Behavioral analysis of exploration and dispersal in *Drosophila*

Thesis by

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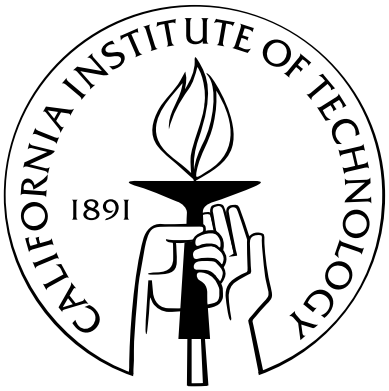
In Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy



California Institute of Technology

Pasadena, California

2010

(Defended August 28, 2009)

To my parents and grandparents, who got me this far.

Acknowledgements

I owe much gratitude to my advisor, Michael Dickinson, who pushed me to become a better scientist, a clearer communicator, and a more careful thinker. I would also like to thank the rest of my committee: Paul Sternberg, Seymour Benzer, Christof Koch, Pietro Perona, and Ralph Greenspan, for their time and helpful suggestions. Working with the late Seymour Benzer was inspiring, and his curiosity was contagious. I will never forget the casually intense discussions of politics, food, and culture that each week were the prologue to his lab's group meeting; nor will I forget the times he invited me into his personal lab space to work late into the night. He is truly missed.

I thank Paul Sternberg, David Anderson and Gilles Laurent for inviting me to carry out rotations in their labs during the first two years of my studies. In addition to getting a chance to experience the differing research programs in each of their respective labs, during these rotations I met many of the folks who guided and helped me during my graduate tenure. I would especially like to thank Nadeem Moghal, René Garciá, Gary Schindelman, Cheryl Van Buskirk, John "DeMo" DeModena, Tim Lebestky, Mark Zylka, Walter Lerchner, Anne Hergarden, Liming Wang, Glenn Turner, Ben Rubin, Alex Bäcker, Vivek Jayaraman, Ofer Mazor, Sijin Cassenaer, Maria Papadopoulou, Anusha Narayan, Bill Ja, David Walker, Dan Tracey, and Greg Suh.

I would also like to thank Mark Konishi, Erin Schuman, and Elliot Meyerowitz for supporting my efforts in starting the animal behavior interest group (ABIG), and

Allyson Whittaker for helping me pull ABIG off. I thank all of the speakers from on and off campus who shared their work and ideas during the group's seminar series.

Thank you fellow Dickinson fly wranglers for a warm, meaningful, and mind-expanding graduate school experience. My experience would not have been as exceptional had it not been for the guidance, support, and camaraderie of Will Dickson, Mike Reiser, Allan Wong, Gaby Maimon, Gwyneth Card, Andrew Straw, Kristin Branson, Wyatt Korff, Seth Budick, and Alice Robie.

I could not have started nor finished this body of work without my family, old friends, and the many friendships born during my time at Tech. I am especially grateful to the following folks: Gaby Mosconi, Mike Vondrus, Rob Lander, Ian Shapiro; my parents who have supported me in every way possible – and are at this moment watching over my daughter as I finish writing this dissertation; my brother and sister out-law who were always there when I needed advice or a reality check; and finally, I would like to thank my wife for reading the many early drafts of the papers making up this thesis, double-checking my math, and adding purpose to my life as I move forward...

Abstract

A fundamentally important decision for all animals is whether to utilize a particular resource or to disperse elsewhere in search of potentially superior resources. Within this dissertation, I present results from laboratory experiments carried out using the experimental genetic workhorse, *Drosophila melanogaster*, to identify and quantify various causal factors contributing to an animal's decision to disperse from food.

With the set of experiments described within the second chapter, I studied the influence of mating experience on the movement priorities of *Drosophila*. From these experiments, I suggest that prior mating experience is a significant and likely an important factor modulating the dispersal of *Drosophila*, and that the change in dispersal results from a change in the fly's priorities rather than simply a change in the general levels of activity. In chapter three, using methods similar to those used to assess the modulatory effects of mating, I explored how the amount and accessibility of food affects the dispersal of hungry *Drosophila*. From these experiments, I suggest that the hunger state of flies can override the visual and olfactory cues from food, and I hypothesize that the observed increase in dispersal resulting from hunger is due to a qualitative change in locomotor behavior related to food search.

With a new machine-vision tracking strategy discussed within the fourth chapter, I studied the exploratory behaviors of individual flies within the environmental chambers discussed in Chapters 2 and 3. I introduced single flies that had recently consumed food

into chambers and tracked their walking and monitored their flying movements as they became hungry. In collaboration, I have attempted to use learning algorithms based on the statistics of each fly's behavior during short windows of time to predict the fly's behavior during the rest of their experimental trial.

I conclude with chapter five by describing a new experimental chamber that I have developed to complement machine-vision methods for tracking individuals within large groups. The motivation behind developing the chamber was to study the changes of social interaction, e.g., courtship and aggressive posturing, of flies near food.

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Chapter 1

Movement patterns of *Drosophila*

The subject of resource-oriented behavior in animals comprises an extensive body of preexisting literature, and a comprehensive review is beyond the scope of the work presented here. I will therefore restrict the following discussion to flies, with particular focus on *D. melanogaster*. For general reading on the subject of resource-orientation behavior, I suggest the following several reviews: Jander offers a comprehensive discussion on the subject of orientation ecology, focusing on the importance of orientation considering an animal's particular life history (Jander, 1975). Readers interested in how changes in an animal's physiology affect its resource orientation behavior may consult the review by Barton Browne (Barton Browne, 1993). Of particular note in the context of *Drosophila*, Hassell and Southwood provide a useful framework for considering the strategies of foraging insects (Hassell and Southwood, 1978), Bell discusses the informational cues guiding the patterns of movement for searching insects (Bell, 1990), and Stinner and colleagues review the dispersal and general movement of insects (Stinner et al., 1983).

I will focus my discussion to studies on *freely* moving flies, emphasizing studies that are significant to the topics addressed within my dissertation – the causal role of hunger, gender, prior mating experience, differences among individuals, and social interactions

on the influence of exploration and dispersal of *Drosophila*. I will begin my discussion by reviewing the studies addressing the search behavior of flies near food. The search behavior of animals in the close proximity of resources has been termed "local search" by Jander (Jander, 1975), and is often referred to as such. Local or area-restricted search is a type of orientation observed in animals that perceive sensory information about a resource, but are unable to localize the resource, or that find a resource and then seek another similar resource in their immediate environment (White et al., 1984). I will then review studies on the general activity of flies, specifically the internally generated movements not structured by external stimuli. This movement has had many names, a few of them are "spontaneous activity," "ranging," "locomotor activity," "general activity," "general locomotor behavior," and "general movement." I will then discuss studies regarding how the general movement of a fly can be modulated by its individual behavioral priorities and intrinsic species-specific preferences. I will conclude this introduction by attempting to synthesize the many studies carried out in the field and laboratory on the dispersal of *Drosophila*.

1.1 Resource-oriented exploration of Diptera

1.1.1 Search movement near resources

To the best of my knowledge, the study of food-oriented behavior for flies started with a description by Vincent Dethier (Dethier, 1957) of the looping locomotor patterns exhibited by the blow fly, *Phormia regina*, as it searched near patches of sugar. He observed that sugar-stimulated flies that had been released onto a surface clear of patches of sugar resources continued to search in the restricted looping manner. This suggested

that aspects of the looping search may be stereotyped. Dethier's suggestion of search stereotypy was strengthened by the observation that the shape of the resource patch did not seem to influence the response of these flies; however, the duration over which a fly exhibited the looping feeding has been observed to decrease as a fly sampled successive drops of resource, suggesting that the search stereotypy is somehow modulated (Fromm and Bell, 1987). A later study by Nelson (Nelson, 1977) augments Dethier's early work by quantifying the looping movement and showing that the looping search can be additionally elicited by water or protein extract (Nelson, 1977). Consistent with Dethier's observations of the search behavior of the blow fly, the house fly, *Musca domestica*, has also been characterized as switching between two "movement tendencies" resulting from specific, quantifiable behaviors (Mourier, 1964). Both of these flies walk faster and straighter when no resource is present, and in contrast display slower and more convoluted looping movement after they find and consume the resource, flattening their legs against the substrate while walking and repeatedly extending and retracting their proboscis to increase the number of chemoreceptors that contact the substrate. The slower looping walk then reverts back to faster and straighter walking after the flies find no further resource within some restricted time and area (Fromm and Bell, 1987). The intensity of the response and the rate of reversion back to the faster, straighter walk have been shown to increase with higher concentrations and greater amounts of the resource, and also the duration of time that has passed since a resource was last consumed (Dethier, 1957; Nelson, 1977; Mourier, 1964). The frequency of, duration between, and rate of switching between these walking modes are together thought to determine the movement on and between patches of food resources (Bell, 1990). The search responses of blow flies and house flies near food are similar but not identical. Light and gravity did not influence the search response of house flies (Mourier, 1964) as was reported for

blow flies (Dethier, 1957), and whereas the search response was comparable between male and female house flies (Mourier, 1964; White et al., 1984), the response was more prevalent for female blow flies (Nelson, 1977).

A study using blow flies that were selected for high and low states of excitability reported that the flies exhibited greater and lesser levels of search response (McGuire, 1986), suggesting that the search response may reflect an internally driven general change in movement activity. However, significant for the work discussed within this dissertation, a series of experiments with parabiotic fly pairs – flies that have been surgically connected so they share hemolymph – have demonstrated that the unfed fly continued the searching response after their partners had fed and stopped searching (Nelson, 1977). This suggested that search behavior is not simply a by-product arising from hormonally controlled changes, as has been shown for general locomotor activity (Green, 1964a,b). The variation between different house flies returning to pre-consumption levels of movement is greater than the variation between repeated runs with the same house fly. This suggests an internal basis for locomotory and turning function and therefore is significant to my work on individualistic exploration (White et al., 1984). Lastly, learning appears to be very restricted in these flies (Nelson, 1971), and it is therefore unlikely that these flies are capable of remembering the particular site of a food source (however see apple maggot flies, *R. pomonella* (Prokopy et al., 1982) and house flies, *M. domestica* (Fukushi, 1983)).[1]

In general, the food-oriented behavior in the fruit fly, *D. melanogaster*, has been described in similar terms as the other Diptera that have been studied (Bell, 1985). Bell and colleagues, however, report that the search tendency of *Drosophila* was not simply

[1]It has been suggested that the restricted looping search is an important factor for re-finding or further finding food nearby the original source and therefore an important factor for these animals to efficiently find food (Nelson, 1977).

a function of switching between a local, restricted search and straighter, faster "ranging" movement (Bell et al., 1985). The post-consumption movement of *Drosophila* did not return to the speed and rate of turning while walking as the pre-feeding rate, but the authors openly admit that the simple switching model may reasonably describe the movement of *Drosophila* if they measured the movement of these flies during a window of time longer after a fly had consumed the resource. Unlike the response of blow flies and house flies, *Drosophila* do respond differently to patch shape (Mayor et al., 1987), and interestingly have also been suggested to process proprioceptive information from their movements, affording them short-term retention of the spatial patterns among foci of resources within patches (Tortorici et al., 1986) (See Fig.1.1). Significant for the

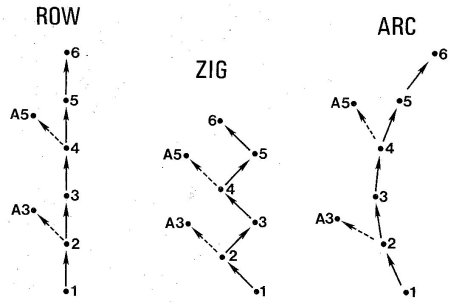

Figure 1.1: Various patterns of sucrose drops used to demonstrate that *Drosophila* possess short-term retention of the spatial patterns of resources making their search for food more efficient. As flies moved along the various patterns, they located the alternative drop, "A5," significantly fewer times than the fifth drop, "5," within the "ROW," "ZIG," and "ARC" patterns. This was not the case earlier on along the patterns for the alternative drop, "A3," which was located comparably to the third drop, "3," suggesting the flies can retain spatial information for the patterns of resources. (Taken from (Tortorici et al., 1986).)

work on social interactions discussed within this dissertation, and currently in preparation, is a study by Tinette and colleagues (Tinette et al., 2004). This study suggests that flies – from a distance – visually assess and use the presence of flies around a resource to aid in their food search, choosing sites containing flies over those that are empty.

As expected for *D. melanogaster*, a major focus with this fly has been on the heritability of its food-oriented behavior. This topic of study began with the observation by Marla Sokolowski that the larvae of *D. melanogaster* collected from natural populations exhibit two distinct foraging strategies, with some larvae foraging comparatively little while others foraged more extensively (Sokolowski, 1980). Sokolowski's findings captured the attention of many researchers interested in relationships between genes and behavior when these strategies were shown to be under the control of a single gene now named *foraging* with two allelic forms, aptly referred to as *sitter* and *rover* (Osborne et al., 1997). Whereas a majority of this work has focused on the behavior of larvae, it has been reported that individual adults also exhibit significant differences in their foraging behavior (Nagle and Bell, 1987; Bell and Tortorici, 1987; Tortorici and Bell, 1988) and this difference has been shown to have a genetic basis (Pereira and Sokolowski, 1993). Nagle and Bell quantified three factors that they suggest explained the restricted, intensive search paths of *sitters* relative to the straighter paths of *rovers*: (1) the initial effect of feeding on locomotor rate, (2) the rate of transition from intensive local search to relatively straight paths, and (3) the tendency to stop during searching (Nagle and Bell, 1987). Further, Tortorici and Bell observed that while adult *sitter* flies rarely left patches of food, *rover* flies left patches quite often (Tortorici and Bell, 1988). The observation that sugar patch concentration and the fly's deprivation level can shift the relative behavior of the flies between the two *foraging* alleles so that they become comparable (Bell and Tortorici, 1987) further underscores the complexity of how genes function within an animal's natural environment. More recently, Shaver and colleagues have reported that adult flies from *sitter* are more attracted to yeast odor than flies from *rover* are attracted to yeast, and suggested that this difference between the two alleles, including their divergent foraging phenotypes, is driven by olfaction (Shaver et al.,

1998). However, for all of the behavioral studies on the local movement of the various flies species, the choice of translucent, non-volatile sugar patches was intentional, so that the search would only reflect local cues driven primarily by a gustatory response (White et al., 1984). With this consideration, it is not immediately clear why Shaver and colleagues used an odiferous stimuli to assay the divergent behavior between the flies from the two alleles. It remains unclear how the differences in the movement of the flies from these alleles on and around food might influence dispersal.

