EVOLUTION AND ECOLOGY OF

LEARNING AND SOCIALITY IN INSECTS

THE EVOLUTION AND ECOLOGY OF LEARNING AND SOCIAL LEARNING IN INSECTS

By ZACHARY T. DURISKO, B.Sc.

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ABSTRACT

Animals utilize information about their environments in order to adaptively modify behaviour. Such information may come from individual experience or from social sources, both of which have costs and benefits to the animal. Here I first show benefits of individual learning with respect to foraging performance, a good proxy of fitness, in bumblebees in a naturalistic setting. Second, I show that despite fitness costs associated with learning, fruit flies do not modify their investment in learning ability due to environmental complexity of larval foraging environment. Third, I show that fruit fly larvae utilize social information in their foraging decisions, including social learning, despite increased competition costs. Fourth, I show that adult fruit flies also use the presence of larvae as a source of social information to find suitable food patches. Finally, I show that larvae spontaneously form small foraging aggregations, one benefit of which may be an improved ability to dig and burrow into the surface of the food. I discuss the costs and benefits of both individual and social learning, as well as the potential for insect model systems in future studies of sociality and learning.

I dedicate this work to the memory of my grandfathers,
Andrew D. Durisko & Walter T. Newdick,
who both passed away while I was off chasing a dream.

Thanks Grandpa, for showing me how far one can get with hard work and a big heart, and Gramps, for showing me that life is to be filled with loved ones and enjoyed.

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I thank my mother and father for emotional support, and for their ability to love and respect who I am without always understanding what I do. I thank my brother for his continued insistence that I follow my heart, and my sister for absolutely unflinching faith and support.

Finally, I have a very special thanks to Sophia Fanourgiakis, soon to be: Sophia Durisko, without whom I would certainly never have made it. You remain an inspiration. Thank you, thank you, *thank you*, for tireless support, reminding me to relax, and keeping me on track.

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DECLARATION OF ACADEMIC ACHIEVEMENT

This dissertation is organized in a sandwich format as approved by McMaster University. Each data chapter is a complete manuscript, either published, submitted, or in preparation for submission. **Chapter 1** contains a brief introduction and overview of the research. **Chapter 2** is a published manuscript. **Chapters 3** and **4** are manuscripts currently in revision. **Chapter 5** is currently under review. **Chapter 6** is a manuscript in preparation for submission. **Chapter 7** discusses the results of the previous experiments in the broad context of other published literature and future avenues of research.

CHAPTER 1 – Overview

Author: Zachary T. Durisko

CHAPTER 2 – Effects of experience on short and long-term foraging performance in bumblebees

Authors: Zachary T. Durisko, Les Shipp, Reuven Dukas

Publication: Ethology 117:49-55 (2011)

Comments: This manuscript was conceived by ZTD and RD. LS provided support. ZTD collected and analyzed the data, and ZTD and RD wrote the MS. Reprinted with permission.

CHAPTER 3 – Effects of environmental complexity during early development on learning ability in fruit flies

Authors: Zachary T. Durisko, Reuven Dukas

Publication: Ethology (in revision – submitted Nov 2012)

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CHAPTER 4 – Social attraction and socially influenced learning in fruit fly larvae

Authors: Zachary T. Durisko, Reuven Dukas

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CHAPTER 5 – Effects of larvae on patch choice in fruit flies

Authors: Zachary T. Durisko, Blake B. Anderson, Reuven Dukas

Publication: Animal Behaviour (in revision – submitted March 2013)

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CHAPTER 6 – Dynamics of social behaviour in fruit fly larvae

Authors: Zachary T. Durisko, Reuven Dukas

Publication: In Prep

Comments: This manuscript was conceived by ZTD and RD. ZTD collected and analyzed data. ZTD wrote the MS under the supervision of RD.

CHAPTER 7 – Discussion

Author: Zachary T. Durisko

CHAPTER 1

Overview

Note that each data chapter includes a more specific introduction, so I therefore keep this general overview brief.

1.1 Introduction

Animals utilize information, cues which reduce environmental uncertainty (Dall et al. 2005), to adaptively modify their behaviour and improve decision making. This information comes from one of two sources. Either an individual explores and experiences their environment first-hand (individual information) or they monitor the interactions of others with their environment (social information). In this dissertation I explore several aspects of the evolution and ecology of individual and social information use in two insect model species: bumblebees (*Bombus impatiens*) and both adult and larval fruit flies (*Drosophila melanogaster*). These species (among others) have become important model organisms for the study of cognition, especially learning and memory (reviewed by Dukas 2008a). In particular, bees and fruit flies have become prominent models for the neuronal and genetic mechanisms of learning (eg. Hammer & Menzel 1995; Davis 2005),

much of which may be conserved among vertebrates (eg. Glanzman 2010). I use these species as models for the study of the evolution of individual learning (bumblebees) and of individual and social learning (fruit flies), focusing on the costs and benefits of each. However, the costs and benefits associated with individual and social information are different and so I begin by discussing each in turn.

1.2 Information and Individual Learning

Individual information use ranges from the simple: an innate behavioural response to an environmental cue, to the more cognitively complex: processing, learning and memory. Every living organism senses and responds to their environment, including prokaryotes like the bacteria, *Escherichia coli* (Baker et al. 2006), or the single-celled protozoan, *Paramecium* (reviewed by Meech & Mackie 2007). The benefits of such responsiveness are somewhat obvious, in that they allow an organism to interact with their environment, for example, finding food, or a site of optimal temperature. Within the animal kingdom, more complex organisms have evolved additional machinery which elaborates their means of environmental interaction, including cells specialized for the transmission, integration, and storage of information called neurons (reviewed by: Meech & Mackie, 2007; Nickel, 2010; Ryan & Grant, 2009).

The value of information rests with its ability to adaptively modify the actions of the organism (Stephens 1989; Dall et al. 2005). Some information is worthless to an individual and does not improve fitness, while other information may prove the difference between life and death, and should be attended to closely. No information is free, however, as there are costs due to the time and energy required for sampling and responding to environmental stimuli (Dall et al. 2005), and so organisms have evolved to attend only to sources of information that are relevant for fitness. For animals with nervous systems, there are additional metabolic costs of building and maintaining the requisite machinery (Niven et al. 2007; Niven & Laughlin 2008), and these costs are even greater for more cognitively complex forms of information use, such as learning and memory.

Learning is a cognitive ability defined as the acquisition of neuronal representations of new information (Dukas 2009), which allow an animal to not simply respond reflexively to stimuli in the present, but modify future behaviour. The simplest forms of learning are habituation and sensitization, defined as the dampening or enhancing of a reflex, respectively, due to stimulus exposure. Such abilities may be present in all animals with nervous systems as they are found even in basal Cnidarians, such as jellyfish (Mackie 1990; Johnson & Wuensch 1994). More complex forms of learning allow a greater deal of behavioural flexibility and precision of environmental interaction such as learning predictive associations between stimuli or actions. Overall, there are a myriad of

contexts in which learning may be beneficial to an animal, but generally, learning is expected to translate into increased individual lifetime performance (Dukas 2008b; Dukas 2009). In Chapter 2 (*Effects of short- and long-term experience on foraging performance in bumblebees*), I quantify some of the aspects of learning in bumblebee foragers that translate into increased performance in a naturalistic setting.

There are also fitness costs associated with learning. As mentioned earlier, learning requires energy, and these costs have been well documented among model species such as fruit flies (reviewed by Burns et al. 2011). The literature on the costs of learning is more thoroughly discussed in Chapter 3. Briefly, both building (*constitutive* or *global* costs) and using (*induced* costs) the neural machinery necessary for learning requires substantial energy. Importantly, the constitutive costs of learning will be experienced regardless of whether the individual utilizes their ability to learn. Given these costs, we do not expect all animals to learn, or animals to learn in all contexts, but for learning to have evolved only when the benefits outweigh the costs. The benefits of learning depend on the environment. Learning affords an animal the ability to cope with environmental change, and in particular, theory predicts that learning will be most beneficial when there exists environmental change that is neither too rapid or too slow (Stephens 1991), and is predictive of a changing optimal response (Dunlap & Stephens 2009). That is, learning is less beneficial when the environment is more constant, or when the optimal behavioural response is always the same.

To illustrate, imagine a hypothetical worker bumblebee emerging from the colony to find only plants with blue flowers, some of which have accessible nectar and others which do not. Similarly, imagine a case where there are many species of plants with a variety of flower colours, but all flowers are of equal quality. In both cases, colour does not predict how best to forage and should be ignored. In the other extreme, imagine a case where flower colour is a perfect indicator of quality, but never changes (eg. blue flowers are always the best). Flower colour becomes important information, but the best response is constant and selection will favour strong attraction to blue flowers and no learning. For learning to be beneficial there needs to be environmental variation which predicts a varying optimal behaviour. For the bumblebee forager, this might be an environment (as is typically the case in nature) where for one bee early in the season white flowers are best, but for another bee later in the season blue flowers are best. Colour varies, but is a reliable cue of quality, and bees benefit by learning to respond to flower colour with different actions.

The optimal amount of learning is different for each environment, therefore, depending on the amount of useful, predictable variation. As environments vary so too will the adaptive value of learning. A species with a fixed ability to learn may find itself in a simple environment with an unnecessary and costly learning ability or in a complex environment with a below-optimal learning ability. In Chapter 3 (*Effects of environmental complexity during early development on learning ability in fruit flies*) we investigated whether fruit fly

larvae can sense early-life cues of environmental complexity and correspondingly adjust their investment in learning ability. Such plasticity would allow an individual to reduce the constitutive costs of learning machinery when in a simpler environment where high levels of learning are not needed.

1.3 Social Information and Social Learning

Acquiring information socially can mitigate many of the costs associated with individual information acquisition, such as the time, energy and exposure to risk. By monitoring the interactions of others with the environment, an individual can adaptively modify its own behaviour without having to personally sample the environment and collect information (for reviews see: Danchin et al. 2004; Kendal et al. 2005; Dall et al. 2005; Valone 2007; Kendal et al. 2009). Such social information can either come in the form of inadvertent cues or intentional signals from conspecifics. The simplest form of social information use is an attraction to others, that is, using others as a location cue, for example, to find a suitable site for foraging. Additionally, rather than simply observing the location, individuals may monitor the performance and outcome of another's interactions to improve their assessment of the quality of a site (so-called "public information", reviewed by Valone & Templeton 2002; Valone 2007). The benefits of such social information use are faster and more accurate estimates of the environment, and therefore more adaptive behavioural responses. Coupled with learning, social

information can have an even greater impact on behaviour as it allows for the rapid spread of information within and between generations, and can lead to the formation of cultural traditions (Galef 1976; Galef 2012).

Social information use and social learning have been well documented in a variety of vertebrates and invertebrates (see reviews by: Heyes 1994; Galef & Laland 2005; Leadbeater & Chittka 2007), however, the use of social information will not always be adaptive. For example, as the frequency of social learning increases in a population, fewer individuals will be generating accurate personal information about the environment, increasing the likelihood of spreading incorrect "information" (Boyd & Richerson 1988; Giraldeau et al. 2002; Laland 2004). Theory predicts that individuals should rely on social learning more when the costs of individual sampling are high, when they do not or cannot accurately assess the environment themselves, and when their current strategy is unsuccessful (reviewed by Laland 2004). Additionally, animals should adopt strategies of selective social learning from those most likely to have valuable information (Boyd & Richerson 1988; Laland & Williams 1998; Giraldeau et al. 2002; Laland 2004; Kendal et al. 2005). Researchers have documented several different social learning strategies across taxa (eg. "*when*" and "*who*" to copy strategies, reviewed by Laland 2004; Kendal et al. 2009), and in general those species and life stages which exhibit frequent interactions among naïve and experienced individuals (eg. due to parental care or overlapping generations) are the best candidates for the evolution of social information use and social learning

(Dukas 2010). However, we still know little about the ecological factors that lead to the emergence of each particular strategy (Laland 2004; Dukas & Simpson 2009; Kendal et al. 2009). Additionally, in the literature there have been some reports of social animals failing to use available social information, despite a presumable publishing bias toward positive results (Dukas & Simpson 2009; Auld et al. 2009; Lancet & Dukas 2011; Racine et al. 2012). Such a "null" strategy may be cases where individual information is comparatively cheap to obtain, or where other individuals are unreliable. Finally, in addition to the costs and benefits afforded by the socially acquired information, there may be other costs and benefits due to the requisite social interactions. Obviously, animals must be at least somewhat *social* in order to utilize social information, and therefore social information use includes some of the costs and benefits associated with sociality. For instance, it is impossible for an animal to copy the foraging site of another without both individuals incurring the costs of increased competition.

In Chapter 4 (*Social attraction and socially influenced learning in fruit fly larvae*) we investigated the extent of social information use in fruit fly larvae. Previous work has documented social learning among adult fruit flies (Sarin & Dukas 2009; Battesti et al. 2012), and the individual learning ability, frequency of social interactions, and overlap of generations exhibited during the larval stage led us to predict that larvae, too, may show social learning (see Gerber & Stocker 2007; Dukas & Simpson 2009; Dukas 2010). We tested for social attraction and social learning among groups of larvae. Additionally, the experimental tractability

of the system allowed us to begin documenting the likely costs and benefits of such social information use. We investigated the costs associated with joining a group, and the potential informational value of a group of foraging larvae.

In Chapter 5 (*Effects of larvae on patch choice in fruit flies*), we investigated whether the larval social cues discovered in Chapter 4 would also be used by adult fruit flies. Although it is more typical that offspring rely on more-experienced adults for information, in this case the larvae are more experienced with the foraging environment, and to some degree are themselves cues of the egg-laying choices of an adult female. We tested social attraction to, and social learning from, larvae by adults.

In Chapter 6 (*Dynamics of social behaviour in fruit fly larvae*) we follow up on the sociality observed during the larval stage with a more in depth analysis of social interactions and tendency to form aggregations throughout the larval stage. Additionally we investigated one non-informational benefit of larval sociality: improved digging.

Taken together, the results in this dissertation contribute to our understanding of learning and information use among insects, particularly fruit flies, which are becoming one of the primary model organisms for future work on the evolutionary causes and mechanisms of learning, sociality and social learning.

CHAPTER 2

Effects of Experience on Short and Long-term Foraging Performance in Bumblebees

Published Manuscript:

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2.1 Abstract

Honeybees in natural settings show a gradual increase in foraging performance similar to the general pattern of lifetime performance seen in a wide variety of animals including humans. To quantify the factors contributing to such gradual increase in foraging success, we studied bumblebees foraging on pepper plants inside a greenhouse. This allowed us to combine the global measure of the net rate of food delivery to the hive with a detailed examination of bees' performance at flowers over time. While bees exhibited short-term improvements in foraging ability during their first few foraging trips, we did not observe the predicted long-term increase in performance over days. Our results suggest that a variety of flower-handling tasks, flower choice, and movements between plants can be learned quickly under the simple greenhouse settings. The long-term increase in performance under natural settings may be caused by factors including spatial

orientation and locating the best plant species, flower patches and individual plants over a large area.

2.2 Introduction

The long-term effects of learning on performance are widely appreciated in humans (Ericsson et al. 2006), but have rarely been closely examined in other species. Long-term studies in several bird species have suggested that learning over years contributes to increased reproductive success (e.g. Daunt et al. 2007; Nol & Smith 1987; Vieyra et al. 2009; Wooler et al. 1990). The avian systems, however, are not amenable to the experimental manipulation necessary for critically examining how long-term experience enhances performance.

In a series of studies, Dukas and colleagues (Dukas 2008b; Dukas & Visscher 1994; Schippers et al. 2006) documented in three different field sites and years that honeybees (*Apis mellifera*) foraging in natural settings exhibit a gradual improvement in performance such that they increase their rate of food delivery to the hive over much of their foraging life. Such lifetime performance curves are similar to the pattern known for birds and mammals including humans (Dukas 1998; Dukas 2008a; Helton 2008). Honeybees, however, only showed a rapid improvement followed by a long-term plateau in performance when they were allowed to forage on feeders placed 400 m from the hive, which provided unlimited volumes of sugar water (Dukas 2008a).

The distinct lifetime patterns of performance in natural vs. artificial settings strongly suggested that bees foraging in the field learn a variety of tasks that together contribute to a gradual, long-term improvement in performance. Such tasks could include long-distance navigation, identification of the most profitable plant species, flower patches and perhaps individual plants in such patches, improved movements between flowers and plants, better flower handling techniques, and superior motor skills. Evidence for short-term improvements owing to the factors just mentioned exists for both honeybees and bumblebees (e.g. Burns & Thomson 2006; Capaldi et al. 2000; Cartar 2004; Heinrich 1979; Laverty & Plowright 1988; Raine & Chittka 2007; Raine & Chittka 2008). The effects of long-term experience, however, have been rarely quantified.

To measure the factors that contribute to long-term improvements in performance, we must integrate direct observations on foragers in the field with data on the weight of food delivered and duration of each trip. Because we cannot follow individual bees initiating foraging to their chosen flowers in natural settings, we compromised by setting up an experiment inside a greenhouse. Another compromise involved using bumblebees (*Bombus impatiens*), which are more suitable than honeybees for foraging in confined spaces (Sabara & Winston 2003; Shipp et al. 1994; Velthuis & van Doorn 2006).

We attempted to identify what features of bees' foraging behaviour contributed to their overall increase in foraging performance. Specifically, in addition to measuring the weight of floral reward per trip and trip duration, we also video

recorded bees from the time they left the hive throughout each foraging trip. We then quantified the durations of orientation flights, flower handling times, lengths of inter-flower flights, frequencies of successive revisits to the same flower and the rate of visits to unrewarding plants. We predicted an overall increase in food delivery rate over a few days and expected both short and long-term improvements in all the foraging components measured.

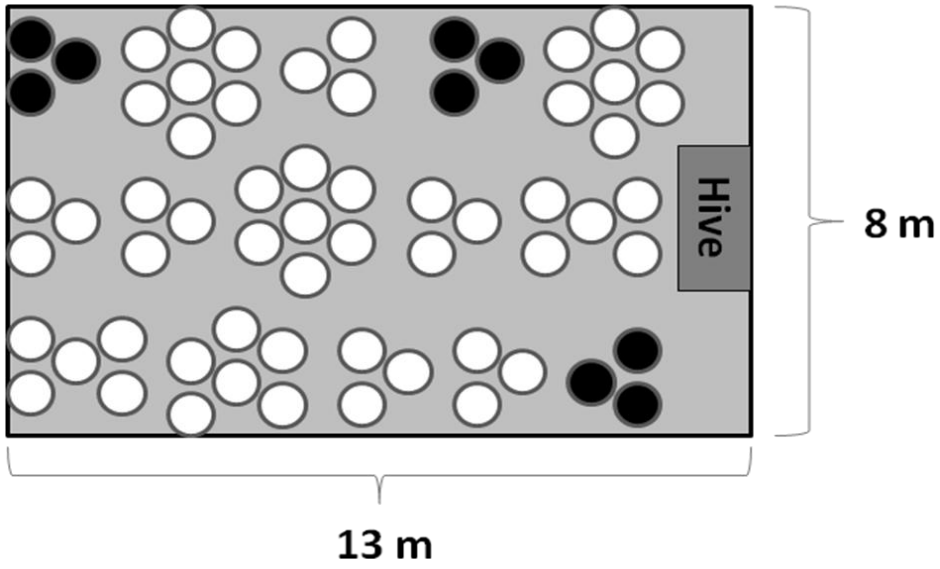
2.3 Methods

We conducted the research inside a climate-controlled greenhouse (13x8x5 m in length, width and height respectively) at the Agriculture and Agri-Food Canada Research Centre in Harrow, Ontario in May - June 2009. Temperature and relative humidity were automatically recorded every 15 minutes. The average (±SE) temperature and relative humidity during data recording (10 AM to 4 PM) were 26 ±0.12 °C and 58 ±0.6% respectively. The greenhouse chamber was decorated with coloured posters and artificial flowers to simulate a natural setting and to provide distinct landmarks.

We used 64 potted sweet pepper plants (*Capsicum annum*) grown according to standard commercial practices (Shipp et al. 1994). For ecological realism, the plants were randomly sorted into 15 distinct patches such that there were 9 patches of 3 plants, 2 patches of 5 plants, 1 patch of 6 plants and 3 patches of 7 plants. The foraging arena was divided into 15 sections and each

section was randomly assigned a patch (Fig. 2.1). This distribution of patches was held constant for the duration of the experiment. The distance between patches was at least 1 m and the distance between adjacent plants within a patch was 0.5 m. Three randomly selected patches of 3 plants were designated as the ‘bad’ patches. In these three patches, we removed pollen and nectar from all flowers each morning before the bees commenced foraging. We plucked the anthers from each flower with tweezers and used the rolled up edge of a Kimwipe to remove the nectar while taking care not to damage the flower. Twice per day, a small drop of water was added to each flower so that any newly secreted nectar would be highly diluted.

Figure 2.1 The distribution of unmanipulated plants (○) and rewardless plants (●) in the greenhouse.



Preliminary investigations revealed that the average nectar secretion rate per unmanipulated flower was 0.194 ±0.035 µL/hour (Mean ±SE; 62 recently-opened flowers sampled over three days, measured from 10am to 2pm, corresponding to peak nectar production). Nectar secretion of the 'bad' flowers was not measured as the added water diluted the sugar concentration of any nectar to effectively zero. Nectar production varied as a function of flower age and time of day (Roldan Serrano & Guerra-Sanz 2004). In the absence of bees, the average nectar volume in 24 flowers on their first day of anthesis was

significantly greater than on the following morning (2.0 ±0.27 µL vs. 0.3 ±0.19 µL, one-tailed paired-samples t-test: $t_{23} = 5.9$, $p < .0001$). Indeed, most flowers (20/24) contained no nectar on the second day. The number of flowers varied naturally throughout the experiment with an average of 7.1 ± 0.13 flowers per plant (Mean ± SE; 64 plants measured across 17 days of the experiment). Thus individual 'good' patches exhibited natural variation in quality, but were always more rewarding than the 3 'bad' patches. During the experiment, we removed early fruits to promote further blooming.

We obtained a small colony of bumblebees (*Bombus impatiens*) from a commercial provider (Biobest Canada Ltd., Leamington, Ontario, Canada). We monitored the colony's food levels and supplemented it with sugar water (~60% w/w) and pollen when necessary. Before the start of the experiment, we marked all bees with a dot of paint on the thorax at night under red light. On the following day, any of these marked bees seen foraging were removed from the hive. Newly eclosed naïve foragers were uniquely marked upon their first attempt to leave the hive and were subsequently allowed to forage one at a time. A Plexiglas observation tunnel (Dukas & Visscher 1994) was attached to the hive such that the weights of foragers could be recorded and flights restricted as necessary.

On each day, we allowed up to four bees to exit the hive and forage one at a time between 10:00am and 4:00pm. Due to the limited number of flowers available in the greenhouse, each bee was restricted to 3 trips per day. We

weighed the bees as they departed and arrived at the hive by placing a removable section of the observation tunnel on an analytical balance (Mettler Toledo, AB54-S) with a precision of 0.1 mg. We followed each bee with a handheld digital video camera (Sony Handycam, DCR-HC42), dictating plant choice and describing behaviour. A single observer (ZD) moved gently and utilized the camera's zoom function to avoid disturbing the bees.

