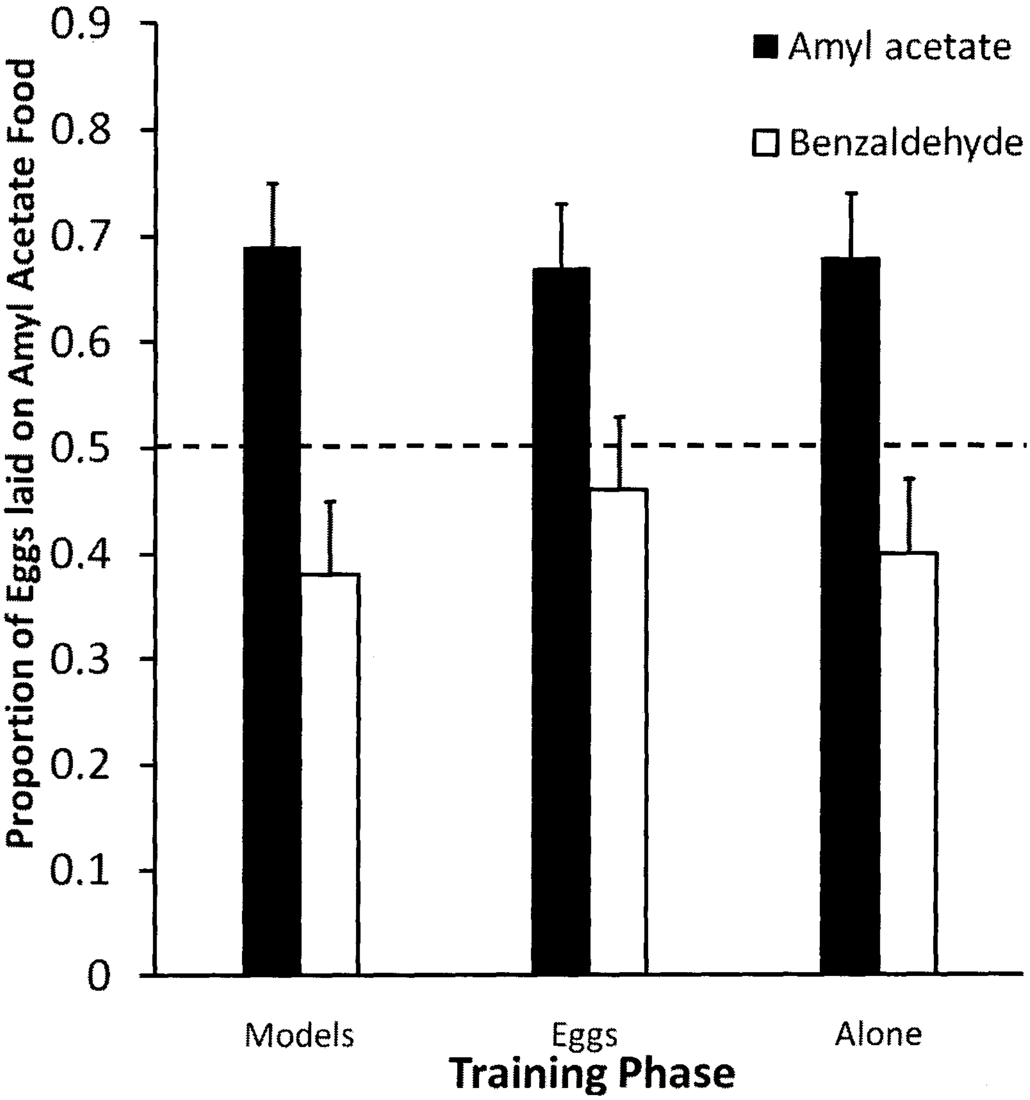

Fig 8: The mean (+1 SE) proportion of eggs laid on amyl acetate flavoured food out of the total numbers of eggs laid by females (n = 406) trained with models on a fresh food vial, food containing eggs from models or alone on either amyl acetate (AA) or benzaldehyde (BA) flavoured foods in Experiment 9.