1.1.2 General locomotor movement

The notion that animals exhibit intrinsic "spontaneous activity," movement independent of any external structure, has long intrigued behaviorists (Richter, 1922). The goal underlying this topic of study is a quantitative tool for characterizing internally driven behavior, a baseline measure that could potentially then be "subtracted off" from the total behavior, thereby permitting subsequent inference of behavioral components that are under the control of a separate physiological or external stimulus. In hindsight, however, many of the studies focused on "spontaneous activity" have instead resulted in measuring movement that was highly dependent upon both the experimental apparatus employed and the duration of the experiment in question. My intent is not to dismiss this body of work, but to reiterate the point made early on by (Ewing, 1963, 1967) and again more recently, (Martin, 2003), that quantitative assessment of general locomotor movements is contextually sensitive to the exact experimental details.

In all of the previously-mentioned studies of search movements by flies near food (with the exception of (Tinette et al., 2004)), single flies, some with and some without wings, were introduced onto a flat horizontal or vertical "open-field" arena in which

movement of the fly was recorded for varying amounts of time as it walked over, around, and away from a resource patch. Flies not restricted in space by an attractive resource, but moving "spontaneously" without containment, would naturally fly away in search of required resources. Therefore, in order to observe the general locomotor movements of flies, several experimental chambers have been developed to confine the movement of flies to varying degrees. To provide some perspective on the development and the particular utility for the various experimental chambers that have been used, I have included a figure (1) noting the various types of chambers used for assaying the general locomotor activity of flies, (2) illustrating the classes of chambers used for studying the various movement patterns of *Drosophila*, and (3) placing some of the important chambers for the study of behavioral genetics and the work discussed within this dissertation into context (See Fig. 1.2).

I have found only a few studies on the subject of general locomotor activity in flies other than *Drosophila*, although these studies highlight the key aspects of what was to become known in great detail after a half-century of study on this subject using *Drosophila*. With a series of chambers connected by funnels designed to bias forward and limit reverse movement, it has been shown that the general locomotor activity of blow flies (Barton Browne and Evans, 1960) and house flies (Arevad, 1963) increased with time after feeding. Significant for the work within this dissertation is the suggestion by Barton Browne and Evans (Barton Browne and Evans, 1960) that the decrease in locomotory activity was due to a factor independent of weight. They established this claim by providing flies with various sugars known to be consumed in different amounts, and subsequently weighed and assayed the flies' activity. Flies fed fructose exhibited less activity than those fed glucose, but were also found to consume less. This demonstrated that the added weight arising from greater consumption of glucose relative

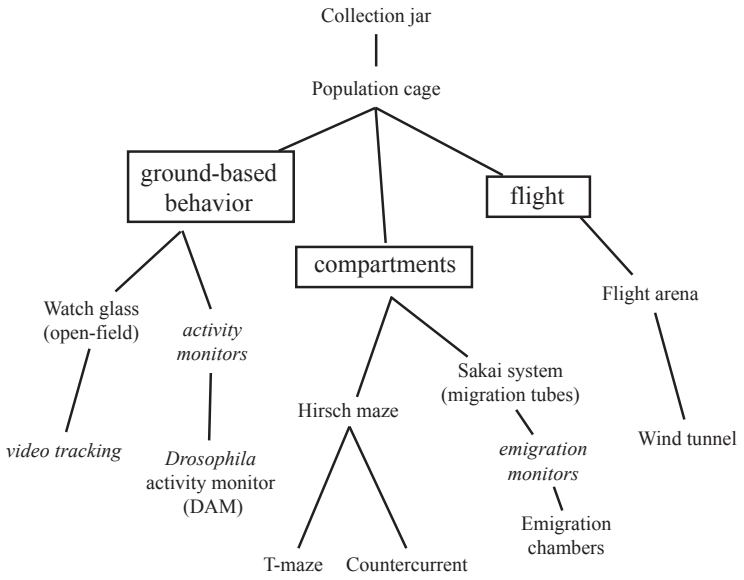

Figure 1.2: Experimental chambers for studying freely moving *Drosophila*. Chambers are categorized into types that have been developed for studying flight, ground-based behaviors, and networks of compartments allowing users to partition flies. The Sakai system was instrumental in shaping the type of studies carried out within this dissertation. The new methodologies described within this dissertation were developed for studying short flights and ground-based movements of *Drosophila* building upon the tradition of observing the behavioral phenotypes of flies in small, restricted chambers in a laboratory setting.

to fructose did not further inhibit the flies' activity. These authors further argued that the hunger-dependent increase in locomotor activity was not the result of the metabolic state of the flies based upon their observation that fructose consumption inhibited the flies' activity as did glucose and mannose, despite the fact that the flies could not utilize fructose metabolically. Finally, through a series of experiments that included weighing the crop of individual flies, these authors suggested that by some mechanism the changing volume of the flies' crop *signaled* a fly to slow or speed up its locomotor activity. This hypothesis motivated Green (Green, 1964a,b) to carry out a series of experiments further characterizing what causal factors might drive the locomotory activity of these

flies. Instead of monitoring the movement of groups of flies through a series of connected chambers, as discussed previously, Green used an apparatus that could record the high-resolution measure of a single fly's activity over its entire life time. This early "activity monitor," a tilting-type actograph, was essentially a small chamber carefully balanced on a beam that would tilt back and forth with even the slightest movements exhibited by a fly. The number of tilts per unit time of the chamber was recorded by closing an electrical circuit by a fine wire at one end of the chamber. Using this methodology, Green showed that the locomotor movement of flies was made up of distinct bouts of actitivity and inactivity, and that it was bout *frequency* that increased over time, not the over-all level of activity. Moreover, by using parabiotic pairs of flies and a series of ablations studies, Green determined that the increase in these bouts of activity over time was under hormonal control of the *corpus cardiacum* and regulated by receptors in the foregut that monitor the presence of food.

Additional conclusions from two early studies using *Drosophila* are also significant for the work within my dissertation. Using flies that had previously been selected for exhibiting fast and slow mating speeds, Manning (Manning, 1960) used a 1-cm graduated 10x10x1-cm "open field" chamber to quantify the number of squares flies visited within a specific period of time. While carrying out these experiments, she observed that the speed at which a fly successfully mates and its general activity were independent and concluded that, "Artificial selection has led to a separation of the two systems and no concept of a 'vigour' which inevitably affects all behavioral levels is adequate" (Manning, 1960). This conclusion agreed with the subsequent findings by Nelson (Nelson, 1977), discussed previously, and strengthened the model that general locomotor activity and appetitive behaviors are both distinct as well as largely independent. In a separate series of experiments, Ewing (Ewing, 1960) selected for flies with big and

small bodies and reported that general activity of flies with both big and small bodies was lower than that of control strains, consistent with the finding by Barton Browne and Evans (Barton Browne and Evans, 1960) which suggested that locomotor activity was independent of body size. Historically significant during this early period were a series of studies carried out by Ewing (Ewing, 1963, 1967) and Connolly (Connolly, 1966b) that failed to directly measure or select for "spontaneous activity." Thereafter the term "spontaneous activity" was largely dropped from the literature and was replaced by "locomotor activity," with both Ewing and Connolly agreeing that the only way to accurately ascertain "spontaneous activity" was to measure "general locomotor activity" by various independent means. Connolly carried this out by selecting for high and low activity strains for 25 generations with a 10x10x0.5-cm "open field" chamber and then confirmed his selections in three independent chambers: (1) a "channel apparatus," a series of long and thin, graduated glass tubes where the speeds of single flies were observed, (2) a "circular runway," a graduated donut shape track for single flies made by sandwiching two half-donuts machined out of clear plastic, and (3) Ewing's original locomotor apparatus, a series of chambers connected by funnels, spacing out and rectifying the movement of groups of flies.

Previous studies of mobile fly activity point toward the dependence of measured behavior upon both elapsed time and individual history, findings that are highly significant to the work discussed throughout this dissertation. Several investigations have reported that locomotor activity of flies increased with food deprivation (Bell et al., 1985). However, the results from these studies remain difficult to interpret due to the array of differing experimental apparatuses used, and how each particular study was carried out. Connolly used a graduated 10x10x0.5-cm "open field" chamber to quantify the area visited during five minute periods through out the day by flies that were deprived of food.

It remains unclear how to interpret the author's conclusions because the increase in activity was reported in relation to flies that were fed continually throughout the day. Flies deprived of food did not display an *absolute* increase in activity, but rather the activity of fed flies *decreased* over the course of the experiment, motivating the author to argue for the relative increase for the deprived flies. The short duration of these experiments is questionable, given that Ewing had previously demonstrated a strong component of "reactivity" when flies were recently introduced into a new environment (Ewing, 1963). In another study reporting the effects of hunger on locomotor activity, Knoppien and colleagues developed a new type of "activity monitor," using radar reflected by moving flies to measure the locomotor activity for groups of flies over longer periods of time. By monitoring half-hour activity of both fed flies and flies deprived of food, this group reported a steady level of increased activity for flies deprived of food (Knoppien et al., 2000), in contrast to the progressively increasing level of locomotor activity reported by Connolly. Some of this confusion was rectified when Jean-René Martin, using a video tracking system, continually measured the locomotor activity of flies over a seven hour period within a 4x4x0.35-cm chamber. These measurements determined that as sated flies become hungry they spend more time moving and tend to travel greater distances. This activity plateaus at a maximum steady level after two hours and does not continue its increase if the flies are further prevented from feeding for longer periods of time (Martin, 2004). Additionally, Knoppien and his colleagues investigated the influence of prior mating experience using the same apparatus described previously and found that, when tested without food, starved mated female flies and virgin males exhibited greater locomotor activity than virgin females and males with prior mating experience (Knoppien et al., 2000). The studies highlighting both the time- and history-dependence of individual fly behavior are of fundamental importance to the work presented in this

dissertation.

In addition to his observations on the effects of food deprivation on general locomotor activity, Martin also reported in this study various measures contrasting the difference between the locomotor behavior among males, females, and virgin females. Martin observed that on average males walked more quickly, turned more frequently, turned more quickly, and spent more time walking within the middle of the chamber than females. Moreover, males also tended to display a lower frequency of switches between stops and starts, moved for shorter durations for each walking period, and during the first two hours travelled less than females. I used these various measures of locomotor activity as a starting point for the work on individual exploration discussed in this dissertation. Also significant for the work presented here within this dissertation, Martin observed that virgin females, on average, moved more during ten minute intervals throughout the entire trial than did females with prior mating experience. Earlier using a simple "activity monitor," a small 4x0.3x0.3-cm rectangular chamber with a pair of light emitting diodes that trigger events when a single fly passes through, Martin and colleagues reported that males had a shorter inter-event interval than females, but the total activity was comparable between males and females (Martin et al., 1999). They also reported that accessible food and dark lighting conditions inhibit total activity. These observations, as well as the new tracking methodologies, motivated Martin and colleagues to review some of the hypotheses, mentioned previously, that were of interest to Barton Browne, Evan, Green, and Nelson several decades ago.

This work started by demonstrating that the less frequent number of start/stops events observed in male *Drosophila* could be made more frequent by utilizing the *transformer* gene to genetically feminize a specific neural loci in the mid-anterior region of the pars intercerebralis (PI) (Gatti et al., 2000). This finding was repeated and then

demonstrated to act hormonally by surgically transplanting the "fem cells" from a female or trans-male fly into the abdomen of a male (Belgacem and Martin, 2002), implying both humoral control of this behavior as well as suggesting the role of the PI neurons as being neurosecretory. This group also reported that a second humoral factor, juvenile homone (JH), that is synthesized within the *corpus allatum* (see references within (Belgacem and Martin, 2005)), could modulate the frequency of starts and stops, demonstrated by feeding males fluvastatin, a JH inhibitor, and then reversing the effect with simultaneous application of methoprene, a JH analog. Belgacem and Martin subsequently followed up this work by: (1) identifying 12 cells in the PI, distinct from the "fem cells," that produce insulin, using immunohistological staining techniques, (2) demonstrating that the *corpus allatum*, a gland in the pro-thorax, possesses insulin receptors, and (3) showed that a disruption in the insulin pathway via the identified cells in the PI or at the receptor level in the *corpus allatum*, increases the start/stop frequency of males to the level of females (Belgacem and Martin, 2005). With these findings, together with independent evidence the JH is produced within the *corpus allatum* (see references within (Belgacem and Martin, 2005)), these authors seem convinced that insulin from the non-fem cells acts on the insulin receptor in the *corpus allatum*, and that in return produces JH and influences the gender-specific walking patterns observed in these flies (Belgacem and Martin, 2007). These groups have uncovered some intriguing correlations, but I believe some of the mechanisms linking the pathway together should be further studied.

Drosophila, like many animals, exhibit crepuscular activity which is readily apparent within a laboratory setting (Roberts, 1956). There exists a rich literature on the subject of circadian rhythm in *Drosophila*. However, since I purposely ran the experiments discussed with this dissertation two hours after the morning activity peak entrained for

my experimental flies and concluded my trials before the onset of their evening peak, I will not include this body of work within my discussion. One study that might be significant for my work on gender differences discussed within my dissertation is the observation that males from several widely-used laboratory strains have a shifted, earlier morning activity peak than females (Helfrich-Förster, 2000). However, since this work also reports similar evening peaks between the genders, these results seems to bear little if any significance, i.e., males flies effectively have a longer "siesta" in the middle of their day.