Following the experiment, we randomized the video files so that the observer was blind to an individual bee's experience and analyzed the videos using The Observer 5.0 computer software (Noldus Information Technology, Wageningen, Netherlands). For each trip, we quantified the duration of orientation flights, the duration of each flower visit, and the duration of flights between flowers. Orientation flights were defined as the interval between leaving the hive and arriving at the first plant. Flower visits were defined as the entire time a bee was in physical contact with a flower. The sum of orientation flights and the time spent in and between flowers constituted the total trip duration. Due to the occasional difficultly that our bees experienced in finding the entrance to the Plexiglas tunnel, we considered the end of a trip to occur when each bee left her final foraging patch, thus excluding the time to return to the hive from our analysis. These return flights were always very direct, never lasting more than a few seconds. We also recorded for each trip the frequency of visits to unrewarding plants, and the frequency of immediate revisits, defined as the number of successive visits to the same flower over the total number of flower

visits per trip. We then coupled the detailed video analyses with the data on net weight of food delivery to the hive (arrival minus departure weight) and calculated the net rate of food delivery (net weight divided by trip duration).

We focused on two levels of analysis: 1) Long-term experience over 5 days, and 2) Short-term experience using the first 50 recorded flower visits and first 30 recorded between-flower flights. We recorded an average of 28.7 ±1.6 (Mean ±SE) flower visits per foraging trip, meaning that short-term experience often spanned a bee's first few trips. Because we allowed only one bee to forage at a time, the short-term experience included all time periods between 10 AM to 4 PM. The data for short-term experience were sorted into blocks of either 5 flower visits or 5 inter-flower flights. The data set for long-term experience included 5 bees that initiated foraging on 4 different days, and the data set for short-term experience had 9 bees that started foraging on 7 different days (except for the between-flower flights, which had only 8 bees owing to missing data). The distinct start dates of bees reduced the chance of confounding day effects with experience. Statistical analyses were conducted with PASW Statistics 18 (SPSS Inc., Chicago, Illinois, USA). The analyses involved repeated-measures ANOVAs, using Huynh-Feldt corrected degrees of freedom when assumptions of sphericity were violated.

2.4 Results

2.4.1 Long-term experience

The average daily rate of food delivery did not improve significantly with experience (repeated-measures ANOVA: $F_{4,16} = 0.6$, $p = .660$, Fig. 2.2). Breaking down the rate information into its components, the average net weight of food and mean trip duration also revealed no significant improvement over 5 days ($F_{2.6,10.3} = 0.2$, $p = 0.8$, $F_{4,16} = 1.2$, $p = 0.3$, respectively). The average duration of flower visits ($F_{4,16} = 0.8$, $p = 0.55$), the average duration of flights between flowers ($F_{4,16} = 0.6$, $p = 0.65$), and the frequency of trips to the 'bad' patches ($F_{4,16} = 2.6$, $p = 0.07$) were all unaffected by long-term experience. Further analyses of these measures comparing day 1 to day 5 revealed no significant differences: rate of food delivery ($F_{1,4} = 1.4$, $p = 0.31$), net weight ($F_{1,4} = 0.3$, $p = 0.61$), trip duration ($F_{1,4} = 3.8$, $p= 0.12$), duration of flower visit ($F_{1,4} = 3.4$, $p = 0.14$), duration of flights between flowers ($F_{1,4} = 0.6$, $p = 0.58$), and frequency of trips to bad patches ($F_{1,4} = 5.0$, $p = 0.09$). In contrast, the average duration of orientation flights declined over the five days ($F_{4,16} = 68.5$, $p < 0.001$; Fig. 2.3), and the rate of immediate revisits showed a non-significant decline with experience ($F_{2.1,8.4} = 2.4$, $p = .15$; Fig. 2.4).

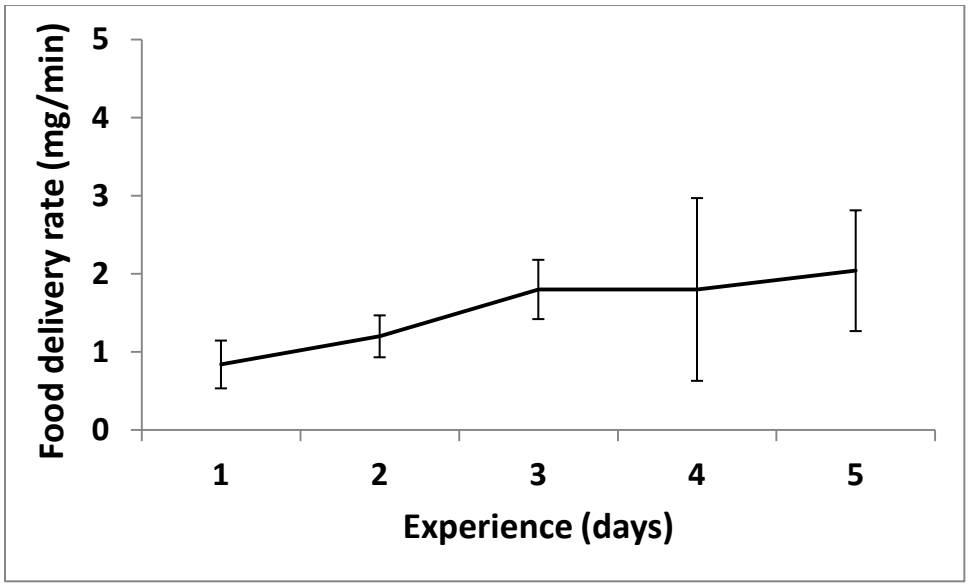


Figure 2.2 Mean (±SE) rate of food delivery across days (N = 5 bees).

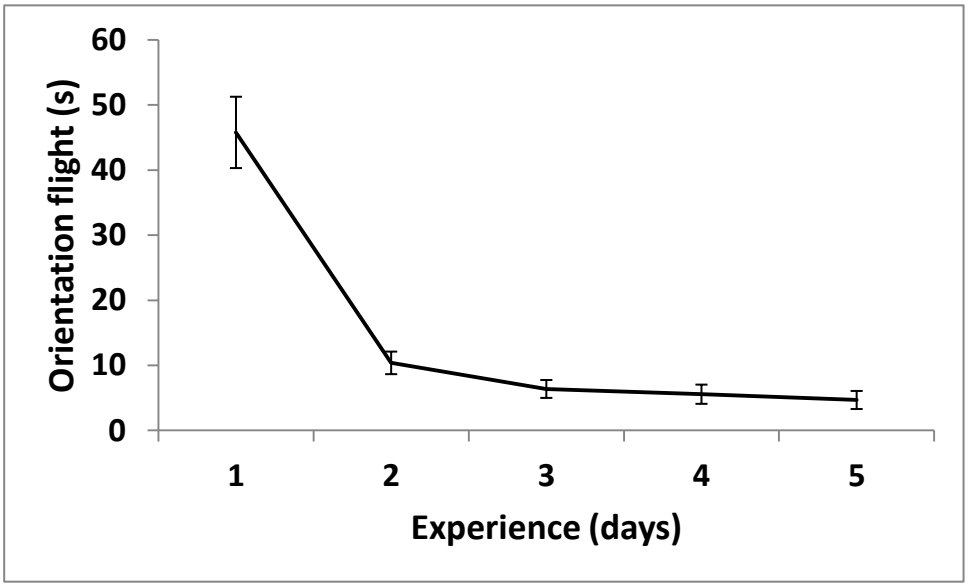


Figure 2.3 Mean (±SE) orientation flight duration over 5 days of foraging (N = 5 bees).

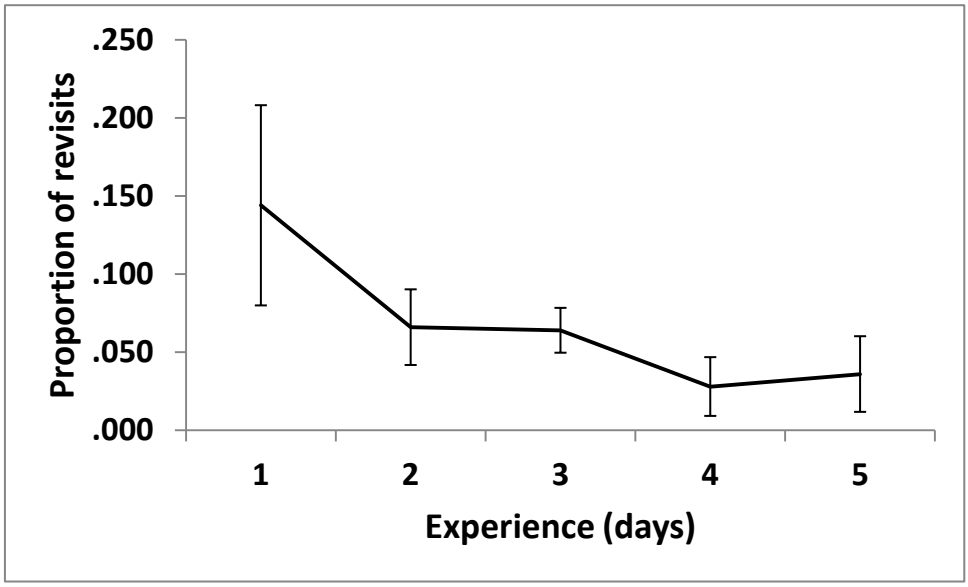


Figure 2.4 Mean (±SE) rate of immediate revisits (N = 5 bees).

2.4.2 Short-term experience

Experience through the first 50 flower visits resulted in a significant reduction in flower visit duration (repeated-measures ANOVA: $F_{9,72} = 5.8$, $p < 0.001$; Fig. 2.5). Post hoc within-subject contrasts revealed that much of this improvement occurred within the first 10 flower visits (Bonferroni adjusted for 9 comparisons, $\alpha = 0.0056$; visits 1-5 vs. visits 6-10: one-way paired $t_8 = 3.4$, $p = 0.005$). A similar analysis using only the 5 individual bees included in the long-term data set indicated a comparable short-term improvement in flower visit duration over the first 50 flower visits ($F_{9,36} = 2.8$; $p = 0.012$). Short-term experience was also associated with a reduction in the rate of immediate revisits (repeated-measures ANOVA: $F_{9,72} = 2.8$, $p = 0.007$; Fig. 2.6). In contrast, bees showed no significant reduction in the duration of flights between flowers ($F_{5,35} = 1.4$, $p = 0.242$, Fig. 2.7).

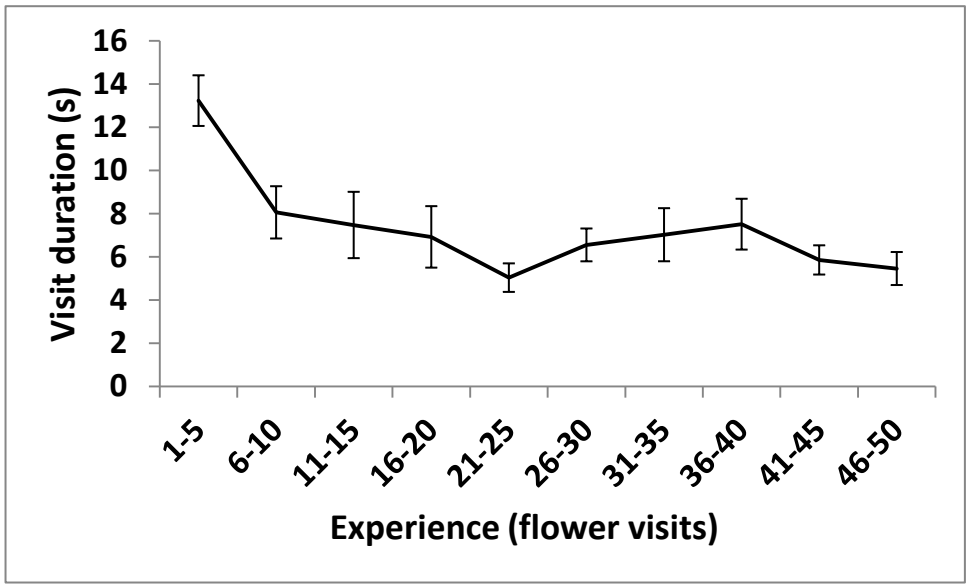


Figure 2.5. Mean (±SE) flower handling duration. Foraging experience is sorted into blocks of 5 flower visits (N = 9 bees).

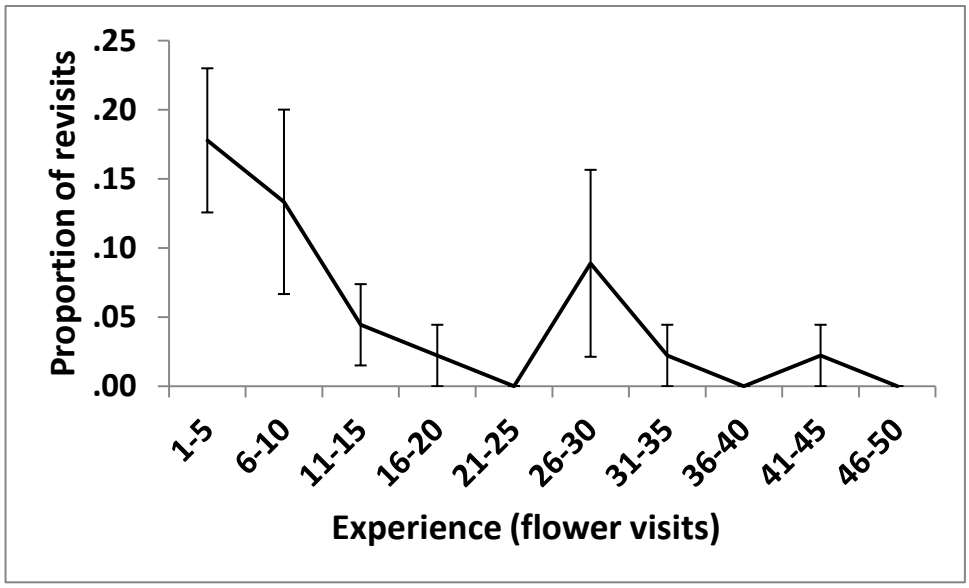


Figure 2.6 Mean (±SE) proportion of immediate revisits per visit. Foraging experience is sorted into blocks of 5 flower visits (N = 9 bees).

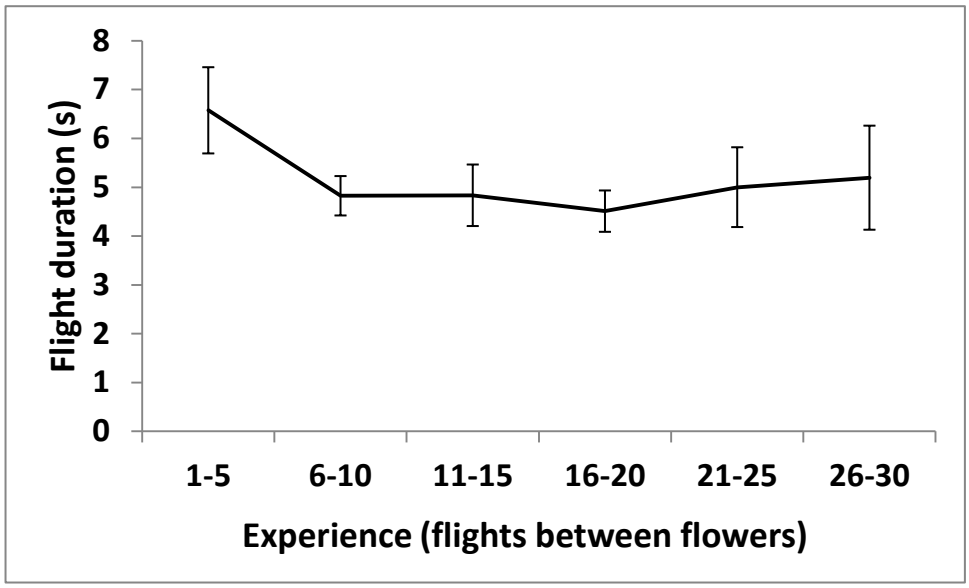


Figure 2.7 Mean (±SE) duration of flights between consecutive flower visits. Foraging experience is sorted into blocks of 5 between-flower flights (N = 8 bees).

2.5 Discussion

Our main goal was to test for long-term improvements in foraging performance and quantify the major contributors to such changes over several days. We found, however, no consistent long-term improvements in the net rate of food delivery to the hive (Fig. 2.2). While our analyses indicated significant reduction in the average duration of orientation flights, this can clearly be attributed to short-term changes over the first few trips (Fig. 2.3). Such rapid decline in the length of orientation flights is also known in honeybees (Capaldi et al. 2000).

In contrast to the analyses over days, our refined examination of each flower visit indicated that bees rapidly reduced their average flower handling time (Fig. 2.5). Such short-term improvement has been well documented in bumblebees (e.g. Heinrich 1979; Laverty 1994; Laverty & Plowright 1988; Raine & Chittka 2007; Raine & Chittka 2008). The bees also quickly improved their ability to orient on plants as indicated by their reduction in the average frequency of immediate revisits (Fig. 2.6). Similar overall reduction in revisits was also observed in bees foraging on artificial flowers (Saleh & Chittka 2007). Contrary to our expectation, bees showed no significant short-term reduction in inter-flower flight durations (Fig. 2.7). It appears, however, that the bees improved over their first several flower visits, but that this improvement was masked by large variation caused by chance differences in inter-flower distances at the vicinity of the flowers visited early on each trip. While the random spatial distribution of flowers would make locating flowers a prime candidate for long-term

improvement owing to learning, the fact that each pepper flower blooms for only two days and is mostly rewarding for a single day probably reduces some of the potential for long-term improvements in inter-flower flight durations.

We can think of three explanations as to why the bumblebees did not show the predicted long-term improvement in foraging performance. First, it could be that, unlike honeybees, which took a long time to improve their foraging performance in natural settings (Dukas 2008b; Dukas & Visscher 1994; Schippers et al. 2006), bumblebees can reach their maximal performance level very rapidly. We believe that this is an unlikely possibility because all species carefully examined under realistic settings show long-term improvements in performance (reviewed in Dukas 1998; Dukas 2008a; Helton 2008). Further studies will be necessary to critically reject this possibility.

The other possible explanation for our results is that the bumblebees could reach asymptotic performance after only a few foraging trips because of the relative simplicity of foraging on a single plant species near the hive inside a greenhouse. That is, prior to our experiment, we could readily envision how a few foraging components would contribute to a gradual increase in performance in bumblebees foraging in a small field of pepper plants. Specifically, such factors include identification of the most profitable individual plants (Burns & Thomson 2006; Cartar 2004; Thomson 1988), improved movements between flowers and plants (Ohashi & Thomson 2009), and better flower handling techniques. The

current study was restricted to a small number of patches confined to a small area compared to natural bee foraging. This allowed us to conduct detailed observations but resulted in a relatively low cost of visiting 'bad' patches. This cost further declined as the rewards available in good patches were depleted. A much larger foraging array consisting of multiple plant species with variation in floral complexity arranged into more realistic patches of different species combinations may allow the observation of further long-term improvements. Such a large array, however, would reduce our ability to closely monitor bees.

Finally, it is possible that our failure to detect long-term improvements resulted from a low statistical power. As noted in the results section, however, the same individual bees that showed no significant long-term improvement did show significant short-term improvement. Thus it is unlikely that we would be able to detect more than a small improvement in performance under our experimental settings even with greater power.

There is good evidence that learning allows bees to reduce flower handling time and reach asymptotic performance after visiting fewer than 100 flowers (e.g. Heinrich 1979; Laverty 1994; Laverty & Plowright 1988). The true measure of foraging performance, however, is the amount of food gathered over time as we have quantified in the current study and previous work with honeybees (Dukas 2008b; Dukas & Visscher 1994; Schippers et al. 2006). Interestingly, perhaps the only other study that examined bumblebees' (*B.*

terrestris) performance measured as floral reward collected over time documented no asymptotic performance even after visiting over 300 flowers (Raine & Chittka 2007). The latter study agrees with our assertion that bumblebees, like honeybees, would show a gradual increase in foraging performance, achieving a peak only after a few days of foraging experience in sufficiently complex settings.

In addition to the factors we could measure in the greenhouse, natural settings add other dimensions of difficulty. First, bees have to locate profitable flower fields, sample a variety of available plant species and then focus on the one or a few most profitable species (Heinrich 1979). Second, unlike the relatively homogeneous greenhouse settings, individual variation in nectar secretion rate within a plant species may be rather high owing to genetic variation and differences in soil type, moisture and herbivory (e.g. Kaczorowski et al. 2008; Nicolson et al. 2007; Pleasants & Zimmerman 1979; Zimmerman 1981). Third, new foragers face a major challenge of locating the best flower patches within perhaps a few kilometres from the nest and navigating successfully back to the hive. Although there have been excellent studies exploring bees' spatial orientation (e.g. Capaldi et al. 2000; Menzel et al. 2005; Osborne et al. 1999), we still do not know whether long-term navigational experience allows bees to locate farther and more profitable food sources. Our results still leave open the possibility that spatial learning and navigational improvements on such a large

scale may be the driving force behind the performance curves observed in natural settings (Dukas, 2008b).

In sum, our attempt to link long-term improvements in foraging performance observed in natural settings with controlled observations in the greenhouse have failed because bees under the simpler settings showed rapid improvement in foraging ability. Our results suggest that long-term improvements in foraging success may be related to complex tasks including spatial orientation and learning to favour the best plant species, patches and individual plants over a large area.

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CHAPTER 3

Effects of Environmental Complexity During Early Development on Learning Ability in Fruit Flies

Manuscript in revision: Ethology (submitted Nov 2012)

3.1 Abstract

Learning and memory require the development, modification and maintenance of brain tissue, which cost time and energy. It may be adaptive for developing animals to adjust such investments based on environmental cues indicating the future utility of learning. The optimal learning ability that maximizes fitness will vary with the degree of complexity or difficulty of the environment, and developing animals may show an adaptive plastic modification of the extent of their learning ability based on early-life cues of environmental complexity. We tested whether fruit fly larvae reared in a “complex” environment, where they had to search, sample, and choose between three foods differing in flavour and bitterness subsequently possessed greater learning abilities than larvae reared in a simple environment with only one food type. We tested learning ability both at

the larval stage and in young adults. Our results suggest that, despite theoretical and intuitive appeal, these environmental factors did not affect learning ability.

3.2 Introduction

It is typically assumed that learning, defined as the ability to acquire neuronal representations of new information (Dukas 2004), will only emerge and persist in a population when its fitness benefits outweigh costs (Stephens 1991; Mery & Kawecki 2005; Dunlap & Stephens 2009). The benefits of learning can often be quite intuitive, but in general, an ability to learn allows an individual to increase its rate of resource acquisition with experience (for example, to increase the units of food or mates encountered per unit time), which typically translates into an increase of individual fitness. Researchers have documented these benefits across several ecological contexts in many laboratory experiments (Siegel & Hall 1979; Gailey et al. 1985; Dukas & Duan 2000; Dukas & Bernays 2000; Dukas 2005a), and under more natural settings (Nager & Noordwijk 1995; Grieco et al. 2002; Raine & Chittka 2008; Dukas 2008b; Dukas 2008c; Durisko et al. 2011).