Discussion

Focals laid similar proportions of eggs on the trained versus untrained foods whether they experienced a food with models, eggs or alone. The difference of orientation of food vials resulted in focal females interacting much more with the food and may have resulted in ceiling effects affecting limiting our interpretation of the results.

GENERAL DISCUSSION

Major Findings

The results reported here provide the first evidence that female fruit flies exhibit socially biased learning of an egg laying site. Focal females exhibited a stronger preference for a food after they briefly experienced it with two mated model females and their eggs than did females who experienced the same food alone (Fig 2, 3). In these experiments females were tested with the food that they had had a brief experience with during the training phase and a novel food. When focal females experienced both foods, one with mated models and their eggs and one alone, focals still showed a preference for the socially experienced food (Fig 7). In contrast to the strong effect of a food experienced with mated models and their eggs, neither a food with virgin models nor the aggregation pheromone (cVA) alone generated socially influenced learning (Figs 3, 5). The ambiguous cues of virgin models who only laid few unfertilized eggs or of aggregation pheromone alone did not provide a strong enough cue to influence focals' behaviour. I further tested whether focal females would be able to learn about a food source without actually experiencing the food itself. Fruit flies who briefly interacted with models that previously fed on and were emitting odours from novel food did not preferentially lay eggs on that food (Fig 6). One plausible explanation for this is that without the presence of the food that models had laid their eggs on, and the eggs themselves, focal females could not have known that the models had found a particular food suitable for egg laying. Note that in the above experiments focal females were allowed only a short, (1 h) experience with food prior to the test.

Behavioral observations during the training phase (experiment 4) revealed that during a typical brief 1 hour training phase with a food, females directly interacted with food for a very short time. This helped avoid ceiling effects as this training generated little to no learning under the solitary condition. In order to increase the amount of time spent in contact with the food, in experiment 9 the orientation of food vials was reversed. My observations indicated that this resulted in focal females interacting with the food during a 1 hour experience. Females that had experienced the food alone now preferentially laid eggs in that food (Fig 8). Thus, the change in orientation of vials may have resulted in an effect.

Adaptive Significance

By examining the life history of fruit flies it is possible to understand why females would pay attention to the egg laying site choice of other mated females. Females' natal experience with a food either directly by eclosion occurring at the food substrate or through smelling residual food odour on the pupal case results in preference for that food over alternative foods of similar quality (Stamps & Blozis, 2006; Stamps et al., 2009). However in nature, by the time females eclose, their natal food may no longer be available or may be unsuitable as an egg laying site. Female fruit flies also exhibit an innate preference for food sites that smell of yeast and decaying fruit but they still assess egg laying sites before ovipositing (Yang et al 2008). Furthermore, fruit flies are strongly attracted to aggregation pheromone that is emitted by recently mated females and deposited by females on an egg laying site. In order for the mated female's use of the pheromone to persist, the fitness benefits due to reduced harassment from courting males and improved larval fitness in aggregations should exceed the costs of reduced oviposition rate through interference in large aggregations, increased larval food competition and increased larval risk of parasitism (Wertheim, 2005).

Here, I replicated results indicating that females prefer to lay their eggs in a food with aggregation pheromone compared to a food without aggregation pheromone (Fig 4). Further, I documented socially influenced learning of an egg laying site (Fig 2, 3, 7). It is important to note that during the testing phase focal females' egg laying site choice was tested by placing females in an environment with two flavored foods that were unused by other females. Thus, females did not choose to lay their eggs on a food substrate due to the direct benefits of aggregated egg laying. These results suggest that female flies are able to pay attention to the egg laying site choices of other females and can subsequently take advantage of this public information by laying eggs on a food that other females have found to be a suitable egg laying site.