On a methodological note, I recently found a brief report referring to an "open field" chamber that had an "develled" edge [sic]. Included within this note was a side-view illustration of a chamber designed for observing sexual isolation, that possessed sloping walls, which included dimensions suggesting that the chamber was 10 cm in diameter and 2.5 cm height (Elens and Wattiaux, 1964). It is unclear to me if the sloped walls of this chamber were modified for this note or had been a design element described earlier that was reported in French (Elens, 1958). This report was unknown to me when I conceived the general purpose observation chamber discussed within this dissertation, and its design was clearly not meant to complement machine vision methodologies.

Significant for the work discussed within my dissertation of individual exploration is an early report that used video tracking to measure the internal structure for walking flies. The movement structure for flies walking within a 0.1x0.06x undisclosed-cm depth chamber were described to have "self-similar" structure, bouts of activity and inactivity that appears the same regardless of the time scale used, motivating the author to compare the walking movement of *Drosophila* to Lévy flights, which produced efficient search behavior (Cole, 1995). Finally, since I started the work discussed within this dissertation several groups have developed software that offer a promising strategy for

automatically tracking and measuring the behavioral phenotypes of flies (Martin, 2004; Valente et al., 2007; Grover et al., 2008; Wolf et al., 2002; Ramazani et al., 2007; Hoyer et al., 2008; Katsov and Clandinin, 2008; Dankert et al., 2009; Branson et al., 2009).

1.1.3 Movement preferences

Two forces largely dictate the movement choices made by all animals. The first of these acts at the level of the individual and within this discussion I will call this process a "behavioral priority." The second acts at the species level and I will call it a "behavioral preference." Examples of a behavioral priority would be the urge for an individual to find food when it is hungry or a mate when it is sexually mature. Behavioral preferences are sculpted over evolutionary time, primarily to keep species distinct through the process of niche separation; it should be understood that behavioral preferences contain, and in fact limit, the possibilities available for an individual's particular behavioral priorities. I have only found a small number of studies on the behavioral priority of flies, some of which address the priorities of *Drosophila*, although there is a rich literature describing the behavioral preferences among various species of *Drosophila*.

Like many animals, flies can be narrowly focused when it comes to their choices. Within the relatively modest body of literature on this topic, most studies discussing fly behavioral proclivities have focused upon food preferences and oviposition site selection displayed by agriculture pests. These studies are often quite detailed in their descriptions of the flies' behavior, but unfortunately rarely provide much information on the ecology or ethology of the particular fly, precluding an understanding of its species-specific behavioral preferences. For example, the *search image* for the cherry fruit fly, *Rhagoletis cerasi*, is a dark, convex, upward facing 10-mm diameter object having a soft, thin,

smooth and dry surface (Prokopy and Boller, 1971). In contrast, the onion fly, *Delia antigua*, seeks a cylindar-shaped object of a specific height and angular orientation, and displays a particular yellow hue and saturation, although the absolute brightness of this object is irrelevant (Harris and Miller, 1983, 1984). Other fly species have been described as simply fixating on a single non-visual feature, a specific chemical compound found within the waxy leaves of the host (e.g., the carrot fly, *Psila rosae* (Städler and Buser, 1982)) or chemical moiety (e.g., the onion fly, *Hylemya antiqua* (Ishikawa et al., 1978; Vernon et al., 1978)).

Of significance to the work discussed within this dissertation is an early report on the difference in feeding priorities among egg-laying females, virgins females, and male house flies, as well as a handful of studies describing the feeding and oviposition priorities of a variety of fly species outside the *Drosophila* genera; these studies have been carried out in the laboratory, outdoors around caged trees, and in experimental plots. In a "population cage" within the laboratory, Greenberg measured the amount of sugar and protein that individual male, virgin female, and egg-laying female house flies consumed. He reported that egg-laying females required $\approx$2-3x more protein than the amount required comparably by virgin females and males (Greenberg, 1959). However, he also reports that all flies, irrespective of their gender or mating status, consume $\approx$7x more sugar than protein, underscoring the importance of extrinsic sugar supplies in the life of this adult fly. Also pertinent in the context of this dissertation are coming-of-age-related behavioral changes in females. These studies describe a behavioral switch displayed by female flies, characterized as a shift in a dietary preference from sugar to protein, dependent upon their maturation state. Females of the Mexican fruit fly, *Anastrepha ludens*, switch from a diet mostly of sugar to a diet requiring 50:50 protein:sugar near their stage of maturation (Robacker, 1991). Female Mediterranean fruit flies, *Ceratitis cap-*

itata, similarly exhibit a switch in preference to protein around maturation (Cohen and Voet, 2002), and mature, fed female apple maggot flies, *Rhagoletis pomonella*, stayed longer and laid more eggs on host fruit oviposition sites containing proteinaceous food (Averill and Prokopy, 1993). Moreover, feeding protein to female Oriental fruit flies, *Bactrocera dorsalis*, switched their preference to fruit odors over protein odors (Cornelius et al., 2000). Lastly, Jang and colleagues have shown both in a laboratory flight tunnel (Jang et al., 1998) and as well in outdoor field cages (Jang et al., 1999) that mating shifts the preference of the female Mediterranean fruit fly, *Ceratits capitata*, from male pheromones to the odor of guava, the fly's host fruit. In contrast, however, immature and mature female Queensland fruit flies, *Bactrocera tryoni*, have been reported to display no visiting preference for host fruit with bacteria-filled vial baits (Prokopy et al., 1991), indicating that this behavioral switch at female maturation may not be a universal phenomenon in flies.

To my knowledge the effect of mating on the movement preferences of *Drosophila* has never been studied. However, there are some studies that describe in general the movement preference of these flies in response to odor plumes. Kellogg and colleagues (Kellogg et al., 1962), and more recently in a pair of papers, Budick and colleagues (Budick and Dickinson, 2006; Budick et al., 2007) have studied the up-wind flight of *Drosophila* towards attractant odors in the laboratory. Kellogg and colleagues used a wind tunnel and time-lapse photography to demonstrate that *Drosophila* depended on visual cues from the ground for upwind guidance and further showed that flies moving out of a filamentous odor plume immediately turned, flying cross wind "at roughly right angles to the wind," presumably attempting to reestablish contact with the plume (Kellogg et al., 1962). In one study, Budick and Dickinson used a wind tunnel and a multiple-camera tracking system for studying the free-flight response

of *D. melanogaster* to attractive odors (Budick and Dickinson, 2006). They showed that the presence of wind was sufficient to initiate the upwind flight of hungry flies. They showed further that when these flies contacted filamentous odor plumes, they actively controlled their flight so as to surge upwind while attempting to maintain contact with the plume. In a second study, Budick and his colleagues studied how visual and mechanosensory cues structured up-wind flight (Budick et al., 2007). They tethered flies to a metal pin and held this pin between two magnets, so the flies could freely rotate about their yaw axis. They then placed flies on this magnetic tether, within an arena display of light-emitting diodes, allowing control over the visual stimuli to the flies, all within a wind tunnel. They showed how wind stimuli could override aversive visual expansion, allowing flies to maintain up-wind flight. In a different line of investigation, Johnston focused on the genetic variation in up- and down-wind movement for laboratory and wild-caught flies (Johnston, 1982). With the laboratory strains, he showed that he could select for wind-directed movements. Interestingly, when he grouped the wild-caught flies into species of flies that specialize on one type of food and those feeding on many types of food, he reasoned that it made sense that the specialist, which may have to move long distances to find its food, exhibited a greater up-wind movement as opposed to the generalists that showed a reluctance to move under windy conditions.

Consistent with the observations mentioned previously for other fly species, *Drosophila* do shift their behavior after mating. A recent study relating directly to the work discussed within this dissertation has shown that mated females feed more, suggesting a shift in the fly's priorities from mating related behaviors to those required for reproduction (Carvalho et al., 2006). However, I have come across only a pair of studies addressing shifts in the behavioral priority of *Drosophila* outside of a post-reproductive context. These studies were carried out in the field and laboratory and assessed how starvation

affects the choice of these flies' feeding and breeding sites. Hoffmann and Turelli show that sated flies from both *D. melanogaster* and *D. simulans* released in laboratory chambers, and as well, released and then recaptured with baits in the field are more commonly found on better resources than starved flies; better in this case being previously determined with each of these species in laboratory choice assays (Hoffmann and Turelli, 1985; Turelli and Hoffmann, 1988). These findings suggest that whereas these species do have preferences for food and oviposition resources, when these flies are stressed – due to starvation in this case – they are adaptively less discriminating. Similar findings documenting the ability for *Drosophila* to adaptively discriminate come from Yang and colleagues (Yang et al., 2008). Capitalizing on the fly's behavioral preference for specific oviposition sites, this group has revealed the fly to be capable of selecting preferred sites from multiple acceptable ones. This observation that the flies are choosey about their oviposition site was not surprising, however, since *Drosophila* have long been known to exhibit specific oviposition site preferences. The major dimensions of preference that are known and well-studied relate to (1) the chemical properties of the substrate, (2) the surface properties of the substrate, (3) the lighting conditions around the substrate, (4) the surface and subsurface temperatures of the substrate, and finally (5) the natal and adult experience of the female laying the eggs. A detailed discussion of the many behavioral preferences of *Drosophila* is beyond the scope necessary for the work discussed within this dissertation. However, I will include this material here, for it should reward those readers interested enough on the topics of fly behavioral preferences to get this far within my introduction, and is a body of literature I would like to have for my own future reference. I will attempt to present these preferences within a ecologically meaningful context.

A fundamental question in ecology is how similar and often closely related species

are capable of coexisting at the same tropic level. Moreover, a major evolutionary driving force fostering coexistence is diversification of these species into separate niches. In this regard, resource partitioning plays a significant role in allowing closely related species to live sympatrically. A text book example of niche partitioning has been observed in "sibling" species of the *D. melanogaster* subgroup. Within this group are three polyphagous "generalists," *D. melanogster*, *D. simulans*, and *D. mauritiana*, that utilize various fruit and vegetable rots, and one monphagous "specialist," *D. sechellia*, that has a sole breeding site – the toxic fruit of *Morinda citritolia*. The separation among these species is significant and appears to be due to *n*-capproic acid contained within the ripe fruit of *Morinda citritolia* (Higa and Fuyama, 1993). By itself, this chemical elicits preferential egg-laying by *D. sechellia*, but strongly repulses both *D. simulans* and *D. melanogaster*. Interestingly, *D. mauritiana* preferentially lays its eggs on morina, despite the fact that its embryos are killed by this toxic fruit. More intriguing still is the fact that the particular acid which attracts *D. sechellia* and repels the other species also equally repels *D. mauritiana*, suggesting the preference for morina in *D. schellia* and *D. mauritiana* are likely mediated by different chemicals, perhaps reflecting their relatedness and/or island adapted ecologies (Moreteau et al., 1994).

Another fascinating and powerful species comparison from this group involves the two genetically tractable "cosmopolitan" species that coexist largely as human commensals worldwide, *D. melanogaster* and *D. simulans*. These species are considered "ecological pairs" – sharing similar breeding sites (Atkinson and Shorrocks, 1977) and having comparable reproductive strategies (Atkinson, 1979) – and have often been studied in an attempt to understand how species live sympatrically. Various investigations of *Drosophila* have reported differences in the spatial and temporal separation of the larvae for species with adults that would otherwise utilize identical resources (see references

within (Nunney, 1990)). However this does not seem the case for *D. melanogaster* or *D. simulans* (see (McKenzie and McKechnie, 1979)). *D. simulans* is considered to be generally more sensitive to stresses than *D. melanogaster* (see (David et al., 2004), and references therein), and it has been suggested that the separation between these species might result from *D. simulans* having a lower tolerance to ethanol than *D. melanogaster*, forcing this species to colonize groves of recently fallen fruit earlier and specializing on the preliminary stages of decay (Nunney, 1990). Neither the differential ethanol tolerance nor the decay-dependant colonization pattern, however, is unique to this ecological pair.

This brings me to an interesting social behavior of *Drosophila*, a much under-considered, multi-species community – the guild of "cosmopolitian" *Drosophila*. This guild is made of *D. melanogaster*, *D. simulans*, *D. immigrans*, *D. hydei*, and *D. busckii*, together with one (or more) species from the obscura group, and coexists almost world-wide. Significant questions remaining in this field are how this coexistence is possible, why these species are not constantly in direct competition with each other, and whether the niche partitioning that permits this coexistence is single- or multi-dimensional.

As in the case for *D. simulans* and *D. melanogaster*, less ethanol tolerance by *D. immigrans* promotes its earlier colonization pattern relative to *D. hydei*, its "ecological pair" (Nunney, 1990). Moreover, all of the members of the guild may be organized from least to most tolerant to ethanol, and this ordering parallels the order of the colonization pattern as observed of these species during field studies here in Southern California (Nunney, 1996). *D. pseudoobscura*, the California obscura member, alone, prefers fresh oranges. The remaining members of the guild have been observed to colonize carefully-aged orange rots, beginning with *D. simulans*, then *D. melanogaster* and *D. immigrans*, followed by *D. hydei* and *D. busckii*, with *D. busckii* being the only member of the

guild preferring rots over 11 days. These field studies are consistent with an older study carried out within the laboratory observing that ethanol has concentration-specific effects on oviposition across 14 *Drosophila* species that have uncorrelated phylogenetic relationships (Richmond and Gerking, 1979). It has also been reported that other chemicals that *Drosophila* are likely to find at breeding sites in the wild were preferred and repelled by various Indian *Drosphila* in species-specific manner (Srivastava and Singh, 1997). There are many additional studies describing the various behavioral preferences that may further restrict the separation among sympatric species, discussed in the following sections.

An egg lying out in the open will either be found and eaten by predators or soon desiccate. It is therefore a reasonable goal for flies to place their eggs into moist refugia. In this regard it has been noted that various *Drosophila* exhibit preferences concerning the surface substrate possible for oviposition. Both *D. melanogaster* and *D. simulans* prefer fresh to old medium (Chiang and Hodson, 1950), although *D. simulans* will oviposit more readily on older crusted subtrates (Moore, 1952). *D. psudoobscura* prefer medium not occupied by previously laid eggs (del Solar, 1970). Various Indian *Drosophila* prefer to lay their eggs in medium rather than paper (Srivastava and Singh, 2001). The hardness of the substrate surface may play a role in the context of niche separation. From reports of tests by Takamura (Takamura, 1984), fly species prefer inserting their eggs into substrates in the following order of preference for substrate surface hardness: *D. teissieri* <*D. melanogaster* <*D. yakuba* <*D. simulans* <*D. mauritiana* <*D. erecta*.[2]

Light has also been suggested as a niche dimension that may separate sympatric *Drosophila* species (Wogaman and Seiger, 1983), and which clearly affects oviposition (Srivastava and Singh, 1996). Whereas it has long been known that flies are attracted to

[2]Or perhaps in order of their lack of strength?

light (Carpenter, 1906) and that light affects their general activity (Cole, 1922), it is not immediately clear how light alone may give rise to separate sympatric species.