There are also fitness costs associated with learning and memory. Learning and memory require brain tissue, which is metabolically expensive for an organism to develop and maintain (Laughlin et al. 1998; Niven & Laughlin 2008). Specifically, learning requires both the initial constitutive, or *global*, investment to develop the brain structure performing the learning and also the *induced* cost of building and maintaining each particular memory (Snell-Rood et

al. 2009; Burns et al. 2011). Artificial selection experiments on fruit flies have shown that an increased learning ability is correlated with a decline in larval competitive ability and a reduction of longevity, regardless of whether an individual utilizes its learning ability, suggesting a cost of the initial investment in the ability to learn (Mery & Kawecki 2003; Burger et al. 2008). Furthermore, the act of forming a long-term memory has itself been associated with a reduction of both survival under environmental stress and fecundity in fruit flies (Mery & Kawecki 2004; Mery & Kawecki 2005). Snell-Rood and colleagues have shown that better learning cabbage white butterflies (*Pieris rapae*) have fewer eggs and also that the learning process itself can reduce fecundity (Snell-Rood et al. 2011). Finally, all learners begin life inexperienced and typically exhibit an initial phase of poor performance, during which the time spent learning instead of acting may constitute an opportunity cost (Stephens 1991; Dunlap & Stephens 2009; Eliassen et al. 2009).

The ecology and neurodevelopment of each particular animal determine the costs and benefits of learning, and the balance of these selective pressures dictates the optimal degree of learning for each environment. Learning is more beneficial in some environments than others (Stephens 1991; Dunlap & Stephens 2009; Eliassen et al. 2009), and therefore each environment may have a different optimal degree of learning ability. A given learning ability may not be sufficient to succeed in environments that are more complex but the same ability may be an excessive waste of time and energy in simpler environments. For

animals that experience variation in environmental complexity, developing an appropriate or optimal degree of learning ability is a challenge with important fitness consequences. One way that animals may evolve to cope with such environmental variation is with adaptive developmental plasticity (Pigliucci 2001; Dukas 2004; Snell-Rood et al. 2010). Avoiding the costs of learning whenever possible would provide an adaptive advantage. If an animal is able to assess the future value of learning, it may be able to adaptively modify the amount of time and energy invested in learning ability (Snell-Rood et al. 2009). For example, an animal experiencing cues that its future environment is likely to be very simple should reduce the energy and time devoted to developing brain tissue associated with learning. In many species, brains develop throughout early life and have the potential to be highly plastic.

We sought to document adaptive plasticity of learning ability in fruit flies (*D. melanogaster*). Female fruit flies seek out appropriate food sources in their local environment and lay eggs directly onto the surface of the food. Larvae spend much of their time eating, and we hypothesized that their foraging experience may be a relevant cue of the future utility of learning. Neurodevelopment continues throughout the larval stage, including neurogenesis in the mushroom bodies, brain structures critical for learning (Ito & Hotta 1992; de Belle & Heisenberg 1994; Tettamanti et al. 1997; Fahrbach 2006; Campbell & Turner 2010), and although the brain undergoes substantial reorganization during metamorphosis (Armstrong et al. 1998), increased neurogenesis due to

environmental complexity could lead to improved learning abilities in later life. We imagined a scenario where environmental cues that indicate the future utility of learning would increase an animal's investment in mushroom body neurogenesis, which has been associated with improved learning ability (Snell-Rood et al. 2009). We hypothesized that the complexity of the larval food environment would be an ecologically relevant cue for the plasticity of learning ability and that fruit flies have evolved to adjust their investment in learning ability such that flies experiencing a challenging early-life environment would subsequently show greater learning abilities than those from a simple environment. Specifically, we predicted that early-life larval exposure to multiple food types of varying flavour and bitterness would result in an increased learning ability compared to early-life larval exposure to a single food type. We conducted two experiments, testing the appetitive learning ability of both larval and adult life stages after exposure to either a *complex* or *simple* early-life environment.

3.3 Methods

3.3.1 General methods

We maintained two population cages of several hundred *Drosophila melanogaster Canton-S* on abundant standard food, one liter of which contained 75 g cornmeal, 20 g agar, 60 g dextrose, 30 g sucrose, 32 g yeast and 2 g methyl paraben. We kept flies at 25°C, 60% relative humidity, and on a 12:12

light/dark cycle with lights on at 11 pm. This irregular light cycle allowed peak egg laying to occur midday so that we could collect experimental eggs within a very small window of time (1.5 h). We collected eggs on 90 mm petri dishes filled with 10 mL of standard food and covered with 0.7 mL of live-yeast suspension to stimulate egg laying (30 g/L of warm water; Sarin & Dukas 2009). We kept egg density low (<200) to optimize larval development. Immediately following egg laying, we moved egg dishes to an incubation chamber maintained at 25°C, high humidity and total darkness. We conducted all further manipulations under red light, which fruit flies cannot see (Bertholf 1932), in order to minimize disturbance. First instar larvae fed on abundant standard food for 24 h. On day 1 of the experiment, we gently rinsed larvae from the food medium with water and moved 50 randomly selected early-second-instar larvae next to the food in each of the treatment dishes (see below).

For Experiment 1, where we tested larval learning ability, it was especially critical to control larval age and stage of development, so we added two additional steps to minimize variation. First, prior to experimental egg collection, we allowed females to lay eggs for 1 h on dishes that we discarded. This ensured that females did not lay partially developed embryos during experimental egg collection. Second, at 7 am on the day following egg laying, two hours prior to the expected start of hatching (expected 22 h following egg laying), we manually removed any early-hatching larvae.

3.3.2 Treatments

To create simple and complex environments, we modified successful insect protocols, which varied the number and qualities of alternative food sources to create simple and complex environments (Dukas & Real 1993a; Dukas & Real 1993b; Bernays 1998; Bernays & Funk 1999; Gegear & Laverty 2005). Each treatment received identical nutrients but different arrangements of food in 60 mm petri dishes containing a thin base of agar and methyl paraben (2 g/L) (Fig. 3.1). We flavoured food with 20 mL/L of commercially available flavour extract: anise, lemon, or mint. The *simple* treatments consisted of a single food flavour and quality in a single patch containing 0.3 mL food. The *complex* treatments consisted of all three flavours, one of which had added quinine (2.5 g/L of quinine hydrochloride, Sigma), which tastes bitter and is aversive to larvae (Dukas 1999), arranged into three separate patches each containing 0.1 mL food located 1 cm apart. On the mornings of days 2 and 3, we added new food to the dishes, directly to the large patch in the *simple* treatments, and by creating new small patches in the *complex* treatments for a total of nine small food patches (Fig. 3.1). Note that all dishes received identical quantities of food. We added food daily instead of providing food in abundance at the outset so that the larvae from the *complex* condition depleted the small patches, forcing them to search, sample and choose whether to feed on the other flavours, likely experiencing the multiple flavours and food qualities in their environment. Larvae typically consumed the entirety of a food patch and thus on subsequent days were forced

to either try a new flavour or search for the previous flavour, both of which may be cues of environmental complexity. In the *simple* dishes, larvae were free to crawl and dig in one abundant food patch. We accounted for all flavour and quinine combinations with six treatments, three *simple* and three *complex*, respectively: (1) anise, *A*; (2) lemon, *L*; (3) mint, *M*; (4) anise, lemon, and mint-quinine, *ALMq*; (5) anise, lemon-quinine, and mint, *ALqM*; (6) anise-quinine, lemon, and mint, *AqLM*. For the test of larval learning ability, larvae experienced these treatments for 72 h (from 24-96 h old) including almost all of their second instar and approximately half of their third instar stage. For the test of adult learning ability, we left larvae in these dishes until eclosion.

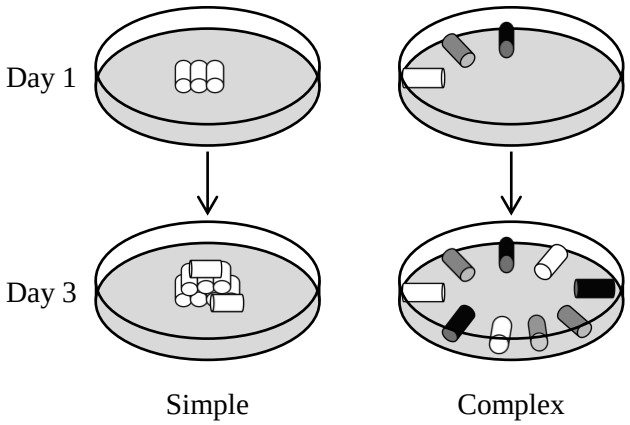

Figure 3.1 We gave larvae identical nutrients in either a *simple* or a *complex* foraging environment. In simple treatments (left), we gave larvae one flavour and quality of food in a single patch. In complex treatments (right), we gave larvae three flavours of food, one of which contained added quinine, which tastes bitter, arranged in separate patches. We added food to all dishes each day, into one large patch for the simple treatments and for a total of nine small patches in complex treatments. Flavours used were lemon, anise and mint.

3.4 Experiment 1 – *Larval Learning Ability*

On the morning of day 4, at 96 h old, we assessed the learning ability of the larvae. We chose this time so that larvae had extensive experience with their foraging treatment while remaining well within the feeding stage of their third instar. We first collected the larvae from the dishes with a soft paintbrush and rinsed them in small droplets of water. We collected all living larvae and were therefore able to calculate the mortality rate for each dish. The overall mean mortality rate was very low, 0.049 ± 0.005 (mean ± SE) larvae per dish, corresponding to 47.5 ± 0.2 living individuals per dish. Mortality did not differ between simple and complex dishes ($F_{1,54}$ = 2.644, p = 0.110) or flavours (nested within complexity; $F_{4,54}$ = 0.161, p = 0.957).

Pupation typically occurs more than 24 h after our chosen time of testing. Approximately 8 h prior to the formation of the puparium, or prepupa, third instar larvae cease feeding and enter the wandering stage (Roberts & Standen 1998). Wandering stage larvae would largely ignore the unconditioned food stimulus in our test of learning ability and would therefore have spuriously lower learning scores. To ensure that we tested larvae prior to wandering, we tested all larvae between 9 AM and 12 PM and after each test we placed the larvae onto plain agar dishes where they could pupate. We reasoned that any larvae in their wandering stage during testing would begin pupation within 8 h without additional food. We counted pupae at 8 PM from 54 dishes (missing six due to experimental error), and again at 9 AM the following day to assess differences

between treatments. Very few larvae entered pupation in the 8 h following the test (1.1 ± 0.4 individuals per dish, or 2.4 ± 0.9% of larvae per dish). There was no difference in the proportion of larvae entering pupation at 8 PM between the simple and complex dishes, (respectively, 2.7 ± 1.7% and 2.1 ± 0.7%; $F_{1,48} = 0.2$, $p = 0.67$) or among the different flavours ($F_{4,48} = 0.9$, $p = 0.50$). At 9 AM the following day, 21-24 h after testing, there were 10.6 ± 1.4 pupae per dish, or 23.8 ± 3.5%, and there continued to be no difference between simple and complex dishes ($F_{1,54} = 1.9$, $p = 0.17$) or flavours ($F_{4,54} = 1.3$, $p = 0.29$).

3.4.1 Larval training and test

The learning test consisted of a group reciprocal conditioning assay with one of two novel odours paired with fructose-flavoured agar (2 mol/L) as a rewarding stimulus (the “rewarded odour”) and the other paired with plain agar (similar to Aceves-Piña & Quinn 1979; Dukas 1998; Scherer et al. 2003; Neuser et al. 2005). We conducted all training and tests under a fume hood. We balanced the odour paired with fructose across replicates to control for any innate odour preference. We rotated the order in which we tested the six treatments and balanced which training odour/food pair we presented first to control for any order effects. We used the chemical odorants 1-butanol (BUT; Fisher) and propyl acetate (PA; Sigma) and we diluted the latter 1:300 in paraffin oil (a concentration at which naïve larvae preferred the two odours approximately

equally in preliminary trials). Both odours are strongly attractive to larvae, and have been used in similar larval learning tests (Kaun et al. 2007). For each training session, we filled a small plastic cup (polypropylene NMR tube caps, Sigma) with 10 uL of odourant and placed it onto the center of a 60 mm petri dish filled either with plain agar or with fructose-flavoured agar. We placed larvae directly into these dishes en masse with a paintbrush. The petri dish lids remained on the dishes during training so that odours vapours collected in the dish, but we perforated each lid with 16 1-mm holes around the perimeter to improve aeration (similar to Neuser et al. 2005). We moved larvae manually between training sessions, alternating between each odour/food pairing. Each training session lasted 5 min, and between each session, we gave the larvae 1 min breaks in a droplet of clean water. This served to rinse any agar or sugar from the previous training session and such breaks improve learning scores (Scherer et al. 2003). Each group of larvae received six training sessions, three of each odour/food pair, lasting a combined total of 35 min.

Immediately following training, we transferred larvae to a clean water droplet for 1 min before giving an odour preference test. We conducted tests in 90 mm diameter petri dishes containing a thin layer of agar. We placed the larvae along the midline, equidistant from two odour cups filled with 10 uL of the respective odours at opposite ends of the dish. Each odour cup sat atop a 1 cm disk of fructose-flavoured agar, which served to reward the larvae so that they did not crawl back across the midline in continued search for food after making a

choice. We perforated the dish lid with holes along the midline to draw the odours toward the center and to prevent the odours from mixing as much as possible. We spun each dish prior to testing to randomize the side of odour presentation and to ensure that the experimenter was blind to odour identity. Larvae crawled freely for a 1 min choice phase, after which we immediately counted the number of larvae on each side of the dish. Larvae within 1 cm of the midline were omitted from analysis. We regarded larvae on either side of the dish as having chosen the corresponding odour, and calculated the proportion of larvae choosing the odour previously paired with fructose. Thus a proportion of 0.5 indicated random choice and 1 indicated perfect learning. For a single replicate, we tested six dishes, one per flavour treatment dish and three for each complexity. We repeated the experiment for 10 replicates (N = 60 total dishes: 10 of each flavour or flavours, 30 of each complexity).

3.5 Experiment 2 – *Adult Learning Ability*

Fly population cages, egg laying, and treatment environments were identical to Experiment 1. Flies underwent pupation and eclosion within the treatment dishes. Since eclosion typically takes place across a few days, we monitored the dishes daily and used any newly eclosing flies from several replicates and days for each test. We gently aspirated the flies into vials of standard food at a density no greater than 20 flies per vial. On the evening before testing, we transferred flies to vials containing plain agar and left them overnight for 16-18 h of starvation. All flies were less than 42 h old at the time of testing. We collected 38.7 ± 2.0 flies per test, and this did not differ between simple or complex tests ($F_{1,42} = 2.1$, $p = 0.150$), or flavours ($F_{4,42} = 1.4$, $p = 0.243$).

3.5.1 Adult training and test

We tested adult learning ability by exposing flies to two novel odours, 3-octanol (OCT, Fluka) and 4-methylcyclohexanol (MCH, Fluka), with one odour paired with a dried filter paper that had previously been soaked in 2M sucrose solution (the “rewarded” odour) and the other odour paired with plain filter paper, followed by a test of odour preference (test adapted from Tully & Quinn 1985; Schwaerzel et al. 2003; Thum et al. 2007). Prior to training, we exposed sugar filter papers to 20 flies for 5-10 min to scent the filter paper and promote the learning of experimental flies (Connolly & Tully 1998). We aspirated flies from the six

treatments into six randomly numbered empty vials and tested in random order, blind to treatment. The testing apparatus consisted of a Plexiglas elevator chamber that moved the flies from a training tube, which was lined with the filter paper, to a point between two choice tubes (similar to Tully & Quinn 1985). Odour concentrations were adjusted by dilution in heavy mineral oil beforehand to 1:50 MCH and 1:250 OCT, concentrations which naïve flies preferred approximately equally in preliminary trials. A vacuum pump drew air through small, 50 mL flasks, bubbling through the odorant-oil mixtures, and then through the training or choice tubes of the experimental apparatus and out of the room. We monitored and controlled odour flow to 14 mL/s per tube. The vacuum pump remained on for the entirety of training and testing so that the flies habituated to the noise and so that clean room air could clear the previous odour between trainings. We aspirated flies into the apparatus and let them rest for 90 s in the elevator chamber. We exposed flies to the first odour/filter-paper pairing for 60 s, gently shook them back into the elevator chamber, gave 30 s rest, and then exposed them to the second odour/filter-paper pairing for 60 s. Following this, we gently shook the flies into the elevator, gave 90 s rest, and finally moved them into a T-maze choice-point at the convergence of the two odour streams for a 60 s choice phase where they could enter the tube containing their preferred odour. We conducted all training and tests under red light with the final rest and choice phases conducted in complete darkness to eliminate any phototactic behaviour. We gave flies one training cycle and test only, the entirety of which took less than

6 min: (1) 60s of OCT, (2) 30s rest, (3) 60s of MCH, (4) 90s rest, (5) 60s choice between the odours. Following the choice phase, we anesthetized flies with CO_2 and counted the proportion of flies choosing each odour. Flies remaining in the center were omitted from analysis. We balanced the odour paired with sugar and the side of odour presentation during the choice phase across replicates. We tested eight replicates of the six treatments for a total of 48 tests, 24 of each complexity.

3.6 Data Analysis

All proportion data were arcsine square root transformed prior to statistical tests (Sokal & Rohlf 1995, p. 419), and met ANOVA assumptions after transformation. We assessed the learning abilities of larvae and adults with an ANOVA on the proportion of larvae choosing PA or MCH respectively, chosen arbitrarily. Significant learning in the larval case was indicated by increased preference for PA while PA was paired with fructose, and decreased preference for PA while BUT was paired with fructose. Similarly, in the adult case, learning was indicated by increased preference for MCH when MCH was paired with sucrose, and a decreased preference for MCH while OCT was paired with sucrose. We assessed differences in learning abilities with an ANOVA of the proportion of larvae or adults choosing the previously rewarded odour. In all analyses, *complexity* was included as a fixed factor and *flavour(s)* as a factor nested within

complexity. For the larvae, we included *rewarded odour, odour presented first during training*, and their interaction in the model. For the adults, we included *rewarded odour* and *side of odour presentation during testing*.

3.7 Results

3.7.1 Larval learning ability

Larvae from complex dishes did not exhibit greater learning than larvae from simple dishes (proportion choosing the rewarded odour, respectively: 0.590 ± 0.029 and 0.572 ± 0.024, N = 60; $F_{1,51}$ = 0.4, p = 0.55; Fig. 3.2A). Overall, the pairing of an odour with fructose had a significant effect on subsequent odour preference ($F_{1,51}$ = 25.3, p < 0.001), indicating significant learning, with an overall mean proportion of 0.581 ± 0.019 choosing the rewarded odour. There was a slight odour preference for PA, despite our attempt to balance odour preference with preliminary tests, as indicated by a significant effect of *rewarded odour* on the proportion of larvae choosing the rewarded odour ($F_{1,51}$ = 13.7, p = 0.001). Nevertheless pairing an odour with fructose increased preference for this odour (Proportion choosing PA when PA+, 0.642 ± 0.026, versus BUT+, 0.480 ± 0.021). There were no significant effects of *flavour(s)* (nested within complexity), *complexity*, *odour presented first during training*, or the interaction between odour order and the odour paired with fructose, on either the proportion of larvae choosing PA or the proportion of larvae choosing the rewarded odour (all F < 1.9,

all $p > 0.178$). Post-hoc ANOVAs within each complexity revealed no significant differences between flavours ($p > 0.350$). Similar analysis showed that the proportion of larvae in the center of the dish (not making a choice) did not differ due to *flavour(s)* ($F_{4,51} = 0.5$, $p = 0.7$) or *complexity* ($F_{1,51} = 0.2$, $p = 0.6$), with an overall mean of 0.381 ± 0.015.

3.7.2 Adult learning ability

Rearing flies in complex larval foraging environments did not result in greater learning than rearing flies in simple environments (proportion choosing the rewarded odour, respectively: 0.600 ± 0.038 and 0.609 ± 0.037, N = 48; $F_{1,40} = 0.02$, $p = 0.891$; Fig. 3.2B). We did observe significant overall learning, however, as pairing of an odour with sucrose increased later preference for that odour ($F_{1,40} = 22.1$, $p < 0.001$), with an overall mean proportion of 0.604 ± 0.026 choosing the rewarded odour. We observed a slight preference for MCH, despite preliminary balancing of odour preference, as indicated by a significant effect of *rewarded odour* on the proportion of flies choosing the rewarded odour ($F_{1,40} = 10.4$, $p = 0.003$), however, pairing an odour with sucrose increased preference for this odour (MCH preference when MCH+, 0.690 ± 0.030, versus OCT+, 0.482 ± 0.035). There was no significant effect of the *side of odour presentation* on the proportion of flies choosing the rewarded odour ($F_{1,40} = 2.1$, $p = 0.150$). Additionally, the proportion of flies remaining in the center of the test apparatus

(not making a choice) did not differ due to *complexity* ($F_{1,40}$ = 0.2, p = 0.9) or *flavour(s)* ($F_{4,40}$ = 0.8, p = 0.5), with an overall mean of 0.180 ± 0.014.

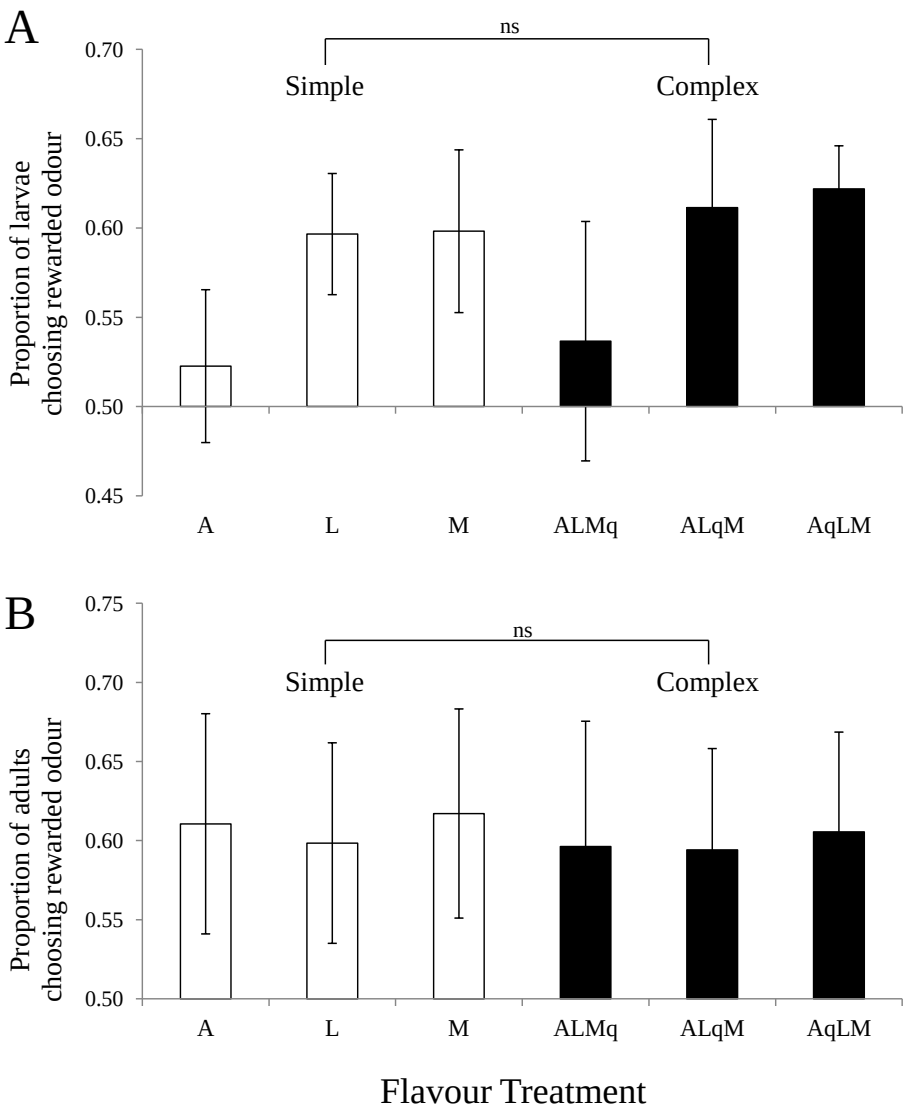

Figure 3.2. Proportion of individuals chosing the rewarded odour (mean ± SE) after rearing in *simple* or *complex* arrangement of foods. Simple treatments (white bars) received one patch of one flavour: anise (A), lemon (L), or mint (M). Larvae from complex treatments (black bars) received nine patches of three flavours, one of which had added quinine. The flavour containing quinine is denoted with a 'q' following the flavour: anise, lemon and mint with quinine (ALMq); anise, lemon with quinine, and mint (ALqM); or anise with quinine, lemon and mint (AqLM). There was no effect of either *flavour(s)* or *complexity* on the learning of **(A)** larvae tested later during the larval stage, or **(B)** young adult flies.

