SOCIAL INFORMATION USE AND ITS CONSEQUENCES IN ADULT AND LARVAL STAGES OF FRUIT FLIES

SOCIAL INFORMATION USE AND ITS CONSEQUENCES IN ADULT AND LARVAL STAGES OF FRUIT FLIES

By

SHANE GOLDEN, H. BSc.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfillment of the Requirements

for the Degree

Master of Science

McMaster University

© Copyright by Shane Golden, July 2014

MASTER OF SCIENCE (2014) McMaster University

Psychology, Neuroscience, & Behaviour Hamilton, Ontario

TITLE: Social information use and its consequences in adult and larval stages of fruit flies

AUTHOR: Shane Golden H. B.Sc. (University of Guelph)

SUPERVISOR: Reuven Dukas, Ph.D.

NUMBER OF PAGES: vii, 60

**Abstract**

Recent evidence has shown that fruit fly adults and larvae are heavily attracted to food sites occupied by larvae. This attraction is especially strong in mated females that are looking for a suitable site for egg laying. In the first set of experiments, we compared the value assigned to social information provided by larvae at a site to the nutritional information that a female has access to by sampling a food. Lowering food quality did decrease egg-laying preference for a food, but females still showed a much stronger preference for occupied foods. We theorized that the social egg-laying preference may be due to an advantage of developing near older larvae. However, eggs that developed near larvae showed lower survival to adulthood, slower development time, and lower adult body mass. Females were also not able to reduce their social egg-laying preference, even when foods were already heavily occupied by larvae. Finally, we found that larvae were not better able to identify a high quality food site than an adult female, and thus the smell of used food was not a reliable cue to the quality of a site. These results provide evidence that the preference for females to lay eggs near larvae is very robust but the exact benefit it provides for the female and her offspring is unknown. We then ran a series of experiments to test larval social information use to see if they value it as heavily as adult females. Our experiments consisted of focal larvae being put on lower quality food and trying to find a higher quality food nearby that was either occupied or unoccupied by a single model larva. Larvae did not reliably use this social information. Overall, it is unclear whether the larvae are using social information to help them find higher quality foods.

**Acknowledgments**

I would like to thank my supervisor, Reuven Dukas, for his constant guidance and wisdom. Our meetings really helped me think more like a scientist. I would also like to thank my committee members, Sigal Balshine and Bennett Galef. Their input has been very valuable and challenged me to think about problems from different angles. I am very grateful to Zachary Durisko, Isvarya Venu, Blake Anderson, Carling Baxter, and all of the undergrads that have helped me in the lab. I couldn’t have accomplished half of what I did without the support and work of my fellow lab members. Finally, I’d like to thank other grad students in the department. It’s been a great 2 years and while the research has been fun, it wouldn’t have been the same without the people. They are what I’ll miss the most.

Table of Contents

Title page ii

Descriptive Note iii

Abstract iv

Acknowledgements v

Table of Contents vi

List of Figures vii

GENERAL INTRODUCTION 2

CHAPTER 1: THE VALUE OF PATCH-CHOICE COPYING IN FRUIT FLIES

Abstract 8

Introduction 9

1.1 Experiment 1: Nutritional titration 11

1.2 Experiment 2: Larval success on social vs. non-social food 14

1.3 Experiment 3: Larval success on abundant food 18

1.4 Experiment 4: Females’ patch choice when the social patches have had high larval densities

1.5 Experiment 5: Adult vs. larval abilities to detect differences in yeast concentration of food23

Discussion 25

References31

CHAPTER 2: THE USE OF SOCIAL INFORMATION IN FORAGING LARVAE

Introduction 36

General Methods 38

2.1 Experiment 1: Rover and sitter social information use 38

2.2 Experiment 2: Social information use among old and young larvae raised on 100% food 40

2.3 Experiment 3: Social information use among old and young larvae raised on 50% food 41

2.4 Experiment 4: Replication of old larvae raised on 50% food 43

Discussion 44

References 48

GENERAL DISCUSSION 50

REFERENCES 56

**List of Figures**

Figure 1.1: Nutritional titration

Figure 1.2: Larval performance as a function of a disc’s nutritional and social status

Figure 1.3: Performance measures of focal larvae on abundant food

Figure 1.4: Social patch choice under high larval densities

Figure 1.5: Patch choice by adult females versus larvae

Figure 2.1: Rover and sitter foraging with and without models

Figure 2.2: Social information use in foraging between young and old larvae raised on 100% food

Figure 2.3: Social information use in old and young larvae raised on 50% food

Figure 2.4: Replicate of old larva social information use raised on 50% food

**GENERAL INTRODUCTION**

**Social information use**

Animals must be able to collect and respond to information in their environment. They can use their own individual information that they gather about the environment themselves. Or, they can rely on the information provided by others, termed social information. Humans are highly social animals and thus we use social information constantly. It is transmitted across and between generations, especially from parent to child. However, many animals do not have parental care or even overlapping generations (Dukas, 2010). There are obvious advantages to using information provided by others. Social animals can save a lot of time and energy just by copying others (Grüter et al., 2010). An individual does not have to go through a lengthy trial and error learning process when it can just observe the outcomes of the behaviour of another individual. Simulations have shown that strategies involving the observation and copying of others tend to be more successful than individual learning strategies (Rendell et al., 2010). However, animals will never ignore their individual learning and will integrate it with the social information they have gathered (Galef & Whiskin, 2001).

A well-studied area in social information use pertains to the transmission of dietary preferences. A study by Galef (1993) found that observer rats were more likely to copy the diet fed upon by a model rat when given the choice between two novel diets. The transmission of dietary preferences can happen much earlier in mammals. Rat pups can detect cues of their mother’s diet in her milk and later prefer that diet (Galef & Sherry, 1973). This was later replicated using rabbits (Bilko et al., 1993). Animals can also use social information to follow a group to a food source that they may not otherwise know about (Lachlan et al., 1998; McClure et al., 2012).

Another use of social information in animals is the transmission of mate preferences. In vertebrates, this is generally used by females to assess the quality of a male without using as much energy or time to evaluate him (Dugatkin, 1992; Galef & White, 1998; Hoglund et al., 1995; Witte & Ryan, 2002). In this instance, a female can rely on social information to supplement her own information on the attractiveness of a mate. However, if a female finds a male very unattractive, it does not matter if another female has previously chosen him (Dugatkin, 1996). Similar to social information use in dietary preference, individuals will never completely ignore their own information in favour of the social information.

**Social information use leading to group formation and group decision-making**

Relying on social information can lead animals to aggregate in the same areas. When animals transmit dietary preferences through social information, they can end up on the same food sources. Animals may then form into groups. There are many advantages and disadvantages to group living. In each facet of group living, there is generally a tradeoff. An obvious disadvantage is that you now have more competition for resources. However, as a group, you may now be able to exploit resources that you could not as an individual. You may also be better able to find resources now that you have the many eyes of the group looking for them (Ward et al., 2011). In terms of mate choice, there would be greater access to members of the opposite sex in a group. Competition would then be fiercer, possibly leading to aggressive encounters.

Groups tend to make fewer false positives, with larger groups making fewer errors (Wolf et al., 2013). Animals can participate in what is known as collective decision-making. Animals in a group can share information and then make the best decision based on the pooled information. Animals can also use a consensus to make accurate decisions. Each individual only makes one choice but the sum of individual choices will hopefully lead the group to make the best decision. This type of process is used when honeybees are trying to find the best site to make their new nest (Seeley & Visscher, 2004). Individuals don’t have access to the same amount of information as a group. The group can share social information so that each individual is better informed. Group decision-making is not limited to eusocial insects. It is found in humans, cockroaches, fish, tent caterpillars, among others (Dussutour et al., 2008; Jeanson et al., 2005; Hoare et al., 2004; Wolf et al., 2013). Any animal that lives in a group, either ephemerally or permanently can gain from sharing information with others and making decisions as a group. Groups tend to find the highest quality foraging sites through exploration and social attraction (McClure et al., 2012).

**Fruit flies as a model system to study behaviour**

Fruit flies (*Drosophila melanogaster*) are a widely used model system in a number of biological fields. Perhaps their most famous use comes from genetics. Many of the genetic pathways involved in development have been conserved across invertebrates and vertebrates and thus any insights gained from fruit fly genetic analysis can be applied to many different animals (Bier, 2005). These genetic insights can also be used to better understand diseases and neurological disorders in humans (Bier, 2005). With the molecular tools becoming available in the last few decades, fruit flies have become a model system for neurogenetics. Researchers can match up genes and alleles to overall behaviour leading to the discovery of natural polymorphisms that govern things such as foraging behaviour (Sokolowski, 1980). Fruit flies are also well known for the different mutations that can be induced and maintained to aid research. There are mutant flies without a sense of hearing (Eberl et al., 1997), touch (Kernan et al., 1994), vision (Pak et al., 1969), taste (Falk & Atidia, 1975), and olfaction (Siddiqi, 1987). One can then investigate how each of these senses plays a role in the formation of social behaviour. Durisko & Dukas (2013) established that fruit fly larvae exhibit social attraction and this is likely due to olfactory cues emanating from occupied food. Testing olfactory mutants, and taste mutants can elucidate the sensory modalities necessary to maintain this behaviour.

From a procedural standpoint, fruit flies are also very convenient in that they have incredibly short generation times, distinct life stages, and breed easily in captivity. An outcome of the short generation times is the presence of overlapping generations (Crow & Chung, 1967). This is thought to be important in the transmission of social information which can lead to the evolution of social learning (Dukas, 2010). However, adult fruit flies do not care for eggs or larvae, which limits the transmission of social information across generations (Azevedo et al., 1997). Based on the neurogenetic tools available and their ease of use in the lab, fruit flies make an excellent model system for studying behaviour.

**Social information use in fruit fly adults**

Fruit fly adults are avid users of social information in their environment. Females show a variety of copying behaviours. Similar to many vertebrates, female fruit flies will copy the mate choices of other females (Mery et al., 2009). They rely on social information to reduce uncertainty about the quality of a male that they may not be able to accurately gauge using their own sensory information (Mery et al., 2009). Female fruit flies will also copy the oviposition site selection of other females through observation, and likely olfaction (Sarin & Dukas, 2009; Battesti et al., 2012). They may do this to reduce the uncertainty about the quality of an egg laying substrate. However, in natural settings, they may not always get the opportunity to view other females while egg-laying. Females can look for the presence of other eggs or larvae as information on the appropriateness of a site for egg-laying. *Drosophila* females tend to lay eggs near larvae (Del Solar & Palomino, 1966; Durisko et al., 2014a). Females are attracted to the smell of feeding larvae (Durisko et al., 2014a) and thus rely on olfactory clues to orient themselves. Females can also learn to associate odours with food occupied by larvae, meaning they can attend to stimuli in their environment that are predictors of attractive sites to lay eggs (Durisko et al., 2014a).

Fruit flies tend to aggregate around a food source, which is mediated by compounds such as cis-vaccenyl acetate (Bartelt et al., 1985). It is unlikely that there is active recruitment of additional flies to a food source as that would just increase the competition for resources. However, individual flies can detect the presence of a group at a food source and know that it is probably suitable for feeding. It is still unclear whether the presence of others serves as a long-range attractant to a food source, or if incoming flies simply detect the food source from afar and then are attracted to the group at shorter distances.

Adults can also use the information provided by others to learn about their environment. In a spatial orientation task, adult flies used a trained group of model flies to find a lower temperature safe spot in a hot environment (Foucaud et al., 2013). On a different kind of learning task, flies tested in groups performed better than flies tested alone (Kohn et al., 2013). In both studies, flies were able to use social information to learn about and navigate their environment more successfully.

**Social information use in larvae**

Fruit fly larvae are socially attracted to foods occupied by other larvae (Durisko & Dukas, 2013). It is likely due to olfactory cues, produced by the gut bacteria of other larvae (Durisko & Dukas, 2013; Venu et al., 2014). Even when larvae are reared in isolation, they still show the same levels of social attraction, implying that it is not a case of them going towards the familiar smell of other larvae (Durisko & Dukas, 2013). Larvae that are unsure of where to forage rely on the social information provided by others to find food in their environment. They can also learn to associate odour cues with the presence of other larvae on a food source (Durisko & Dukas, 2013). In nature, groups would be likely to find the highest quality food sites (Dussutour et al., 2008), so individuals could gain by joining the group. Of course, they may be better off trying to find their own food source to reduce competition. Larvae can effectively discriminate between artificial lab diets of varying nutritional quality when these foods are close by and they have time to sample both (Durisko & Dukas, 2013).

Larvae also use social information when forming aggregations. Groups of larvae form modest aggregations on a food medium (Durisko et al., 2014b). They form the closest aggregations in the middle to late second instar, when digging behaviour is just emerging (Durisko et al., 2014b). The exact mechanisms of the aggregation are unknown, but the larvae can likely detect each other through mechanosensory (Durisko et al., 2014b), olfactory (Durisko & Dukas, 2013), and visual cues (Justice et al., 2012). Since larvae tend to aggregate most at the commencement of digging behaviour, they likely rely on social information to coordinate and facilitate digging into the substrate. Digging is an important defensive behaviour to avoid parasitoid wasps (Carton & David, 1985). Parasitoids could be a strong enough selective force to cause larvae to pay attention to social information so they can aggregate and dig more effectively.