Comparison to other species

Social learning about what, where, when and how to eat has been most well studied in vertebrates (see Galef & Giraldeau (2001) for a review of the social influences on foraging in vertebrates). In particular, social learning of what to eat after experiencing the breath of conspecifics has been well studied in rats and other rodents (Galef, 1976; Valsecchi & Galef 1989; Galef et al., 1998). After briefly interacting with a previously fed demonstrator rat, an observer rat will exhibit an enhanced preference for the food the demonstrator previously ate (Galef, 1976; Galef & Stein, 1985). These socially induced food preferences are very robust and can last for weeks (Galef, 1989). Furthermore, social interactions with conspecifics can result in rats socially learning to reverse innate food preferences (Galef, 1986; 1989) and even reverse previously learned food aversions (Galef, 1986).

Among social insects, most social learning research has focused on signals that have been shaped by selection specifically for communication (e.g., the waggle dance in honeybees) as opposed to instances where animals learn through inadvertent social information (Leadbeater and Chitka, 2007). However, an example of social learning through inadvertent social information has been observed in bumblebees (*Bombus terrestris*). Bees accompanied by experienced models are quicker to learn which of two flower species is actively producing nectar than when they are alone (Leadbeater and Chitka, 2007). I am only aware of one study in non-social insects which tested for social learning of what to eat. Recent experiments with locusts suggest that they exhibited rapid individual learning about what food to eat but no social learning (Dukas & Simpson, 2009). Locusts preferred diets they ate for a single meal of only 20 min over another diet of equal nutritional quality. Locusts, however, did not show stronger preference for novel food they had previously consumed while in the presence of a conspecific experienced with that food.

Future research

I believe that the protocol in this thesis should be utilized to further study social learning in fruit flies. Below I discuss a few potential avenues of future research that I think would be particularly fruitful. Before females deem a food substrate suitable for egg laying, they exhibit a search behaviour that is highly variable in duration (Yang et al, 2008). From the experiments presented here it is unclear how a social influence with food could affect subsequent search times. If social information is used to offset the costs associated with individual exploration, a reasonable hypothesis would be that search times would be shorter after a social experience with a food than after a solitary experience. The further examination of such behaviors could help us learn more about the benefits of social learning in flies.

Other future experiments could also examine the value of social experience when varying food quality. Previous studies have suggested that the benefits of aggregated egg laying are most important for egg laying on low quality substrates (Wertheim, Dicke & Vet, 2002). In this case quality was defined by the amount of live yeast on the food substrate. Wertheim, Dicke & Vet (2002) hypothesized that when many adults feed on a food substrate the larvae benefit because the adults act as vectors to transport yeast to the egg laying site where larvae will eventually feed and thus the benefit of aggregated oviposition was more important on a low quality food substrate. One way to further test this hypothesis would be to examine the extent to which females exhibit socially biased learning of egg laying site while systematically varying food quality (amount of live yeast on the food substrate). This is analogous to Dugatkin (1996) who studied whether female guppies would copy the mate choice of other females while systematically varying the perceived quality of males. If adults act as vectors to transport yeast and the benefits of aggregated egg laying are more important for low quality food substrates (as defined above), then I predict that the females should exhibit stronger socially influenced learning with foods of low quality.

Other pertinent questions revolve around how robust this social learning is in fruit flies. Given that learning is known to be costly in fruit flies (Mery and Kawecki, 2003; 2004) and that animals should be selective in their reliance on social information (Laland, 2004; Kendal et al., 2009) future experiments may further elucidate the situations under which socially biased learning has evolved.

For example, one way that females may offset the costs associated with learning is to be primed to learn about egg laying sites at certain times of day. Further experiments may also address how long after a training phase focal females retain their preference for a food.

Conclusions

This thesis presents strong evidence that female fruit flies' exhibit social learning. Specifically, female fruit flies exhibit a socially biased learning of an egg laying site. Although fruit flies are non-social insects, the presence of social learning in an aggregating insect species provides evidence that social learning may be more widespread among insects than the current literature indicates. I believe that further research utilizing fruit flies as a model system may help us gain a better understanding of the evolution and neurogenetics of social learning.

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