3.8 Discussion

We found no evidence for adaptive plasticity of learning ability in fruit flies. Our early-life treatments differed in complexity of foraging environment while controlling for relevant developmental factors such as nutrition and temperature. In our “complex” environments, we forced the larvae to search, sample and choose from multiple food sources of different bitterness. We thought that these factors represented an ecologically valid manipulation of environmental complexity at the larval stage as larvae attend to such environmental variation and similar variation has proven effective in previous assays of larval learning (Dukas 1999; Scherer et al. 2003; Neuser et al. 2005; Kaun et al. 2007; Gerber & Stocker 2007). Furthermore, similar manipulations of food types for varying environmental complexity have been used successfully in a few insect taxa (eg. Dukas & Real 1993b; Bernays 1998; Gegear & Laverty 2005). Contrary to our prediction, developing in such complex environments did not result in greater learning abilities than simple environments, either later as larvae or as young adults. We did, however, replicate previous studies showing robust learning in both larval and adult fruit flies (reviewed by Gerber et al. 2009), and it is interesting that, while larvae attend to food sweetness/bitterness and associated flavours and odours, variation in the complexity of these factors did not noticeably affect the development of later learning ability.

The brains of fruit flies are highly plastic, especially within the mushroom bodies, structures critical for learning and memory, and so they remain a good

model for future study of the plasticity of learning. Adult experience with a wide range of social and environmental stimuli can modify neuropil volume and fiber number in the mushroom bodies, but it is unclear how such changes relate to learning ability (Technau 1984; Balling et al. 1987; Heisenberg et al. 1995; Barth & Heisenberg 1997; Fahrbach 2006). Larval mushroom bodies, since they possess functioning neuroblasts throughout the larval stage (Technau & Heisenberg 1982; Ito & Hotta 1992), may be a site where brain development can adaptively respond to environmental variation. For instance, Heisenberg et al. (1995) observed that larval development under high density resulted in increased fiber number and larger mushroom bodies at eclosion, though this effect was limited to females. Additionally, both pharmacological and environmental (sporadic heat shock) disruption of larval neurogenesis impairs adult learning and memory (de Belle & Heisenberg 1994; Wang et al. 2007). The question remains whether ecologically relevant environmental factors can cause increased mushroom body neurogenesis or improve learning ability.

Fruit flies may exhibit adaptive plasticity of learning ability for other environmental cues that we did not test. For example, the availability of adequate nutrition may be a more relevant cue of environmental difficulty. In several songbird species, malnutrition is an early-life stressor that results in cognitive deficits of quality and quantity of song learning (Nowicki et al. 2002; Searcy & Nowicki 2009), and spatial memory (Pravosudov et al. 2005; Pravosudov 2009). Although such deficits may be maladaptive in birds (Pravosudov 2009), one can

readily imagine a case where an individual experiencing a barely-adequate food supply invests more in the cognitive abilities that will better prepare it to find a novel source of food for itself and its offspring. Among fruit flies, it has recently been shown that larval nutritional adversity can affect other foraging related behaviours, increasing the tendency to explore among so-called *sitters* but not *rovers*, flies possessing different variants of the *foraging* gene, for^{s} and for^{R}, respectively (Burns et al. 2012). That is, nutritional stress effectively makes the *sitters* more like *rovers*. Interestingly, this gene has also been implicated in a trade-off between short and long-term memory, with *rovers* possessing better short-term and *sitters* possessing better long-term memory (Kaun et al. 2007). It would be interesting to test whether developmental nutritional stress can adaptively improve the short-term memory of *sitters*. Additionally, cues of predation or competition could trigger an adaptive developmental shift away from learning ability and toward faster development in order to out-compete others on a dwindling resource, or in order to leave a vulnerable site as soon as possible. Indeed, any environmental cues of expected longevity may be particularly relevant (Eliassen et al. 2007).

Alternatively, fruit flies may not possess adaptive plasticity of learning ability. It could be that the larval food environment is not predictive of the adult's future environment because adults possess greater mobility and can more readily find novel sources of food. Another alternative is that although the larval environment is predictive of the future adult environment, adults utilize their

learning abilities in a wide range of contexts, not just foraging. For example, learning plays a role in mate-choice (Dukas 2005a; Dukas 2005b) and flies may benefit from learning regardless of foraging environment. Finally, the global fitness cost of developing unnecessary brain structures may be small compared to the potential costs of plasticity (DeWitt et al. 1998; Snell-Rood et al. 2010b), such as a developmental error or environmental mismatch. Learning could be so crucial to fitness that instead of being plastic, it is a highly canalized developmental priority regardless of environment (Pravosudov 2009; Roth et al. 2010; Roth et al. 2012).

Despite our failure to find evidence for plasticity of learning ability in fruit flies, our results add to a growing body of literature on the ecology, evolution, and development of fruit fly cognitive abilities (Burger et al. 2008; Dukas 2008a; Kolss & Kawecki 2008; Reaume et al. 2010). Fruit flies are an important model system for the neurogenetics, ecology, and evolution of learning and memory (Dukas 2008a; Gerber et al. 2009; Busto et al. 2010; Burns et al. 2011), and our protocols and results are highly relevant for future research. In general, negative results are also important to shed light on the evolution and ecology of cognitive plasticity because we do not expect plasticity to evolve under all conditions (DeWitt et al. 1998; Snell-Rood et al. 2010b). Comparing related species that do and do not exhibit cognitive plasticity in different contexts will further highlight the associated ecological and neurodevelopmental factors.

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CHAPTER 4

Social Attraction and Socially Influenced Learning in Fruit Fly Larvae

In press: Proceedings of the Royal Society B: Biological Sciences

Note that *Supplementary Materials* are included in section 4.10, after the MS.

4.1 Abstract

We examined the use of social information in fruit fly larvae, which are an ideal model system owing to their robust learning abilities, small number of neurons and well-studied neurogenetics. Focal larvae showed attraction to the distinct odour emanating from food occupied by other larvae. In controlled learning experiments, focal larvae preferred novel odours previously paired with food occupied by other larvae over novel odours previously paired with unoccupied food. When we gave groups of larvae a choice between food patches differing in quality, more larvae aggregated on the higher quality food, suggesting that attraction to and learning about cues associated with other larvae can be beneficial. Furthermore, larvae were more likely to find the best available food patch in trials when that food patch was occupied by other larvae than in trials

when that food patch was unoccupied. Our data suggest, however, that the benefits from joining others may be at least partially offset by the fitness costs of increased competition because larvae reared in isolation did as well as or better than larvae reared in groups on three key fitness parameters: developmental rate, survival rate and adult dry body mass. Our work establishes fruit fly larvae as a highly tractable model species for further research on the mechanisms that modulate behaviour and learning in a social context.

4.2 Introduction

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

research on social learning has focused on vertebrates and eusocial insects [10-12].

As a part of a series of experiments on the evolution of social learning in insects [13], we examined social behaviour and social information use in fruit fly (*Drosophila melanogaster*) larvae. Adult fruit flies are moderately social. Most notably, the pheromone, *cis*-vaccenyl acetate (cVA), produced by males and transferred to females during copulation, serves as a long-distance attractant promoting adult aggregation [14-16]. Both cVA and an individual's cuticular hydrocarbons modulate aggression between males [17, 18]. Social experience also influences fruit flies' circadian rhythms and the expression of cuticular hydrocarbons [19-21]. Finally, adult fruit flies show social learning in the contexts of egg laying and mate choice [22-25]. Because adult female fruit flies tend to aggregate and lay eggs at a single site, many larvae typically share a food substrate and thus social behaviour may occur at the larval stage as well. Identifying social interactions among larvae opens opportunities for analyzing social behaviour and the use of social information in a simple and tractable model system with well-studied learning abilities [26-28] and neurobiology [29-31]. We began by examining social attraction in the larvae. We then tested whether larvae learn to prefer cues associated with other larvae. Finally, having found both social attraction to and learning from social cues, we assessed some of the benefits and costs larvae incur from joining other larvae.

4.3 Material and Methods

4.3.1 General

We maintained three population cages each containing several hundred *Drosophila melanogaster Canton-S* on abundant standard food at 25°C, 60% relative humidity, and on a 12:12 light/dark cycle with lights on at 1 am. This irregular light cycle placed peak egg-laying midday so that we could collect experimental eggs within a very short time window of about 1 hour. We collected eggs on 85 mm diameter petri dishes filled with 10 mL of standard food and covered with 0.7 mL of live-yeast suspension (30 g dry live yeast / L of warm water) to stimulate egg laying [22]. Immediately following egg laying, we transferred these dishes to an incubation chamber maintained at 25°C, high humidity and total darkness. We conducted all further manipulations and tests under far red light, which fruit flies cannot see [32], in order to minimize disturbance and phototaxis.

4.3.2 Food preparation

In several experiments we created *social* and *nonsocial* food disks. We placed disks of food (ranging from 1.15 to 2.5 mL, depending on the experiment) in 85 mm petri dishes containing a thin layer of agar. To social disks, we added groups of 20-30 randomly selected larvae, which fed on the disks for 18-42 hours prior to testing, depending on the experiment. After such feeding, we considered food to

be *used*, as opposed to unused *fresh* food, which was identical in quality and age, but had not been occupied by larvae. Used food has a noticeably different texture, smell, and presumably taste, than fresh food. Because the larvae on social stimuli may have provided social cues to the focal larvae, we refer to them throughout as “models”.

4.4 Social Attraction

4.4.1 Methods

We began our investigations by testing for simple social information use, attraction to a substrate frequented by others. We placed a social and nonsocial food disk on opposite sides of a petri dish containing a thin layer of agar (Figure 4.1A). We tested each focal larva individually by placing it through a 1-cm hole in the lid at the centre of the petri dish, equidistant to either disk, and recording its choice, defined as making contact with a disk within 5 minutes. We placed larvae parallel to the midline, facing perpendicular to either disk so that they could not make a choice by simply crawling straight ahead. Typically, larvae crawled along the midline before turning and contacting a food disk, with the mean (±SEM) latency to make a choice ranging from 37.4 ± 4.9 seconds in Experiment 1C to 88.8 ± 7.1 seconds in Experiment 1A. We alternated the side of social and nonsocial disks between trials to control for side bias. We tested each focal once and always on a fresh dish of agar to prevent larvae from following a trail

established by others. All larvae were in the feeding stage of their third-instar at the time of testing, approximately 90 hours after egg laying. In all experiments we analyzed only those larvae that made a choice during the test phase, comparing social and nonsocial choices with generalized linear models (hereafter GzLM) with a binomial distribution and logit link function, including side of social disk as a factor. Wald χ^2 statistics are reported.

First, in Experiment 1A, we gave focal larvae a choice between a social and a nonsocial disk of 2.5 mL of standard food, 2.3 cm in diameter and 6 mm thick. Social disks contained 30 model larvae that had been feeding on that substrate for 42 hours. We conducted tests in 60 mm agar petri dishes with the food disks placed on opposite sides. Focals were placed 7 mm from either disk. In this experiment, however, we reared focals with others for the first 2 days of life and so they may have learned to prefer the familiar cues associated with others. To eliminate this possibility, in Experiment 1B, we reared each focal larva individually by placing each egg into its own 60 mm petri dish containing 0.3 mL of standard food, which is abundant for a single larva. These isolated larvae experienced no other larvae prior to testing. Social and nonsocial food disks for this and subsequent experiments were 1.25 mL of standard food, 3.4 cm diameter and 1.4 mm thick, which were thinner and made it easier to locate and observe larvae. Social disks contained 30 model larvae reared on these disks for 18 hours prior to testing. We conducted tests similar to the previous experiment,

but in 85 mm agar petri dishes with the social and nonsocial food disks 10 mm apart, and placed larvae 5 mm from either disk.

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

In Experiment 1D, we tested which cue served as the attractant for the focal larvae: the distinct odour emanating from food previously occupied by larvae for about a day or some cue directly originating from the model larvae. We compared larval attraction to social foods in two tests each involving a choice between foods: (1) *used food without models* versus *fresh food*, and (2) *fresh food with models* versus *fresh food*. As a control, we also included our baseline test involving *used food with models* versus *fresh food*. Used food consisted of a

food disk consumed by 20 early third-instar model larvae for 24 hours. Depending on the treatment, we left the models, removed models from used food disks, or added models to fresh food disks immediately before testing.

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

4.4.2 Results

Focal larvae reared both in groups and in isolation showed significant attraction to the social food disks of larvae and used food (Groups: 66.7%, N = 126, GzLM intercept: $\chi^2_1 = 13.8$, $p < 0.001$; Figure 4.1B, Experiment 1A. Isolation: 71.6%, N = 67, $\chi^2_1 = 11.7$, $p = 0.001$; Figure 4.1B, Experiment 1B). Similarly, focal larvae from a wild population showed significant attraction to the banana slice that had been used by larvae overnight (68.4%, N = 76, $\chi^2_1 = 10.0$, $p = 0.002$; Figure 4.1B, Experiment 1C). In the test of the nature of the attractive cue, focal larvae showed significant attraction to the used food without model larvae but not to fresh food containing model larvae (respectively: 63.0%, N = 81, $\chi^2_1 = 5.5$, $p = 0.019$; and 48.4%, N = 64, $\chi^2_1 = 0.03$, $p = 0.874$; Figure 4.1C, Experiment 1D). As before, focal larvae showed significant attraction to used food occupied by models (72.0%, N = 82, $\chi^2_1 = 14.6$, $p < 0.001$; Figure 4.1C, Experiment 1D). Larval attraction to the social food was similar in the tests consisting of *used food with models* and *used food without models* ($p = 0.245$). Attraction to *fresh food with models* was significantly lower than attraction to *used food with models* ($p = 0.004$). Attraction to used food persisted even when the alternative food was similarly textured artificially “used” food (68.0%, N = 50, $\chi^2_1 = 6.2$, $p = 0.013$; Figure 4.1C, Experiment 1E).

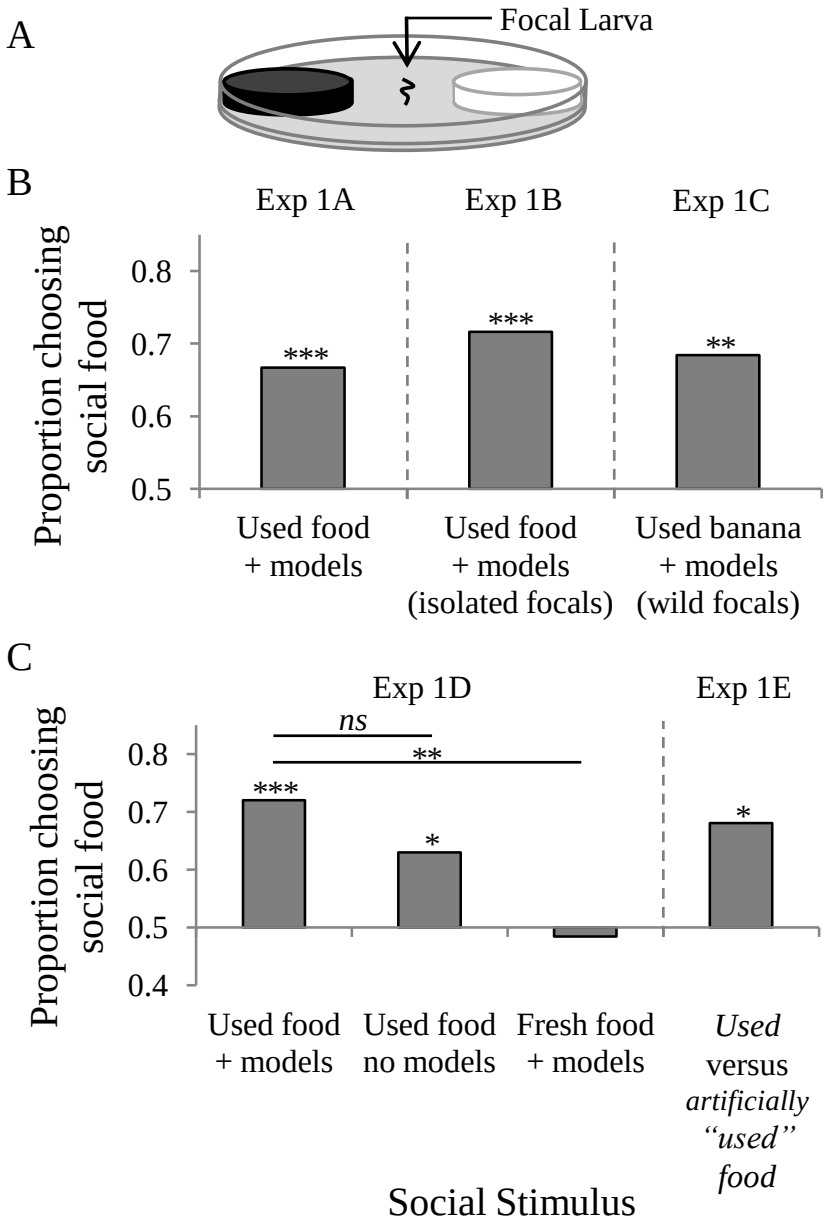

Figure 4.1 (**A**) In a series of experiments we gave larvae a binary choice between a social food (black disk) and a nonsocial food (white disk). (**B-C**) Social foods and larval treatment varied between experiments as noted on the X-axis legend. 'Focals' refers to the larvae being tested and 'models' refers to the larvae providing social cues (see methods for details). Dashed lines separate experiments. Asterisks indicate significant deviation from random (0.5): $p<0.05$ (*), $p<0.01$ (**), and $p<0.001$ (***), and "*ns*" indicates no significant difference.

4.5 Socially Influenced Learning

4.5.1 Methods

Next we asked whether larvae learn to prefer novel cues associated with other larvae. All experiments consisted of pairing one novel odour with a social food and another novel odour with a nonsocial food and then testing the subsequent odour preference (Figure 4.2A). Prior to training, focal larvae fed on standard food dyed with blue food colouring so that they were easily distinguishable from model larvae. Training and preference test were adapted from previous larval learning assays [27, 28, 33]. We gave focals six 3-minute training sessions alternating between odour/food pairings with 1-minute breaks between sessions, during which we rinsed larvae in a droplet of fresh water and placed them on an empty petri dish. For each training session, we placed larvae directly on top or in the center (depending on experiment) of the food disk between two small cups (polypropylene NMR tube caps, Sigma) each containing 10 uL of chemical odourant, either 1-butanol (BUT; Fisher) or propyl acetate (PA; Sigma), the latter diluted in paraffin oil prior to each experiment to a concentration that naïve larvae prefer equally (ranging from 1:300 to 1:1000; data not shown). The vapours of both odours are strongly attractive to larvae [29, 34]. We alternated the odours paired with each food type between tests to control for odour preference. The odour cups had lids made of mosquito-net mesh, which allowed ample evaporation of the odours but prevented larvae from making contact with the chemicals. The petri-dish lids remained on the dishes during training so that

odour vapours could collect, but each lid had a series of small holes along the midline of the dish to improve aeration [28]. In all cases, we trained and tested focal larvae individually and used training and test dishes only once. We tested larval odour preference immediately following training. We placed each focal larva on the midline of an 85 mm petri dish, between two fresh odour cups filled with 10 uL of the respective odours on opposite sides, each atop a 1 cm diameter disk of fresh food. We placed focals 3 cm from each odour, parallel to the midline, perpendicular to both odours, so that they could not make a choice by simply crawling straight ahead. We gave focals up to 10 minutes to choose an odour, defined as contacting the corresponding food disk underneath an odour cup. We shuffled odour cups before testing to randomize sides of chemicals and to ensure that the observer was blind to odour identity. As in training, we perforated the lids of the petri dishes along the midline to improve aeration, draw odours to the center and minimize odour mixing. In all experiments we analyzed only those larvae that made a choice during the test phase. We assessed odour choice (BUT or PA) using GzLMs with a binomial distribution and logit link function. As factors, we included the identity of the social odour (BUT or PA), the order of training (social or nonsocial first), the side of odour presentation, and relevant interactions. We compared learning between treatments with a GzLM on the frequency of choices (social or nonsocial) including the identity of the social stimulus as a factor.

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

The results from Experiment 2B indicated that focal larvae learned to prefer novel odours associated with both used food with no larvae and models on fresh food (Figure 4.2B). In Experiment 2C, we directly tested which factor was more important to the larvae: used food or other larvae. We tested whether focal larvae preferred an odour previously paired with (a) *used food without models* or an odour previously paired with (b) *fresh food with models*. As a control, we simultaneously replicated Experiment 2A. If larvae do not learn from their direct interactions with others, they should prefer an odour paired with used food over

an odour paired with others on fresh food. If, however, direct interactions with others improve the perceived quality of a food, they should prefer the odour paired with used food less strongly than controls. Additionally, we observed a subset (71%) of the *fresh food with larvae* training dishes to quantify social interactions between focals and models. We recorded the proportion of time (out of the 9 total possible minutes) that each focal larva spent within 2 mm (approx. 1 body length) of a model larva. Typically, focal larvae crawled beside and remained in contact with other larvae. Once a focal was near models, it usually stayed close to them for the remainder of the training session.