**Consequences of social information use in fruit flies**

Based on work in Durisko et al. (2014a), females have a strong preference for laying eggs near developing larvae. This can create crowded living conditions that would increase the density and the intensity of competition. However, there are not only negative effects of laying eggs near larvae. There can be some benefits as well.

One important advantage of larvae being in a group is the suppression of harmful fungal growth at higher larval densities (Wertheim et al., 2002). Mould can render a food source unusable for larvae so they have to compete with it. Groups of larvae are better able to stop mould growth on decaying fruit, which is the main food source of fruit fly larvae. Another advantage to being in a group has to do with allee effects. Allee effects are an increase in individual fitness due to group living. Rohlfs & Hoffmeister (2003) found that larval survival was actually highest at intermediate densities on natural decaying fruit substrates. Artificial lab diets show a different trend. Larval survival tends to be highest at the lowest densities, and then decreases as density increases (Durisko & Dukas, 2013). Thus, the lab diet shows no allee effects. Another important advantage to being in a group is maintaining beneficial yeast species on a substrate (Stamps et al., 2012). Both fruit fly larvae and adults rely on yeast as their primary protein source. Larvae can even survive on a diet of just yeast but develop faster when it is supplemented with sucrose (Schwarz et al., 2014). There are disadvantages other than competition. One important one is the buildup of toxic ammonia waste products by groups of larvae (Borash et al., 1998). This could make conditions especially inhospitable for young larvae that may not have much tolerance for ammonia. There could also be toxicity problems for the eggs. Females must find a tradeoff between these advantages and disadvantages to maximize their offspring’s success.

**Major questions we wanted to answer**

Chapter 1:

1. How do adult female fruit flies weigh social information and individual information in their oviposition choice?
2. What are the developmental consequences of eggs developing near older larvae?
3. Can females detect sub-optimal densities of larvae and reduce their social egg-laying preference?
4. Are larvae better able to tell the difference between foods of varying nutritional qualities compared to adult females?

Chapter 2:

1. Do larvae use social information to find better quality food in their environment?
2. Is the use of social information in larvae dependent on foraging morph, developmental instar, or rearing conditions?

**CHAPTER 1: The value of patch-choice copying in fruit flies**

The following is a submitted manuscript authored by Shane Golden and Reuven Dukas. SG and RD designed the experiments, which were conducted by SG. Abstract, introduction, and discussion were written by RD. Methods and results were written by SG. The format has been slightly modified for ease of reading but the content remains the same.

**ABSTRACT**

Many animals copy the choices of others but the functional and mechanistic explanations for copying are still not fully resolved. We relied on novel behavioural protocols to quantify the value of patch choice copying in fruit flies. In a titration experiment, we quantified how much nutritional value females were willing to trade for laying eggs on patches already occupied by larvae (social patches). Females were highly sensitive to nutritional quality, which was positively associated with their offspring success. Females, however, perceived low-nutrition patches (33% of the nutrients) with the same value as high nutrition ones (100% of the nutrients) when the former were social. In follow up experiments, we could not, however, either find informational benefits from copying others, or detect what females’ offspring may gain from developing with older larvae. Because patch choice copying in fruit flies is a robust phenomenon in spite of potential costs due to competition, we suggest that it is beneficial in natural settings, where fruit flies encounter a complex dynamics of microbial communities, which include, in addition to the preferred yeast species they feed on, numerous harmful fungi and bacteria. In agreement with our proposition, fruit flies show attraction to volatiles from beneficial microbes and aversion to volatiles from harmful ones. We suggest that microbial ecology underlies many cases of copying in nature.

**INTRODUCTION**

In many animal species, individuals copy the choices of others. Examples include choices of feeding sites (Thorpe, 1963; Krebs, 1973; Waite, 1981), territories (Stamps, 1987; Betts et al., 2008), egg laying substrates (Prokopy and Roitberg, 2001; Fletcher and Miller, 2008; Raitanen et al., 2014) and mates (Dugatkin, 1992; Galef and White, 1998; Alonzo, 2008). Depending on the system, copying can have substantial effects on organismal ecology and evolution. For example, aggregation at feeding and egg laying sites can promote species coexistence (Shorrocks and Sevenster, 1995; Krijger and Sevenster, 2001) and mate choice copying can influence the intensity and direction of sexual selection (Wade and Pruett Jones, 1990; Kirkpatrick and Dugatkin, 1994; Agrawal, 2001).

While it is widely agreed that copying can influence animal ecology and evolution, it is often unclear how the possible fitness benefits from copying outweigh the likely costs. For example, patch-choice copying typically involves a focal individual choosing a feeding or egg laying site that is either that is either occupied by other individuals (models) or contains products left by these individuals. There are probably only two non-mutually exclusive explanations for such copying. The first explanation involves pure information: a focal can either find a satisfactory patch faster, or locate a better patch among the available alternatives by copying others than by exploring on its own (Stamps, 1987; Danchin and Wagner, 1997; Valone and Templeton, 2002; Danchin et al., 2004). That is, the first explanation focuses on two related difficulties that animals have in locating optimal resource patches. Either the patches are hidden, so it takes time to find them, or it is difficult and time consuming to assess the multitude of features that determine patch quality. Given individuals’ limited time horizon, focals that copy others can shorten the time devoted to exploration and hence increase the time spent exploiting without compromising on the quality of the patch utilized. This proposition, of course, is based on the tenuous assumption that the models indeed have chosen the optimal patch.

The other explanation for patch-choice copying involves material benefits that focals can gain from joining others, which include reduced per capita risk of attack by predators and parasitoids, enhanced foraging efficiency and thermoregulation (Allee, 1931; Arnold, 1988; Rohlfs and Hoffmeister, 2004; Wertheim et al., 2005; Willis and Brigham, 2007; Beauchamp, 2014). It is worth noting that, when patch-choice copying involves joining others, focals and models might face asymmetric payoffs: while a focal can gain more from joining than from settling alone, the models might lose from having another individual joining (Pulliam and Caraco, 1984). The obvious costs from joining others are competition for resources and reduced patch quality caused by accumulating waste products (Allee, 1931; Danchin and Wagner, 1997; Prokopy and Roitberg, 2001).

While there are numerous reports of copying in a wide variety of species and contexts, the value of copying has been rarely quantified. We have recently developed protocols for quantifying patch-choice copying in fruit flies (*Drosophila melanogaster*). Larvae and adults from both established laboratory strains and recently caught wild populations copy the choices of others: adult females prefer the egg laying substrates chosen by other females (Sarin and Dukas, 2009; Battesti et al., 2012), both male and female adults are attracted to volatiles emanating from conspecific larvae, females show a strong preference for laying eggs in patches with larvae over unoccupied alternatives, and larvae also show significant attraction to patches already occupied by larvae (Durisko and Dukas, 2013; Durisko et al., 2014a; Venu et al., 2014). The establishment of fruit flies as a model system for research on patch-choice copying offers new opportunities. First, the fruit fly system allows one to conduct highly controlled experiments assessing the factors that influence patch choice copying. Second, findings from the behavioural analyses of patch-choice copying can be extended to research on the genetics and neurobiology of such behaviour given the numerous neurogenetic tools available in fruit flies and the increased interest in establishing simple model systems for research on the mechanisms that control social behaviour as well as behavioural decisions in general (Robinson et al., 2008; Yang et al., 2008; Sokolowski, 2010; Durisko et al., 2014b).

To elucidate the value of patch-choice copying in fruit flies, we conducted a series of experiments. We began with a titration experiment designed to quantify the perceived value that females assign to food occupied by larvae. This involved testing female preferences between reference patches and test patches of varying food qualities, which were either occupied or unoccupied by larvae. In follow-up experiments, we compared larval success on occupied and unoccupied patches of relevant food qualities. This allowed us to translate patch-choice copying by females into the consequent success of their offspring. Because females showed strong patch-choice copying even when nutritionally superior patches were readily available and in spite of the expected costs owing to larval competition, we wished to assess whether females would moderate their strong tendency to copy when the occupied patches either have had numerous larvae or have already experienced heavy consumption by larvae. Finally, in order to distinguish between the two explanations for patch-choice copying (information versus group benefits), we tested whether larvae were better than adult females at assessing food quality. If larvae are better able to identify higher quality foods, larvae could be a reliable cue for high quality patches that the female may be unable to assess on her own. The larvae would provide social information to a female that a site is suitable for egg-laying.

**Experiment 1: Nutritional titration**

**MATERIALS AND METHODS**

We maintained two population cages of several hundred *Drosophila melanogaster Canton-S* following standard protocol (Sarin and Dukas, 2009). To quantify the value that females assign to patches already occupied by larvae, we placed each of 192 recently mated female inside a 60 mm Petri dish lined with agar and containing one reference disc and one test disc both 1.1 cm in diameter and each containing 0.5 ml food (Fig. 1.1a). The reference disc always had standard food in which 1 litre contained 60 g dextrose, 30 g sucrose, 32 g yeast, 75 g cornmeal, 20 g agar and 2 g methyl-paraben. The test disc was either fresh (non-social) or contained five early second instar larvae that had fed on that disc for 24 h (social). The test disc had standard food or one of two lower food concentrations containing either 33% or 11% of the nutrients (dextrose, sucrose, yeast, and cornmeal) available in the standard food. The reference and test discs were 3 cm apart with the central 2 cm being a trough filled with fine sand (Fig. 1.1a) to prevent larvae located on the social discs from crossing to the reference discs. We housed all dishes in a chamber kept at 25o C and 90% relative humidity and left the females in the Petri dishes for 14h to lay eggs. Then we discarded the females and counted the number of eggs laid on each disc. We used a generalized linear model with a Tweedie distribution and identity link and conducted pairwise comparisons with Bonferroni corrections and 95% Wald confidence intervals (IBM-Corp., 2011).

**RESULTS**

Females laid significantly higher proportions of eggs on the test food when it was social than non-social at all three food concentrations (Wald χ²1=49, P<0.001 for the main effect and P<0.01 for the three pairwise comparisons with Bonferroni corrections, Fig. 1.1B).

**Figure 1.1**. **Nutritional titration.** (A)Each dish always contained a reference discand one of six types of test discs varying in nutritional concentration and larval presence. Sand at the centre of the dish prevented larval crawling to the reference food. (B) The average proportion of eggs (±1 SE) laid on the test disc as a function of its nutrient concentration and presence or absence of larvae (social or non-social). N=30 replicates per treatment. Females laid more eggs on the test food in the presence than absence of larvae.

**Experiment 2: Larval success on social vs. non-social food**

**METHODS**

Our nutritional titration experiment indicated that females perceive social food with about one third the nutritional value as valuable as the non-social reference food (Fig. 1.1b). We thus wished to quantify the success of females’ eggs on social vs non-social food discs of distinct nutritional concentrations. We had a total of 6 treatments involving 2 food concentrations, 100% and 33%, and 3 social treatments, non-social, social and previously social (see below). We omitted the 11% food concentration because females in experiment 1 mostly avoided it even when it was social (Fig. 1.1b). The food discs were identical in constitution and volume to the 100% and 33% food discs in experiment 1.

The non-social discs contained unmodified food. To generate the social and previously social discs, we placed on each disc 5 24-hour old first instar larvae and allowed these larvae to feed for 24 hours. In the social disc treatment, we kept the now second instar, 48 h old larvae on each disc. In the formerly social disc, we removed the larvae. That is, both the social and previously social discs were equally modified by the five larvae prior to the placement of focal eggs. Then the focal larvae emerging on the formerly social disc could reap potential benefits from such previous food modification without experiencing competition with the older larvae on the social disc. Thus the formerly social disc gave us a greater power for quantifying possible benefits of prior food modification by larvae.

We placed each food dish inside a 35 mm Petri dish lined with agar, added to each dish five focal eggs and housed all the dishes in a chamber kept at 25o C and 90% RH. When the five older larvae in the social dishes pupated, we removed these pupae. We then monitored the number of focal larvae reaching pupation. We sexed all eclosing adults, dried them in an oven at 70°C for 3 days and weighed the flies in groups of five on a microbalance.

Because no larvae survived in the social 33% treatment, we conducted two separate analyses. First, we omitted the social treatment and compared larval performance in the four treatments of non-social and formerly social on 33% and 100% food. Second, we compared larval performance in all three treatments of non-social, formerly social and social on the 100% food.

We analyzed larval development rate and the proportion of eggs surviving to adulthood using a generalized estimating equation with a gamma distribution and log link function (Durisko and Dukas, 2013). We had sufficient sample sizes for analyzing adult dry mass only for the 100% food (Fig. 1.2E, F). These data met ANOVA assumptions and we thus used a two-way ANOVA with a Tukey HSD. All post-hoc pairwise comparisons were conducted using the sequential Bonferroni method adjusting for multiple comparisons.