In less than one and one-half hours, internal temperature measured in fruit from the wild during the summer 1994 in Cook Country, Il exceeded 35°C (Feder, 1994); temperatures greater than 40°C were not uncommon and measured values reached 50°C within tomatoes. On entering direct sunlight, the temperature of a 10-mg fly can rise by 10°C in 10 seconds (Heinrich, 1993), and a fly weighing merely a tenth this amount will surely heat up even more rapidly. Given that the reproductive success of many species of *Drosophila* depends on their larvae and adults forms utilizing fruit and vegetable rots, it is a reasonable conjecture that the internal and surface temperatures of these rots are important. Several studies report that cool temperatures inhibit oviposition; the oviposition of various Indian *Drosophila* is reduced at 19°C (Srivastava and Singh, 1998). At $<$12°C *Drosophila* from the Australian temperate region do not oviposit, are inactive, and do not mate (Parsons, 1978). In an attempt to assess how temperatures might contribute to the niche widths for oviposition, Schnebel and Grossfield (Schnebel and Grossfield, 1986) used a laboratory hot plate, capable of establishing a 3-38°C temperature gradient (modified after (Fogleman, 1978)), to test the oviposition preferences of an array of species from various ecological backgrounds. They found, perhaps as expected, that the oviposition preferences common to groups of species reflect their ecological distribution. While testing at a 100% relative humidity, the temperate-montane *virilis* group (*D. virilis*, *D. americana*, *D. montana*) has the lowest temperature limits (9-32°C), the desert *repleta* group (*D. arizonensis*, *D. mojavensis*, *D. mulleri*) has the highest limits (12-36°C), and the cosmopolitan *melanogaster* group (*D. melanogaster*, *D. simulans*, *D. ananassae*) has the broader temperature limits (10.5-34°C) than the endemic tropical *willistoni* group (*D. paulistorum*) semispecies-Amazonian, Interior,

Transitional (10.5-30°C). In reporting these findings, the authors point out that for some species the observed oviposition temperature range is wider than the preferred mating temperature range of the species, suggesting a multidimesional model for the niche partitioning that includes a temperature dimension.

For those interested in *how* a fly senses its preferred temperature, I suggest examining Sayeed and Benzer's genetic study of thermosensation and hygrosensation (Sayeed and Benzer, 1996). Briefly, to assays a fly's temperature preference, they used a thermal plate, capable of producing a thermal gradient, comparable to the plate used and discussed previously, and for both temperature and humidity they used a modified "T-maze." For the thermal assay, a band heater was wrapped around one of the arms of the maze; for the humidity assay, moist or dry air was delivered to one of the two arms. Using a series of genetic and physical ablations, they determined that (1) the sensory mechanisms subserving thermosensation and hygrosensation were independent and (2) that the temperature resulting in the fly's preference is sensed by the 3rd segment of the antennae and that humidity is sensed more distally by the antennal arista. Finally, significant for the work discussed within this dissertation on individual as well as social behavior, I mentioned a study testing the preference of light and temperature on the spatial distribution of *Drosophila*. Using a round-bottom flask submerged into water that was either 10°C or 20°C, Navarro and del Solar observed that flies in both mixed and single gender groups aggregated towards each other, suggesting a non-mating related clustering preference for these flies (Navarro and Solar, 1975).

The fact that the niche dimension for a particular species may be modified by the behavior of the individuals within the species (Jones et al., 1987) further highlights the complexity of interacting factors influencing an animal's behavioral preferences. A comprehensive overview of this topic is beyond the scope of the present discussion;

however, for those that are interested in the subject I recommend a primer by Feder that nicely reviews this complex regulatory phenomenon drawing from his knowledge on the behavioral and physiological responses of animals, including flies (Feder, 1996).

H. Hirsch and Tompkins review the dependence of developmental experience on the behaviors of *Drosophila* (Hirsch and Tompkins, 1994); however, largely their perspective is as if flies were *just little humans* and mention little of the literature presenting the flies' behavior within an ecological context. The ecological literature on the dependence of past experiences in these flies is interesting. For example, various strains of *D. tripunctata* exhibit strong and consistent strain-specific preference when choosing between mushrooms and tomatoes. Females from this species show augmented preference for the type of food they were kept on [experienced] before release, although males do not (Jaenike, 1985). The influence of natal and adult experience of oviposition sites appears variable. As mentioned previously, *D. melanogaster* exhibit strong oviposition preference within a continuous gradient for a particular substrate temperature; it has been noted that flies raised at hot and cold temperatures prefer to oviposit on either hot or cold substrates, respectively. Interestingly, adults shifted to a temperature different than their rearing temperature resulted in intermediate oviposition temperature preferences, with the adult temperature having a greater effect than the larval temperature (Fogleman, 1979), a response that makes sense for animals living in ecological niches with transient resources. The effect upon oviposition by environmental odorants is complex and seems largely dependent on the species tested. Jaenikie found no sign that larval environmental odors influenced the adult's oviposition preference, although prior exposure to peppermint oil, a chemical commonly used for olfactory conditioning, significantly reduced the aversion to follow-up presentation of the oil in *D. melanogaster D. pseudoobscura*, *D. immigrans*, but not *D. recens* ((Jaenike, 1982)

and references therein.) The broad-niched comsopoliton species, *D. melanogaster*, became habituated to 7% ethanol, a concentration normally repulsive to this species, when exposed. When exposed as adults, *D. immigrans* were induced to prefer a medium containing piperidine, an alkaloid often encountered in breeding sites of *Drosophila*. Given the importance of fruit and vegetable rots to *Drosophila*, as well as the aforementioned aversion and specialization to temperatures, I was surprised to learn that female *Drosophila melanogaster* presented with previously heated necrotic fruit or the presence of heat-killed larvae, do not respond to this stimuli experience (Feder, 1997). While some *Drosophila* species may truly be "specialists" having a narrowly-defined niche, such as e.g., *D. sechellia* in this case of smell, many others have a complicated, presumably multidimensional and *evolving* niche. The inherent complexity of natural environments results in great difficulty separating behavioral contributions from numerous individual and interacting environmental factors using field studies alone.

A recent study by Stamps and colleagues is the first I have read of a group attempting to reconstruct model environments within a large, room-sized volume presenting various realistic but carefully placed features of the fly's natural world, so that a fly's preference among multidimensional niches may be quantified (Stamps et al., 2005). This type of study is important if we are to connect the behaviors measured in restrictive experimental chambers with those observed in the wild. For example, significant to the work discussed here is the observation by this group that more males than females were present on food (banana) and more females were perched on leaves around the food, as often in seen in the wild. Fully understanding why these flies express their specific movement-based behavioral priorities necessitates carefully constructed experiments that build upon preexisting observations of how they search for, assess, utilize, and disperse from resources.

1.2 *Drosophila* dispersal

Drosophila have adapted to living around the world in a variety of habitats, from deserts and swamps to cohabitating with humans. Due to their interesting life histories, facile study, and potential impact on human welfare, *Drosophila* have become one of the most studied organisms to date. For nearly three-fourths of a century, there has been a focus on their movement and a corresponding immense body of literature on their dispersal. I will not attempt an exhaustive discussion of their dispersal, but will instead present a brief overview using examples from field and laboratory studies that I believe have had the most influence on this subject. In perhaps a dangerously simplistic generality, from my readings, it seems that if the resources required for a particular species of *Drosophila* are present and available, these flies will move very little; however, if conditions change, and the resources required for the flies' livelihood are not present, these flies can and will move over great distances in search of the required resources. For a more complete introduction to this topic, I suggest a synopsis by Dobzhansky (Dobzhansky, 1973) and a review by Grossfield (Grossfield, 1978).

Dobzhansky uses data from prior literature and his personal observations to distinguish three types of movement, two of which are exhibited by *Drosophila*. He describes directional migration as the movement of many individuals in more or less the same direction, occurring on any time scale. While this type of movement is found to occur in other insect species, I have not in my readings ever found evidence for this type of group movement in any drosophilid. Dobzhansky describes active dispersal as the uncorrelated movement of individual flies from their birthplace to where they might find the resources required for their life histories, e.g., food, water, mates, shelter, and oviposition sites. Dobzhansky does not mention repulsive movement, but I assume that he would

have also considered movement away from heat, noxious materials, competition, and predators as components of active dispersal. Finally, he describes passive dispersal and suggests that the transport of *Drosophila* by air currents is the most important means of passive movement. He also proposes that the transport of *Drosophila* by human agencies might be important for some domestic species. Grossfield recounts many of same studies described by Dobzhansky, but also includes studies on the dispersal of *Drosophila* conducted within the laboratory.

1.2.1 Field studies on dispersal

The earliest study on the dispersal of *Drosophila* that I have found was a short report by Gordon (Gordon, 1935). In this investigation, Gordon released a population of nearly 40,000 flies marked with the cuticle-darkening gene, *ebony*, and four months later sampled the frequency of this gene in wild-caught flies at various distances from the original release site. Timofeff-Ressovskys' report on their studies of releasing laboratory mutants of *Drosophila* on to an experimental plot near Berlin, Germany (Timofeeff-Ressovsky and Timofeeff-Ressovsky, 1940) and Dobzhansky and Wright's report on their releasing of laboratory mutants into the mountain forests of Southern California (Dobzhansky and Wright, 1943), provide the first in-depth attempts at analyzing the rates, distances, and diffusions for the dispersive movements of flies released into the wild. An additional influential study on the dispersal of *Drosophila* was that of Dubinin and Tiniakov (Dubinin and Tiniakov, 1946), who released a natural population of *Drosophila* with a recognizable karyotype that did not carry a potentially deleterious genetic mutation as those used for marking flies in previous studies. Dobzhansky and Wright (Dobzhansky and Wright, 1947) released and followed the dispersion of

Drosophila over a longer period of time, much longer than previous studies, including several seasons. Dyson-Hudson (Dyson-Hudson, 1956) collected *Drosophila* from various habitats throughout the course of a day while monitoring the ambient temperature, humidity, light levels, and wind velocity, and attempted to infer the effects that changes in these environmental factors might have on the movement of flies. Finally, Crumpacker and Williams (Crumpacker and Williams, 1973) captured, marked with micronized dust, and released small numbers of wild *Drosophila* back into the natural habitats from where the flies were captured. Together, these studies provide a starting point and framework for future studies on the dispersal of *Drosophila*.

In addition to the principal studies mentioned above, there are many other studies contributing to a basic understanding of the dispersal of *Drosophila*. Both the long-distance and short-range movements of these flies have been studied. Coyne and his colleagues have studied the dispersal of *Drosophila* over large distances from favorable areas, or at least currently populated areas, over regions that are less favorable, e.g., from an oasis into the surrounding desert (Coyne et al., 1982, 1987) and from a fruit orchard into the surrounding fields and deciduous forest (Coyne and Milstead, 1987). Toda and Wallace studied the movements of more than two dozen species from natural populations of drosophilid found and studied in the arboretum of the botanical garden at Hokkaido University (Toda, 1974). Wallace studied the movements of several laboratory mutants he released into a variety of spaces, e.g., an empty lot near his home in New York, in a greenhouse at Cornell University, and near his hotel at the Marine Biological Institute in Venice (Wallace, 1970).

Many studies have focused on which factors influence the movement of flies. Studies have focused on the influence of environmental factors, some of which are abiotic – e.g., temperature (Dobzhansky and Wright, 1947; Burla et al., 1950), humidity (McCoy,

1962), and active dispersal (Richardson and Johnston, 1975) or passive dispersal (Gressitt et al., 1962) in response to air movement – while others are biotic, e.g., preference to particular vegetations (Heed, 1973), response to ephemeral resources (Johnston and Heed, 1976), and effects of inter-species competition (Richardson, 1974). A handful of studies address the dispersal between different species of *Drosophila* (Dobzhansky and Powell, 1974; Mckenzie, 1974; Powell et al., 1976; McInnis et al., 1982; Taylor et al., 1984). Others studies focus on the physiological restrictions limiting dispersal, e.g. the upper limit for durations of flight as restricted by the total reserves of a fly's energy stores (Wigglesworth, 1949), the calculated maximum ranges for flights using these known upper limits (Hocking, 1953), and the total distances flies have traveled upwind (Yerington and Warner, 1961). These studies, together with the studies mentioned before, have inspired and guided the studies on the dispersal of *Drosophila* carried out within the laboratory.

1.2.2 Laboratory studies of dispersal

Although studies on the movement of *Drosophila* conducted within the laboratory will miss some subtlety of a fly's ecology, what they lack in realism they can make up for by providing the possibility of conducting experiments that are very difficult or impossible in the field. The ability to hold constant any one factor believed to influence the movement of flies, while systematically and simultaneously manipulating others, enables attempts to disentangle the complex interactions driving the movement of flies in natural conditions. Moreover, studies controlling the genetic make up of a population of flies are only possible in a laboratory setting. Since I discuss various experimental chambers throughout this body of work, I provide here for those readers not as familiar

with the type of chambers used for studying the behavior of *Drosopila* a figure with images or simplified illustrations for some of the important experimental chambers (See Fig. 1.3).

The development of a series of connected chambers by Kan-Ichi Sakai and colleagues (Sakai et al., 1958) influenced the experimental approach taken in this dissertation. Until this work, studies within the laboratory on the dispersive movement of *Drosophila* were carried out within a closed experimental space, the "population cage," with no place for the flies to actually disperse. For an interesting example of such work, see the last report from a series of studies on migration carried out by Dobzhanksy and his colleagues (Dobzhansky et al., 1972). Flies in these experiments could not move freely into a population, but were systematically introduced or removed as if they in fact had emigrated or immigrated from the test population. Dobzhanksy was interested in how genes underlying behavioral phenotypes moved within and affected the dynamics of a population.