4.5.2 Results

In Experiment 2A, focal larvae chose the odour previously paired with social food (*used food with models*) more frequently than the odour paired with nonsocial food (77.5%, N = 71, GzLM: $\chi^2_1 = 15.4$, $p < 0.001$; Figure 4.2B, Experiment 2A). In Experiment 2B, focals chose the odour previously paired with the social food more frequently in all treatments: when the social food was *used food with models* (65.6%, N = 61; GzLM: $\chi^2_1 = 5.5$, $p = 0.019$; Figure 4.2B, Experiment 2B)*, used food without models* (66.2%, N = 65, $\chi^2_1 = 6.4$, $p = 0.012$), and *fresh food with models* (61.3%, N = 62; $\chi^2_1 = 3.8$, $p = 0.050$). There was no overall difference in the frequency of social choices between the three tests (GzLM: $\chi^2_2 = 0.429$, $p = 0.807$), and pairwise comparisons revealed no significant differences between the three tests (all $p > 0.528$; Figure 4.2B, Experiment 2B). In Experiment 2C, focals did not differ in preference for odours previously paired with *used food without models* or *fresh food with models* (50%, N = 48, GzLM: $\chi^2_1 = 0.01$, $p = 0.937$; Figure 4.2C), and the presence of model larvae on the fresh food significantly reduced preference for the odour paired with used food in test trials compared to controls (GzLM: $\chi^2_1 = 4.1$, $p = 0.044$; Figure 4.2C). We replicated our previous results from Experiment 2A, with larvae choosing an odour previously paired with *used food with models* significantly more often than an odour previously paired with fresh food alone (68.6%, N = 51; GzLM: $\chi^2_1 = 7.1$, $p = 0.008$). Our quantification of social interactions revealed that focal larvae spent 52.4 ± 3.8% (N = 41) of their time within 2 mm of model larvae.

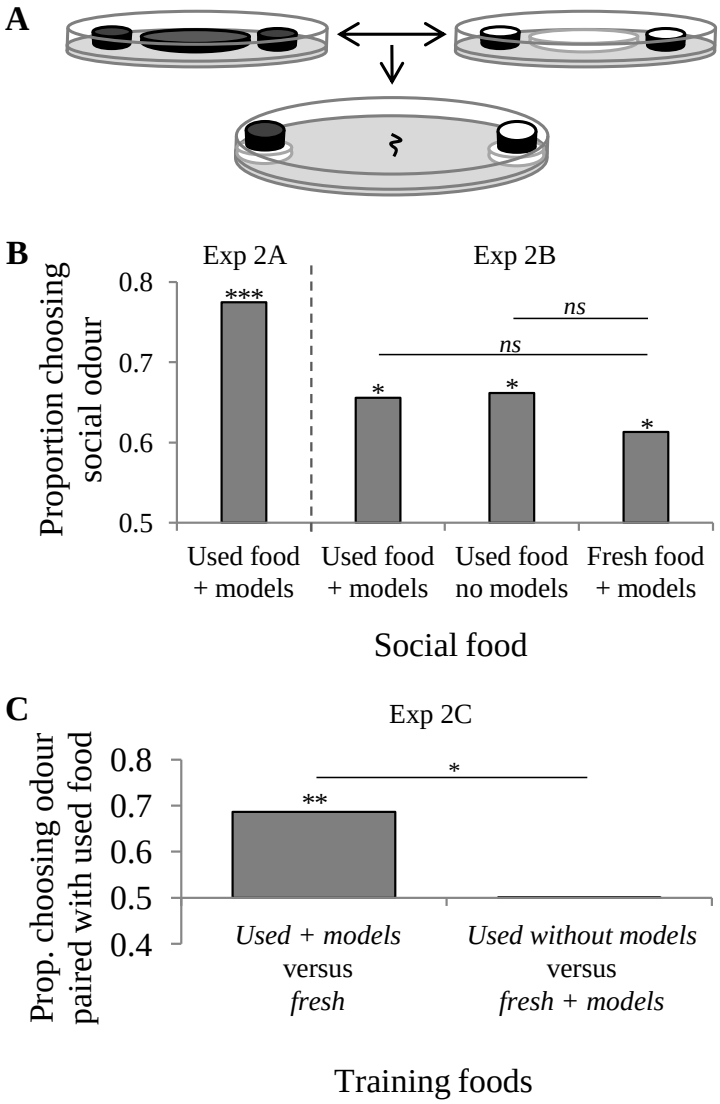

Figure 4.2 **(A)** We trained larvae with one odour paired with a social food (black odour cups and black disk), and another odour paired with nonsocial food (white odour cups and white disk), then gave them a choice between the two odours. **(B)** Social foods varied between experiments as noted on the X-axis legend (see methods). The dashed line separates experiments. **(C)** We directly tested which factor was more important to the larvae: used food or other larvae. In control trials (left bar), we gave larvae a choice between an odour previously paired with *used food with models* and an odour paired with *unused food without models*. In test trials (right bar; at 0.5), we gave larvae a choice between an odour paired with *used food without models* and an odour paired with *unused food with models*. Asterisks indicate significance from random chance (0.5): $p<0.05$ (*), $p<0.01$ (**), and $p<0.001$ (***), and "*ns*" indicates no significant difference.

4.6 Benefits and costs of joining others

4.6.1 Methods

In our final three experiments, we addressed the ultimate evolutionary question of *why* focal larvae prefer to join others. First, we asked whether an aggregation of larvae can be a valuable source of foraging information to other larvae. If groups of larvae tend to aggregate at the best sites in their environment, individuals can rely on the cues of foraging conspecifics to quickly locate high quality sites. In Experiment 3A, we tested whether groups of larvae are more likely to aggregate on the best available food in their environment. We collected eggs for experimental larvae on 85 mm petri dishes containing 10 mL of food with 50% of the sugar and yeast of our standard recipe (henceforth, “50% food”). We left larvae to develop normally on these dishes until late second instar. We tested two different combinations of food quality, with one food always containing twice the nutrients as the other. We randomly selected 30 larvae and placed them at the edge of an 85 mm agar dish, 3 cm from two 2.5 mL disks of food (2.3 cm diameter, 6 mm thick). Dishes contained either (a) one disk of standard food (100%) and one disk of 50% food, or (b) one disk of 50% food and one disk of 25% food. Additionally, the food disks were presented in one of two possible configurations, either touching or separated by 1 cm. We alternated the side of food disks between replicates in order to control for any side bias. We left larvae for 18 h to forage freely, after which, we separated the disks, placed them in the freezer for 15 minutes to immobilize the larvae and counted the number of larvae

on each disk. All proportions were arcsine square root transformed prior to statistical analyses to meet assumptions of normality. We compared whether the proportion of larvae feeding from the higher quality food differed from chance levels with one-sample t-tests and tested for differences due to treatment, side of presentation and distance apart with an ANOVA. We tested 200 dishes of larvae, 50 from each combination of foods and configuration. Additionally, we confirmed the relative quality of the foods by monitoring pupation rates and adult body mass of individuals reared on 100, 50 or 25% (see supplementary material).

In Experiment 3B, we tested whether individual larvae were better at locating the best locally available food patch when that patch was occupied by other larvae than when it was unoccupied. We allowed focal larvae to choose between a low and high quality food in one of two conditions. In the models-absent condition, individual focal larvae could choose between the two food patches based on food-derived cues only. In the models-present condition, we placed 30 larvae on the higher quality food disk 18 hours prior to testing. In short, we gave larvae a choice between (a) low quality food and (b) either social or nonsocial high quality food. We analyzed the frequency of choices with a generalized linear model with a binomial distribution and logit link function including factors for the presence/absence of model larvae, foods available, side of food disks and relevant interactions.

Finally, in Experiment 3C, we assessed the developmental effects of group foraging. We measured key parameters related to fitness as a function of larval group size. We transferred 1, 3, 10 or 50 eggs to dishes with 2.5 mL of standard food immediately after egg laying. As a reference, fruit fly laboratories typically rear a few dozen flies per vial containing 5 mL of standard food [35, 36]. We recorded larval developmental rate, egg-to-adult survival, and adult body mass. See supplementary material for further details. If foraging aggregations improve fitness in this context, we would expect moderately sized groups of larvae to develop faster, larger, and with lower mortality rates than either larvae reared alone or in large groups with increased competition.

4.6.2 Results

In Experiment 3A (group choice), larvae showed significant preference for aggregating on the higher quality food for all food combinations and configurations (all $t_{49} > 3.7$, all $p < 0.001$; Figure 4.3A). For the 100% versus 50% nutrition food tests, the proportion of larvae choosing the 100% food was 0.808 ± 0.024 when the foods were touching and 0.617 ± 0.031 when 1 cm apart. For the 50% versus 25% food tests, the proportion choosing the 50% food was 0.917 ± 0.011 when touching and 0.708 ± 0.031 when 1 cm apart (Figure 4.3A). When the disks were touching, a significantly greater proportion of larvae chose the higher quality food than when the disks were 1 cm apart ($F_{1,192} = 56.3$, $p <$

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

In Experiment 3B (individual choice), focal larvae chose the higher quality food more often in the presence than the absence of model larvae (GzLM: $\chi^2_1 = 6.7$, $p = 0.009$). In the presence of models, focal larvae chose the higher nutrition food significantly more frequently in both the 100% versus 50% and the 50% versus 25% food conditions (respectively, 76.9%, N = 39; GzLM: $\chi^2_1 = 10.0$, $p = 0.002$; and 66.7%, N = 39, $\chi^2_1 = 4.1$, $p = 0.042$; Figure 4.3B). Without model larvae on the higher quality food, focals did not differ from chance (respectively, 48.6%, N = 37, $\chi^2_1 = 0.4$, $p = 0.842$; and 54.1%, N = 37, $\chi^2_1 = 0.2$, $p = 0.619$; Figure 4.3B). There was no significant effect of food types available, side of food disk presentation, or the interaction between available foods and the presence of model larvae (all $p \geq 0.288$). The presence of model larvae did not affect choice latency (58.7 ± 6.3 versus 61.0 ± 8.0 seconds, with and without larvae, respectively; $t_{150} = 0.2$, $p = 0.815$).

In Experiment 3C, larval density negatively affected developmental rate, survival, and adult body mass (Figure 4.4). Larval density decreased developmental rate (Kaplan-Meier survival analysis with Mantel-Cox log rank chi-square: $\chi^2_3 = 29.6$, $p < 0.001$; Figure 4.4A). Post-hoc comparisons revealed that

larval development was significantly slower in the density of 50 larvae than all others (all $p < 0.001$), and that 1 and 3 vs. 10 approached significance (respectively, $p = 0.090$ and $p = 0.059$). Density negatively affected egg-to-adult survivorship ($F_{3,36} = 6.4$, $p = 0.001$; Figure 4.4B). Post-hoc comparisons showed that the density of 10 larvae had the lowest survivorship, significantly lower than densities of 1 and 3 larvae (Tukey HSD, respectively, $p = 0.025$ and $p = 0.001$). A planned contrast of *low density* (1 and 3) versus *high density* (10 and 50) revealed a significantly lower survivorship in the higher density than the low density treatments ($t_{36} = 3.7$, $p < 0.001$). Increasing density also significantly reduced adult body mass in both males ($F_{3,98} = 118.5$, $p < 0.001$) and females ($F_{3,79} = 69.3$, $p < 0.001$; Figure 4.4C). See supplementary materials for further details.

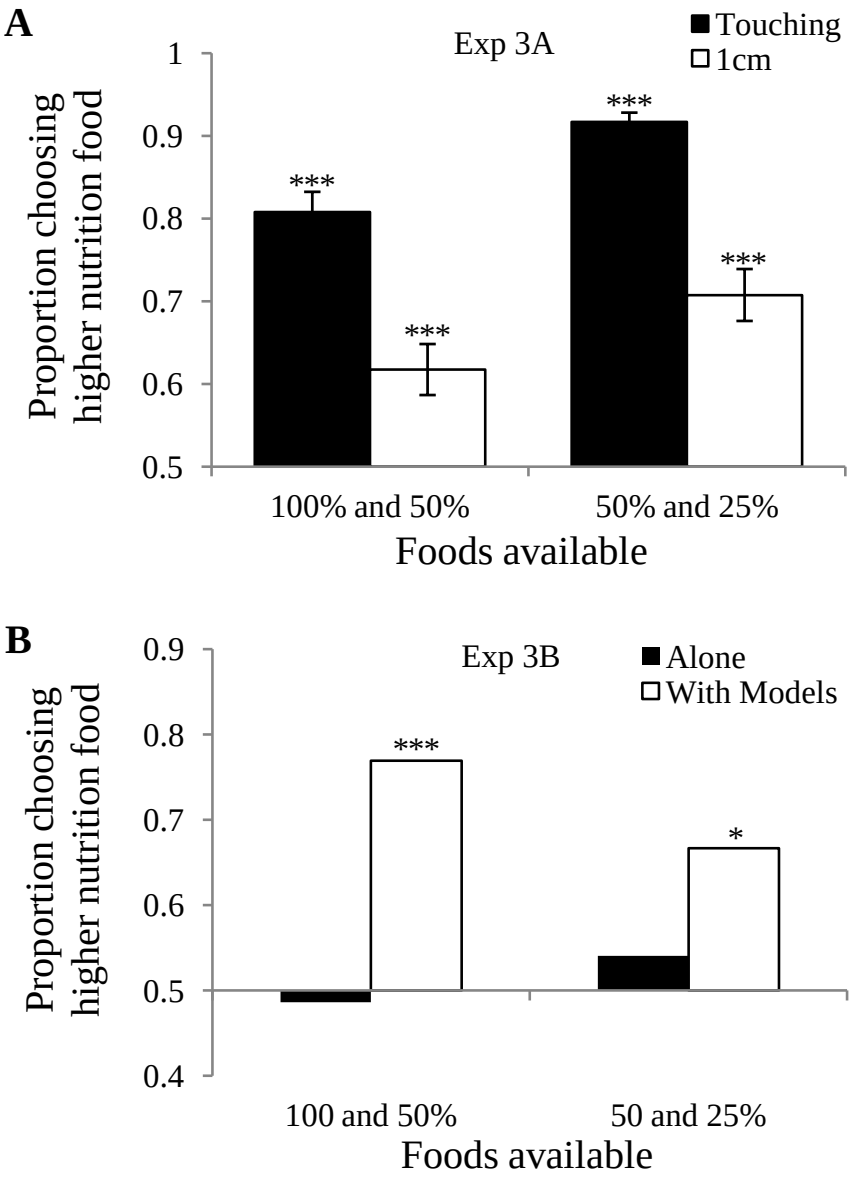

Figure 4.3 **(A)** We gave groups of larvae a choice between two food disks of different quality placed together (black bars) or 1 cm apart (white bars), and recorded the proportion feeding from the higher quality food after 18 hours. **(B)** We gave individuals a choice between two food disks of different quality, either alone or with a group of larvae on the higher quality food. Asterisks indicate significant difference from chance (0.5) or significant differences between treatments: $p<0.05$ (*), $p<0.01$ (**), and $p<0.001$ (***).

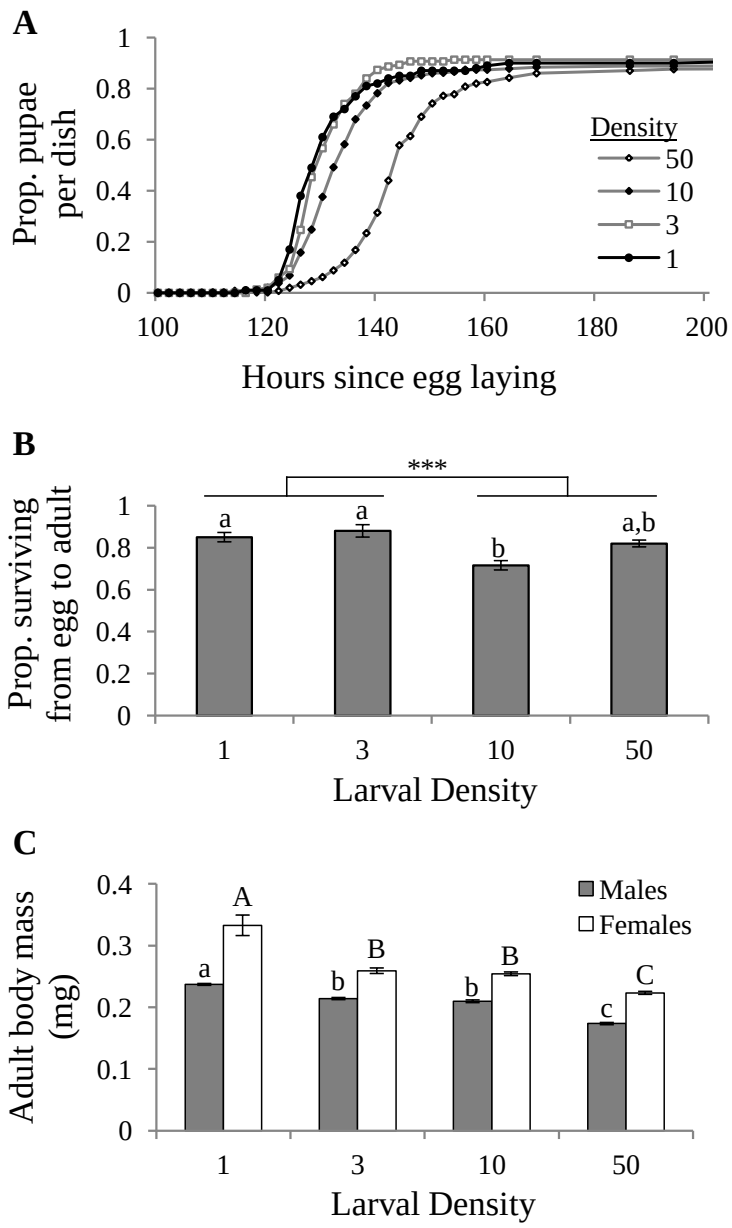

Figure 4.4 We monitored **(A)** larval developmental rates, **(B)** egg-to-adult survival (mean ± SE), and **(C)** adult body mass of flies reared at different larval densities. Letters above bars indicate significant differences in post-hoc tests, with upper and lower case in panel C reflecting independent comparisons within females and males, respectively.

4.7 Discussion

Our main findings were that (1) fruit fly larvae are attracted to odours emanating from food used by other larvae, (2) larvae prefer novel odours previously associated with other larvae over novel odours previously associated with nonsocial alternatives, (3) for a foraging larva, other larvae can be a useful source of social information about high quality food, and (4) when larvae join others, they may incur costs owing to competition. We discuss each of these results in turn.

4.7.1 Social attraction

In our first series of experiments, we found that focal larvae showed significant attraction to food patches occupied by other larvae and this was consistent whether or not we reared focal larvae in a group or isolation (Figure 4.1B). This indicates that focal larvae did not merely show attraction to an already-familiar group setting. Furthermore, we replicated the social attraction results using larvae from a recently collected wild population reared on natural fruit (Figure 4.1B). Larvae far away from food rely on cues that lead them back to food, and cues of other feeding larvae are especially relevant because they indicate that others have found a site with sufficiently high quality food. Moreover, food patches that have been occupied by larvae for several hours develop a distinct odour. Experiment 1D suggests that larvae are attracted to this odour (Figure

4.1C) and not to the direct presence of larvae at a food site. Finally, Experiment 1E indicates that the attractive odour is associated with feeding larvae rather than with mere mechanical disturbance of the food. The tendency of animals to join others and form aggregations has been studied for a long time [37-39]. Our experimental work on fruit fly larvae allows us to link work on social attraction to simple cases of social information use in a leading model system highly amenable to experimental manipulation in both the evolutionary ecological and neurogenetic arenas.

One could argue that the larvae in our experiments (Figure 4.1) did not actually show social attraction in the strict sense because they were not attracted directly to others, but instead to the volatiles in food consumed by others. However, social attraction should always be based on the most relevant and salient cues available, and the ultimate cause of all social attraction is some fitness benefit such as the opportunity to locate and feed on higher quality food [see 38, 39].

4.7.2 Socially influenced learning

To assess the magnitude of social information use by larvae, we asked whether larvae assigned higher values to novel odours associated with relevant social settings. In agreement with the data for social attraction, we found that the larvae preferred novel odours previously associated with either used food occupied by

larvae or used food from which we had removed the larvae (Figure 4.2B). Interestingly, larvae also preferred odours paired with fresh food occupied by larvae over odours paired with fresh, unoccupied food (Figure 4.2B), and larvae did not prefer odours paired with used food over odours paired with fresh food containing models (Figure 4.2C), which indicates that experiencing direct interactions with other larvae on a food increases the perceived quality of that food.

4.7.3 Benefits and costs of joining others

Our model system is somewhat unique because it allows us to quantify potential benefits and costs of social information use. We found that, given a choice between foods of different quality, groups of larvae were more likely to settle on the better option (Figure 4.3A). Importantly, the distance between the high and low quality food patches had strong effects on larval choice, with fewer larvae settling on the high quality food when the inter-patch distance was greater (Figure 4.3A, white versus black bars), suggesting that limited mobility and perception may prevent larvae from readily locating the best available food patches. Given such limitations, it may be highly beneficial for larvae to be attracted to odour cues associated with others and to learn about novel cues associated with others. Indeed, we found that focal larvae were significantly

better at locating a higher quality food when that food was occupied by larvae than when it was unoccupied (Figure 4.3B).

While the information gleaned by seeking others has obvious benefits, we also documented some costs. Isolated larvae had the heaviest adult dry body mass (Figure 4.4C). This can translate into higher fitness because males prefer larger females, which are more fecund [40, 41], and larger males have a mating advantage owing to both superior fighting ability and female preference for larger males [42-44]. Moreover, isolated larvae did as well as or better than a modest group of 10 larvae in terms of developmental rate and survival from egg to adult (Figure 4.4A, B). Costs associated with aggregation are well known from a large variety of species [37, 39] and our results are consistent with those showing such costs among *D. melanogaster* in both laboratory and natural settings [45, 46].

One can imagine some benefits from being in a small group, including suppressing mould, enhancing the growth of preferred species of yeast and bacteria, and improved ability to dig into the substrate [46-50]. Such benefits, however, may not be important in our laboratory settings, where we provide larvae with a diet containing yeast and a mould inhibitor. We cannot yet provide an estimate of the net benefit larvae may gain from joining others in natural settings. Overall though, our results are in agreement with previous work, which highlighted the tradeoffs involved in joining others: individuals searching for the best available site may rely on the inadvertent social information of others who

have already found such a site. By joining others, however, an individual increases the level of competition at that site [37, 39].

4.7.4 Conclusions and prospects

We have established fruit fly larvae as a simple, highly tractable model system for studying social behaviour and socially influenced learning. This is especially exciting given that larvae have only about 3,000 functional neurons and that there are powerful tools available for studying their neurogenetics [30, 51, 52]. The most logically consistent explanation for our results is that focal larvae use cues of others as a guide to superior feeding sites. Learning about novel cues associated with others and then preferring such cues over alternatives constitutes social learning, defined as the acquisition of new information by an individual (observer) through interaction with either another individual (model) or cues left by that individual [22]. While one can question whether such simple social learning can inform us about elaborate cases of social learning among vertebrates, experience clearly indicates that simple, tractable behaviours and brain functions identified in fruit flies have been instrumental for furthering our understanding of behaviour and cognition in more complex animals including humans [53, 54]. Further work on fruit fly larvae can elucidate the social cues or signals they rely on, and the neurobiological pathways that modulate behaviour and learning in a social context.

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4.10 Supplementary Material

4.10.1 Benefits and costs of joining others

Exp 3: Larval Aggregation and Food Quality, Supplemental Methods

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

Exp 3: Larval Aggregation and Food Quality, Supplemental Results

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

Exp 4: Developmental effects of foraging density, Supplemental Methods

For the analyses of larval developmental rate and egg-to-adult survival, dishes were analyzed in groups assigned *a priori* to give N = 10 for each density: dishes of 50 larvae were counted singly, dishes of 10 and 3 larvae were counted in groups of 5, and dishes of single larvae were counted in groups of 10. This

categorization enabled us to analyze proportions of larvae in each group either reaching pupation or surviving.