**RESULTS**

*Larval performance across food qualities*

Owing to 100% mortality in the social 33% food treatment, we could compare larval performance across food qualities only for the non-social and previously social treatments. Larvae developed much faster (Wald χ²1=474.74, P<0.001; Fig. 1.2A,B) and had higher survival rates on the 100% than 33% food (Wald χ²1=75.6, P<0.001; Fig. 1.2C,D). Similarly, larvae developed much faster (Wald χ²1=33.361, P<0.001; Fig. 1.2A,B) and had higher survival rates in the non social than formerly social treatments (Wald χ²1=75.769, P<0.001; Fig. 1.2C,D) .

Because survival rates in the 33% food treatment were low, we could only compare the non-social treatment across food qualities. Adults in the 100% food quality were much heavier than those in the 33% food quality (Wald χ²1=512.96, P<0.001; Fig. 1.2E,F).

*Larval performance across social treatments*

This analysis could include only the 100% food owing to 100% mortality in the social 33% food treatment. Larvae developed significantly faster in the non-social treatment, intermediate in the formerly social treatment, and slowest in the social treatment for the 100% food (Wald χ²2=1700, P<0.001; Fig. 1.2A). Post-hoc pairwise comparisons showed that each treatment was significantly different from the other two (P<0.001).

Survival to adulthood was significantly affected by the social treatment (Wald χ²2=13.9, P=0.001; Fig. 1.2C). Survival was similar in the non-social and formerly social treatment (post-hoc pairwise comparison, P=0.709) but higher in each of these treatments than in the social treatment (post-hoc pairwise comparisons, P=0.002 and 0.005 for the non-social and formerly social treatment respectively).

Adult mass was significantly affected by the social treatment (F2,61=85.184, P<0.001; Fig. 1.2E). In both males and females, adults of the non social treatment were heavier than those of the social and formerly social treatments (Tukey HSD, P<0.001). While males of the formerly social treatment were lighter than those in the social treatment (P=0.007), females of the formerly social and social treatments had similar weights (P=0.438).

****

**Figure 1.2.** **Larval performance as function of a disc’s nutritional and social status.** The left panels (A, C, and E) refer to the 100% nutrients while the right panels (B, D, and F) refer to the 33% nutrients. (A) and (B) show the time it takes for the larvae to develop from egg into pupae . (C) and (D) show the proportions of eggs that survived to adulthood (mean+SE). In (D), survival in the social treatment was 0. (E) and (F) show the adult dry mass (mean+SE). N= 30 replicates for each treatment. The number of eclosing adults is shown above the bars in panels E and F.

**Experiment 3: Larval success on abundant food**

**METHODS**

In our previous larval success experiment, larvae were reared on 0.5 ml of food. Because the results indicated strong effects of competition, we tested larval success on social and non-social discs each containing 2.5 ml of 100% food. By providing abundant food, we wished to maximize our ability to detect possible benefits that larvae may gain from developing on social food. All other protocol details were similar to those detailed above.

**RESULTS**

Larvae developed faster in the non-social condition than in the social condition (Wald χ²1=34.683, P<0.001; Fig. 1.3A). However, the same proportion of focal eggs survived to adulthood (Wald χ²1=0.014, P=0.905; Fig. 1.3B). Adult flies in the non-social condition were heavier than adults in the social condition (Wald χ²1=4.515, P=0.034; Fig. 1.3C).



**Figure 1.3. Performance measures of focal larvae on abundant food**. Discs were either social or non-social (n=30 replicates per treatment). (A) Time from egg laying to pupal formation (B) The proportion of eggs surviving to adulthood (mean+SE). (C) The adult dry mass of females and males in both conditions (mean+SE). Adults in the non-social condition were significantly heavier than their social counterparts. Numbers in brackets above the bars indicate the number of adults in each group.

**Experiment 4: Females’ patch choice when the social patches have had high larval densities**

**METHODS**

In our titration experiment (Fig. 1.1B), females showed a strong preference for laying eggs near larvae even though this reduced their offspring success in our laboratory settings (Figs 1.2, 1.3). Because larval crowding and the consequent lower larval success are prevalent in nature as well (Atkinson, 1979; Grimaldi and Jaenike, 1984), we expected females to make egg laying decisions that balance their perceived benefit from laying next to larvae versus the expected cost due to larval overcrowding. We allowed females to choose between either a non-social patch and a social patch occupied by 5 larvae, or a non-social patch and a social patch occupied by 20 larvae. We predicted that females would lay a lower proportion of eggs on the social food when it was more crowded.

We placed each recently mated female inside a plastic cages (15 cm wide, 30 cm long, and 15 cm high), which contained two 35 mm Petri dishes placed at the opposite far corners of each cage. One dish was non-social and the other was social. Both dishes contained 0.5 ml food discs composed of 100% standard lab diet. The dishes were lined with a layer of agar to prevent desiccation. Non-social food discs were unoccupied. The social food discs had either 5 or 20 middle second instar larvae, which we had added 6 h before the addition of females.

We allowed the females to lay eggs overnight. In the following morning, we removed the females from the cages and counted the number of eggs on each food disc. We also counted the number of eggs laid on the agar layer adjacent to the food. These eggs were within 1cm of the food. We analyzed the proportion of eggs laid in the social dish compared to the total number of eggs that a female laid using a generalized linear model with a tweedie distribution and log link function.

The experiment above tested females’ sensitivity to larval density. It is feasible however, that females are more sensitive to the condition of food as indicated by volatiles from microbes and waste products rather than to the sheer number of larvae already on the food. To test this possibility, we allowed females to choose between either a non-social patch and a social patch that had been previously occupied by 5 larvae, or a non-social patch and a social food patch that had been previously occupied by 20 larvae. Again, we predicted that females would lay a lower proportion of eggs on the social food that had been more crowded.

Forty-eight hours before the experiment, we transferred groups of either 5 or 20 middle second instar larvae to social food discs and kept them in 35mm Petri dishes lined with agar. We also kept unoccupied food discs in Petri dishes lined with agar. All food discs contained 0.5 ml of 100% standard lab diet.

By the day of the experiment all larvae on the social discs had pupated. We then placed one non-social food disc and one social disc in 60mm Petri dishes lined with agar. The social disc had been previously consumed by either 5 or 20 larvae but was free of larvae and pupae by the time of the test. Discs were placed 2cm apart. We then added a recently mated female to each 60 mm dish through a hole in the lid which was then plugged with a foam. We allowed the females to lay eggs overnight. In the morning, we removed the females from the dishes and counted the number of eggs on the social and non-social food discs. We analyzed the proportion of eggs on each type of social food using a generalized linear model with a Tweedie distribution and log link function.

**RESULTS**

Females laid a similar proportions of eggs in the social dish regardless of it being currently occupied by 5 or 20 larvae (Wald χ²1=0.204, P=0.651; Fig. 1.4A). However, females placed a greater proportion of their eggs on the agar rather than on the food disc itself in the social discs with 20 than 5 larvae (Wald χ²1=4.649, P=0.031; Fig. 1.4B). When females had a choice between non-social and previously occupied social dishes, they laid a similar proportion of their eggs on the social food regardless of the number of larvae that had previously occupied it (Wald χ²1=0.472, P=0.492; Fig. 1.4C).



**Figure 1.4: Social patch choice under high larval densities.** The proportion (mean+SE) of eggs laid on the social disc when it has had currently (A, B) or previously (C) either five or 20 larvae. In each case, females could choose between laying on a social or non-social dish. (A) The proportion of eggs laid in the social dish. (B) The proportion of eggs that were laid on agar rather than on the food disc in the social dish. N=24 replicates per treatment. (C) Females laid a similar proportion of eggs on a food that had been heavily used by 20 larvae or more lightly used by 5 larvae. No larvae were present on the food at the time of egg laying. N=28 replicates per treatment

**Experiment 5: Adult vs. larval abilities to detect differences in yeast concentration of food**

**METHODS**

Because we documented a lower larval success of eggs laid at social patches, we wished to test whether the benefit of patch choice copying is related to information rather then the cost of joining. To this end, we tested whether larvae could detect pertinent patch characteristics that adult females could not. We had two treatments testing larval and adult females’ abilities to detect differences in yeast content between adjacent patches. One test involved a reference 100% standard fly medium vs standard medium with only 33% of the yeast content, and the other test involved a reference 100% standard medium vs standard medium with only 50% of the yeast content. All other medium ingredients were identical.

We added either one recently mated adult female or five mid-second instar larvae to Petri dishes containing one reference food disc and one food disc with lower yeast concentration (either 33% or 50%) placed 2 cm apart. We added the focal larvae and adults in the evening at an identical location 1 cm between the food discs. We gave them 14 hours to decide where to lay eggs or feed. In the following morning, we counted the number of eggs laid on each food disc in the adult female treatments and counted the number of larvae on each food disc in the larval treatments. We then calculated the proportion of eggs laid and the proportion of larvae on the reference 100% disc and analyzed the data with a generalized linear model with a Tweedie distribution and identity link function.

**RESULTS**

The proportion of eggs that females laid on the 100% food and the proportion of larvae choosing the 100% were similar when the alternative had only 33% of yeast concentration (Wald χ²1=0.227, P=0.634; Fig. 1.5). When the alternative was 50% yeast concentration, females showed a greater preference than larvae for the higher quality food (Wald χ²1=3.835, P=0.05; Fig. 1.5).

****

**Figure 1.5.** **Patch choice by adult females versus larvae**. In one experiment (black bars), adult females or larvae had a choice between a disc containing the regular yeast concentration (100%) or a disc containing 33% of the regular yeast concentration. In the other experiment (white bars), adult females or larvae had a choice between a disc containing the regular yeast concentration (100%) or a disc containing 50% of the regular yeast concentration. N=80 replicates per nutrition for larvae, and N=60 replicates per nutrition for the adult females. Adults and larvae are equally good at finding the higher quality site when the alternative is 33% yeast concentration. When the alternative is 50% yeast concentration, adults are better than larvae at finding the high quality site.

**DISCUSSION**

Our titration experiment (Fig. 1.1) indicated that, while females were highly sensitive to the nutritional values of alternative patches, they equated the reference (100% nutrients), unoccupied patches (non-social patches) with low-nutrition patches (33%) occupied by larvae (social patches). The larval success experiment (Fig. 1.2) indicated that the females’ sensitivity to nutrient concentration was highly justified: their larvae developed significantly faster, had higher survival rates and produced larger adults on the non-social 100% than non-social 33% patches. Because females were willing to trade the nutritional quality of patches for the opportunity to lay eggs at patches already occupied by larvae, we expected that such choice would translate into some larval benefit. However, we failed to find such advantage in a few attempts. First, in all cases, larval success on the social patches was lower than that on non-social patches (Fig. 1.2). Second, in the previously social treatment, we removed the larvae that had occupied the patches before placing focal eggs. This allowed us to test for possible benefits that females could gain from laying eggs at patches that have been occupied by larvae while eliminating the negative effects of competition from such larvae. Even in this case, however, we failed to identify any benefit from laying on previously occupied patches (Fig. 1.2). Finally, one could argue that our larval to food-volume ratio was too high so that larval competition obscured a gain occurring when food is abundant. To address this possibility, we repeated the larval success experiment with a much lower larval to food-volume ratio. Even in this case, however, larvae performed better under the non-social then social treatment (Fig. 1.3).

To further assess the egg laying decisions by females, we wished to quantify females’ responses to clear signs of current and future competition in social patches due to either the previous or current presence of many larvae. Although we expected females to reduce their preferences for the social patches when they were either crowded or heavily exploited, we found no such moderation (Fig. 1.4). Finally, although the sense of taste provides important information about the nutritional quality of food, it is insufficient for assessing whether all nutrients required for optimal larval development are available (Vosshall and Stocker, 2007; Yarmolinsky et al., 2009; Masek and Scott, 2010; Stafford et al., 2012). While prospecting females cannot fully assess nutritional quality, the presence of feeding larvae is probably the most relevant cue indicating that a substrate is nutritionally sufficient. First, the substrate is adequate for sustaining the larvae as indicated by the fact that they are alive. Second, the larvae are highly mobile and are adept at exploring and settling at the best locally available food (Durisko and Dukas, 2013; Schwarz et al., 2014). Contrary to our expectation, however, we found in two experiments that adult females were as sensitive as larvae to realistic variations in nutritional qualities (Fig. 1.5).

To summarize our key results, we have strong evidence that females assign high values to patches already occupied by larvae as we quantified by titrating the nutritional quality of the patches (Fig. 1.1) and we could translate these values into the relevant currency of larval success (Figs 1.2, 1.3). Our data, however, indicated neither informational gain (Fig. 1.5) nor direct benefits from patch choice copying (Figs 1.2, 1.3). How can this puzzle be resolved? We propose four non-mutually exclusive explanations related to fruit flies’ natural history features that do not occur under laboratory settings.