Another important body of study on the movement choices of *Drosophila* was conducted by Jerry Hirsch, focusing on light and gravity-oriented movements of flies within an elaborate apparatus made up of an expanding maze of *one-way* channels, allowing him to separate individuals from within a population that exhibit subtle differences in their movement preferences (Hirsch, 1963). This apparatus, and the studies carried out with it, inspired Benzer to conceive his famous "countercurrent" apparatus – the basis for his powerful assays used for investigating the connection between genes and behavior (Benzer, 1967). There are several other important apparatuses used for studies of freely moving animals. The "T-maze," which I believe was first suggested for working with *Drosophila* by Murphey (Murphey, 1967), and which is used to assay forced choices. The "water moat," an open field arena surrounded by water that, after clip-

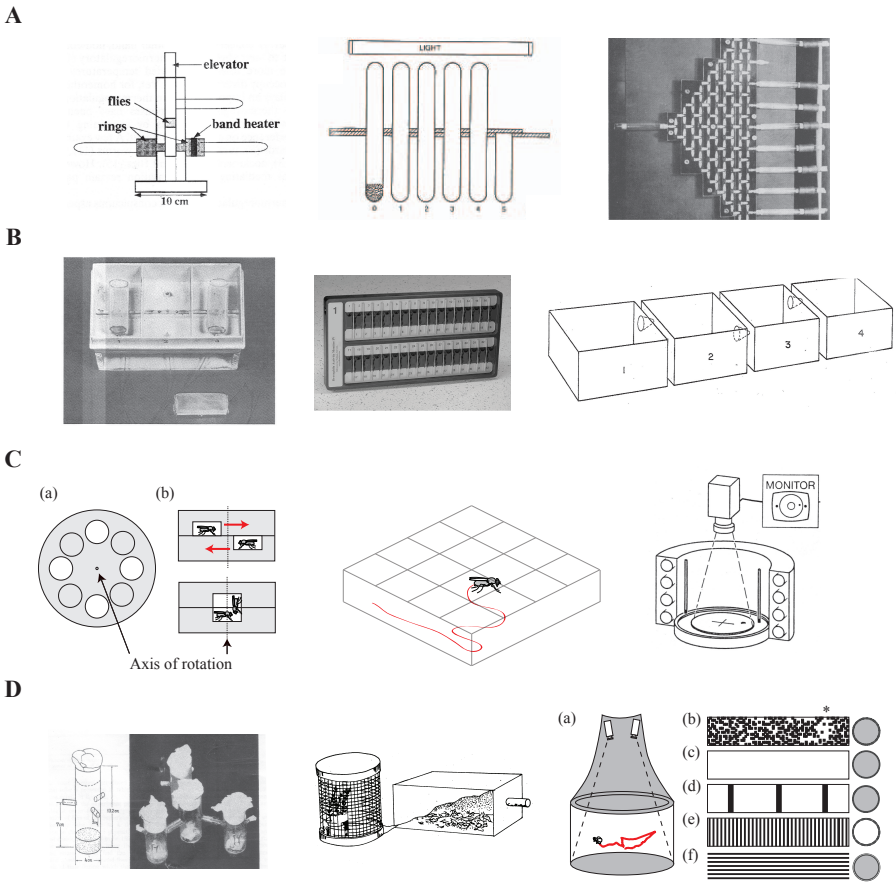

Figure 1.3: Images and illustrations for various experimental apparatuses used for studying the behaviors of freely moving *Drosophila*. (A) Apparatuses designed for assessing forced choices: "T-maze" (taken from (Sayeed and Benzer, 1996)), "Countercurrent" (taken from (Benzer, 1973)), and "Hirsch maze" (taken from (Hirsch, 1963)). (B) Apparatuses used for measuring general activity: "Tilting-type actograph" (taken from (Green, 1964a)), "*Drosophila* activity monitor (DAM)" (taken from (DAM, 2005)), and "Funnel-connected chambers" (Taken from (Barton Browne and Evans, 1960)). (C) Apparatuses designed for studying ground-based behaviors: "Mating wheel," "Open-field" chamber, and "Water moat" (taken from (Bülthoff et al., 1982)). (D) Apparatuses designed for studying movements within complex environment: "Sakai migration tubes" (taken from (Sakai et al., 1958)), "Population cage" (taken from (Open Schooling, 2009)), and "Flight arena" (modified from (Frye et al., 2003)).

ping off the flies' wings, restrict the flies to moving within a specific region (Bülthoff et al., 1982), allowing their study over longer period of time than previously. And fi-

nally, the "Mating wheel," (Hotta and Benzer, 1976) a clever apparatus consisting of two connected disks that may be rotated with respect to each other to introduce many pairs of flies simultaneously so their behaviors might be carefully studied. My plan is not to discuss these various important arenas or the science carried out with them, but rather limit my discussion to studies for which flies could move freely between distinct experimental regions.

I am aware of only one author, (Koch, 1967), who followed up on measurements made from his observation of dispersal rates in the laboratory with later studies carried out in the field. Using a system inspired by the experimental setup developed by Sakai and colleagues, Koch and Burla tested the effects of temperature, humidity, food quality, hunger, age, and gender on dispersal (Koch and Burla, 1962). Several years later, Koch examined the effects on the movement of *Drosophila* for various factors in the field and reexamined some of these factors within the laboratory (Koch, 1967). Koch's work demonstrates that laboratory studies may be used for examining the ecological influences on the dispersal of *Drosophila*.

I have come across several studies that are significant for the discussion on the difference in dispersal between genders. In a set of Sakai "migration tubes," Mikasa and Narise tested whether temperature affects the migratory movements of males and females similarly, and observed that at the optimum temperature for *D. melanogaster*, 20°C≈25°C, males from laboratory strains migrated at a higher rate than females; however, he observed the reverse was true for recently collected natural isolates with the females being more vagile (Mikasa and Narise, 1980). There is an enigmatic study by Mikasa in which he looked at 140 lines and claims to have observed no differences in the movements of males and females (Mikasa, 1992). More recently, a group studying two recently isolated strains of *D. melanogaster*, one from a mesic environment and the

other from a xeric desert, reported to have measured higher rates for female migration, even though the general locomotor activity of the genders appear to be similar (Iliadi et al., 2002). Of particular note, it has been reported that mated female *Drosophila* emigrated at a lower rate than unmated females between chambers containing food (Mikasa, 1998); moreover, the degree of the difference measured between these mated and unmated females was twice as great as that measured between isofemale lines, suggesting that mating status modifies the motivation to emigrate. I know of no studies on the effects of prior mating experience on the movement preferences of male *Drosophila*.

Critics might claim that Sakai's "migration tubes" are simply elaborate "locomotor activity monitors." However, with a series of studies, Rockwell and colleagues report findings and argue that the two types of experimental chambers are distinct (Rockwell et al., 1978). A major motivation behind Rockwell's laboratory studies is to parameterize and characterize the interactions between two "behavioral preferences," an exercise that would be quite difficult or impossible in the field setting. Rockwell is interested in how light and geometry, specifically the height of the exits leading from the chambers, influence the flies' movement. He carried out his experiments in a series of studies with migration tubes that have exits either along the floor, level to the surface of the food in the chambers, or exits that are higher up leading from the middle of the chambers. The different placement of the exit serves to distinguish between flies accidentally *bumping* into the exit that is level to the surface of the food, and flies intentionally *finding* the exit that is higher up. He studied the movements of wild-type and blind flies and uses dark to illustrate and quantify the component of migration that might be due to a fly's general activity, and also the component of presumably visual exploration. He observes that flies moving through a series of connected chambers in the dark, or flies that are blind, exhibit dispersive movements that are greater when these flies are tested in chambers

with low exits, compared to the higher exits. However, visually intact flies disperse at a significantly higher relative rate than blind flies in the light through the elevated exits, suggesting that their enhanced migration stems from their ability to search and find the higher exit. Using these alternative model environments, "high light," "high dark," "low light," and "low dark," Rockwell and Levine carry out several studies from which they conclude that *Drosophila buskii* – not exhibiting improved or diminished dispersal with and without light – has a more restricted behavioral plasticity compared to *Drosophila melanogaster* (Rockwell, 1979; Rockwell and Levine, 1986); however, this may also reflect the stronger attraction to light as has been shown by *D. melanogaster* compared to *D. simulans* (McDonald and Parsons, 1973).[3]

Since Sakai's early study on the effects of group density for the movement of flies (Sakai et al., 1958), many groups have carried out studies within the laboratory showing effects of various factors on the migration of *Drosophila*: genetics (Narise, 1962; Tantawy et al., 1975; Rockwell et al., 1983; Mikasa and Narise, 1986; Rockwell and Levine, 1986; Mikasa, 1990), species (Takada, 1959), and temperature (Tantawy et al., 1975; Mikasa and Narise, 1979, 1983a,b, 1986), none of which are particularly relevant to the work discussed within this dissertation. However, I will share some of the more interesting stories from these many studies.

In one series of studies on the possible ecologically relevant phenomenon measured within the laboratory, Mikasa and Narise report on the variability of the response of movement to temperature for island and mainland strains of *Drosophila* collected from regions differing in temperature ranges (Mikasa and Narise, 1979, 1983b). They pro-

[3]Inspired by Rockwell's finding that species varying in their ratio of general activity to dispersal activity within his model "high/low," "light/dark," environments, I suggest the following line of investigation – mapping out the general activity-to-dispersal movement ratio among the various fly species found within the "cosmopolite guild" described previously, as an attempt to quantify niche specialization among these sympatric species.

pose from these and other findings that the different sensitivities to temperature between strains might be related to the environmental conditions from their sites of origin (Mikasa and Narise, 1983a). They carried this work further to show and propose that within a natural population there is genetic variation sufficient to cope with changing temperature conditions (Mikasa and Narise, 1986).

One important factor that I have not discussed here, and that is relevant for both the mating studies discussed within this dissertation and as well my current focus on social behaviors, is the role of gender-specific secreted chemicals. The role of secreted chemicals in arthropod communication is well established (Howard and Blomquist, 2005) and has been a topic of many studies using *Drosophila* (Ferveur, 2005). While there are several studies that have focused on the effects of secreted chemicals on the movement of *Drosophila* (Narise and Narise, 1991a,b), the authors of these studies limit their focus to how secreted chemicals affected emigration activity among genetically different strains and not the differential movement between genders. Secreted chemicals deposited on food patches could influence the movements of both males and females from *Drosophila* and is a quality of olfactory preference that would be worth studying.

Finally, the most intriguing studies I have read on the dispersal of *Drosphila* have been those related to the influence of mixtures of types of flies on the movement of groups. del Solar's early work, mentioned previously, and more recently (Tinette et al., 2004; Lefranc et al., 2001), suggest that flies do not move completely independently from each other. Whereas the studies just mentioned pertain to *like* flies interacting, there is an interesting series of studies by Takashi Narise on mixtures of flies among different types: among strains of *D. ananassae* (Narise, 1966); between the sympatric species *D. melanogaster* and *D. simulans* (Narise, 1967); among wild strains (Narise and Mikasa, 1984); and finally, between wild strains and laboratory strains (Narise,

1968, 1969, 1974). Here is a list of the interesting findings suggested by Narise from laboratory studies: (1) Dispersive activity is negatively correlated with fitness (Narise, 1974); (2) The competitive ability of strains that were selected for greater migration was far lower than the progenitor stock; although the fitness, as measured by the number of emerged flies in the next generation, was similar between selected and progenitor stock (Narise, 1967); (3) The more distant two strains were from each other, the stronger their strength at driving each other away, as assessed geographically (i.e., presumably naturally genetically divergent) (Narise and Mikasa, 1984) and genetically (comparing wild, lab and their F1 hybrids) (Narise, 1969); further (4) this effect scaled with ratio of the mixture; and finally – perhaps the most interesting laboratory study that I have read – (5) Narise showed that inferior laboratory mutant strains can survive, albeit at very low levels, in the refugia that a network of connected "migration tubes" provided as compared to their being completely eliminated under mixed population competition experiments with wild strains in standard "population cages" (Narise, 1968).

Chapter 2

Prior mating experience modulates the dispersal of male *Drosophila* to a greater extent than females

2.1 Summary

An important decision in an animal's life is whether to stay put or move somewhere potentially more desirable. Cues from both an animal's internal physiological state and its local environment influence the decision to disperse. Identifying and quantifying the causal factors underlying the initiation of dispersal is difficult, and often impossible, using traditional field research practices. Within this report, we describe a new technology that we have designed to automatically monitor the movement of large groups of the fruit fly, *Drosophila melanogaster*, between model environments, thereby facilitating studies of the movement and the behavioral priorities of this genetic model organism. Using this system, we carried out experiments within a laboratory setting from which we suggest that prior mating experience modulates the dispersal of these animals. Flies with mating experience stayed longer within distinct environments when food and was available, but dispersed to adjoining environments at a higher rate than unmated flies when no food

was available. Males tended to stay longer in environments containing food than did females, but dispersed at a higher rate, moved more between environments, and were more active than females when food was unavailable. We found no significant relationship between weight and activity, suggesting the behavioral difference between males and females is caused by an intrinsic factor relating to gender and not simply to body size. The standard laboratory strain Canton-S dispersed at a considerably lower rate than the natural isolate used throughout this study, and the magnitude of this difference was more apparent in the presence of food.

2.2 Introduction

Rotting vegetable matter spotted with fungal and microbial growth offers all of the known resources required for the livelihood of *Drosophila* (Throckmorton, 1975). A single vegetable rot provides the nutrients needed for the growth and development of both the larvae and the adult forms of these flies, a source of protein for egg maturation, a site suitable for oviposition, and a location for pupariation. Moreover, staying near this rot increases the potential for finding mates and may grant shelter from predators and at least some adverse environmental conditions (but see (Feder, 1997)). Because *Drosophila* obtain multiple resources from a single location, it would seem logical that they would stay at a patch of resources indefinitely, but even under constant ambient environmental conditions they disperse. Presumably, cues from the flies' internal physiological state and stimuli from the local environment combine to influence the probability that the flies disperse from a patch of resources rather than remain (Dethier, 1964; Kennedy, 1978). The dispersal of *Drosophila* provides a promising model for studies of behavioral priory, both because of the extensive literature on the dispersal of these flies

(Dobzhansky, 1973; Grossfield, 1978), and also the potential for using the available genetic technologies to examine the neural mechanism underlying the regulation of their behavior (Callaway, 2005; Zhang et al., 2007; Luo et al., 2008).