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

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

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

CHAPTER 5

Effects of larvae on patch choice in fruit flies

Manuscript in revision: Animal Behaviour (submitted April 2013)

5.1 Abstract

We investigated social information use in fruit flies (*Drosophila melanogaster*), which can be an excellent model species for research into the ultimate and proximate mechanisms underlying social behaviour and social learning. Adult male and female fruit flies were attracted to odours emanating from foraging larvae, and females preferred to lay eggs on food patches occupied by larvae over similar unoccupied patches. Females subsequently preferred to lay eggs at patches with novel flavours previously associated with feeding larvae over patches with novel flavours previously associated with no larvae. However, when we controlled for the duration of exposure to each flavoured patch, females no longer preferred the flavour previously associated with feeding larvae. Our results suggest that adult males may rely on the distinct odour emanating from feeding larvae to locate females. Females have limited abilities to assess food quality but larvae are highly mobile and tend to aggregate over time at the best available

food patch. Females can therefore rely on the presence of larvae as a social cue for optimizing patch choice.

5.2 Introduction

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

consumed by other larvae and prefer cues associated with other larvae (Durisko & Dukas, in press).

Our recent findings that fruit fly larvae rely on social information from other larvae led us to predict that the presence of larvae and food consumed by larvae would be a reliable indicator of high quality sites for adults. The presence of larvae at a site indicates that both a previous egg-laying female and the larvae themselves, which are highly mobile and tend to aggregate at the best available local site (Sokolowski 1980; Gerber & Stocker 2007; Gomez-Marin et al. 2011; Durisko & Dukas, in press), have found the site to be of sufficient quality. Specifically, adult males may utilize larval cues as social information to locate food and mates while females may use these cues to find suitable egg-laying sites. Additionally, by learning cues such as the specific fruit odour associated with sites commonly occupied by larvae, an individual could quickly identify similar sites without devoting the time and energy required for individual sampling. Such social learning can allow females to locate high quality sites efficiently without exposing their offspring to increased competition at sites already occupied.

We assessed whether adult flies use larval social cues in their patch choice decisions. First, we tested whether females prefer to lay eggs at patches occupied by larvae over unoccupied patches. Second, we assessed the attractiveness of the odours emanating from food occupied by larvae to both males and females. Third, we tested whether females would prefer to lay eggs at

patches with flavours previously experienced with larvae over patches with flavours previously experienced without larvae.

5.3 General Methods

We maintained three population cages each containing several hundred *Drosophila melanogaster Canton-S* on abundant standard food at 25°C, 60% relative humidity, and on a 12:12 light/dark cycle with lights on at 1 am. This irregular light cycle placed peak egg laying midday so that we could collect experimental eggs within a very short time window of about 1 h. We collected eggs for experimental larvae on 85 mm diameter petri dishes filled with 10 ml of standard food, one liter of which contained: 60 g dextrose, 30 g sucrose, 32 g yeast, 75 g cornmeal, 20 g agar and 2 g methyl paraben dissolved in 20 ml ethanol. Immediately following egg laying, we transferred these dishes to an incubation chamber maintained at 25°C and high humidity. For experimental adults, we collected and sexed flies with light CO_2 anesthesia within 8 h of eclosion. We stored the males in small cages (24x11x11 cm) and females in standard vials at a density no greater than 20 per vial. We provided flies with abundant 2M sucrose solution diets hardened with agar (20 g/l), and gave females an additional sprinkle of live yeast as a protein supplement to encourage egg development. In all female oviposition or choice experiments, we added females to the cage of males for mating about 16 h prior to experiments so that

they did not have experience with larvae before testing as larvae hatch from eggs after about 22 h. All flies were 3-4 days old during testing.

5.4 Experiment 1 – *Oviposition and Social Attraction*

5.4.1 Methods

We began our investigations by testing for simple social information use: oviposition preference for and attraction to a site containing larvae. In Experiment 1A, we had small cages (20x12x13 cm) each containing one social and one nonsocial 35 mm diameter petri dish containing 4 ml of standard food. Social dishes each contained 30 early 3nd instar larvae, which had been feeding at the dishes for 24 h prior to the test so that the food ("used food") had a noticeably different surface texture and smell. As a control, we treated the nonsocial dishes identically, including a sham addition of larvae with the same paintbrushes. Food contained a few drops of blue colouring to increase the visibility of eggs. We placed dishes in opposite corners, furthest from a lamp in the center of the room scattering diffuse light to the ceiling on a timer that turned off at 10 pm. We alternated the locations of social and nonsocial dishes between tests to control for side bias. Testing began at 6 pm with each focal female introduced into the cage through a hole in the centre of the side opposite the food to minimize any bias (Fig. 5.1A, left). We left females to lay eggs overnight for a total of 16 h. At 10 am the following morning, we removed females, randomized the dishes and

counted eggs while blind to female identity. For each female we calculated the proportion of eggs laid on the social dish. Due to violations of normality, we compared these proportions to random chance (0.5) with a One-Sample Wilcoxon Signed-Ranks test. In Experiment 1B, we repeated this protocol but with continuous lighting throughout the night in order to ensure that the females could perceive both food dishes. We analyzed only those females that laid eggs during the test phase.

In Experiment 1C, we tested whether females were attracted to the olfactory cues emanating from larvae. We used vials of food capped with funnels, effectively forming a 1-way trap (Fig. 5.1A, right). Vials contained 5 ml of standard food, either with or without larvae, as before. Flies had to enter the vials via the funnels and, once inside, could not exit (R. Dukas, unpublished data). Each female therefore made a single choice of either the social or nonsocial vial, and we analyzed the frequency of choices with a binomial test. We analyzed only those flies that entered a vial. Since females may have been attracted not to the smell of larvae, but to the smell of live yeast that had been transferred within the gut of larvae (Coluccio et al. 2008; Stamps et al. 2012), we repeated this protocol in Experiment 1D with a modified recipe of food containing no live yeast. We used this new diet for all subsequent experiments. In Experiment 1E, we repeated this protocol with virgin male fruit flies.

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

5.4.2 Results

Females strongly preferred to lay eggs on dishes containing larvae and used food both under standard photoperiod (Experiment 1A; mean proportion of eggs on social dishes ± SE: 0.995 ± 0.004; N =128, One-Sample Wicoxon Signed-Ranks Test: W = 8128, $P < 0.001$; Fig. 5.1B) and under continuous lighting (Experiment 1B; 0.978 ± 0.022; N = 46, One-Sample Wicoxon Signed-Ranks Test: W = 1034, $P < 0.001$; Fig. 5.1B). In these two experiments, each female laid on average 35.2 ± 1.7 and 26.5 ± 2.5 eggs, respectively. In Experiment 1C, females significantly preferred vials containing larvae and used food over vials containing unused food (Proportion choosing social vial: 0.643; Binomial test: N = 56, P = 0.044; Fig. 5.1C). Females showed a similar preference when we

controlled for the potential presence of live yeast in Experiment 1D (0.660; Binomial test: N = 53, P = 0.027; Fig. 5.1C). In Experiment 1E, males also preferred vials containing larvae and used food over vials containing unused food alone (0.594; Binomial test: N = 155, P = 0.024; Fig. 5.1C). Finally, in Experiment 1F, wild-caught females also chose vials containing larvae and used banana significantly more often than the vials containing only banana (0.620; Binomial test: N = 108, P = 0.016; Fig. 5.1C).

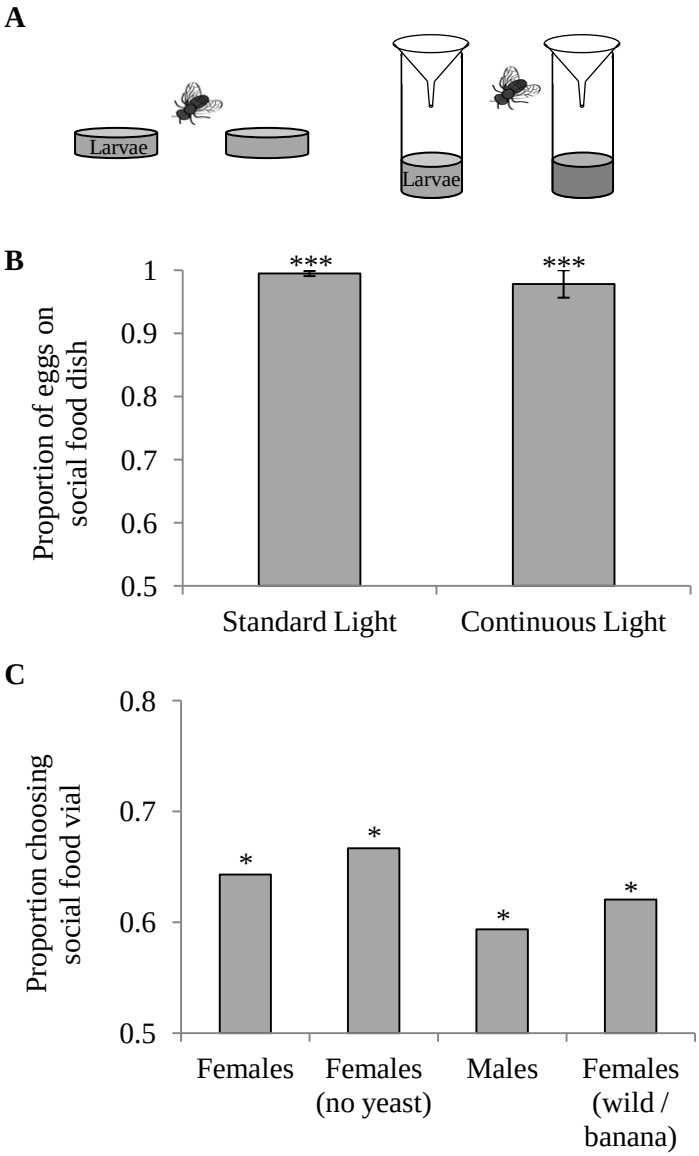

Figure 5.1 We gave individual adult flies a choice between two sources of food, one of which contained larvae. **(A)** The experiments used either dishes (left) from which we calculated the proportion of eggs laid by each female on the social dish containing larvae, or vial-traps (right) from which we calculated the proportion of flies entering the social vial. **(B)** Females strongly preferred to lay their eggs on dishes containing larvae in both standard (lights off from 10 PM to 10 AM) and continuous lighting conditions. **(C)** More females and males entered vials containing larvae and food than entered vials containing food alone.

5.5 Experiment 2 – *Socially Biased Learning*

5.5.1 Methods

In Experiment 2A, we asked whether attraction to foods occupied by larvae could result in female learning, which would bias future oviposition decisions in the absence of larvae. We collected and stored males, females and larvae as before. We transferred mated females individually into training cages each containing a social and a nonsocial dish of standard food, one flavoured with cherry and one with orange (sugar-free Kool-Aid drink mixes, 3 and 4.3 g/l, respectively, which flies preferred approximately equally in preliminary tests), placed in opposite corners at the rear of the cage. Each female received a 4-hour training session (2 pm – 6 pm) with the two flavours, one of which was associated with larvae and used food. We counted the number of eggs laid on each dish during training as an indication of each female's experience. We alternated the side of flavour presentation and the flavour paired with larvae across females to control for bias. For testing, we replaced the training dishes with one new dish of each flavour without larvae. The location of each flavour was the same during training and testing. We also spread 0.8 ml of yeast suspension (3 g live yeast / l warm water) on the surface of each test dish and allowed it to dry for at least 1 h before testing. This amount of yeast is sufficient to stimulate egg laying but does not overpower the orange and cherry flavours. Females laid eggs overnight (6 pm – 10 am, with lights off at 10 PM). We randomized the dishes to ensure that observers were blind to female identity, and then counted the number of eggs

laid on each flavour. We analyzed only those females that laid eggs during the test phase. Many females (52%) exclusively preferred one flavour during testing. This resulted in highly non-normal data, so we analyzed oviposition flavour preference (proportion of eggs laid on cherry flavoured food) with a nonparametric permutation ANOVA (using the R package 'lmPerm', version 1.2; Wheeler 2010). We included factors for the side of cherry presentation, identity of the social flavour, whether or not females laid eggs during training, and the interaction between social flavour and whether females laid eggs during training. A parametric ANOVA revealed similar results.

Experiment 2A simulated realistic settings in which females are attracted to and lay eggs on a substrate with larvae and used food. In Experiment 2B, we tested whether a female would prefer the flavour paired with larvae during training if we exposed the female to a social patch with one flavour and a nonsocial patch with another flavour. All collection, storing, training, and testing of the females was identical to Experiment 2A, except that we trained females with the two distinct patches and flavours in succession. We gave each female a 4-hour training session (10 am – 2 pm) with a food dish of the first flavour, and then a second training session (2 pm – 6 pm) with a dish of the other flavour, only one of which contained larvae, followed by a test phase (6 pm – 10 am) with both flavours without larvae. During training, we alternated the order and side of flavour presentation, as well as which flavour contained larvae, between females. As in Experiment 2A, results were non-normal with 40.4% of females laying eggs

exclusively on one flavour. We again analyzed oviposition flavour preference (proportion of eggs laid on cherry flavoured food) with nonparametric permutation ANOVA, here including factors for the flavour paired with larvae, the order of training flavours, the side of flavour presentation, whether or not females laid eggs during training, and relevant interactions.

Having found no evidence of social learning in Experiment 2B when we controlled for exposure, in Experiment 2C, we tested whether a female experiencing a novel cue with larvae would show a stronger preference for this cue than another female experiencing the cue without larvae (similar to Sarin & Dukas 2009). All collection, storing, training, and testing of the females was identical to the previous two experiments, except that we trained each female with one flavour only. Each female received a single 8-hour (10 am – 6 pm) training session with a single flavoured food dish, either with or without larvae, followed by a test phase (6 pm – 10 am) with both flavours without larvae. Again, results were non-normal with 94.0% of females exclusively preferring one flavour during the test, and so we analyzed oviposition flavour preference (proportion of eggs laid on cherry flavoured food) with nonparametric permutation ANOVA, here including factors for the flavour experienced, the presence or absence of larvae during training, the side of flavour presentation, whether or not females laid eggs during training, and relevant interactions.

5.5.2 Results

In the test phase of Experiment 2A, females significantly preferred to lay eggs on the flavour that had been paired with larvae and used food during training (N = 254; Permutation test: P = 0.004; Fig. 5.2A). Side of the flavour presentation, whether females laid eggs during training, and the interaction had no significant effect (all P > 0.255). In Experiment 2B, females did not prefer the flavour previously experienced with larvae over the flavour previously experienced without larvae (N = 114; Permutation test: P = 1.000; Fig. 5.2A). There was no significant effect of the side of flavour presentation, the order of training, whether or not females laid eggs during training, or interactions (all P > 0.141). In Experiment 2C, the presence of larvae on a flavour during training did not increase female preference for this flavour during the test (N = 67; Effect of larvae X flavour experienced, Permutation test: P = 0.157; Fig. 5.2B). We did, however, observe a main effect of the flavour experienced during training on later preference (Permutation test: P = 0.027). There was no significant effect of the side of flavour presentation, whether or not females laid eggs during training, or interactions (P > 0.170). Overall, females showed socially influenced learning when we simulated natural settings, but no social learning when we controlled for exposure duration to social and nonsocial flavours.

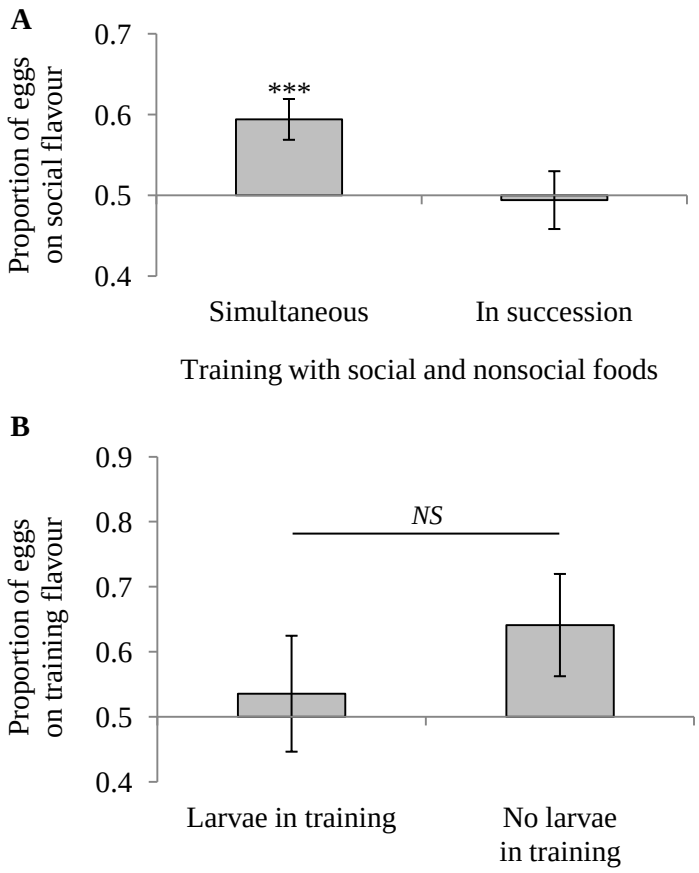

Figure 5.2 **(A)** We trained females with a dish of each flavour, only one of which contained larvae, either simultaneously (left), or successively (right), with the latter controlling for duration of exposure to each flavour. During a subsequent test, females significantly preferred to lay eggs on the flavour paired with larvae only if they had been trained with both flavours simultaneously. When we controlled for exposure duration, females no longer preferred the social flavour. **(B)** We trained females with a single flavoured dish, which either did or did not contain larvae. Females trained with a flavour containing larvae did not prefer that flavour more than females experiencing the flavour without larvae.

5.6 Discussion

In this study we showed, first, that female fruit flies strongly prefer to lay eggs on a food substrate already occupied and consumed by larvae (Fig. 5.1B). Second, both female and male adult fruit flies are attracted to odours emanating from food that is occupied by larvae (Fig. 5.1C). Third, females learn to prefer novel cues associated with food consumed by larvae over novel cues associated with unused food of similar quality (Fig. 5.2A). Fourth, if females experience a flavour associated with food used by larvae *for the same duration* as another flavour associated with food without larvae, they do not subsequently prefer the flavour associated with larvae (Fig. 5.2A). Finally, females that experience only a single flavoured food used by larvae do not subsequently show a stronger preference for that flavour compared to females that experience a single flavoured food without larvae (Fig. 5.2B). Altogether, this suggests that female experience with a flavour regardless of the presence of larvae can explain the later preference for that flavour, but that larval presence is attractive and can bias female experience.

Our easiest result to explain is the fact that males are attracted to food that has been used by larvae. The odour emanating from such food, probably produced by microbiota associated with the larvae, is likely a cue for males that can lead them to a food source and to sexually receptive females. Females' attraction to such food patches with larvae is more complex, as choosing an oviposition site that already contains larvae will have both costs and benefits. Like with males, the odour associated with foraging larvae will guide a female to

a good site that has already been chosen by both other females and the larvae themselves. Our experiments have indicated that, given a choice between food patches of varying qualities, larvae will settle on the better alternative (Durisko & Dukas, in press), suggesting that the presence of larvae may be a particularly informative cue. Additionally, larvae may actually improve the quality of the substrate for subsequent larvae owing to changes in texture, suppression of mould and facilitation of favourable microbes, including beneficial yeast species (Wertheim et al. 2002; Rohlfs & Hoffmeister 2003; Stamps et al. 2012; Durisko & Dukas, in press).

While the informational value of odours emanating from feeding larvae is probably substantial, there are obvious costs as well. Because it takes about a day for eggs to hatch, a female laying eggs on substrates already occupied by larvae guarantees that her larvae will encounter a substrate containing harmful waste products (Borash et al. 1998) and likely competition for food. We recently showed that increased larval density slows development rate, increases mortality, and decreases adult body mass. Even with abundant food, adult body mass, which has been shown to be an important determinant of fitness (Partridge et al. 1987; Lefranc & Bundgaard 2000), was significantly lower when larvae were reared in a density of 3 versus 1 larvae (Durisko & Dukas, in press). Similar effects of density on larval success under different experimental settings have been previously reported (Sang 1949; Wertheim et al. 2002).

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

Given that larval presence at a food patch is perhaps the best indication that it is highly suitable for larval development, it is clear why females that were attracted to substrates occupied by larvae learned and subsequently sought out similar substrates (Fig. 5.2A). However, in contrast to our previous data from adult (Sarin & Dukas 2009) and larval (Durisko & Dukas, in press) fruit flies, females did not show social learning under strictly controlled conditions that equalized the duration of fly exposure to either social or nonsocial flavoured food patches (Fig. 5.2A, B). It is possible that direct cues from the larvae diminish the effect of novel flavours under such controlled experimental settings.

The costs and benefits of social behaviour and aggregations have been appreciated for a long time (Allee 1931; Danchin & Wagner 1997), striking the balance between the benefits of obtaining information by copying and the costs of increased competition is likely a widespread evolutionary phenomenon throughout the animal kingdom. How such tradeoffs affect the evolution of social learning is an interesting question, and fruit flies, which are a growing model for the study of social information use and social learning, can help shed light on the mechanisms and evolution of social attraction and social information use.

5.7 Acknowledgements

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CHAPTER 6

Dynamics of Social Behaviour

in Fruit Fly Larvae

Manuscript in prep.

6.1 Abstract

Fruit fly larvae have great potential as a model organism for the study of both the evolution and neurogenetic mechanisms of social behaviour. We show first that larvae are more social than previously thought, and that both laboratory and wild-caught strains of larvae form foraging aggregations on standard food. This aggregation behaviour exhibits an inverse-quadratic trend throughout the larval stage, declining with the wandering stage before pupation. To our knowledge this is the first documentation of such behaviour. Additionally, we show that one potential benefit of larval aggregations is an improved ability to dig and burrow into the food substrate. Larvae aggregate more on harder food, and at sites where we have previously broken the surface of the food, and interestingly, even pairs of larvae placed on a dish together stay near one another until they begin digging.

6.2 Introduction

Social behaviour can have enormous impacts on the fitness and evolution of animals (Allee 1927; Wilson 1975), but its neurogenetic underpinnings and the mechanisms by which it evolves are only beginning to be understood (Robinson et al. 2005; Toth & Robinson 2007). Crucial for such research is the use of simple model systems (Sokolowski 2010; Schneider et al. 2012), and to this end we have established behavioural protocols for examining social interactions in fruit fly larvae, *Drosophila melanogaster* (Durisko & Dukas, in press), an ideal model system owing to their simple brains, which contains only ~2,000 functional neurons (Nassif et al. 2003; Younossi-Hartenstein et al. 2003), and amenability to neurogenetic manipulation. While studies on fruit fly larvae have been immensely successful in furthering our understanding of foraging, locomotion, and the mechanisms of taste, olfaction, and learning (Gerber & Stocker 2007; Gomez-Marin et al. 2011; Iyengar et al. 2011; Huser et al. 2012), the study of larval social behaviour has been relatively limited. Wu et al. (2003) noted that older (wandering stage) larvae are more 'clumpy' and seem to engage in cooperative burrowing, adopting a vertical drilling motion, which they suggest may help larvae locate safer sites to pupate, although this remains to be studied closely. Some of the neural mechanisms involved in this social burrowing have been identified (Wu et al. 2003; Xu et al. 2008). Recently, it has been shown that larvae are attracted to the visual cues of other larvae exhibiting similar writhing

and digging behaviour (Justice et al. 2012). Finally, in a previous study, we have shown that feeding stage larvae can be used to examine social information use and social learning. We found that larvae are attracted both to groups of other foraging larvae and to cues previously experienced in the presence of others (Durisko & Dukas, in press). Larval social behaviour, therefore, appears to be a robust phenomenon with great potential for future studies. We, however, still do not understand the dynamics of social interactions during larval development as they would occur in nature.