**Competition with microbes**

While fruit flies feed on yeast species growing on fallen fruit (Begon, 1982), such fruit are also consumed by numerous other fungi as well as bacteria. This means that the other microbes can adversely impact yeast through exploitation competition. Furthermore, microbial interference competition involves a rich arsenal of compounds toxic to other microbes as well as to animals. That is, such compounds can either hamper yeast growth, thus reducing the amount of food available to larvae, or have direct negative effects on larval survival and growth (Janzen, 1977; Arndt et al., 1999; Demain and Fang, 2000; Janisiewicz and Korsten, 2002; Lacey and Shapiro-Ilan, 2008; Sharma et al., 2009). Although highly pertinent for understanding the behaviour of larval and adult fruit flies, the microbial ecology relevant to fruit flies remains mostly unexplored. A notable exception is work by Rohlfs and colleagues (Rohlfs, 2005; Rohlfs et al., 2005), which quantified negative effects of three mould species on fruit fly larvae and indicted that groups of five and 10 larvae were more effective at suppressing mould growth than single larvae. Another relevant observation is that fruit flies possess a dedicated olfactory circuit tuned to geosmin. Fruit flies rely on this circuit to avoid feeding and egg laying on substrates containing geosmin-producing microbes, which are harmful to fruit flies (Stensmyr et al., 2012). This indicates that fruit flies are sensitive to the constitution of microbial communities at prospective egg laying sites. It is thus likely that, by preferring to lay eggs at patches already occupied by larvae over unoccupied patches, females in natural settings ensure that their newly hatched larvae will be better protected from microbes harmful to their larvae or their yeast food.

**Group enhancement of favourable yeasts**

There appear to be mutualistic interactions between some yeast species and fruit flies. Adults and larvae inoculate fruit with yeast and larval activity promotes the growth of certain yeast species (Wertheim et al., 2002; Stamps et al., 2012). While some of the positive effects of larvae on yeast can be modulated through churning of the substrate, the larval gut bacteria also produce antifungals, which could selectively suppress mould and thus enhance the growth of the preferred yeast food (Schnürer and Magnusson, 2005; Mauch et al., 2010; Crowley et al., 2012; Venu et al., 2014). Intriguingly, adult and larval fruit fly attraction to food inhabited by larvae is mediated by volatiles emitted from gut bacteria (Venu et al., 2014). Hence it is likely that females in nature lay eggs in occupied patches because such patches are more favourable for further growth of yeast food than are unoccupied patches.

**Microbial information**

While we found no evidence that larval presence provides superior nutritional information about patch quality that females cannot readily assess, the discussion above suggests that larval presence is the best indicator that the microbial ecology is favourable to larval growth. That is, it is likely that different fruit patches allow for the optimal growth of different microbial species with only some of them being hospitable to fruit flies. For example, substrates may vary in their ability to sustain the growth of harmful mould and bacteria versus the yeast species favoured by fruit flies. Assuming that females cannot assess all the relevant ecological settings that would influence fungal growth, the presence of thriving larvae may be the best cue indicating that a patch is providing the appropriate microbial environment.

**Parasitoid avoidance**

Larval parasitoids are a major source of fruit fly mortality in natural settings and fruit flies possess a suite of behavioural and physiological adaptations for reducing parasitoid success (Carton et al., 1986; Fleury et al., 2004; Hwang et al., 2007; Kacsoh et al., 2013). One way by which larvae can avoid parasitism is through hiding in micro-sites inaccessible to parasitoids. Although newly hatched larvae are not proficient at burrowing, older larvae, especially ones in the third instar stage, have stronger and larger mandibular hooks containing several teeth (Bodenstein, 1950) and they spend much of their time tunnelling deep inside the substrate (Durisko et al., 2014b). It is thus possible that, by laying eggs close to larvae, females ensure that their hatching offspring can hide in burrows dug by the older larvae. Limited evidence indeed indicates that larvae hidden deep in natural fruit experience lower rates of parasitoid attacks (Rohlfs and Hoffmeister, 2004).

**Patch choice copying in other species**

Our work on the value of patch choice copying in fruit flies can inform and be informed by research on copying in other species. Perhaps the best studied and most relevant system involves the economically important bark beetles (Scolytidae), which aggregate at host trees. While there are many species of bark beetles, we focus here on obligate parasites, which attack and kill trees (Paine et al., 1997). Long-distance attraction to host trees in bark beetles is mediated by pheromones. Early colonizers benefit from attracting others because a critical mass of beetles and perhaps associated fungi are necessary for overcoming tree defences (Wood, 1982; Raffa and Berryman, 1983; Paine et al., 1997). Because prospective females gain from joining patch occupiers, the adaptive function of patch choice copying is clear.

There are at least two major differences between the fruit fly and bark beetle systems. First, in the bark beetles, there is active recruitment by early colonizers, which is crucial for the early colonizers’ success because a critical mass of beetles is necessary for overcoming the massive defence mounted by the host tree (Wood, 1982; Raffa et al., 2008). In fruit flies, cis-vaccenyl acetate (cVA), has been referred to as an aggregation pheromone (Bartelt et al., 1985; Wertheim et al., 2002). However, cVA is produced only by males, who transfer it during copulation to females (Brieger and Butterworth, 1970), in which it signals to prospective males that the females are recently mated and unreceptive. Indeed females emitting cVA are much less attractive to males than females with no cVA (Ejima et al., 2007; Dukas and Dukas, 2012; Keleman et al., 2012). It is thus likely that cVA has a relatively negligible role in long-distance attraction compared to the dominant role of microbial volatiles (Stökl et al., 2010; Becher et al., 2012; Venu et al., in press). That is, there is no critical evidence indicating active recruitment of conspecifics in fruit flies.

The second and somewhat related difference between the bark beetle and fruit fly systems is the change in patch attractiveness with density. In the bark beetle system, there is a clear decline in tree attractiveness once a threshold beetle density has been reached. Such decline can readily be explained. Functionally, the occupiers no longer require further individuals once the tree is dying. Mechanistically, the occupiers can readily modulate patch attractiveness by ceasing to emit the aggregation pheromone (Wood, 1982; Raffa et al., 2008). In the fruit fly system, we failed to identify the predicted decline in patch attractiveness with density. It is likely, however, that, in natural settings, cues from microbes associated with high density could decrease patch attractiveness or even repel females, as does geosmin discussed above.

Most other systems in which patch choice copying occurs are not as well studied as bark beetles. We suggest, however, that fruit flies can serve as an excellent general model system for further research on the topic owing to their amenability to research in the ecological, evolutionary and mechanistic domains. Our work so far suggests that direct benefits from joining others are likely in many systems even when such benefits are not observed under controlled settings. The most likely reason for such outcomes is an involvement of harmful microbes in natural settings, which a group is more likely to overcome than an individual. Similarly, because the microbial ecology and dynamics is complex, prospective individuals probably gain the best available information from relying on others, because the other’s presence indicates a suitable microbial setting. For example, is it possible that egg laying butterflies copy the choice of others in spite of obvious competition costs (Raitanen et al., 2014) owing to benefit incurred from group suppression of harmful microbial growth? Our proposition about the central importance of microbes will require extensive experimental work in collaboration with microbial ecologists.

**References**

**Agrawal, A. F.** (2001). The evolutionary consequences of mate copying on male traits. *Behav. Ecol. Soc.* **51**, 33-40.

**Allee, W. C.** (1931). Animal Aggregations. A Study in General Sociology. Chicago: University of Chicago Press.

**Alonzo, S. H.** (2008). Female mate choice copying affects sexual selection in wild populations of the ocellated wrasse. *Anim. Behav.* **75**, 1715-1723.

**Arndt, C., Cruz, M. C., Cardenas, M. E. and Heitman, J.** (1999). Secretion of FK506/FK520 and rapamycin by *Streptomyces* inhibits the growth of competing *Saccharomyces cerevisiae* and *Cryptococcus neoformans*. *Microbiology* **145**, 1989-2000.

**Arnold, W.** (1988). Social thermoregulation during hibernation in alpine marmots (*Marmota marmota*). *Journal of Comparative Physiology B* **158**, 151-156.

**Atkinson, W. D.** (1979). A field investigation of larval competition in domestic *Drosophila*. *J. Anim. Ecol.* **48**, 91-102.

**Bartelt, R. J., Schaner, A. M. and Jackson, L. L.** (1985). *cis*-vaccenyl acetate as an aggregation pheromone in *Drosophila melanogaster*. *Journal of Chemical Ecology* **11**, 1747-1756.

**Battesti, M., Moreno, C., Joly, D. and Mery, F.** (2012). Spread of social information and dynamics of social transmission within *drosophila* groups. *Curr. Biol.* **22**, 309-313.

**Beauchamp, G.** (2014). Social Predation: How Group Living Benefits Predators and Prey. London: Elsevier Academic Press

**Becher, P. G., Flick, G., Rozpędowska, E., Schmidt, A., Hagman, A., Lebreton, S., Larsson, M. C., Hansson, B. S., Piškur, J. and Witzgall, P.** (2012). Yeast, not fruit volatiles mediate *Drosophila melanogaster* attraction, oviposition and development. *Func. Ecol.* **26**, 822-828.

**Begon, M.** (1982). Yeasts and *Drosophila*. In *The Genetics and Biology of Drosophila*, vol. 3b eds. M. Ashburner H. L. Carson and J. N. Thompson), pp. 345-384. London: Academic Press.

**Betts, M. G., Hadley, A. S., Rodenhouse, N. and Nocera, J. J.** (2008). Social information trumps vegetation structure in breeding-site selection by a migrant songbird. *Proceedings of the Royal Society B: Biological Sciences* **275**, 2257-2263.

**Bodenstein, D.** (1950). The postembryonic development of *Drosophila*. In *Biology of Drosophila*, (ed. M. Demerec), pp. 275-367. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

**Brieger, G. and Butterworth, F. M.** (1970). *Drosophila melanogaster*: Identity of male lipid in reproductive system. *Science* **167**, 1262.

**Carton, Y., Bouletreau, M., Alphen, J. J. M. v. and Lenteren, J. C. v.** (1986). The *Drosophila* parasitic wasps. In *The Genetics and Biology of Drosophila*, eds. M. Ashburner H. L. Carson and J. N. Thompson), pp. 347–934. London: Academic Press.

**Crowley, S., Mahony, J. and van Sinderen, D.** (2012). Comparative analysis of two antifungal *Lactobacillus plantarum* isolates and their application as bioprotectants in refrigerated foods. *Journal of Applied Microbiology* **113**, 1417-1427.

**Danchin, E. and Wagner, R. H.** (1997). The evolution of coloniality: the emergence of new perspectives. *Trends Ecol Evol* **12**, 342-347.

**Danchin, E., Giraldeau, L.-A., Valone, T. J. and Wagner, R. H.** (2004). Public Information: From Nosy Neighbors to Cultural Evolution. *Science* **305**, 487-491.

**Demain, A. and Fang, A.** (2000). The natural functions of secondary metabolites. In *History of Modern Biotechnology I*, vol. 69 (ed. A. Fiechter), pp. 1-39: Springer Berlin Heidelberg.

**Dugatkin, L. A.** (1992). Sexual selection and imitation: females copy the mate choice of others. *Am. Natur.* **139**, 1384-1389.

**Dukas, R. and Dukas, L.** (2012). Learning about prospective mates in male fruit flies: effects of acceptance and rejection. *Anim. Behav.* **84**, 1427-1434.

**Durisko, Z. and Dukas, R.** (2013). Attraction to and learning from social cues in fruit fly larvae. *Proc. R. Soc. Lond. B. Biol. Sci.* **280**, 20131398.

**Durisko, Z., Anderson, B. and Dukas, R.** (2014a). Adult fruit fly attraction to larvae biases experience and mediates social learning. *J. Exp. Biol.* **217**, 1193-1197.

**Durisko, Z., Kemp, B., Mubasher, A. and Dukas, R.** (2014b). Dynamics of social interactions in fruit fly larvae. *PLOS ONE*.

**Ejima, A., Smith, B. P. C., Lucas, C., van der Goes van Naters, W., Miller, C. J., Carlson, J. R., Levine, J. D. and Griffith, L. C.** (2007). Generalization of courtship learning in *Drosophila* is mediated by cis-vaccenyl acetate. *Curr. Biol.* **17**, 599-605.

**Fletcher, R. J. and Miller, C. W.** (2008). The type and timing of social information alters offspring production. *Biol. Lett.* **4**, 482-485.

**Fleury, F., Ris, N., Allemand, R., Fouillet, P., Carton, Y. and Boulétreau, M.** (2004). Ecological and genetic interactions in Drosophila–parasitoids communities: a case study with D. *Melanogaster, D. Simulans* and their common *Leptopilina* parasitoids in south eastern France. *Genetica* **120**, 181-194.

**Galef, B. G. and White, D. J.** (1998). Mate-choice copying in Japanese quail, *Coturnix coturnix japonica*. *Anim. Behav.* **55**, 545-552.