Most insects, in addition to needing food and water, require a mating partner and an oviposition site for successful reproduction. Priority among foraging for food, mates, and egg-laying sites depends on both the level of hunger and the mating status of an individual (Barton Browne, 1993). Many studies suggest that hungry insects modify their behavior to increase their probability of finding food (see (Barton Browne, 1993) and references there within); however, there is no obvious general rule explaining the influence of mating on the movements of insects or other arthropods (see Table 2.1).

Mating status has severe effects on the reproductive biology of many insects including *Drosophila* (Chapman et al., 2003) and is known to affect the general movement of many insects (Johnson, 1969). It is known from the study of flies other than *Drosophila* that females shift their preference among sex pheromones, and nutritive (carbohydrates) and proteinous foods odors after mating (Jang et al., 1998; Cornelius et al., 2000). In addition, females from one of these species have been observed to shift their behavioral priorities from mating to oviposition activities (Jang et al., 1999). Of particular note, it has been reported that mated female *Drosophila* emigrated at a lower rate than unmated females between chambers containing food (Mikasa, 1998); moreover, the degree of the difference measured between these mated and unmated females was twice as great as that measured between isofemale lines, suggesting that mating status modifies the motivation to emigrate. We know of no studies on the effects of prior mating experience on the movement preferences of male *Drosophila*.

The primary goal of this work was to investigate if and to what extent mating experience influenced the dispersal of *Drosophila* from food. Additionally, we considered

whether changes in the general locomotor activity of these flies were sufficient to explain the effects we observed of mating on their dispersal. We carried out these studies within the laboratory using a system of custom-built connected chambers. The technology allowed us to regulate and automatically quantify the movement of large groups of flies between controlled sensory environments. This new system will make possible high-throughput studies of complex behavioral phenotypes, such as food and habitat selection, social interactions, and emigration.

Table 2.1: Known effects of mating on movement for a sample of arthropods

Order	Species (common name)	Male	Female	Behavior	Reference
Acari	*Tetranychus urticae* (Twospotted spider mite)	n.r.	=	dispersal	(Suiter and Gould, 1992)
Coleoptera	*Leptinotarsa decemlineata* (Colorado potato beetle)	+	-	flight	(Alyokhin and Ferro, 1999)
Diptera	*Aedes aegypti* (Mosquito)	n.r.	-	flight	(Jones, 1981)
Diptera	*Aedes aegypti* (Mosquito)	n.r.	0	post blood meal/ pre oviposition activity	(Jones, 1981)
Diptera	*Anopheles balabacensis* (Mosquito)	n.r.	+	flight to blood source; biting	(Iwanaga-Sawabe and Kanda, 1990)
Diptera	*Anopheles gambiae* (Mosquito)	n.r.	-	flight (dusk)	(Jones and Gubbins, 1978)
Diptera	*Anopheles gambiae* (Mosquito)	n.r.	+	flight (night)	(Jones and Gubbins, 1978)
Diptera	*Anopheles gambiae* (Mosquito)	n.r.	-	post blood meal/ pre oviposition activity	(Jones and Gubbins, 1978)
Diptera	*Anopheles stephensi* (Mosquito)	n.r.	+	flight (dusk and evening)	(Rowland, 1989)
Diptera	*Anopheles stephensi* (Mosquito)	n.r.	0	post blood meal/ pre oviposition activity flight	(Rowland, 1989)
Diptera	*Glossina morsitans* (Tsetse fly)	n.r.	+	post reproductive activity	(Rowcliffe and Finlayson, 1982)
Diptera	*Glossina morsitans* (Tsetse fly)	n.r.	-	pre larvaposition activity	(Brady and Gibson, 1983)
Diptera	*Toxotrypana curvicauda* (Papaya fruit fly)	n.r.	=	flight (plumb tracking, hovering)	(Landolt and Heath, 1988)
Hemiptera	*Oncopeltus fasciatus* (Milkweed bug)	-	?	flight	(Dingle, 1966)
Hemiptera	*Lygus lineolaris* (Tarnished plant bug)	=	=	flight	(Stewart and Gaylor, 1994)
Hymenoptera	*Eretmocerus eremicus* (Wasp)	-	-	dispersal	(Bellamy and Byrne, 2001)
Hymenoptera	*Nasonia vitripennis* (Wasp)	n.r.	+	walking, hopping, and flight	(King, 1993)(King et al., 2000)
Hymenoptera	*Trichogramma brassiae* (Wasp)	+	-	flight	(Pompanon et al., 1999)
Lepidoptera	*Agrotis ipsioln* (Black cutworm, adult moth)	=	-	flight	(Sappington and Showers, 1992)
Lepidoptera	*Helicoverpa armigera* (Bollworm, adult moth)	n.r.	-	flight	(Armes and Cooter, 1991)
Lepidoptera	*Rhyacionia buoliana* (European pine shoot moth)	n.r.	+	flight initiation	(Green, 1962)

$^{+}$Increase, $^{-}$Decrease, $^{=}$No change, ^{0}No movment, $^{?}$Inconclusive, $^{n.r.}$Not reported.

2.3 Materials and Methods

2.3.1 Animals

We performed experiments on 3- to 4-day-old adults from two laboratory colonies of the fruit fly, *Drosophila melanogaster* (Meigen). The first colony descended from a wild-caught population of 200 females. The second colony came from the original Canton-S stock of the late Ed Lewis. We reared, entrained, and tested all flies on a 16 h: 8 h light: dark photoperiod. Transitions between light and dark were immediate, and the light-on phase started at 7AM PST. We maintained fly stocks at $25\,^{\circ}\mathrm{C}$ and at a relative humidity of either 30% or 60% on Lewis food medium in standard 250 mL bottles (Lewis, 1960).

2.3.2 Animal handling

Unless otherwise noted, we housed groups of 50 flies in vials (AS-515; Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA), on a 2 mL aliquot of food from a food medium (Ralph Greenspan, personal communication) consisting of 30 mL Karo® dark corn syrup, 15 g sucrose, 15 g Torula yeast (Lake States, Wisconsin, USA), 10 g agar, and 1.0 L distilled water.

In order to compare mated and virgin flies of a similar age that had been reared and housed at a similar temperature and humidity, we collected virgins $<$7 hours post-hatching and divided the collected individuals into three groups: 50 males per vial, 50 females per vial, and a mixture of 25 males and 25 females per vial. To keep housing densities equivalent, three days later we combined the two vials that each contained a mixture of 25 males and 25 females and then sorted them by gender into two new vials. The result was two vials, the first containing 50 mated males and the second

containing 50 mated females. The following day, we tested these mated flies along with the previously collected virgins. To help with counting and sorting, we immobilized flies by cooling them to $4\,^{\circ}\mathrm{C}$ on a Peltier stage (Marlow Industries, Inc., Dallas, Texas, USA).

2.3.3 Experimental test chambers

We developed a system of hardware and software to help automate studying the movement of flies between controlled sensory environments. The building blocks of this system were opaque, white cylindrical chambers, 9 cm high and 9.5 cm in diameter (inner dimensions), which interconnected in a modular fashion (Fig. 2.1A). Flies placed within a pair of connected chambers moved between adjacent chambers through narrow tubes that had an inner diameter of 0.55 cm. These tubes ran for 5.5 cm from each chamber into the opposite sides of a 3.5 cm-long channel drilled through a block sitting between the two chambers. The cross-sectional diameter of this channel narrowed to 0.20 cm. This diameter was large enough to allow a single female to pass through, but small enough to prevent the simultaneous passage of two males. Along this channel sat a solenoid-driven gate and two pairs of emitter/detector diodes (Fig. 2.1B). We used these blocks to control and detect the passage and direction of flies moving between chambers (Fig. 2.1 and Fig. 2.6).

We designed and built circuit boards with programmable ATmega8 microcontrollers (Atmel, Inc., California, USA) to control the gates and monitor the movement of flies passing through each counting block sitting between chambers. We include as a supplementary figure a diagram of the operational logic and examples of behavior near the detector illustrating how the counting blocks work (Fig. 2.6A-J). We configured a single

personal computer to control the opening or closing of an array of 16 gates flanked by 16 pairs of chambers. We used the same computer to query the controllers monitoring when a fly passes any of the 16 counting blocks. While each microcontroller detected events triggering the counting blocks at a rate of 100 kHz, the processing load of our system limited our capacity to query the movement of flies to approximately every 26 s. Each additional counting block would slow this processing capacity by 1.6 s per counter.

To test the accuracy of our counters, we introduced groups of 50 flies to the first of two connected chambers, each containing only water, and monitored their movement between chambers for 30 hours. At the end of this test, we counted the number of flies observed in the second chamber and compared this number to the number of flies determined by our automated system to be in this second chamber. On two separate occasions, we tested each of the 16 counters used in these studies. The average accumulated errors after 30 hours were 7.2% and 6.7%. In both tests, the ratio of under-counting to over-counting error was approximately 4:5 to 1:5, and thus our system tended to undercount. To avoid this counting error in all studies, we report rates of dispersal, a measure more resilient to the accumulation of error over time.

The entire array of chambers sat within a temperature-, light-, and humidity-controlled room. Average light levels inside chambers were around 360 lux. (See supplementary Table S2.2 for the measures of temperature and humidity from specific experiments.) We acquired the results for the movement of flies and configured the specifics of each experiment (e.g., when or which door opens and closes) with custom software written in C and Matlab (Mathworks Natick, MA, USA). We designed this technology to be flexible so that it would be possible to reconfigure the experimental chambers to create various model habitats for further studies.

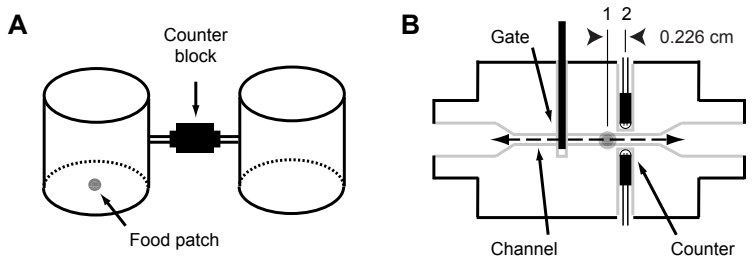

Figure 2.1: Instrument devised to study the movement of *Drosophila* between controlled sensory environments. (A) Illustration showing two experimental chambers connected by tubes feeding into the opposite sides of a counter block. For dispersal experiments, we either did or did not provide a patch of food in the middle of the chamber floor. (B) Schematic of a gate and bi-directional counting block. We drove each gate with a solenoid (push-pull type) motor and monitored the transition of flies through a channel within the counting blocks with two pairs of infrared emitter/detector diodes, denoted as 1 and 2. Note that the second pair of diodes is offset from the first pair by 0.226 cm (measured between diode centers) and are not shown in the drawing. The second set of diodes would project normal to the plane of the drawing (gray dot).

2.3.4 Dispersal assay protocol

Characterizing the movement of *Drosophila* through a specific experimental setup provided a starting point for studying dispersal in the laboratory. We introduced groups of 50 individuals or single flies into the first of two connected chambers. In all experiments we deprived flies of food, but not water, by transferring 50 flies into single vials containing 2 mL of 0.5% agar for 12 hours preceding a given trial. If an experiment included food, we used the same recipe as we had for rearing. All chambers provided access to a 2 mL plug of 0.5% agar to prevent dehydration. We introduced flies into chambers at 9AM and waited 1 hour for them to settle down before starting experiments. We programmed the solenoid-driven gates to all open precisely at 10AM and monitored the movements of flies until 4PM. In these and all subsequent experiments, we ran trials during this midday, 6 hour time window to avoid confounding interactions with crepuscular morning and evening peaks in activity.

By approximately the fourth hour, the flies' dispersal reached a state of equilibrium between chambers that both contained only water (see Fig. 2.2 for sample data, equilibrium denoted by arrowhead in C). The system reached an equilibrium more quickly if we decreased the length of the channel connecting the chambers or widened the diameter of the exit hole leading into this channel (See supplementary Fig. B.2). Conversely, if we increased the length of the channel or narrowed the diameter of the exit hole leading into the channel, we could lengthen the time required to reach a movement equilibrium between chambers (see supplementary Fig. B.2). We chose a length of channel connecting the chambers as well as a width of exit hole leading into this channel that would minimize the interference of cross traffic on the movement between chambers.

To demonstrate that the level of cross traffic for the chosen channel length and exit hole width would not appreciably interfere with the interpretation of the movement of flies, we ran experiments similar to those described in Fig. 2.2A-D, but modified the experiments by removing the lid covering the second chamber (Fig. 2.2E, F). Flies escaping through the lid of the second chamber decreased the rate of flies returning to the first chamber. This modification increased the level of dispersal (ANOVA, cross traffic, $p < 0.05$), but did not alter the relative difference in dispersal from chambers containing water or food (Fig. 2.2; compare C, D to E, F; ANOVA, interaction between food and cross traffic, $p = 0.856$).

Flies that had moved to the second chamber returned to the first chamber at a statistically similar rate irrespective of the presence of food in the first chamber. (For an example, see Fig 2.2C, D; Mann-Whitney U, $p = 0.147$.) Also, the amount of food used in this study was not enough to attract flies from adjacent chambers. The mean rates for flies dispersing from water towards chambers containing only water (9.4 ± 1.3 exit h^{-1}, $n = 14$), 65 μL of food and water (10.3 ± 0.9 exit h^{-1}, $n = 14$), and even 100 μL of

food and water (10.1 $\pm$ 0.9 exit h^{-1}, n = 15) were similar over the first hour (ANOVA, p = 0.816). However, once flies found food in the second chamber, their return to the adjacent chamber was inhibited. Compared to water alone, both 65 μL and 100 μL patches of food significantly inhibited the flies' movement (65 μL, Mann-Whitney U, p = 0.002; 100 μL, Mann-Whitney U, p $<$0.0001). From these observations we conclude that the flies could not perceive food in adjacent chambers and responded only to food nearby. For all experiments, we ran simultaneous trials in 16 pairs of connected chambers. Within a given experiment, we pooled results from trials run over several days. Unless otherwise indicated, all data within this report were reported as mean $\pm$ s.e.m. exit rates per hour and were averaged over 6 hours for statistical analyses (SPSS, SPSS, Inc., Chicago, Illinois, USA).