Typically, females lay clusters of eggs on exposed sections of rotting fruit, which, due to the deposition of attractive pheromones and transferred yeast species, as well as the attractive odour of larval residues, draw additional females which will copy this egg-laying site choice (Bartelt et al. 1985; Wertheim et al. 2005; Reaume & Sokolowski 2006; Stamps et al. 2012; Durisko, Anderson & Dukas, submitted). The result is a competitive foraging environment where larvae must cope with dwindling food and the build-up of toxic waste products like ammonia (Borash et al. 1998). Larvae, however, are surprisingly mobile and will explore their local environment searching for new, higher-quality foraging sites (Sokolowski 1980; Gomez-Marin et al. 2011; Durisko & Dukas, in press; Schwarz, Durisko & Dukas, submitted). Such aggregations of larvae also allow frequent opportunities for social interaction. Additionally, we've observed that, when placed in a dish with others, feeding-stage larvae are attracted to and spend much of their time very close to others (Durisko & Dukas, in press),

indicating that larval sociality may extend to physical interactions in the context of foraging. We still do not know, however, if larvae will spontaneously form foraging aggregations, the patterns and dynamics of such sociality, the ecological factors that influence this social behaviour, or how social interactions benefit the larvae. Here, we expand our understanding of larval sociality by closely monitoring aggregation behaviour throughout larval development to identify the pattern and critical periods of social interaction. Additionally, by manipulating ecological factors and observing the pattern of aggregation, we investigated one potential benefit of larval sociality: improved digging ability.

6.3 General Methods

We maintained fly populations in large cages each containing several hundred *Drosophila melanogaster* on abundant standard food, one liter of which contained: 60 g dextrose, 30 g sucrose, 32 g yeast, 75 g cornmeal, 20 g agar and 2 g methyl paraben dissolved in 20 ml ethanol, in an environmental chamber at 25°C, 60% relative humidity, and on a 12:12 light/dark cycle with lights on at 1 am. This irregular light cycle placed peak egg laying midday so that we could collect eggs within a short window of time by providing flies with an 85 mm petri dish containing 10 ml standard food. Since females may hold developing embryos while searching for a suitable egg-laying substrate, prior to experimental egg collection we provided females with a fresh dish with a sprinkle of live yeast for 1 h, which we discarded. We then collected eggs for experimental larvae

within 45 min on dishes without live yeast. We immediately transferred these eggs one at a time to experimental dishes with a soft paintbrush. All experimental dishes were stored in incubation chambers maintained at 25°C and high humidity. All manipulations were conducted under red light, which larvae cannot see (Bertholf 1932). On the following day, we replaced any unhatched eggs (typically less than 20% of eggs per experiment, which may have been damaged or slower to develop) with age-matched larvae in order to keep the number of larvae per dish constant. We used the *Canton-S* strain of wild type flies in all cases except Experiment 1B, where we compared *Canton-S* to a wild-caught population of flies from Southern Ontario.

6.3.1 Aggregation assay

For each experiment we utilized a novel behavioural assay which allowed us to quantify larval aggregation over time. We filled a 3cm x 3cm x 2cm Plexiglas dish with 9 ml of standard food, 1 cm thick with a smooth, uniform surface. Each dish was covered with loose-fitting lid, which allowed some airflow. We divided the dish into nine equally-sized 1cm x 1cm quadrats (Fig. 6.1A), and, taking care not to damage the eggs or the surface of the food, placed one egg in the center of each quadrat, except for Experiment 1A where, in half of the dishes, we placed all nine eggs in one quadrat. Beginning at 5 pm on the day following egg laying (6 h following hatching), we counted the number of larvae in each quadrat 3

times per day (9 am, 1 pm, and 5 pm) until pupation. Larvae moved freely throughout the experiment, and we took care not to disturb them during observations. In cases where larvae were crossing between quadrats at the time of observation, we recorded the location of their mouth. In the very rare case where this was still ambiguous, we watched the larva for a few seconds until it chose one quadrat.

For each dish and time we calculated an *Aggregation Index* (*AI*), defined as the variance-to-mean ratio (Krebs 1999). For each experimental treatment, we compared larval aggregation to the null model of random motion, defined by a Poisson distribution where the mean equals variance, *AI* = (variance/mean) = (1/1) = 1. Indices greater than 1 indicate aggregated or "clumpy" distributions, and indices lower than 1 indicate more uniform distributions. Note that with this protocol, *AI* ranged from 0, a perfectly uniform distribution (one larva per quadrat), to 9, a perfectly aggregated distribution (all larvae in one quadrat).

Due to violations of normality associated with our Aggregation Index, we tested the dynamics over time with Generalized Estimating Equations (GEE) with a gamma distribution and log link function. In all experiments, *time* was included as a within-subject factor. Wald χ^2 values are reported for these analyses. In experiments where larvae were initially placed in a uniform distribution, the expected climb in *AI* from 0 to 1 due to random motion could have resulted in spurious trends, so we modified indices from this time point to a value of 1 for analyses, which represented the null hypothesis of random motion. In cases

where we observed a significant effect of time, we conducted one-sample t-tests at each time point to test for significant deviance from the null hypothesis of 1.

6.4 **Experiment 1 – Initial Distribution and the Dynamics of Aggregation**

6.4.1 Methods

In Experiment 1A, we examined how the two extreme initial starting distributions affected the dynamics of larval aggregation. The starting distributions were either uniform (one egg in each quadrat), or aggregated (all eggs in one quadrat). We first analyzed the initial distributions separately, and seeing that the Aggregation Index of the two rapidly converged within the first 22 h after hatching (Fig. 6.1B), we compared the pattern of aggregation from this convergence onward. We conducted additional analyses to assess two alternative hypotheses: (a) that larvae are merely attracted to one particular site in the dish (eg. the corners), or one particular quadrat in the dish (eg. the top-left quadrat) due to external environmental factors, and (b) that larvae are not directly attracted to one another *per se* but form aggregations as a result of one larva improving a quadrat, which others then find attractive. First, we compared the sites of greatest aggregation, defined as the site where we observed the highest number of larvae. In the event that a dish had multiple sites with the same degree of aggregation, we chose the quadrat with the greatest total number of larvae throughout the experiment. We compared the frequency of types of quadrats (corners, sides, or the middle) to the distribution expected by random chance (4:4:1, respectively) with a chi-

square goodness of fit test. We compared the frequency of particular quadrats of greatest aggregation (numbered 1-9) with a “meta”-aggregation analysis where we compared the different locations of greatest aggregation to our null hypothesis of random distribution, similarly defined as an index of dispersion equal to 1. Larval dishes from both initial distributions had similar results from our “meta”-aggregation analysis, so we combined their results. Finally, to show that larvae are not merely attracted to one higher quality site in the dish, we noted the total number of quadrats per dish where larvae formed aggregations and counted the number of times an aggregation shifted quadrats throughout the experiment. For this analysis, we defined an aggregation as four or more larvae per quadrat because aggregations of five or more larvae were not sufficiently common for statistical analyses.

In Experiment 1B, we directly compared the pattern of aggregation between our laboratory fly strain (*Canton-S*) and a wild-caught population. We caught a few hundred *D. melanogaster* from natural populations in several locations around southern Ontario using plastic bottle traps with slices of banana seeded with a sprinkle of live yeast. We maintained these flies on our standard lab diet and tested them within 8 months of collection. We analyzed several dishes of the wild-caught and CS simultaneously, with observers blind to population.

6.4.2 Results

In all of our experiments, larvae showed a tendency to aggregate, which peaked between 40-80 h after hatching (Fig. 6.1B–D; Fig. 6.2). In Experiment 1A, after convergence of the two initial distributions (Fig. 6.1B & C), the average maximum Aggregation Index from each dish reached 3.33 ± 0.17, (N = 40, mean ± SEM), corresponding to ~5 out of 9 (55.6%) individuals in one quadrat. Aggregations were highly variable within a dish, however, as the larvae are highly mobile. We observed a strong decline in aggregation coincident with the onset of the larval wandering and pupation.

Larvae showed a similar pattern of aggregation throughout the larval stage regardless of whether we placed eggs in a perfectly uniform or perfectly aggregated distribution, suggesting that this is their preferred pattern of distribution. Larvae from both initial distributions rapidly (within 22 hours) converged on similar small aggregations ($AI \approx 1.5$), which then increased throughout the second and third instar stages before declining with the onset of larval wandering and pupation (Fig. 6.1B & C). From the point of convergence onward (22 h, dropping the first two time points), aggregation did not differ between the two initial distributions (Main effect of initial distribution, GEE: $\chi^2_1 = 1.6$, N = 40, $p = 0.209$; Interaction between initial distribution and time: $\chi^2_9 = 10.4$, $p = 0.321$; Fig. 6.1B & C), and both showed a similar quadratic trend (Effect of time, GEE: $\chi^2_9 = 81.1$, $p < 0.001$; Quadratic: $\chi^2_1 = 53.4$, $p < 0.001$).

In Experiment 1A we assessed how variable the site of greatest aggregation was within and between dishes. Larval aggregations were not merely due to preference for one particular site in the dish (eg. corners), or one particular quadrat. Larvae did not prefer one area of the dish over others, forming greatest aggregations in corner (52.5%), side (45.0%) and middle (2.5%) quadrats no differently than expected by chance (4:4:1 ratio, respectively; χ^2_2 = 3.3, p = 0.196). The location of greatest aggregation of each dish did not differ from random, and larvae formed aggregations in all quadrats (Index: 1.24; χ^2_8 = 10.0, less than $\chi^2_{critical}$ = 17.5). Finally, larval aggregations moved throughout the experiment (Fig. 6.2), and cannot readily be explained by attraction to or remaining in one site that has been improved by another larva. All dishes had at least one aggregation of four or more larvae at some time, and in most dishes the site of greatest aggregation shifted throughout the experiment (eg. Fig. 6.2). When starting from a uniform distribution, clumps of four or more larvae formed in 2.4 ± 0.2 different quadrats, with the site of aggregation changing locations an average of 1.7 ± 0.3 times per dish (see Fig. 6.2 for example). When starting with a perfectly aggregated distribution (dropping the first two time points that had artificially high aggregation), larvae formed clumps of four or more larvae in 2.5 ± 0.2 different quadrats, with the site of aggregation moving 1.9 ± 0.3 times per dish. In 60% of dishes (12/20), larvae formed their first aggregation of four or more larvae in the quadrat where the eggs had hatched. In 25% of dishes, larvae never formed an aggregation at this site after hatching.

In Experiment 1B, wild-caught and *Canton-S* larvae showed similar aggregation behaviour, with no significant main effect of population (GEE: χ^2_1 = 1.3, N =19, p = 0.258; Fig. 6.1D). Dishes of the wild and CS populations reached average maximum indices of 2.3 ± 0.1 and 2.7 ± 0.4, respectively, corresponding to ~4 larvae in one quadrat. We observed a significant overall effect of time and also a significant interaction between time and population (χ^2_{11} = 562.0, p < 0.001, and χ^2_{11} = 34.7, p < 0.001, respectively), indicating that the aggregation behaviour of the two populations changed differently throughout development (see Fig 6.1D for comparison). However, analyzing both populations independently revealed that both best fit a quadratic trend (CS: χ^2_1 = 11.0, N = 9, p = 0.001; and Wild: χ^2_1 = 9.5, N = 10, p = 0.002), where the tendency to aggregate increases before declining prior to pupation, suggesting that the two populations exhibit similar patterns of aggregation.

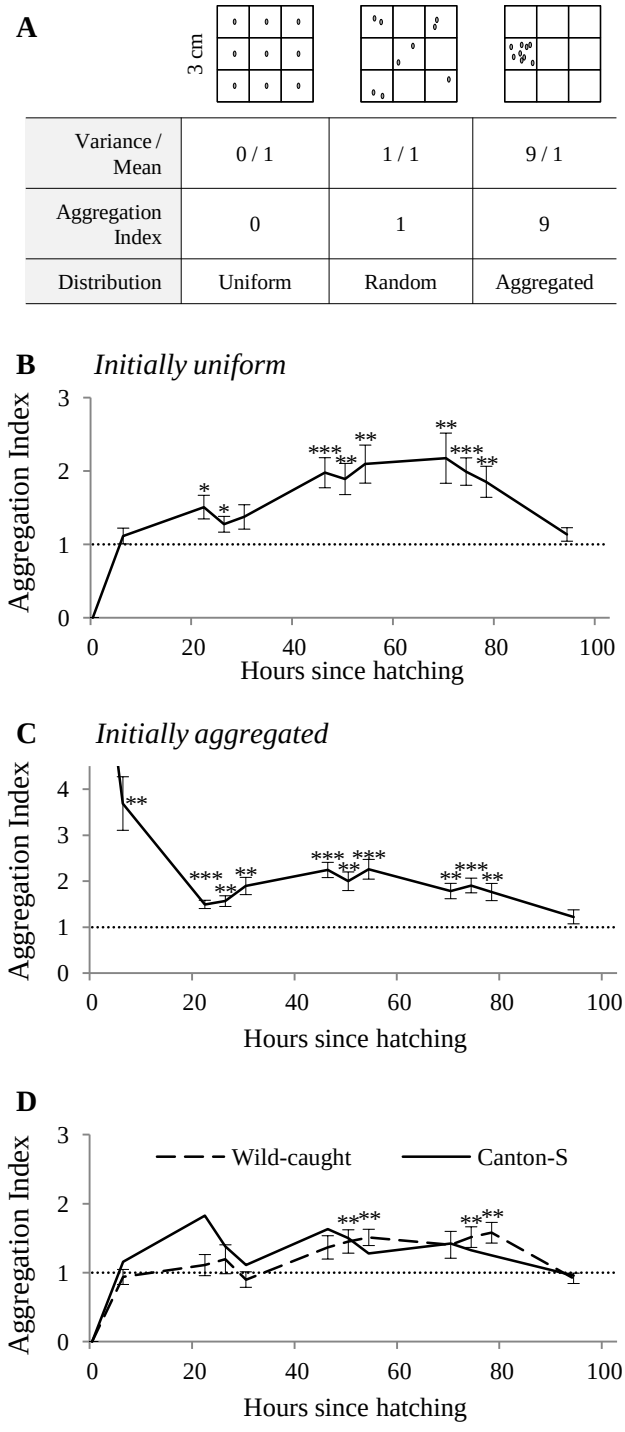

Figure 6.1 (A) We monitored larval aggregation behaviour in 3 cm x 3 cm dishes containing nine larvae each, and calculated an *Aggregation Index*, defined as the variance-to-mean ratio. We started the larvae in either a **(B)** perfectly uniform or **(C)** perfectly aggregated distribution, and observed aggregation three times per day until pupation. Asterisks indicate significance from the null hypothesis of random motion, AI = 1: $p \leq 0.05$ (*), $p \leq 0.01$ (**), and $p \leq 0.001$ (***). **(D)** We compared aggregation behaviour between two populations of flies, our laboratory *Canton-S* and a wild-caught strain. For clarity, we only show the error bars and asterisks for the wild-caught larvae.

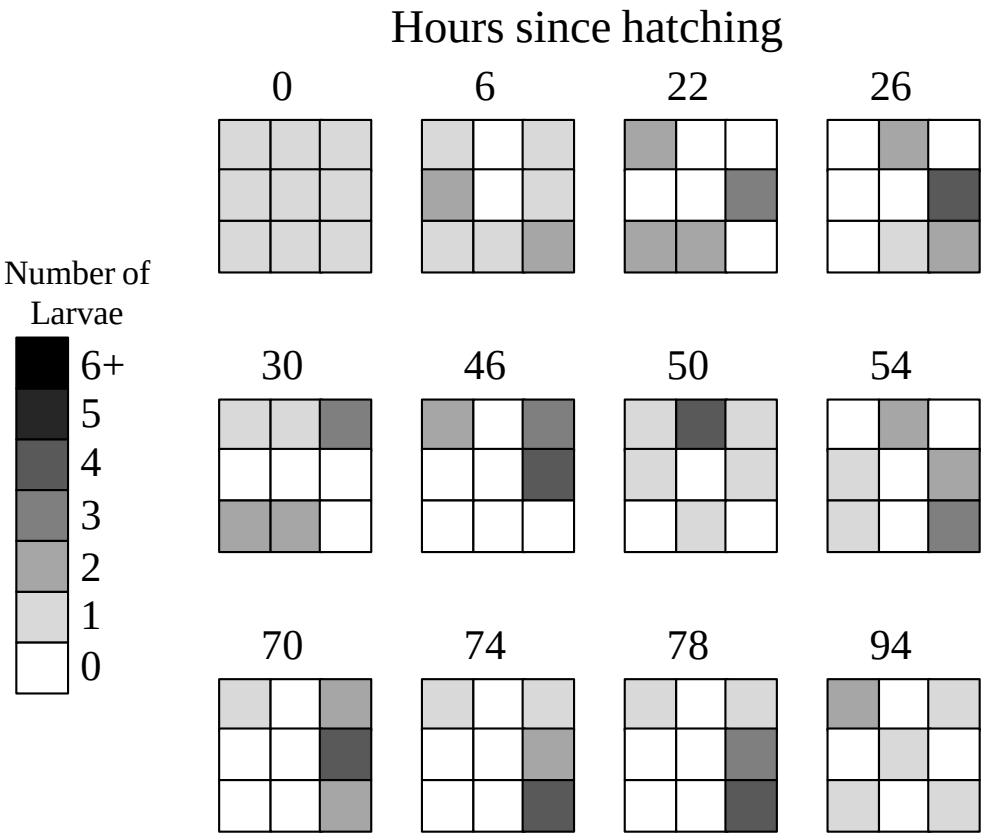

Figure 6.2 An example dish showing typical larval aggregation and movement behaviour. Larvae are highly mobile and formed modest aggregations that moved over time. Colour corresponds to the number of larvae per quadrat.

6.5 Experiment 2 – Food Hardness

6.5.1 Methods

In the previous experiment, larvae exhibited an inverted-U quadratic pattern of aggregation behaviour which peaked in late-second and early-third instar stages (approximately 40-70 h after hatching). These times roughly correspond with the onset of digging behaviour on our standard food, when larvae break the surface and spend less time crawling (*personal observation*). In Experiment 2A we tested whether the ability of larvae to dig in their food affected their tendency to aggregate. We predicted that, if larvae are aggregating in order to improve digging, when the food is tougher and therefore more difficult to dig, we would see increased aggregation. Conversely, we predicted that when the food is softer and easier to dig, we would see decreased aggregation. We simultaneously monitored the aggregation of larvae on dishes where we altered the toughness of the food by changing the concentration of agar in our standard recipe. We tested (a) our standard food recipe, (b) food in which we had doubled the agar, making the food harder and more difficult to dig, and (c) food in which we halved the agar, making it much easier for larvae to dig (2, 4, and 1% agar weight/volume, respectively). The surface texture of the food was similarly smooth in all treatments. We placed one egg per quadrat and monitored dishes as in Experiment 1.

Having found an effect of the food hardness in Experiment 2A, in Experiment 2B we directly assessed whether breaking the surface of the food *per se* affected aggregation behaviour. We predicted that, if larvae aggregate in order to dig into the food (for instance, in order to hide from parasitoids), larvae given one quadrat with the surface already broken would show high levels of aggregation at this site, and conversely, larvae where every quadrat has the surface of the food already broken would show little aggregation. We simultaneously monitored dishes with different surface textures. We either (a) left the surface of the food smooth, as in previous experiments, (b) dug away the surface of the food with a shallow 0.5 cm x 0.7 cm x 0.2 cm “trench” in the center of one randomly selected side quadrat, or (c) dug trenches in all quadrats. Breaking the surface of the food with trenches increased the rate of larval development, probably due to the softer food beneath which is easier to ingest, and so pupation in these treatments began several hours earlier than in previous experiments.

6.5.2 Results

In Experiment 2A, larvae on harder food aggregated significantly more than larvae reared on standard food ($p < 0.001$) and softer food ($p = 0.002$; GEE, effect of food hardness: $\chi^2_2 = 17.8$, $N = 36$, $p < 0.001$; Fig. 6.3A). Larvae on hard food reached an average maximum Aggregation Index of 4.4 ± 0.5 (N = 12), corresponding to ~6 out of 9 larvae in one quadrat. Larvae in both standard and soft food treatments formed smaller but significant aggregations (compared to random chance) which did not differ ($p = 0.801$), reaching average maximum Aggregation Indices of 2.6 ± 0.2 (N= 12) and 2.7 ± 0.3 (N = 12), respectively, corresponding to ~4 out of 9 larvae in one quadrat. There was a significant interaction between food hardness and time ($\chi^2_{22} = 165.4$, $N = 36$, $p < 0.001$), but in all three food conditions: hard, standard and soft, there was a significant effect of time (all $p < 0.001$), all of which best fit a quadratic trend (all $p < 0.001$), just as in previous experiments where aggregations initially increased before declining with the onset of larval wandering.

In Experiment 2B, larvae aggregated significantly more when one quadrat contained a trench than when all or none of the quadrats were trenched ($p < 0.001$, both comparisons; GEE, effect of surface texture: $\chi^2_2 = 54.9$, $N = 20$, $p < 0.001$; Fig. 6.3B). Larval aggregation in the all- and none-trenched dishes did not differ ($p = 0.541$). Additionally, the pattern of aggregation behaviour over time differed between the three different treatments, (GEE, interaction between surface texture and time: $\chi^2_{17} = 4890.7$, $p < 0.001$). When only one quadrat was

trenched, the pattern of aggregation over time best fit a quadratic trend ($p < 0.001$), similar to previous experiments. When all or none of the quadrats were trenched, the trends of best fit were 4th-order ($p = 0.002$), and linear ($p = 0.003$), respectively. Several time points of both the one-trench and no-trenches treatments reached significance from random motion (AI = 1), but for the all-trenched condition, only the peak at 22 h was significantly different from 1 ($p = 0.009$). Aggregation in the one-trench condition peaked with 59.7% ± 5.8% of larvae (5.4 out of 9) at 54 h after hatching.