**Grimaldi, D. and Jaenike, J.** (1984). Competition in natural populations of mycophagous *Drosophila*. *Ecology* **65**, 1113-1120.

**Hwang, R. Y., Zhong, L., Xu, Y., Johnson, T., Zhang, F., Deisseroth, K. and Tracey, W. D.** (2007). Nociceptive neurons protect *Drosophila* larvae from parasitoid wasps. *Curr. Biol.* **17**, 2105-2116.

**IBM-Corp.** (2011). IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.

**Janisiewicz, W. J. and Korsten, L.** (2002). Biological control of postharvest diseases of fruits. *Annual Review of Phytopathology* **40**, 411-441.

**Janzen, D. H.** (1977). Why fruits rot, seeds mold, and meat spoils. *The American Naturalist* **111**, 691-713.

**Kacsoh, B. Z., Lynch, Z. R., Mortimer, N. T. and Schlenke, T. A.** (2013). Fruit flies medicate offspring after seeing parasites. *Science* **339**, 947-950.

**Keleman, K., Vrontou, E., Kruttner, S., Yu, J. Y., Kurtovic-Kozaric, A. and Dickson, B. J.** (2012). Dopamine neurons modulate pheromone responses in *Drosophila* courtship learning. *Nature* **489**, 145-149.

**Kirkpatrick, M. and Dugatkin, L. A.** (1994). Sexual selection and the evolutionary effects of mate copying. *Behav. Ecol. Soc.* **34**, 443-449.

**Krebs, J. R.** (1973). Social learning and the significance of mixed-species flocks of chickadees (*Parus* spp.). *Can. J. Zool.* **51**, 1275-1288.

**Krijger, C. L. and Sevenster, J. G.** (2001). Higher species diversity explained by stronger spatial aggregation across six neotropical *Drosophila* communities. *Ecol. Lett.* **4**, 106-115.

**Lacey, L. A. and Shapiro-Ilan, D. I.** (2008). Microbial control of insect pests in temperate orchard systems: potential for incorporation into IPM. *Ann. Rev. Entomol.* **53**, 121-144.

**Masek, P. and Scott, K.** (2010). Limited taste discrimination in *Drosophila*. *Proceedings of the National Academy of Sciences* **107**, 14833-14838.

**Mauch, A., Dal Bello, F., Coffey, A. and Arendt, E. K.** (2010). The use of *Lactobacillus brevis* PS1 to in vitro inhibit the outgrowth of *Fusarium culmorum* and other common *Fusarium* species found on barley. *International Journal of Food Microbiology* **141**, 116-121.

**Paine, T. D., Raffa, K. F. and Harrington, T. C.** (1997). Interactions among Scolytid bark beetles, their associated fungi, and live host conifers. *Ann. Rev. Entomol.* **42**, 179-206.

**Prokopy, R. J. and Roitberg, B. D.** (2001). Joining and avoidance behavior in nonsocial insects. *Ann. Rev. Entomol.* **46**, 631-665.

**Pulliam, H. R. and Caraco, T.** (1984). Living in groups: is there an optimal group size? In *Behavioural Ecology*, eds. J. R. Krebs and N. B. Davies), pp. 122-147. Oxford: Blackwell.

**Raffa, K. F. and Berryman, A. A.** (1983). The role of host plant resistance in the colonization behavior and ecology of bark beetles (Coleoptera: Scolytidae). *Ecol. Monog.* **53**, 27-49.

**Raffa, K. F., Aukema, B. H., Bentz, B. J., Carroll, A. L., Hicke, J. A., Turner, M. G. and Romme, W. H.** (2008). Cross-scale drivers of natural disturbances prone to anthropogenic amplification: the dynamics of bark beetle eruptions. *BioScience* **58**, 501-517.

**Raitanen, J., Forsman, J. T., Kivelä, S. M., Mäenpää, M. I. and Välimäki, P.** (2014). Attraction to conspecific eggs may guide oviposition site selection in a solitary insect. *Behav. Ecol.* **25**, 110-116.

**Robinson, G. E., Fernald, R. D. and Clayton, D. F.** (2008). Genes and Social Behavior. *Science* **322**, 896-900.

**Rohlfs, M.** (2005). Density-dependent insect-mold interactions: effects on fungal growth and spore production. *Mycologia* **97**, 996-1001.

**Rohlfs, M. and Hoffmeister, T. S.** (2004). Spatial aggregation across ephemeral resource patches in insect communities: an adaptive response to natural enemies? *Oecologia* **140**, 654-661.

**Rohlfs, M., Obmann, B. and Petersen, R.** (2005). Competition with filamentous fungi and its implication for a gregarious lifestyle in insects living on ephemeral resources. *Ecol. Entomol.* **30**, 556-563.

**Sarin, S. and Dukas, R.** (2009). Social learning about egg laying substrates in fruit flies. *Proc. R. Soc. Lond. B. Biol. Sci.* **276**, 4323-4328.

**Schnürer, J. and Magnusson, J.** (2005). Antifungal lactic acid bacteria as biopreservatives. *Trends in Food Science & Technology* **16**, 70-78.

**Schwarz, S., Durisko, Z. and Dukas, R.** (2014). Food selection in larval fruit flies: dynamics and effects on larval development. *Naturwissenschaften* **101**, 61–68.

**Sharma, R., Singh, D. and Singh, R.** (2009). Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. *Biol. Cont.* **50**, 205-221.

**Shorrocks, B. and Sevenster, J. G.** (1995). Explaining Local Species Diversity. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **260**, 305-309.

**Sokolowski, M. B.** (2010). Social interactions in "simple" model systems. *Neuron* **65**, 780-794.

**Stafford, J. W., Lynd, K. M., Jung, A. Y. and Gordon, M. D.** (2012). Integration of taste and calorie sensing in *Drosophila*. *The Journal of Neuroscience* **32**, 14767-14774.

**Stamps, J. A.** (1987). Conspecifics as cues to territory quality: a preference of juvenile lizards (*Anolis aeneus*) for previously used territories. *The American Naturalist* **129**, 629-642.

**Stamps, J. A., Yang, L. H., Morales, V. M. and Boundy-Mills, K. L.** (2012). *Drosophila* regulate yeast density and increase yeast community similarity in a natural substrate. *PLoS ONE* **7**, e42238.

**Stensmyr, Marcus C., Dweck, Hany K. M., Farhan, A., Ibba, I., Strutz, A., Mukunda, L., Linz, J., Grabe, V., Steck, K., Lavista-Llanos, S. et al.** (2012). A conserved dedicated olfactory circuit for detecting harmful microbes in *Drosophila*. *Cell* **151**, 1345-1357.

**Stökl, J., Antonia Strutz, Amots Dafni, Ales Svatos, Jan Doubsky, Markus Knaden, Silke Sachse, Bill S. Hansson and Stensmyr, M. C.** (2010). A deceptive pollination system targeting Drosophilids through olfactory mimicry of yeast. *Curr. Biol.* **20**, 1846-1852.

**Thorpe, W.** (1963). Learning and Instinct in Animals. London: Methuen and Co.

**Valone, T. J. and Templeton, J. J.** (2002). Public information for the assessment of quality: a widespread social phenomenon. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **357**, 1549-1557.

**Venu, I., Durisko, Z., Xu, J. P. and Dukas, R.** (2014). Social attraction mediated by fruit flies’ microbiome. *J. Exp. Biol.*

**Vosshall, L. B. and Stocker, R. F.** (2007). Molecular architecture of smell and taste in *Drosophila*. *Ann. Rev. Neurosci.* **30**, 505-533.

**Wade, M. J. and Pruett Jones, S. G.** (1990). Female Copying Increases the Variance in Male Mating Success. *P Natl Acad Sci USA* **87**, 5749-5753.

**Waite, R. K.** (1981). Local enhancement for food finding by Rooks (*Corvus frugilegus*) foraging on grassland. *Z. Tierpsychol.* **57**, 15-36.

**Wertheim, B., Dicke, M. and Vet, L. E. M.** (2002). Behavioural plasticity in support of a benefit for aggregation pheromone use in Drosophila melanogaster. *Entomol. Exp. Appl.* **103**, 61-71.

**Wertheim, B., van Baalen, E.-J. A., Dicke, M. and Vet, L. E. M.** (2005). Pheromone-mediated aggregation in nonsocial arthropods: an evolutionary ecological perspective. *Ann. Rev. Entomol.* **50**, 321-346.

**Willis, C. R. and Brigham, R. M.** (2007). Social thermoregulation exerts more influence than microclimate on forest roost preferences by a cavity-dwelling bat. *Behav. Ecol. Soc.* **62**, 97-108.

**Wood, D. L.** (1982). The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. *Ann. Rev. Entomol.* **27**, 411-446.

**Yang, C.-h., Belawat, P., Hafen, E., Jan, L. Y. and Jan, Y.-N.** (2008). *Drosophila* egg-laying site selection as a system to study simple decision-making processes. *Science* **319**, 1679-1683.

**Yarmolinsky, D. A., Zuker, C. S. and Ryba, N. J. P.** (2009). Common Sense about Taste: From Mammals to Insects. *Cell* **139**, 234-244.

**CHAPTER 2: The use of social information in foraging larvae**

**The following experiments were designed by Shane Golden and Reuven Dukas. They were conducted by SG. All sections were written by SG.**

**INTRODUCTION**

Social information can be invaluable to animals to reduce the uncertainty in their environment. Paying attention to others can reduce the time and risk associated with exploring the environment. Social information can also be integrated with individual information to reconcile any errors (Galef & Whiskin, 2001). Generally, strategies associated with observing others tend to be more successful, and less costly than strategies associated with individual learning (Rendell et al., 2011).

In the fruit fly larval system, there is strong evidence for them being socially attracted to food sources containing larvae or the cues left behind by larvae (Durisko & Dukas, 2013; Venu et al., 2014). This means that they must be able to detect and orient themselves toward the cues associated with other larvae. A likely candidate for the socially attracting cue is the gut bacterium *Lactobacillus brevis* (Venu et al., 2014). Olfactory cues emanating from the presence of this bacterium cause larvae to move towards a food, even in the absence of any larvae occupying that food (Venu et al., 2014). This movement towards others is unsurprising as larvae naturally form loose aggregations (Durisko et al., 2014b). Egg laying females are attracted to sites with larvae where they can lay many eggs at a time (Durisko et al., 2014a; Del Solar & Palomino, 1966). Thus, in the absence of long distance dispersion, the larvae tend to be near one another during development. According to other animal systems, groups should tend to find and settle on the highest quality patches (Ward et al., 2011; McClure et al., 2012). Groups of larvae should therefore be a reliable predictor of a high quality foraging site.

Larvae may want to aggregate for other reasons as well. Groups of larvae churn the substrate more effectively which limits fungal growth (Wertheim et al., 2002). They are also better at sustaining beneficial yeast species on decaying fruit by breaking more of the surface of a fruit, allowing more yeast to settle (Stamps et al., 2012). Avoiding parasitoids may also be easier in a group, which facilitates digging into the substrate (Durisko et al., 2014b).

We wanted to test a number of variables that are associated with differences in social information use. The first one was a genotypic effect of foraging morph. The foraging allele gives rise to rover and sitter morphs which are characterized by differences in movement on and between food sources (Sokolowski, 1980). In adult fruit flies, it was found that sitters seemed to rely more on social information than rovers when tested in a spatial learning task (Foucaud et al., 2013). Due to their lower overall movement within a food patch and tendency not to explore and find other food patches, sitters are theorized to form tighter aggregations. This could lead to a heavier reliance on social information use, at least within the larvae. However, work in our lab has not found any differences in aggregation between rover and sitter larvae (Anderson, unpublished data). We would still predict that sitter larvae pay more attention to social information, matching the results found with the adult flies.

The next variable we wanted to test was the developmental stages of the larvae and how those affect social information. Theoretically, younger, more inexperienced larvae should pay closer attention to social information, as they know less about the environment (Dukas, 2010). We therefore predict that our younger (early second instar) larvae would pay more attention to social information than our older (early third instar) larvae.

A third variable that we tested was the rearing conditions of the larvae. This was to motivate them to explore when we moved them from an optimal food to a sub-optimal one. With additional exploration in our test dishes, we expected a greater use of social information because they could come in closer contact with a model larva. Conversely, we also wanted to see if raising them on sub-optimal food and then moving them to another sub-optimal food inhibited exploration and perhaps stopped them from using social information.