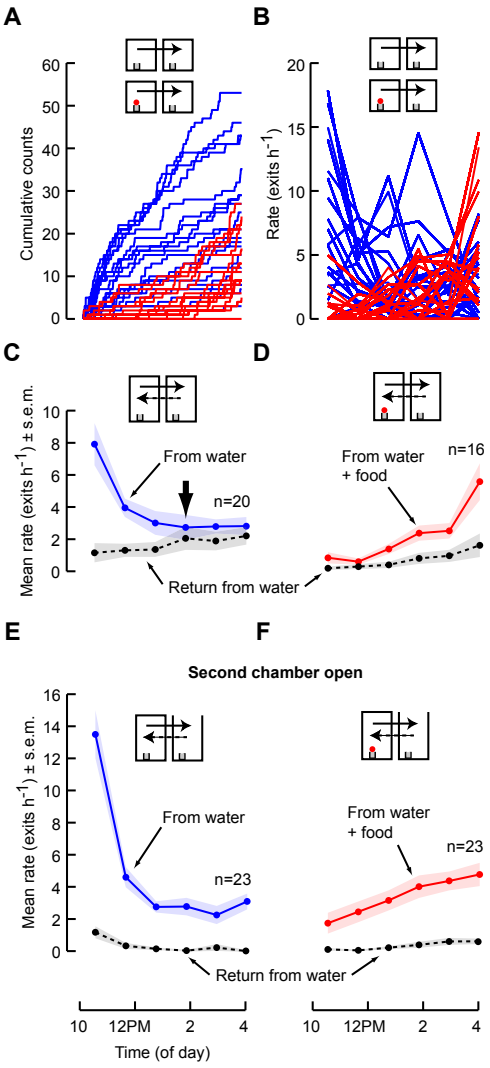

Figure 2.2: Sample data showing the movement of flies between two connected chambers containing either water and a patch of food or water alone. (A, B) Cumulative forward counts and corresponding forward rates from independent trials for transitions from only water (blue, 2 mL 0.5% agar) and water and a patch of food (red, 65 μL on 2 mL of 0.5% agar) contained in a first chamber to a second chamber containing only water. (C) Means $\pm$ s.e.m. for forward (blue solid) and reverse (black dashed) rates each hour from trials where both chambers contained only water. Flies reached equilibrium movement between chambers in this particular experiment after 4 hours (arrowhead). (D) Means $\pm$ s.e.m. for forward (red solid) and reverse (black dashed) rates from trials where the first chambers contained water and a patch of food and the second chamber contained only water. (E, F) Flies introduced to the first of two connected chambers moved comparably from the first to a second chamber whether or not the lid to the second chamber was closed or open (compare A-D to E, F). (E) Means $\pm$ s.e.m. for forward (blue solid) and reverse (black dashed) rates from trials where both chambers contained only water (2 mL of 0.5% agar), and the second chamber was open. (F) Means $\pm$ s.e.m. for forward (red solid) and reverse (black dashed) rates from trials where the first chamber contained water and a patch of food (65 μL on 2 mL of 0.5% agar) and a second chamber contained water and was open.

2.3.5 Justification for using a natural isolate

Many studies on the behavior of *Drosophila* have been carried out using derivatives of the standard laboratory wild-type Canton-S strain (CS). We report a large difference in the dispersal behavior between our natural isolate (NI) and CS from a chamber containing food to a second chamber containing no food, and a subtle, yet significant, difference in dispersal from chambers containing only water (Fig. 2.3). The dispersal rate for NI from food was greater than 1-exit-per-hour by the third hour of the experiment (One-Sample T-test, p = 0.003), but did not exceed this level until the ninth hour for CS (One-Sample T-test, p = 0.048, 1-tailed). From chambers containing only water, the dispersal rate for the NI (13.4 ± 1.4 exit h^{-1}) was significantly higher than that from CS (7.5 ± 1.0 exit h^{-1}) during the first hour (T-test, p = 0.001), but not for the remainder of the experiment. Dispersal rates during the second hour, for example, were 6.2 ± 0.7 exits h^{-1} (NI) and 8.1 ± 1.0 exits h^{-1} (CS), and were not significantly different (T-test, p = 0.127). We chose to continue our experiments using our own laboratory strain because it is likely to be less affected by genetic bottlenecks and we have accumulated a large set of behavioral experiments on this strain.

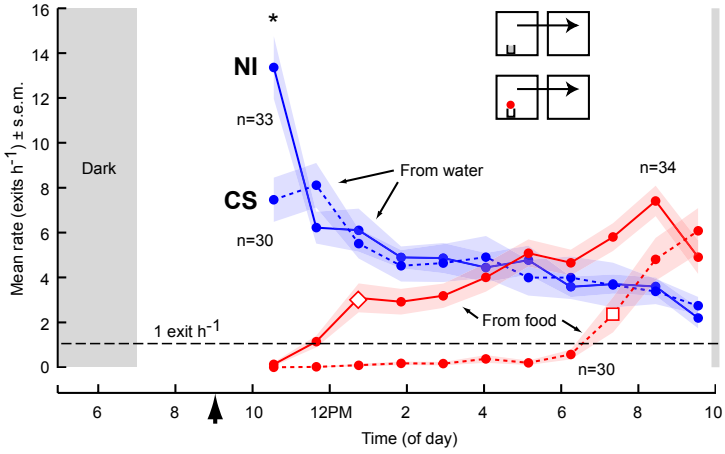

Figure 2.3: Genetic background is an important factor in the dispersal of *Drosophila* from food. The figure shows the dispersal rates of the natural isolate (NI, solid) used throughout this report and Canton-S (CS, dashed) from chambers that contained water and a patch of food (red, 65 μL on 2 mL of 0.5% agar) and chambers containing only water (blue, 2 mL of 0.5% agar). From food, the mean dispersal rates were greater than 1-exit-per-hour for the NI by the 3^{rd} hour (open diamond) and did not exceed this level until the 9^{th} hour for CS (open square). For the first hour, groups of the NI dispersed at a significantly higher rate than groups of CS from water (asterisks). The gray rectangles indicate lights-off periods. We started experiments 1 hour after introducing flies (arrowhead) into chambers, allowing them time to settle.

2.3.6 Activity experiments

To test whether a change in the intensity of a fly's general locomotor activity might have contributed to the differences we have observed in their dispersal, we measured the effects of gender, weight, and mating status on their general locomotor activity using commercially available *Drosophila* Activity Monitors (DAM) (TriKinetics, Inc., Waltham, Massachusetts, USA). Unless otherwise specified, we reared, housed, entrained, and handled flies, as well as ran experiments over the same midday, 6 hour time window, as we had in the dispersal experiments. To weigh flies, we placed them in Eppendorf tubes of known weight on a standard chemical balance (Sartorius Corp., Edgewood, New York, USA) and then placed each fly for 12 hours into separate vials containing food. The following day, after allowing flies to settle for 1 hour, we measured their activity using the TriKinetics monitors. Unless noted, flies began experiments sated, and during trials had access only to water. When the channel from the monitor for a particular fly stopped registering events, and continued not registering events throughout the rest of the experiment, we assumed that this marked the death of the fly. We adjusted the calculation for mean activity for each 5 min period throughout the experiment, taking into account the death of the individuals making up the mean.

2.4 Results

2.4.1 Mating inhibits dispersal from food yet increases dispersal from water.

A recent study reported that mated females seek ideal sites for oviposition (Yang et al., 2008). Mated females have also been shown to move less between chambers containing food than unmated females (Mikasa, 1998). It is unknown, however, to what extent mating influences the basic movement preferences of male and female flies from food or water. To test the effect of mating on their dispersal, we introduced groups of mated or virgin flies to chambers containing either food and water or only water and monitored their movement.

We observed that prior mating experience strengthened the inhibitory effect of food on movement. After being introduced to chambers containing food, mated males dispersed at a significantly lower rate than virgin males during the first hour (Fig. 2.4A, B; E, F; T-test, $p = 0.037^{\dagger}$) and mated females dispersed at a significantly and nearly significantly lower rate during the first and second hours than virgin females (Fig. 2.4A, B; E, F; T-test, $p = 0.015$; Mann-Whitney U, $p = 0.058^{\dagger}$, 1-tailed). In the absence of food, we found that mating experience had the opposite effect. Mated flies dispersed at a significantly higher rate than groups of virgins from water (Fig. 2.4C, D; G, H; All-male group, Mann-Whitney U, $p = 0.009^{\dagger}$; All-female group, ANOVA, $p = 0.003^{\dagger}$). As stated above, the general effect of mating was similar for both males and females. However, whereas the dispersal of both virgin males and those with mating experience was inhibited by the presence of food (Fig. 2.4B, D; F, H; T-test, $p = 0.003^{\dagger}$), the dispersal rate of virgin females was unaffected by the presence or absence of food (Fig. 2.4B,

D; F, H; Mann-Whitney U, p = 0.543) (†Bonferroni correction).

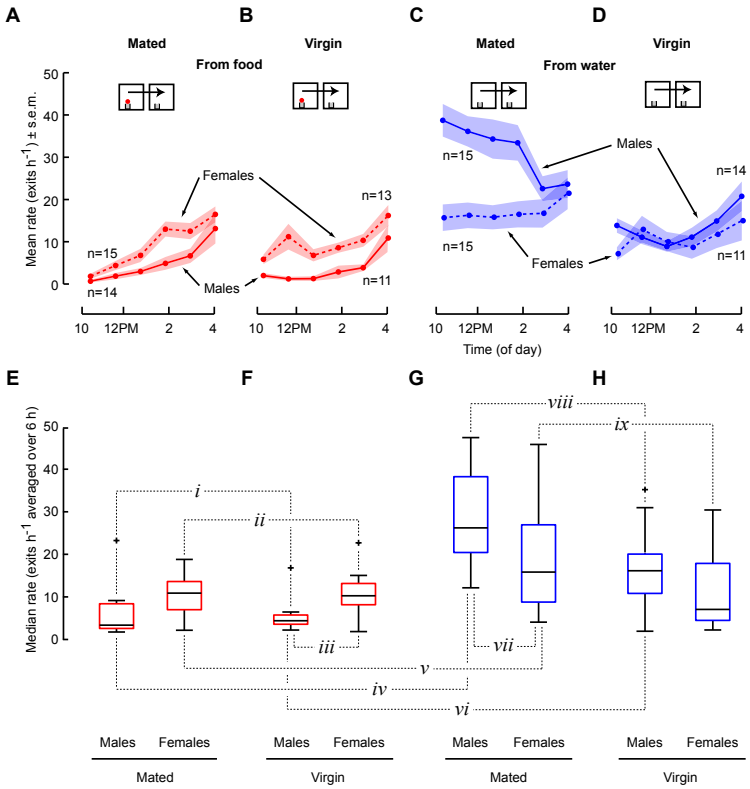

Figure 2.4: Mated flies are more sensitive than virgins to both the presence and absence of food. (A, B) In the presence of food (red, 65 μL on 2 mL 0.5% agar), (A) previously mated males (solid) and females (dashed) dispersed slower than (B) virgin males (solid) and virgin females (dashed). (C, D) In the absence of food (blue, 2 mL 0.5% agar), both (C) previously mated males (solid) and females (dashed) dispersed at a higher rate than (D) virgin males (solid) and females (dashed). (E, F) Mating significantly inhibits the dispersal of males (*i*) and females (*ii*) from food (see text). (F) Virgin males disperse from food at a significantly lower rate than virgin females (*iii*). (E, G) Food significantly inhibited the dispersal of mated males (*iv*) and mated females (*v*). (F, H) Virgin males, but not virgin females, dispersed from food at a significantly lower rate than water (*vi*). (G) Mated males dispersed from water at a significantly higher rate than mated females (*vii*). (G, H) Mating significantly increased the dispersal of males (*viii*) and females (*ix*) from water. (E-H) Median hourly rates averaged over 6 hours. The top and bottom edges of the boxes represent 75^{th} and 25^{th} percentiles; the whiskers extend to the most extreme point not considered outliers, and outliers are plotted individually (+).

2.4.2 Males disperse at a higher rate than females.

Gender differences in the nutritional requirements of flies are well documented (Greenberg, 1959) and have recently been shown in *Drosophila* (Carvalho et al., 2006). It is therefore likely that different factors may modulate the dispersal of females and males from patches of food. To test whether gender plays any role in dispersal, we introduced groups composed of only males or only females to chambers with food and water or only water and monitored their dispersal.

We report that flies from both genders dispersed from water rapidly, as expected, but with groups of males dispersing at a higher rate than groups of females (Fig. 2.4C, G; Males, 29.2 $\pm$ 3.0 exit h^{-6}; females, 18.0 $\pm$ 3.0 exit h^{-6}; T-test, p = 0.036†). This was not an emergent property of being in a group. When tested individually, single male flies also dispersed from water at a higher rate than single females (Males, n = 43; Females, n = 51; Mann-Whitney U, p = 0.023). Moreover, as expected, the presence of food inhibited the dispersal of both males and females. Again, this was observed whether flies were tested as single individuals or in groups (Fig. 2.4A, C; E, G; Males, Mann-Whitney U, p $<$0.0001†; For females, inhibition was statistically significant for only the first three hours: first, Mann-Whitney U, p $<$0.0001†; second, Mann-Whitney U, p $<$0.0001†; third, T-test, p = 0.028†). Single flies never left chambers containing food during 12 hours of observation (12 males and 12 females). In contrast to their higher rate of dispersal from water, males dispersed at an inhibited, lower rate than females from food. This lower dispersal for males was non-significant for mated flies (Fig. 2.4A) and statistically significant when flies had no prior mating experience (Fig. 2.4B, F; T-test, p = 0.045†) (†Bonferroni correction).