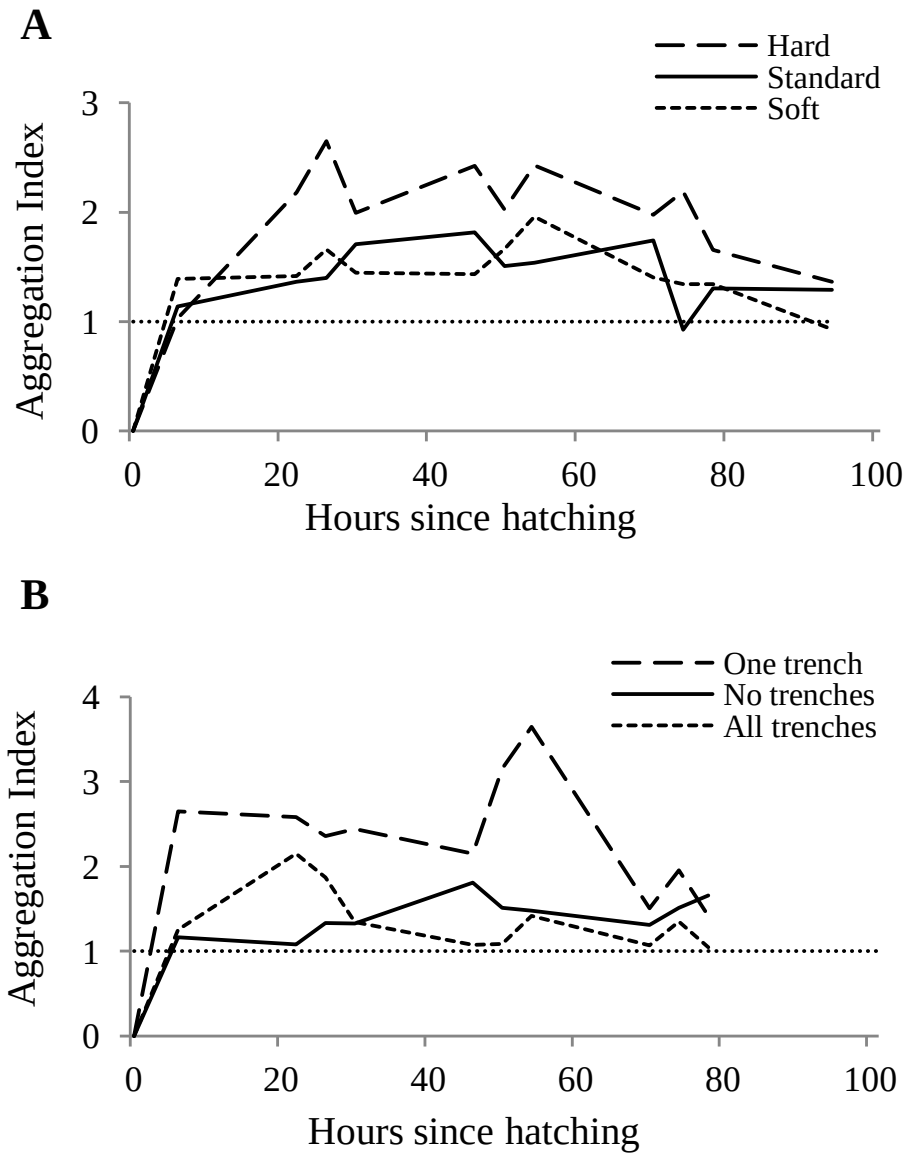

Figure 6.3. We monitored larval aggregation behaviour while manipulating ecological factors. **(A)** Larvae exhibited greater aggregation behaviour on harder substrates than on standard or soft substrates, **(B)** and aggregated more at sites where the surface had been broken with an artificial "trench". Error bars and asterisks were omitted for clarity. Note that even when food was uniformly soft or trenched at every quadrat, larvae still showed significant aggregation.

6.6 Experiment 3 – Detailed Behavioural Observations

6.6.1 Methods

In the previous experiments we only quantified larval aggregation over time. Here we sought to monitor the larvae more closely to better understand their social interactions. For ease of observation, we placed only two eggs per dish, one each into two randomly chosen side quadrats. Starting six hours after hatching, we observed each dish closely for ten minutes twice per day (10 am & 5 pm) until pupation. We recorded the duration of time that larvae were within 5 mm (1-2 body lengths) of one another, which we chose to approximate social interaction, and whether larvae were moving along the surface of the food or digging. Additionally, we recorded the frequency of larvae physically making contact with one another and the duration of time that the larvae spent in contact with one another. For duration data, we calculated the proportion of each ten minute session per dish. We also recorded the latency to the first observation of digging in each dish, and the latency until the first observation of social interaction (larvae within 5 mm of one another).

6.6.2 Results

Larval social interactions increased before declining in the fourth day following hatching. Both the proportion of time the two larvae spent within 5 mm of each other (Fig. 6.4A) and the proportion of time larvae were in physical contact with

one another (Fig 6.4B) increased in the first 24 hours before falling at 70 h post-hatching. Additionally, larvae touched each other approximately once per 10 minute session for the first two days after hatching, but never touched after 70 h post-hatching (Fig 6.4B). Larvae typically find each other and then remain mobile before digging, and we observed larvae to crawl within 5 mm of each other in 2.2 ± 0.4 quadrats across the experiment (N = 10). Digging behaviour increased steadily, with larvae spending almost all of their time digging from 50 h after hatching until digging declined dramatically with the onset of pupation (Fig. 6.4A). Dishes which we observed to be social earlier began digging earlier, though this correlation was only significant at the $p = 0.10$ level (Pearson's $r = 0.619$, $N = 10$, $p = 0.056$; Fig 6.4C), given our small sample size.

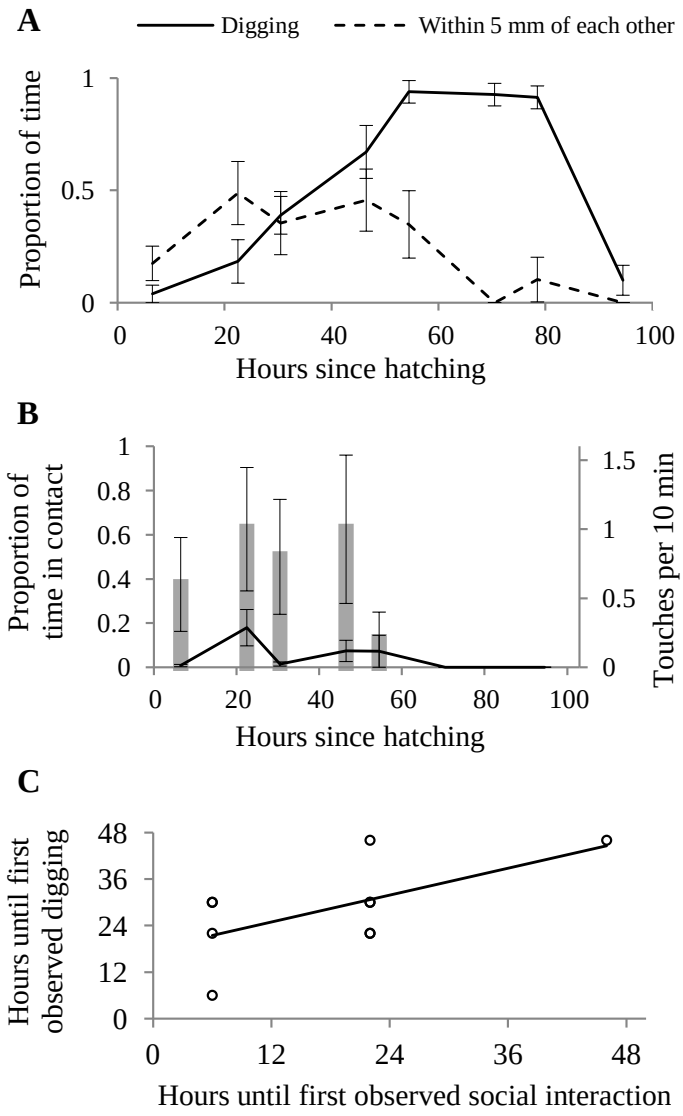

Figure 6.4 We monitored two foraging larvae for ten minutes twice per day from hatching until pupation. **(A)** Initially, both larval social interaction, as measured by time observed within 5 mm of each other (dashed line), and digging behaviour (solid line) increased steadily. Sociality declined at 70 h post hatching, whereas digging continued until pupation. **(B)** Larvae initially spent some of their time in physical contact (black line, left axis), on average touching each other almost once per ten minutes (mean ± SE, grey bars, right axis). **(C)** The latency until the first observed "social interaction", defined as being within 5 mm of one another, was positively correlated ($p = 0.056$) with the latency to begin digging.

6.7 Discussion

We have developed a novel protocol for the study of social behaviour in a widely-used model organism well-suited for future neurogenetic investigations. We have shown that larvae form modest foraging aggregations of four or five out of nine individuals, with social interactions peaking in the late-second-instar stage, regardless of initial distribution (Fig. 6.1). These aggregations are not simply due to larvae preferring one site or quadrat of the dish, and form in different quadrats over time, suggesting that the larvae are not merely aggregating at the best site, or at a site that has been improved by others. Even a pair of larvae placed in a relatively large (9 cm^2) dish will crawl alongside each other through multiple quadrats, often physically touching, which may suggest cooperative foraging rather than site improvement. To our knowledge, this is the first documentation of such social behaviour among fruit fly larvae. Hence our novel protocol can serve for future work on the evolution and mechanisms of social behaviour.

Additionally, we have shown several lines of evidence indicating that larval aggregations allow for improved digging ability, which is important for fitness (see below). First, larvae aggregate more on harder substrates (Fig. 6.3A), and more in sites where the surface has already been broken and thus is easier to dig (Fig. 6.3B). Second, pairs of larvae spend 40-50% of their time within 5 mm of each other until 70 h after hatching, and this corresponds to a steady increase in digging behaviour (Fig. 6.4A). Finally, pairs of larvae that are observed to be social earlier, initiate digging sooner (Fig. 6.4C). Taken together, these results

suggest that larvae may benefit from foraging aggregations with an improved ability to dig into the substrate. Interestingly, other reports of cooperative digging and burrowing have observed the behaviour during the wandering stage prior to pupation (Wu et al. 2003; Xu et al. 2008), whereas our larvae typically exhibited a reduction in aggregation and digging behaviour at this time.

Digging and burrowing may be important to the larvae for several non-mutually exclusive reasons. Probably the greatest benefit that digging affords is an ability to hide from parasitoid wasps. Larval mortality from parasitoids can be enormous (up to 90% in some instances; Fleury et al. 2004), and digging allows an individual off the surface of the food where they are most vulnerable to some species of parasitoids (Carton & David 1985). Second, digging may allow larvae to better maintain homeostasis in variable environments (Reaume & Sokolowski 2006). In particular, the temperature and humidity inside a fruit are much less variable than the surface. Third, larval digging may serve to break down and soften food, making it easier to ingest. Finally, larval digging may function to churn the food substrate, which can fight off competitive mould growth (Rohlfs 2005a; Rohlfs 2005b), and can facilitate the growth of beneficial yeast species (Stamps et al. 2012).

Digging, however, is not the only reason for larval aggregations. We observed aggregations even when foods were uniformly very soft and easy to dig (Fig. 6.3A), or uniformly pre-dug (Fig. 6.3B), indicating that larvae form small

aggregations even when they are able to dig alone. Indeed, in our previous work we have suggested that larvae may benefit from copying the site choices of others, using the presence of others as social information to find higher quality sites (Durisko & Dukas, in press). Accordingly, an individual larva may have a modest, innate attraction to others even when the site currently occupied is of sufficient quality.

Larval behaviours have been less well-studied than those of adults, yet for many researchers the larvae may prove a simpler model system. For the study of social behaviour in particular, the quantification of sociality among adults typically requires more complex apparati due to the adults' greater mobility and ability to fly (Saltz 2011; Simon et al. 2012), sometimes also including advanced computer tracking programs (Dankert et al. 2009; Branson et al. 2009; Ardekani et al. 2013). Our new protocol for the quantification of larval sociality is simple and can further research into the evolution, ecology, and mechanisms of social behaviour.

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CHAPTER 7

Discussion

This dissertation has examined several aspects of learning and social learning in two insect species: bumblebees (*Bombus impatiens*) and fruit flies (*Drosophila melanogaster*). We first showed some of the targets of individual learning that contribute to improved foraging performance, a proxy for fitness, in bumblebees (Chapter 2). Next we showed that despite costs associated with developing an ability to learn, fruit flies did not modify their investment in learning ability due to the complexity of larval foraging environment (Chapter 3). Finally, in a series of experiments, we discovered new examples of social information use among adult and larval fruit flies (Chapters 4 and 5), and that fruit fly larvae are more social than previously thought, possessing an ability to socially learn (Chapter 4), and spontaneously forming aggregations (Chapter 6). I discuss these results in turn.

7.1 The Costs and Benefits of Learning

The value of information to an organism depends on the degree to which that information improves fitness. Information that does not improve fitness should be ignored, while information that improves performance should be monitored closely. For foraging bees, selection favours learning in contexts which improve

the total lifetime rate of pollen and nectar return to the hive, which is closely tied to fitness (Raine & Chittka 2008; Dukas 2008c). Although the learning abilities of bees in several different contexts have been well documented (see literature cited in Chapter 2), the degree to which improvement in each individual task contributes to overall performance across the lifespan is unknown. Although we failed to document the expected long-term improvements seen by honeybees in more natural contexts (Dukas 2008b; Dukas 2008c), we did show significant and remarkably rapid learning in several particular tasks (eg. orientation flights, flower handling, avoiding flower revisits; Chapter 2), suggesting the learning abilities of bees are well-tuned to their foraging performance. Interestingly, our failure to document the long-term improvements observed in natural settings suggests that bees are able to learn more in more complex environments. Our greenhouse environment simplified many of the contexts in which learning may improve foraging performance in nature (eg. long-distance spatial navigation). In other words, the simplistic greenhouse setting reduced the degree of variation in the environment and therefore likely reduced the *value of learning* (the degree to which learning could improve fitness), which is something we expanded upon in Chapter 3.

The value of learning depends on the environment. Since learning is always associated with some costs due to the metabolic demands of building and using the required brain structures, recent models have suggested that for learning to be more beneficial than non-learning fixed-behaviour alternatives

there needs to be (a) environmental variation that (b) reliably predicts a (c) varying optimal response. That is, if an animal's optimal behaviour is constant, or if there is no environmental variation that indicates the optimal behaviour, learning will not be able to improve an individual's fitness (Stephens 1991; Dunlap & Stephens 2009). Thus, the net benefit of learning may fluctuate across environments, or between generations. In Chapter 3 we tested the prediction that early-life cues of environmental complexity are an indicator of the future value of learning, and that such cues are used by fruit fly larvae to modify investment in learning. Despite our inability to document such plasticity, our results may be useful to future research as follows. While we do not know whether our null results were due to larvae failing to perceive our treatments as *complex*, or whether they do not exhibit plasticity of this sort, similar protocols in other species, or modified protocols with flies, may reveal such plasticity. Our results are the first step toward investigating this kind of adaptive cognitive plasticity in fruit flies, a popular model for the study of the evolution and mechanisms of learning (eg. Mery & Kawecki 2005; Davis 2011). Generally, we expect animals to have evolved this and other mechanisms to ameliorate the substantial costs of learning where possible.

7.2 Social Attraction and Information

We identified two novel cases of social attraction in fruit flies. Both larvae (Chapter 4) and adult flies (Chapter 5) are attracted to sites containing volatile cues associated with foraging larvae, likely originating from larval excreta, or "*frass*". One benefit of such attraction is likely informational: a reduction in the search costs associated with finding a suitable site, either for foraging or oviposition. We showed that groups of foraging larvae will tend to form aggregations at the highest quality sites in their local environment (Chapter 4), and therefore may be reliable indicators of local optima. Even in the absence of local variation, cues of larval foraging indicate that at least one other female found the site to be suitable for egg laying, and that it has been of sufficient quality to sustain other larvae. Interestingly, we've shown that this social attraction occurs in spite of potential fitness costs incurred by the larvae, such as decreased body size and slower development (Chapter 4), associated with increased competition and the build-up of toxic waste products like ammonia (Borash et al. 1998). These costs likely increase for the demonstrators (models) when observers (focals) join the group, which suggests that such social attraction may be a case of informational parasitism (Giraldeau et al. 2002; Danchin et al. 2004), where the odour cues are inadvertent byproducts of successful foraging.

In general, aggregation pheromones are common among insects (reviewed by Wertheim et al. 2005), and many insect species use larval frass as a social indicator of site quality, although many use it to *repel* further oviposition

and reduce larval competition, even between species (eg. Li & Ishikawa 2004). The deterrent effects of frass have been well studied among *Lepidoptera* (eg. moths) and *Coleoptera* (eg. beetles) species due to their potential use for pest control (Hilker & Klein 1989; Renwick & Chew 1994; Anbutsu & Togashi 2002; Agarwala et al. 2003; Li & Ishikawa 2004). On the other hand, in both locusts (*Schisocerca gregaria*; Order: *Orthoptera*) and German cockroaches (*Blatella germanica*; Order: *Blattodea*), aromatic compounds in the fecal pellets help maintain aggregations (Scherkenbeck et al. 1999; Dillon et al. 2000; Dillon et al. 2002). Presumably, the balance of the costs and benefits of larval aggregations drive the evolution of the relative attractiveness or repulsiveness of larval odours, but in either case, larval odours are relevant social cues across taxa. By comparing the ecologies of these different species, one may gain insight into the factors relevant for the evolution of social attraction (or repulsion). It would be particularly interesting, for example, to see if those species which exhibit social attraction have lower competition costs, greater costs of individual information acquisition (eg. due to predation), or additional benefits of sociality.

7.3 Other Benefits of Larval Sociality

Animals across taxa exhibit social behaviour. We speak of swarms of bees, herds of deer, flocks of geese, schools of fish, troops of monkeys, and crowds of people. We may add to this list: aggregations of larvae (although I prefer “clumps”). We have shown that larvae spontaneously form aggregations (Chapter 6), on a uniform source of food, and that such aggregation is likely to be associated with increased competition (Chapter 4). In the previous section we discussed some of the potential informational benefits of larval sociality, but other benefits may arise from food conditioning (the churning of food, and seeding with the most beneficial microbes) which can increase foraging efficiency and reduce competitive fungal growth (Wertheim et al. 2002; Rohlfs & Hoffmeister 2003; Rohlfs 2005a; Rohlfs 2005b; Weiss 2006; Stamps et al. 2012). Also, feeding on excrement, or coprophagy, can be a source of mutualistic gut fauna, microbial protein and detoxified or broken-down undigested food (reviewed by Weiss 2006). These benefits may exist in nature, however, we only observed evidence of *costs* to fitness (eg. reduced body mass, slowed development and increased mortality) associated with increasing density (Chapter 4), which suggests that any increases to foraging efficiency among similarly aged larvae are offset in our foraging conditions by competition and waste build-up. We were unable to assess the benefits of reduced mould growth due to our use of a hygienic environment and anti-fungals. In nature, the mould-fighting ability of larval aggregations could provide a substantial benefit (Rohlfs 2005a). The benefits of

acquiring mutualistic gut fauna and broken-down food are likely to be greater among younger larvae, which may not possess the appropriate digestive bacteria, and may not be able to process larger pieces of raw food. This potential benefit may be especially relevant for explaining female preference to lay eggs on food occupied by larvae (Chapter 5). Future studies will need to examine such benefits of larval sociality in more natural settings, especially among younger larvae.

We showed some evidence suggesting that another benefit of larval sociality may be an improved ability to dig (Chapter 6). We proposed that one of the primary benefits of such digging is that it allows larvae to hide from parasitoid wasps. Interestingly, parasitoid wasps of different species may employ different searching strategies such as *vibrotaxis* (sensing the vibrations of larvae) and *ovipositor searching* (probing the substrate frequently in search of larvae) (Carton & Sokolowski 1992). As Carton & Sokolowski (1992) point out, digging is an effective strategy against wasps utilizing vibrations because a burrowed larva moves much less. On the other hand, digging makes it easier for *ovipositor searching* wasps to locate larvae, and in this case a better strategy may be increased mobility. It would be interesting to see whether larvae from populations which are exposed to wasps exhibiting different search strategies differ in their degree of sociality and digging.

7.4 Social Learning

Social learning is not one trait, but an umbrella term describing many different types of social information use. We can think about the simple cases of indirect, socially biased learning, where social attraction leads to subsequent individual learning (eg. Chapter 5; "*local enhancement*"*;* Thorpe 1963; Heyes 1994), or cases where interacting with others directly increases the perceived quality of a stimulus (eg. the palatability of food, Galef 2012), or, finally, more active forms of social learning such as *teaching* (Caro & Hauser 1992). How these different forms of social learning evolve, and whether they require different and increasingly complex (respectively) neural mechanisms is unknown.

The biggest difference between the larval and adult uses of social information described in Chapters 4 and 5 are that focal larvae exhibited more direct social learning from other larvae, while adult females did not. While we must be careful not to over-interpret our null result, which may have been due to flaws in experimental design rather than the actual absence of learning, adult females have been shown across labs with similar protocols to engage in robust social learning from other adults (Sarin & Dukas 2009; Battesti et al. 2012), and so we may speculate on why females do not learn directly from interactions with cues of larvae. Given that the females possess the necessary learning abilities and can perceive both the cues of larvae and the food flavours, presumably they would have evolved to learn directly from larvae if it were in their interest to do so. This suggests that the costs of more direct social learning may be higher

(perhaps due to more elaborate neural mechanisms required to integrate larval social cues with individual information), and/or the benefits lower, for example, because the ecological outcome of strong social attraction and individual learning is very similar to that of more direct social learning in this context (both result in future attraction to similar foods).

If indeed adults are attracted to, yet do not socially learn from, larval cues (Chapter 5), this means that in fruit flies, a fantastic model organism for neurogenetic mechanisms, there are different contexts wherein the adults show (a) individual learning, (b) attraction to social cues without direct social learning (from larvae), and (c) more direct social learning (from other adults; Sarin & Dukas 2009; Battesti et al. 2012). By comparing across these contexts it may be possible to see what (and how costly) additional mechanisms are required for social learning compared to social attraction and individual learning, or compared to individual learning alone.

One very interesting avenue of future research is the integration of individual and social information. Both larvae and adults have been shown to learn both individual and social information, and it would be interesting to study how they respond in natural settings where both sources are available, sometimes simultaneously, sometimes in conflict. In contrast to one report of stickleback fish ignoring social information when they have relatively recent individual information (van Bergen et al. 2004), in both rats and fruit flies,

individuals incorporate social information even when they “know better” (Galef & Whiskin 2008; Battesti et al. 2012). Further study is needed to know the ecological conditions which adjust the relative weighting of social and individual information. With such a simple model as the fruit fly (adults and larvae), the neural mechanisms of such information integration may also be tractable.

7.5 Conclusions

Rather than being “just another animal”, in which to study these relatively complex cognitive abilities, insects are especially useful. First, as has been highlighted throughout this dissertation, the simplicity of invertebrate models makes them highly tractable for future studies on the mechanisms of such cognition. Second, insects represent the other side of a major divide in the animal kingdom (invertebrates and vertebrates). With the exception of ecologically special cases like eusocial bees, the analysis of the ecological and evolutionary factors contributing to social learning has been largely restricted to vertebrates: mammals, birds and fish (reviewed by Brown & Laland 2003; Galef & Laland 2005), all of which are separated from insects by several hundred million years of evolution (Hedges et al. 2006). A comparison of cognitive abilities across such diverse taxa, therefore, may be particularly telling about the importance of various ecological factors and the deep evolutionary roots of behavioural mechanisms. Social learning among non-colonial invertebrates is a

very recent discovery (Coolen et al. 2005; Sarin & Dukas 2009), and this dissertation extends our knowledge in this area. Altogether, the results in this dissertation contribute to our understanding of learning and information use among insects, particularly fruit flies, and will hopefully contribute to future research.

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