**GENERAL METHODS**

Populations of fruit flies were maintained in either cages (Canton-S) or vials (rovers and sitters). Populations were raised on standard lab diet (for recipe, see methods in Chapter 1). To quantify how larvae respond to social information, we placed a single focal larva in each 60mm test dish lined with agar, containing one 100% standard lab diet food disc and one 50% lab diet food disc. Food discs were placed 1cm apart. Dishes were non-social if they contained only the focal larva, or social if they contained a model larva in addition to the focal. Social dishes had a model larva placed on the 100% lab diet food disc 7 hours prior to the addition of any focals. These dishes were kept at 25**°**C and 90% relative humidity. The 100% lab diet disc had an artificial pre-dug burrow to keep the models on that food. Seven hours after the addition of the models, a single focal larva was added to each test dish, on to the 50% food disc. We then waited for one hour, giving the focal time to explore the test dish. At the end of the one hour, we recorded where the focal had moved. We also ensured that models had stayed within the burrow on the 100% food disc. Any dishes where the model had moved were discounted. Focals could end up in one of three spots; still on the 50% food disc, exploring on the agar, or foraging on the 100% food disc. We wanted to see the proportion of focal larvae that moved over to the 100% food disc after the one hour in the dish.

We analyzed the results using a generalized linear model with binomial distribution and logit link function. This was appropriate as we treated the data as a focal being either on the 100% food disc, or off of it.

**Experiment 1: Rover and sitter social information use**

**METHODS**

Unlike the general methods, the 100% food disc and 50% food disc were placed 2cm apart because of some preliminary testing that showed very high rates of the focal moving to the 100% food disc. We also only gave focals a half hour to explore, instead of the one hour in the general methods. In social dishes, rover focals were matched with rover models, and sitter focals were matched with sitter models. All focal and model larvae were raised on 100% standard lab diet. Focals and models were both early third instar at the time of testing. We ran a total of 96 dishes, divided evenly among the four treatments.

The statistical model included genotype and social treatment as factors, as well as the interaction between them.

**RESULTS**

A greater proportion of rovers than sitters moved over to the 100% food disc after the half hour exploration period (Wald χ²1=4.053, P=0.044; Fig. 2.1). However, neither rovers nor sitters had a greater proportion of focals move over to the 100% food disc when it was social compared to non-social (Wald χ²1=0, P=0.997; Fig. 2.1). There was also no interaction between genotype and social treatment (Wald χ²1=0.351, P=0.554; Fig. 2.1).

**Figure 2.1: Rover and sitter foraging with and without models.** We tested the differences in social information use between rover and sitter genotypes. If focal larvae were on their own in a test dish, it was considered non-social (black bars). If there was a model on the 100% food disc in the dish with the focal, it was considered social (white bars). Food discs were 2cm apart and focals were given a half-hour to explore. N=24 replicates per treatment.

**Experiment 2: Social information use among old and young larvae raised on 100% food**

**METHODS**

For this experiment, we switched over to Canton-S larvae, having found no differences in social information use between rovers and sitters. Canton-S larvae were found to be less avid explorers than the rovers and sitters with some preliminary testing. Thus, we placed the food discs only 1cm apart. We also gave the focals one hour to explore the test dishes before recording where they ended up. All focals and models were raised on 100% standard lab diet. All models were early third instar at the time of testing. Preliminary testing with young models showed that they were likely just providing a weaker social cue than the older models. We did not use any young models in this experiment. Old focals were also early third instar. Young focals were early second instar at the time of testing. 240 total dishes were used but four were omitted due to models leaving the 100% food disc giving a total sample size of 236.

The statistical model included focal age and social treatment as factors in addition to the interaction between these two variables.

**RESULTS**

A greater proportion of the young focals moved over to the 100% food disc than the old focals (Wald χ²1=4.992, P=0.025; Fig. 2.2). However, the social treatment did not cause more focals in either condition to forage on the 100% food (Wald χ²1=0.008, P=0.927; Fig. 2.2). Additionally, there was no interaction between focal age and social treatment (Wald χ²1=0.561, P=0.454; Fig. 2.2).

**Figure 2.2: Social information use in foraging between young and old larvae raised on 100% food.** Non-social dishes (black bars), had just a single focal in the test dish. Social dishes (white bars) had either a young or old focal, and an old model. Food discs were 1cm apart and focals were given one hour to explore. Models were given 7 hours to eat the 100% food before the addition of focals. Total sample size was N=236, spread across the 4 treatments evenly.

**Experiment 3: Social information use among old and young larvae raised on 50% food**

**METHODS**

The protocol is identical to experiment 2 except for raising both models and focals on 50% food. Since we place focals directly on the 50% food disc, this should inhibit exploration more than if we raised them on 100% food. They are being placed on a familiar food that is the same quality as the only one they have known thus far in their development. We ran 240 test dishes and had to discount one due to a model moving to the 50% food, giving a total sample size of 239. Again, focal age and social treatment were the factors used in the statistical analysis.

**RESULTS**

Old and young focals reached the 100% food at about the same proportion (Wald χ²1=0.15, P=0.698; Fig. 2.3). However, dishes in the social treatment had a higher proportion of focals finding the 100% food than dishes in the non-social treatment (Wald χ²1=4.771, P=0.029; Fig. 2.3). Similar to the previous experiment, there was no interaction between focal age and social treatment (Wald χ²1=1.38, P=0.24; Fig. 2.3).

**Figure 2.3: Social information use in old and young larvae raised on 50% food.** Non-social dishes are shown in black bars and social dishes shown in white bars. All larvae were raised on 50% food. Focals were either early third instar (old) or early second instar (young). All models were early third instar. Food discs were 1cm apart and models were given 7 hours to eat the 100% food before the addition of focals. Total sample size was N=239.

**Experiment 4: Replication of old larvae raised on 50% food**

**METHODS**

Having finally found a significant effect of having a model on the 100% food, we decided to replicate the results. Since we found the strongest effect of social treatment in the old focal larvae, we tested just the old focals. The food discs were still 1cm apart and only early third instar models were used in the social dishes. Only early third instar focals were used as well. All larvae were raised on 50% food. We ran 120 total dishes and had to discount four, meaning a sample size of 116 total. For the statistical analysis, we only had social treatment as a factor because we were no longer testing different focal ages.

**RESULTS**

Unlike the previous experiment, there was no difference in the proportion of focals making it on to the 100% food in the social and non-social treatments (Wald χ²1=1.663, P=0.197; Fig. 2.4). The proportion that moved over to the 100% food was much higher for this replicate than the previous one (Wald χ²1=37.854, P=<0.001; Fig. 2.3, 2.4).

**Figure 2.4: Replicate of old larva social information use raised on 50% food.** Only old (early third instar) models and focals are used. This is a replicate of experiment 3 excluding the young larva treatments. The sample size is N=116 dishes. These proportions are quite a bit higher than those in the previous replicate. Very few larvae moved over to the 100% food originally, but in this replicate, a majority of the larvae moved over. Foods are 1cm apart and models have been feeding for 7 hours prior to the addition of focals.

**DISCUSSION**

In the rover and sitter social information experiment, we found no evidence for the use of social information. Instead, we found evidence for the differences in movement between patches for rovers and sitters. This was expected based on earlier research (Sokolowski, 1980; Sokolowski, 2001). Rovers are considered to be more mobile and will move between patches more frequently. We could not find differences in social information use between rover and sitter larvae (Fig. 2.1). This is in contrast to findings in rover and sitter adults. Sitter adults supposedly pay more attention to social information in a spatial learning task (Foucaud et al., 2013). Rovers were theorized to rely more on their own individual information in that task, as they did not pay as much attention to a group of models (Foucaud et al., 2013). There are differences between rover and sitter adults and larvae. Rover and sitter larvae respond similarly to yeast olfactory cues but rover and sitter adults respond differently (Shaver et al., 1998). In our experiment, the two foods in the test dish did have different yeast concentrations but we do not know if rovers and sitters differ in their ability to detect the difference. Likely, the greater proportion of rovers making it to the 100% food was due to their foraging strategy of moving between food patches more often (Sokolowski, 2001). Sitters were more content than rovers to stay on the 50% food. Since both rovers and sitters were raised on 100% food, they should have both been motivated to leave the 50% food after they were placed on it. That was the case in our preliminary testing where foods were only 1cm apart and focals were given one hour to explore. A very high proportion of rovers and sitters moved over to the 100% food. We had to double the distance and halve the time of exploration so that we did not get ceiling effects and could find an effect of social treatment if there was one. Unfortunately, the main conclusion from the rover and sitter experiment was that rovers rove and sitters tend to sit. While useful to confirm the identity of our two lines, it did not illuminate any possible differences in social information use.

After the lack of success with the rovers and sitters, we then used our standard Canton-S lab strain of larvae. Based on a lot of preliminary work, we knew they would not be as avid explorers as our rover and sitter line. That is why we decided to place the foods 1cm apart and give the focals one hour to explore. In Durisko & Dukas (2013), Canton-S larvae could tell the difference between 100% food and 50% food when these foods were very close together and would tend to settle on the higher quality food. Also, Canton-S larvae exhibited social attraction to occupied foods (Durisko & Dukas, 2013). Based on these findings, we expected our Canton-S larvae to use the social information provided by a model on the 100% food. We also expected our younger focals to rely more on the social information, as they are more inexperienced (Dukas, 2010). However, when larvae were raised on 100% food, they did not seem to use any social information (Fig. 2.2). They were able to rely on their individual information to reach the 100% food at the same rate with or without social information available. Raising larvae on 100% food should be a reasonably strong motivator to have them leave the 50% food and try to find something better. What we did end up finding was that younger focals were better than older focals at finding the 100% food. This was a bit surprising because older focals are much larger than younger focals and should have better developed sensory structures. All of the work in Durisko & Dukas (2013) used third instar larvae for food nutrition discriminations. Similarly, Schwarz et al. (2014) found that larvae were highly mobile and able to discriminate foods of different protein and sugar amounts. The larvae used were also third instar. Recent focus in our lab has turned toward the mobility of very young larvae. First to second instar larvae are actually highly mobile and can orient themselves toward patches containing yeast (Abbott, unpublished data).

We still are not sure if the differences between young and old focals making it to the 100% food are due to differences in exploration or a difference in the ability to actually find the 100% food. Focals are given one hour, which is more than enough time to explore a large proportion of the dish. Young focals may just be exploring for a greater amount of time which increases the chance of them finding the 100% food. Or, it could be the case that young focals explore just as much if not less than the old focals, but are better at finding the 100% food quickly. This is a limitation of the protocol. We do not get any fine details in the movements of the larvae. We are simply asking whether or not a larva has made it to the 100% food after one hour.

Old and young focals may be facing a different exploitation vs. exploration tradeoff. Young focals are small, but still highly mobile so they may face a lower cost for exploration and are not as motivated to exploit right away. When young focals are raised on 100% and moved to 50% they are good at leaving the 50% and finding the 100% food when it is close by. Old focals are not quite as good at doing this. Perhaps they are more motivated to exploit and less motivated to explore. Again, the protocol used does not keep track of when and where these larvae are moving. Had the results been more promising, this would have been the next set of experiments. If the larvae were biased by a single model to move over to the 100% food, the next step would have been to see if the model larva is causing the focal larva to explore more, or is it causing the focal larva to explore more successfully. The differences in exploration were only found when focals were raised on 100%, giving them an incentive to explore when placed on 50%.

When raised on 50% food, focals of both ages probably explored very little. There was little incentive for them to leave the 50% food when placed on it. It was familiar and what they had developed on. Due to this, a much lower proportion of focals moved over to the 100% food than when they were raised on 100% food. However, this was the one experiment that actually found a significant effect of social information use. Having a model larva on the 100% food caused more focal larvae to move over to it than when there was no model larva. This was especially pronounced with the older focals. This was contrary to our prediction that would’ve had the younger focals relying more on the social information. They are less experienced and thus should’ve relied more on the information provided by others. Since it was actually the older focals detecting and moving towards the model, perhaps this is due to social attraction, or ability to detect others based on development. Larvae in the late second to early third instar do tend to show the highest instances of aggregation (Durisko et al., 2014b). This could be for communal digging which would help them avoid parasitoids (Carton & David, 1985). Parasitoids may be a greater concern for larger larvae as they are easier to detect and oviposit into.

There is still the interesting question of why old focals would rely on social information when raised on 50% food but not when raised on 100% food. Perhaps they were unmotivated to move from the 50% food unless there was a model in the dish. The logical next experiment would’ve been to test for time of exploration and the time it took to leave the 50% food. That might have provided more detailed results in addition to having more statistical power.

Being raised on 50% food, they should be more content to stay on the 50% food when placed into the test dish (Fig. 2.3). This was the case in experiment 3. However, in experiment 4, many of the old focals moved over to the 100% food even though they were raised on 50% food. This failure to replicate brought into question the effect of raising the larvae on 50% or 100% food. In fact, the replicated results in experiment 4 match the results from experiment 2, when the larvae were raised on 100% food (Fig. 2.2, 2.4). There are a few possible confounds that may be responsible for this failure to replicate. The first would be the weather and conditions in the lab. The two experiments were run several weeks apart and there could’ve been large atmospheric pressure differences, which can cause the larvae to behave differently. The second source of error would be in the natural variation found in the larvae. One batch of larvae may be less likely to explore than another. In experiment 3, we might have had a batch of larvae that hardly ever explore, and then in experiment 4, we might have had a batch of larvae that are avid explorers.

A recent study corroborates some of the findings of this research. Niewalda et al. (2014) found that in an olfactory learning task, larvae tended to rely on their individual learning and ignored what the larvae around them were doing. Even if a group of larvae moved to one odour source, an individual who had learned the opposite association would not follow the group. If larvae relied heavily on social information, they would be more likely to follow the group than to follow their individual information. Or, they would at least be conflicted in which odour to go towards.

Overall, it is unclear from these experiments whether larvae do rely on the social information provided by a single model to inform their foraging decisions. We got some strong evidence for social information use but then were unable to replicate it. Had we chosen to use multiple models, instead of just one, we may have gotten a stronger effect of social treatment. The food would’ve been heavily used which could attract the focals over. However, this would’ve been quite similar to the social attraction experiments from Durisko & Dukas (2013). We decided against that, as it would not have provided much new information. Using only a single model would prove that it does not take very strong social cues for a larva to pay attention to others in its environment. Work on social information use in fruit fly larvae still needs to be refined in order to produce more meaningful, replicatable results.

**REFERENCES**

**Carton, Y. and David, J. R.** (1985). Relation between the genetic variability of digging behaviour of *Drosophila* larvae and their susceptibility to a parasitic wasp. *Behavior Genetics*, **15**, 143-154.

**Del Solar, E., & Palomino, H.** (1966). Choice of Oviposition in Drosophila melanogaster. *The American Naturalist*, **100**, 127–133.

**Dukas, R. 2010**. Insect social learning. *In: Encyclopedia of Animal Behavior* (Breed, M. & Moore, J., eds). Academic Press, Oxford, pp. 176—179.

**Durisko, Z. and Dukas, R.** (2013). Attraction to and learning from social cues in fruit fly larvae. *Proc. R. Soc. Lond. B. Biol. Sci.* **280**, 20131398.

**Durisko, Z., Anderson, B. and Dukas, R.** (2014a). Adult fruit fly attraction to larvae biases experience and mediates social learning. *J. Exp. Biol.* **217**, 1193-1197.

**Durisko, Z., Kemp, B., Mubasher, A. and Dukas, R.** (2014b). Dynamics of social interactions in fruit fly larvae. *PLOS ONE*.

**Foucaud, J., Philippe, A., Moreno, C., Mery, F.** (2013). A genetic polymorphism affecting reliance on personal versus public information in a spatial learning task in Drosophila melanogaster. *Proceedings of the Royal Society B*, **280**.

**Galef, B. G. and Whiskin, E. E.** (2001). Interaction of social and individual learning in food preferences of Norway rats. *Animal Behaviour*, **62,** 41-46.

**Mcclure, M., Morcos, L., & Despland, E.** (2012). Collective choice of a higher-protein food source by gregarious caterpillars occurs through differences in exploration. *Behavioral Ecology*, ***24*(1)**, 113–118.

**Niewalda, T., Jeske, I., Michels, B. and Gerber, B.** (2014). ‘Peer pressure’ in larval *Drosophila*? *Biology Open*.

**Rendell, L., Boyd, R., Cownden, D., Enquist, M., Eriksson, K., Feldman, M. W., Fogarty, L**. (2010). Why copy others? Insights from the social learning strategies tournament. *Science*, **328(5975**), 208–13.

**Schwarz, S., Durisko, Z. and Dukas, R.** (2014). Food selection in larval fruit flies: dynamics and effects on larval development. *Naturwissenschaften* **101**, 61–68.

**Shaver, S. A., Varnam, C. J., Hilliker, A. J. and Sokolowski, M. B.** (1998). The foraging gene affects adult but not larval olfactory-related behaviour in *Drosophila melanogaster*. *Behavioural Brain Research*, **95**, 23-29.

**Sokolowski, M. B.** (1980). Foraging strategies of *Drosophila melanogaster*: a chromosomal analysis. *Behavior Genetics*, **10**, 291-302.

**Sokolowski, M. B.** (2001). *Drosophila*: Genetics meets behaviour. *Nature Reviews Genetics*, **2,** 879-890.

**Stamps, J. A., Yang, L. H., Morales, V. M. and Boundy-Mills, K. L.** (2012). *Drosophila* regulate yeast density and increase yeast community similarity in a natural substrate. *PLoS ONE* **7**, e42238.

**Venu, I., Durisko, Z., Xu, J. P. and Dukas, R.** (2014). Social attraction mediated by fruit flies’ microbiome. *J. Exp. Biol.*

**Ward, A. J. W., Herbert-Read, J. E., Sumpter, D. J. T. & Krause, J.** (2011). Fast and accurate decisions through collective vigilance in fish shoals. *Proceedings of the National Academy of Sciences*, **108**, 2312-2315.

**Wertheim, B., Dicke, M. and Vet, L. E. M.** (2002). Behavioural plasticity in support of a benefit for aggregation pheromone use in Drosophila melanogaster. *Entomol. Exp. Appl.* **103**, 61-71

**GENERAL DISCUSSION**

This thesis has investigated the use of social information among fruit fly adults, and larvae of various ages. In each case, fruit flies had to weigh their own individual information against the social information available. Sometimes the social information led them to make poor choices. This is indicative of the value assigned to social information in the fruit fly system. Adult females were heavily biased by social information even though this caused them to make seemingly sub-optimal decisions in the lab (Chapter 1). Competition proved to be a deciding factor in larval development so females should have actually been trying to avoid laying eggs near larvae but their innate preference for that was too strong. This preference was so strong that it seemed to override cues for poor nutrition, overcrowding, and food covered in larval nitrogenous waste products. Social information use in the larvae was less reliable (Chapter 2). Based on a simple protocol of choosing to leave a sub-optimal food in favour of a better quality food nearby, we could not find consistent use of social information. Clearly there are differences in the way adult and larval fruit flies attend to, and use social information.

**Costs and benefits of social information use in adult fruit flies**

Previous research has shown that adult fruit flies do use social information for several aspects of their lives. It is used in mate choice (Mery et al., 2009), oviposition site selection (Sarin & Dukas, 2009; Battesti et al., 2012; Durisko et al., 2014a), spatial learning tasks (Foucaud et al., 2013), and deciding where to aggregate and forage (Bartelt et al., 1985). Many of these behaviours have strong fitness consequences. Fruit fly females want to maximize fitness by selecting the best oviposition sites for their offspring, selecting the highest quality mates, and foraging at the highest quality sites. Their individual information may not fully inform them of different aspects of their environment. Relying on social information allows access to the wisdom of the group. Groups are generally less likely to make errors, and thus make more accurate decisions (Wolf et al., 2013). This assumes some level of information sharing, or observation of others, which is present in fruit flies (Mery et al., 2009; Sarin & Dukas, 2009). Fruit flies can generally gain by observing and copying others as they save time and energy needed to extensively sample their environment.

The copying behaviour investigated in chapter 1 is that of oviposition site selection. Based on previous research in our lab, we knew that females greatly prefer laying eggs near actively feeding larvae (Durisko et al., 2014a). Just based on this strong preference, one can infer that there must be a fitness benefit to copying the egg laying choices of another female in the wild. While Durisko et al. (2014a) discovered and quantified this strong preference, we wanted to expand on it. The first thing we wanted to see was how much value females assigned to a food occupied by larvae when there was a high quality unoccupied site nearby. In essence, how do females weigh individual and social information when evaluating an egg laying substrate? We know that oviposition site selection is an effortful process (Yang et al., 2008). Thus, females should extensively sample their environment before settling on a substrate. We lowered the nutritional quality of the occupied food and saw if females still laid eggs on it. We found that they roughly equated an occupied food one-third the nutritional quality of an unoccupied food. Their individual information should have been telling them that this food was sub-optimal, and to lay eggs on the higher quality food nearby. However, the social information from the lower quality food biased them to lay a bit less than half of their eggs on it. When the food was incredibly low quality (one ninth the quality of the unoccupied food), females were generally good at avoiding laying eggs on it. Similar to Dugatkin (1996), females had a breaking point. At a very low nutritional quality, it didn’t matter if there were larvae on a food. Females sampled it and determined that it was unsuitable for egg-laying.

Having found that females do heavily value the social information provided by larvae at a possible egg-laying site, the next step was to see the consequences of the decisions made by the female. Perhaps the females were laying eggs near larvae because these larvae condition the food in a way that makes it more hospitable for future generations. Based on work in Durisko & Dukas (2013), we expected a negative effect of competition at higher densities, at least on artificial lab diets, which contains anti-fungal ingredients. On more natural food sources, intermediate densities of larvae seem to be more successful (Rohlfs & Hoffmeister, 2003). Using our artificial lab diet, we found that eggs develop much more slowly when surrounded by older larvae. Even when there is no direct competition and the older larvae are removed after 24 hours occupying the food, the residues they leave behind cause the eggs to develop slower. These results were only made more extreme by lowering the nutritional quality to 33%. Eggs developing in the presence of older larvae never made it to adulthood. In the lab, it seemed as though females laying eggs near larvae was actually costly. This was a counter-intuitive result given the strong preference for females to lay eggs on occupied foods. We ran the experiment again, giving the larvae abundant food. Again, eggs developing near older larvae developed more slowly than their non-social counterparts. The artificial lab diet that we use is hypernutritious and has anti-fungal compounds in it. This may have been part of the reason for seeing slower development in the social condition. Groups of larvae are better able to fight off fungal growth, which may inhibit development much more than competition (Wertheim et al., 2002). We decided not to use natural food substrates, as they are more difficult to control. Some may have had rampant mould growth whereas others may have had none. Based on the larval development experiments, we were not able to find a benefit to patch choice copying. We were only able to find a cost.

We then wanted to see if females could reduce their preference for an occupied food if it was too densely occupied. Perhaps there was a benefit to laying eggs near larvae, but only at lower densities. However, we found that females were unable to distinguish between a medium, and very high density. This suggests that the benefit for a female’s offspring to be around older larvae may be density independent up to a point. We did only test two densities. Further work could test very low densities as well to see if only a single larva can bias a female to lay eggs on a food.

Since we could not find a developmental benefit to laying eggs near larvae, we thought there could be an informational benefit. Though unlikely, it was possible that the larvae knew something about the quality of a food that a female did not. Or, that the larvae being there were indicative of quality foods for larvae, but possibly not for female flies as well. Adult females and larvae require similar nutrients but do not have identical dietary requirements (Church & Robertson, 1966). We tested the ability of larvae, and the ability of adult females to detect differences in yeast concentration, the major protein source for fruit flies. When the difference in yeast concentration was high between two foods, both adults and larvae were good at detecting the difference. When the difference was low, adult females were better than larvae at detecting it. This provided evidence against any possible informational benefits of having larvae occupying a food. Through all of our experiments, we found that fruit fly females rely heavily on social information and that there is a cost due to competition involved. We could not find a benefit in our lab, but theorize that laying eggs near larvae allows them to more easily regulate and react to their microbiome (Stamps et al., 2012; Rohlfs et al., 2005; Rohlfs, 2005). Mould and yeast growth are difficult to control in the lab, but could have provided much greater insight into the possible benefits and costs of social information use.

**Larval social information use**

Unlike the adults, it is less clear how larvae are using social information. We could not find a strong preference for being near others, like was found with adult females being socially attracted to feeding larvae (Durisko et al., 2014a). Larvae do show robust social attraction to occupied foods, likely facilitated by larval gut bacteria growing on the food (Durisko & Dukas, 2013; Venu et al., 2014). However, this generally takes a larger group of larvae (approximately 20-30 on a 2.5ml food disc) feeding for 24 hours. In our protocol, we used only a single larva as a model on a 0.5ml food disc, having fed for only 7 hours. Our goal was to show the use of social information using less obvious social cues. We zeroed in on several variables that we thought might affect the use of social information in our protocol. These were foraging allele genotype, larval age, and nutritional rearing quality. Based on work in adult flies, we expected sitters to pay more attention to social information (Foucaud et al., 2013). We also predicted that rovers would be better at the task based on their exploration and foraging strategies (Sokolowski, 2001). Only one of our predictions was supported by the data. We found no evidence for sitters paying more attention to social information than rovers. In fact, we found no evidence of either rovers or sitters using the social information to find the high quality food. We did find that rovers tended to find the high quality food more often than sitters.

When investigating the effect of focal age on social information use, we switched over to our Canton-S lab strain of flies. This is the same strain used in Durisko & Dukas (2013), and Venu et al. (2014). Those studies found social attraction to foods occupied by larva using the Canton-S strain and early third instar larvae. Thus, for our models and old focals, we used early third instar larvae. For our young focals, we used early second instar larvae, which are maybe one-fifth the size of the old larvae. We predicted that the young focals would pay more attention to social information, as they are less experienced and thus would benefit more from it (Dukas, 2010). This was not the case in either of the two rearing conditions. Focals and models were raised on either 50% food or 100% food. When raised on 100% food, neither old nor young focals seemed to pay attention to the social information. Young focals did seem to be better explorers however. When raised on 50% food, old focals did seem to be paying attention to social information whereas young focals seemed to be slightly better explorers, but indifferent to social information. When raised on 50% food, focal larvae are going from familiar to familiar in terms of nutritional quality. When raised on 100% food, focal larvae are going from optimal, to sub-optimal. However, it is worth noting that larvae raised on 50% food and larvae raised on 100% food develop at about the same rate. Based on the exploration rates between the two types of rearing conditions, it is clear that larvae are able to tell the difference between 100% food and 50% food. At the least, they are able to tell the relative difference between them.

Unfortunately, we were unable to replicate the results of the old focals reared on 50% food. This may have been due to natural variation within the larvae. Even in Durisko & Dukas (2013), only about 65-70% of larvae showed social attraction to the occupied food. The other 30-35% were indifferent to the social information, unaware that it was there, or were motivated to explore using only their individual information. Those percentages were found in later replications in Venu et al. (2014) as well. Though I used a relatively large sample size in all of my social information larval experiments, I could have been unknowingly using a batch of larvae that were indifferent or unaware of social information. Or, larvae may just be unwilling to use social information when they have individual information available. Perhaps they only rely on social information when they have no other information available. This theory is supported by recent results in Niewalda et al. (2014), which found that larvae actually ignore the actions of a group in favour of their own information and learning. In the social attraction experiments in Durisko & Dukas (2013) and Venu et al. (2014), larvae were put in an unfamiliar dish and given very little time to select a food. They likely had very little individual information available to them to make a decision. In my experiments, focals were given at least a half-hour to decide which food they wanted to be on. This was enough time for them to explore and accrue individual information about their environment. They may not have had any need to pay attention to social information as they could make an informed decision on their own.

**Conclusions and prospects**

This thesis provides strong evidence of social information overriding individual information in adult female fruit flies. It also shows potential costs of this behaviour and theorizes about some of the benefits in the natural ecology of fruit flies. There is not much evidence for social information use in the larvae but they are capable of social attraction so they must be picking up on social cues (Durisko & Dukas, 2013). It is likely that larvae do use some elements of social information in their foraging decisions but our protocol was not able to capture them. Overall, I hope that I’ve shown that fruit flies can be an excellent model system for the study of social behaviour and specifically the study of social information use.

Time permitting, for my next area of research, I’d like to see what larvae do if they are given a choice to remain on a heavily occupied food or move to a nearby unoccupied one. Larvae may be able to avoid the costs incurred by females laying near developing larvae by just dispersing to unoccupied areas. However, this may run in to the same problem of there actually being a benefit in the wild of larvae remaining in groups. It would also be odd for the female preference for where to lay eggs to not match up with the larval preference of where to forage. There should be strong selection for females to take into account where a larva will be most successful. Further investigation into the dispersal of larvae will provide greater understanding of the natural oviposition preferences of adult females.

**REFERENCES**

**Azevedo, R. B. R., French, V. and Partridge, L.** (1997). Life-history consequences of egg size in *Drosophila melanogaster*. *The American Naturalist*, **150**, 250-282.

**Bartelt, R. J., Schaner, A. M. and Jackson, L. L.** (1985). *cis*-vaccenyl acetate as an aggregation pheromone in *Drosophila melanogaster*. *Journal of Chemical Ecology* **11**, 1747-1756.

**Battesti, M., Moreno, C., Joly, D. and Mery, F.** (2012). Spread of social information and dynamics of social transmission within *drosophila* groups. *Curr. Biol.* **22**, 309-313.

**Bier, E.** (2005). *Drosophila*, the golden bug, emerges as a tool for human genetics. *Nature Reviews Genetics*, **6**, 9-23.

**Bilko, A., Altbacker, V. and Hudson, R.** (1994). Transmission of food preference in the rabbit: The means of information transfer. *Physiology & Behavior*, **56**, 907-912.

**Borash, D.J., Gibbs, A.G., Joshi, A., Mueller, L.D.** (1998). A genetic polymorphism maintained by natural selection in a temporally varying environment. *American Naturalist* **151**, 148–156.

**Carton, Y. and David, J. R.** (1985). Relation between the genetic variability of digging behaviour of *Drosophila* larvae and their susceptibility to a parasitic wasp. *Behavior Genetics*, **15**, 143-154.

**Church, R. B., Robertson, F. W.** (1966). A biochemical study of the growth of *Drosophila melanogaster*. *J. Exp. Zoo,* **162**, 337-351.

**Crow, J. F. and Chung, Y. J.** (1967). Measurement of effective generation length in Drosophila population cages. *Genetics****,* 57**, 951-955.

**Del Solar, E., & Palomino, H.** (1966). Choice of Oviposition in Drosophila melanogaster. *The American Naturalist*, **100**, 127–133.

**Dugatkin, L. A.** (1992). Sexual selection and imitation: females copy the mate choice of others. *Am. Natur.* **139**, 1384-1389.

**Dugatkin, L. A.** (1996). The interface between culturally-based preferences and genetic preferences: female mate choice in *Poecilia reticulata*. *Proceedings of the National Academy of Sciences USA*, **93**, 2770-2773.

**Dukas, R. 2010**. Insect social learning. *In: Encyclopedia of Animal Behavior* (Breed, M. & Moore, J., eds). Academic Press, Oxford, pp. 176—179.

**Durisko, Z. and Dukas, R.** (2013). Attraction to and learning from social cues in fruit fly larvae. *Proc. R. Soc. Lond. B. Biol. Sci.* **280**, 20131398.

**Durisko, Z., Anderson, B. and Dukas, R.** (2014a). Adult fruit fly attraction to larvae biases experience and mediates social learning. *J. Exp. Biol.* **217**, 1193-1197.

**Durisko, Z., Kemp, B., Mubasher, A. and Dukas, R.** (2014b). Dynamics of social interactions in fruit fly larvae. *PLOS ONE*.

**Dussutour, A., Nicolis, S. C., Despland, E. and Simpson, S. J.** (2008). Individual differences influence collective behaviour in social caterpillars. *Animal Behaviour*, **76**, 5-16.

**Eberl, D. F., Duyk, G. M. and Perrimon, N.** (1997). A genetic screen for mutations that disrupt an auditory response in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA*, **94**, 14837-14842.

**Falk, R. and Atidia, J.** (1975). Mutation affecting taste perception in *Drosophila melanogaster*. *Nature*, **254**, 325-326.

**Foucaud, J., Philippe, A., Moreno, C., Mery, F.** (2013). A genetic polymorphism affecting reliance on personal versus public information in a spatial learning task in Drosophila melanogaster. *Proceedings of the Royal Society B*, **280**.

**Galef, B. G.** (1993). Functions of social learning about food: a causal analysis of effects of diet novelty on preference transmission. *Animal Behaviour*, **46**, 257-265.

**Galef, B. G. and Sherry, D. F.** (1973). Mother’s milk: A medium for transmission of cues reflecting the flavor of mother’s diet. *Journal of Comparative and Physiological Psychology*, **83**, 374-378.

**Galef, B. G. and Whiskin, E. E.** (2001). Interaction of social and individual learning in food preferences of Norway rats. *Animal Behaviour*, **62,** 41-46.

**Galef, B. G. and White, D. J.** (1998). Mate-choice copying in Japanese quail, *Coturnix coturnix japonica*. *Anim. Behav.* **55**, 545-552.

**Grüter, C., Leadbeater, E., & Ratnieks, F. L. W.** (2010). Social learning: The importance of copying others. *Current Biology*, **20**, R683-R685.

**Hoare, D. J., Couzin, I. D., Godin, J.-G. & Krause, J.** (2004). Context-dependent group-size choice in ﬁsh. *Animal Behavior*, 67, 155–164.

**Hoglund, J., Alatalo, R. V, Gibson, R. M., & Lundberg, A.** (1995). Mate-choice copying in black grouse. *Animal Behaviour*, **49**, 1627–1633.

**Jeanson, R., Rivault, C., Deneubourg, J. L., Blanco, S., Fournier, R., Jost, C. and Theraulaz, G.** (2005). Self-organized aggregation in cockroaches. *Animal Behaviour*, **69**, 169–180.

**Justice, E. D., Macedonia, N. J., Hamilton, C. and Condron, B.** (2012). The simple fly larval visual system can process complex images. *Nature Communications*, **3**, 1156.

**Kernan, M., Cowan, D. and Zuker, C.** (1994). Genetic dissection of mechanosensory transduction: mechanoreception-defective mutations of Drosophila. *Neuron*, **12**, 1195-1206.

**Kohn, N. R., Reaume, C. J., Moreno, C., Burns, J. G., Sokolowski, M. B. and Mery, F.** (2013). Social environment influences performance in a cognitive task in natural variants of the *Foraging* gene. *PLOS ONE*.

**Lachlan, R. F., Crooks, L. and Laland, K. N.** (1998). Who follows whom? Shoaling preferences and social learning of foraging information in guppies. *Animal Behaviour*, **56**, 181-190.

**Mcclure, M., Morcos, L., & Despland, E.** (2012). Collective choice of a higher-protein food source by gregarious caterpillars occurs through differences in exploration. *Behavioral Ecology*, ***24*(1)**, 113–118.

**Mery, F., Varela, S. A. M., Danchin, E., Blanchet, S., Parejo, D., Coolen, I. and Wagner, R. H.** (2009). Public Versus personal information for mate copying in an invertebrate. *Current Biology*. **19**, 730-734.

**Niewalda, T., Jeske, I., Michels, B. and Gerber, B.** (2014). ‘Peer pressure’ in larval *Drosophila*? *Biology Open*.

**Pak, W. L., Grossfield, J. and White, N. V.** (1969). Nonphototactic mutants in a study of vision of *Drosophila*, *Nature*, **222**, 351-354.

**Rendell, L., Boyd, R., Cownden, D., Enquist, M., Eriksson, K., Feldman, M. W., Fogarty, L**. (2010). Why copy others? Insights from the social learning strategies tournament. *Science*, **328(5975**), 208–13.

**Rohlfs, M.** (2005). Density-dependent insect-mold interactions: effects on fungal growth and spore production. *Mycologia* **97**, 996-1001.

**Rohlfs, M., & Hoffmeister, T. S.** (2003). An evolutionary explanation of the aggregation model of species coexistence. *Proceedings of the Royal Society of London B*, **270**, S33–5.

**Rohlfs, M., Obmann, B. and Petersen, R.** (2005). Competition with filamentous fungi and its implication for a gregarious lifestyle in insects living on ephemeral resources. *Ecol. Entomol.* **30**, 556-563.

**Sarin, S. and Dukas, R.** (2009). Social learning about egg laying substrates in fruit flies. *Proc. R. Soc. Lond. B. Biol. Sci.* **276**, 4323-4328.

**Schwarz, S., Durisko, Z. and Dukas, R.** (2014). Food selection in larval fruit flies: dynamics and effects on larval development. *Naturwissenschaften* **101**, 61–68.

**Seeley, T. D. and Visscher, P. K.** (2004). Quorum sensing during nest-site selection by honeybee swarms. *Behav. Ecol. Sociobiol.* **56**, 594-601.

**Siddiqi, O.** (1987). Neurogenetics of olfaction in *Drosophila melanogaster*. *Trends in Genetics*, **3**, 137-142.

**Sokolowski, M. B.** (1980). Foraging strategies of *Drosophila melanogaster*: a chromosomal analysis. *Behavior Genetics*, **10**, 291-302.

**Sokolowski, M. B.** (2001). *Drosophila*: Genetics meets behaviour. *Nature Reviews Genetics*, **2,** 879-890.

**Stamps, J. A., Yang, L. H., Morales, V. M. and Boundy-Mills, K. L.** (2012). *Drosophila* regulate yeast density and increase yeast community similarity in a natural substrate. *PLoS ONE* **7**, e42238.

**Venu, I., Durisko, Z., Xu, J. P. and Dukas, R.** (2014). Social attraction mediated by fruit flies’ microbiome. *J. Exp. Biol.*

**Ward, A. J. W., Herbert-Read, J. E., Sumpter, D. J. T. & Krause, J.** (2011). Fast and accurate decisions through collective vigilance in fish shoals. *Proceedings of the National Academy of Sciences*, **108**, 2312-2315.

**Wertheim, B., Dicke, M. and Vet, L. E. M.** (2002). Behavioural plasticity in support of a benefit for aggregation pheromone use in Drosophila melanogaster. *Entomol. Exp. Appl.* **103**, 61-71.

**Witte, K., Ryan, M. J.** (2002). Mate choice copying in the sailfin molly, Poecilia latipinna, in the wild. *Animal Behaviour,* **63**, 943-949.

**Wolf, M., Kurvers, R. H. J. M., Ward, A. J. W., Krause, S. & Krause, J.** (2013). Accurate decisions in an uncertain world: collective cognition increases true positives while decreasing false positives. *Proceedings of the Royal Society B: Biological Sciences*, **280**.

**Yang, C.-h., Belawat, P., Hafen, E., Jan, L. Y. and Jan, Y.-N.** (2008). *Drosophila* egg-laying site selection as a system to study simple decision-making processes. *Science* **319**, 1679-1683.