2.4.3 Males are more active than females.

We reported above that mated males dispersed at a higher rate than females from chambers containing only water. One possible explanation for this difference in dispersal is that it represents a fundamental difference in the levels of their general movement rather than a difference in locomotory behaviors related to dispersal. To determine whether males moved more between connected chambers than females, in addition to examining their forward dispersing movement, we examined the return movement of these same flies, where both chambers contained only water. Both groups of males returned at a higher rate (26.9 ± 3.0 exit h^{-6}) than groups of females (15.7 ± 2.8 exit h^{-6})(T-test, p = 0.012), and single males returned at a higher rate than single females (Mann-Whitney U, p = 0.045, 1-tailed). From these comparisons, it was reasonable to propose that males, in general, moved more than females.

To support the hypothesis that the difference in dispersal between mated males and females could be explained by a difference in their general activity, we introduced individual flies fed *ad libitum* food into a simple activity monitor (*Drosophila* Activity Monitor, TriKinetics) and measured their activity until all flies had died from starvation. We found that males did exhibit a significantly higher level of activity than females throughout the same 6 hour period we had tested in our dispersal assay. During the fourth hour, on average, males (8.25 ± 0.29 beam crosses h^{-1}, n = 96) were twice as active as females (4.14 ± 0.21beam crosses h^{-1}, n = 96)(Fig. 2.5A, B; T-test, $p < 0.0001$).

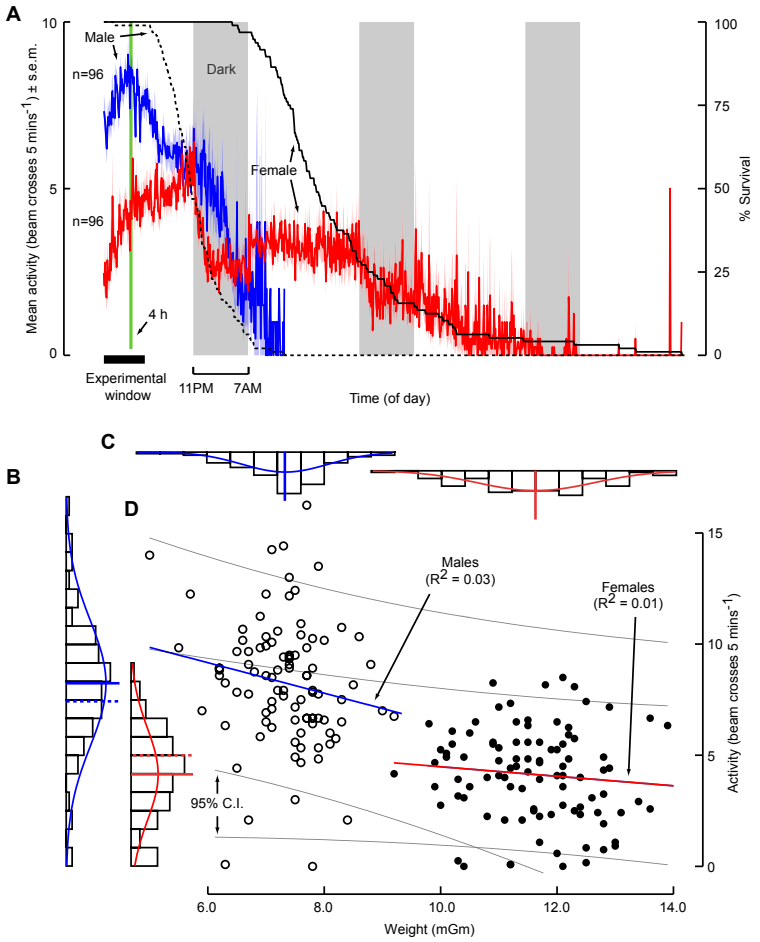

Figure 2.5: Gender and not weight explains why males are more active than females. We measured the activity of individual flies using the commercially available *Drosophila* Activity Monitor (DAM). (A) Male flies (blue) exhibited a significantly greater mean activity level than females (red) during the same time window as the experiments on dispersal carried out in this study (horizontal black bar). (B) During the 4^{th} hour (vertical green line in A), males were approximately twice as active as females. (C) Females weighed nearly twice that of males. (D) We measured the activity of individual males (open circles) and females (closed circles) and compared their weight with their activity during the 4^{th} hour of the experiment (vertical green line in A). The contribution of weight to the activity of flies was minimal and insignificant. By adjusting the measures of activity for weight (dashed lines near respective mean activity), we illustrate that gender contributes to why males are more active than females.

2.4.4 Weight does not explain the greater activity of males.

A functional explanation for why males were more active than females was that they were morphologically smaller and activity is correlated with body size (Fig. 2.5C). Males (7.3 $\pm$ 0.1 mGm) weighed nearly half that of females (11.6 $\pm$ 1.0 mGm) (Mann-Whitney U, $p < 0.0001$). To test whether the difference in activity between males and females might be due to body size, we weighed individual flies and then subsequently measured their activity. We found no significant relationship between weight and activity for either male or female flies (Fig. 2.5D). The contribution of weight to the activity of flies was minimal and insignificant (Regression; males, $p = 0.082$; females, $p = 0.326$). We conclude that something intrinsic to a fly's gender explains their difference in activity (ANCOVA; gender, $p = 0.01$; weight, $p = 0.064$), and suggest that this gender-specific difference also underlies their difference in dispersal.

2.4.5 Level of activity does not explain mating-induced increase in dispersal.

A difference in the level of general locomotor activity was sufficient to explain the difference in dispersal we observed between males and females (compare Fig. 2.4 with Fig. 2.5). It is possible that a change in the level of activity could also explain the increased rates of dispersal observed in mated flies. To test this hypothesis, we introduced individual flies, which were either mated or virgin, into activity monitors and measured their activity for 6 hours. These flies were deprived of food, but not water, for 12 hours preceding a given trial. We repeatedly did not observe an increase in the activity of mated flies. For example, from one repetition of the experiment we observed that the activity of virgin females (4.2 $\pm$ 0.4 beam crosses h^{-6}, n = 23) was similar to mated

females (5.4 $\pm$ 0.7 beam crosses h^{-6}, n = 22; T-test, p = 0.149) and the activity of virgin males (4.1 $\pm$ 0.7 beam crosses h^{-6}, n = 20) was similar to mated males (4.9 $\pm$ 0.7 beam crosses h^{-6}, n = 24; T-test, p = 0.405). Together with the independent observation that virgin females were more active than mated females (Martin, 2004), we suggest that a change in general activity, as measured with widely utilized *Drosophila* activity monitors, cannot directly explain the increase that we have observed in dispersal due to prior mating experience.

2.5 Discussion

Based on a laboratory assay, we provide evidence suggesting that mating experience modulates the dispersal of *Drosophila*. We observed that mated flies dispersed at a lower rate from food and an increased rate from water as compared to unmated flies (Fig. 2.4). A functional explanation for why mated females disperse from food at a lower rate than virgins is that they require extra food for egg production (Carvalho et al., 2006) and suitable sites to oviposit (Yang et al., 2008). Their higher dispersal from water presumably reflects their requirement to find food for feeding and laying eggs. We observed that virgin females dispersed at a similar rate whether or not food was present, consistent with the notion that virgin females prioritize finding mates over feeding (Fig. 2.4B, D, F, H). However, the presence of food inhibited the dispersal of males whether or not they had previously mated, suggesting that feeding is a constant priority for males (Fig. 2.4). In general, flies with mating experience behave as if they are hungrier than unmated flies, staying longer when food is available and leaving at a greater rate when it is not. A possible explanation for the elevated dispersal observed of mated males from water is that mating increases their requirement for food.

We observed that males within both single-gender groups and as individuals dispersed from food at a lower rate than females (Fig. 2.4 and in Results). These results are consistent with previous findings for the relative dispersive movements between males and females from natural isolates tested at the optimum temperature for these flies, $20\,^{\circ}$ to $25\,^{\circ}\mathrm{C}$ (Mikasa and Narise, 1980; Iliadi et al., 2002); however, Mikasa later adds that the gender differences are influenced by genetic variability of a particular population (Mikasa, 1992). Males, both within single-gender groups and as individuals, dispersed from chambers with water but without food and moved, back and forth, between two chambers at higher rates than females (Fig. 2.4 and in Results).

The results from our study indicate that the greater dispersal observed in males may reflect an intrinsic difference in the locomotor activity between genders (Fig. 2.5). Early accounts reporting on gender differences in locomotor behavior focus on the "reactivity" of flies (*sensu* (Connolly, 1967)). In Connolly's work, males were observed to move faster than females during the first 10 minutes following their introduction into an experimental arena (Burnet et al., 1988). Recent video-tracking methods provide detailed quantitative measures of differences in locomotor behavior between males and females over long periods of time (Martin, 2004) and in a group context (Branson et al., 2009). We report that the difference in activity cannot merely be explained by the disparity between their weights (Fig. 2.5D), and suggest that the differences observed in dispersal result from intrinsic differences between the genders (however, see (Belgacem and Martin, 2007)). Results from measuring the general locomotor activity of mated and unmated flies indicate that a change in their general locomotor activity alone was insufficient to explain the effect of mating on dispersal (see Results section on activity).

Laboratory studies of the dispersive movements of *Drosophila* are not new. After the development of a series of connected chambers by Sakai and colleagues (Sakai

et al., 1958), many studies have been carried out within a laboratory setting attempting to identify the various abiotic and biotic factors contributing to the movement patterns of *Drosophila*. For a review, see (Grossfield, 1978).

One important factor that we have not discussed within this study is the role of gender-specific secreted chemicals. The role of secreted chemicals in arthropods' communication is well established (Howard and Blomquist, 2005) and has been a topic of many studies using *Drosophila* (Ferveur, 2005). While there are several studies that have focused on the effects of secreted chemicals on the movement of *Drosophila* (Narise and Narise, 1991a,b), the authors of these studies limit their focus to how secreted chemicals affected emigration activity among genetically different strains and not the differential movement between genders. The effect of secreted chemicals on the movement patterns of males and females would be an interesting line of investigation in the future. In this current study, we have lumped together the chemical labeling of a food with the rest of the sensory stimuli arising from food. A recent study by Stamps and her colleagues (Stamps et al., 2005) observed the movement patterns and space use of marked individuals from patches of food within a large population cage. The focus of this work was on natal experience on habitat preference, but the authors additionally report differences in how male and female flies position themselves in relation to patches of food.

We observed a considerable difference in the dispersal between the standard laboratory strain Canton-S and the natural isolate used throughout this study (Fig. 2.3). This observation was not surprising, for it has been shown previously that laboratory strains exhibit lower levels of dispersal than wild strains (Tantawy et al., 1975; Mikasa and Narise, 1980). However, the magnitude of this difference raises concern when evaluating studies carried out with Canton-S (or any stocks derived from this stock) and

perhaps any stock cultivated too long within the laboratory.

From these studies, we suggest that prior mating experience is a significant and likely important factor modulating the dispersal of *Drosophila*. However, a richer description of dispersal is required before making conclusions regarding the mechanisms underlying the various factors contributing to this complex behavior. It would be informative to directly observe both the movement of single flies and individual flies behaving within groups as they disperse from patches of food. This is a direction of research that we are currently pursuing.

2.5.1 Supplementary Figure and Table

Table 2.2: Ambient environmental conditions from experiments within this study and from a representative sample of studies published from the 1970s until present on the behavior of *Drosophila melanogaster*

Table S1. Ambient environmental conditions from experiments within this study and from a representative sample of studies published from the 1970s until present on the behavior of *Drosophila melanogaster*.

Experiment	Figure	Year	Duration (days)	Temperature (°C)*	% Relative Humidity*
Sample data	2.2A-D	2005	10	25.7±0.3	43.3±2.7
Cross traffic	2.2E-F	2006	3	26.4±0.2	63.1±2.3
Genetic background	2.3	2004	16	≈25$^{\tau}$	n.r.
Proximity to food	Methods	2005	3	26.1±0.3	60.7±5.8
Mating history	2.4	2005	8	26.1±0.5	60.8±3.0
Single flies	Results	2008	21	21.2±1.0	38.0±5.7
Activity, weight	2.5	2007	3	24.5±0.2	29.2±3.5
Activity, mating	Results	2007	5	25.6±0.3	34.0±0.9
Literature‡	>1970			24.1±1.8	62.8±9.4

*Mean±s.t.d., $^{\tau}$Incubator, $^{n.r.}$Not recorded, ‡From 62 articles.

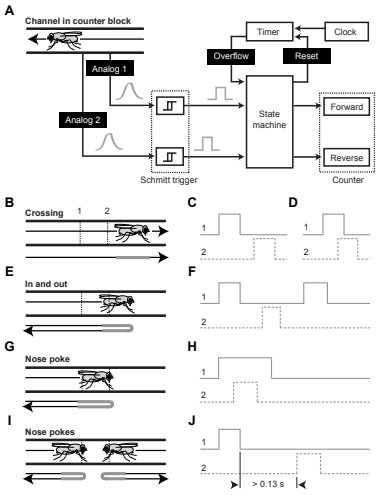

Figure 2.6: Diagram of the operational logic and examples of behavior near the counter. (A) Block diagram illustrating how the counter detects and assigns the bi-directional movement of flies. As a fly walks through the channel, it triggers two pairs of emitter/detector diodes, each pair producing an analog signal, denoted as Analog 1 and Analog 2. We used Schmitt triggers to create an all-or-none pulse based on a threshold of these analog signals. The coincidence of these two inputs and a time input pass into a state machine. In order to avoid registering false crossing events, if no additional signal from either of the emitter/detector pairs reached the state machine within 0.13 s after a previous signal, then the progression towards registering a crossing event was stopped and the timer was reset. This window of time between signals captured true crossing events, but minimized false signals from two flies approaching the counter from opposing directions (see I, J below). A counter tallied the forward and reverse crossing events registered from the state machine. (B) Illustration showing a typical crossing event. A fly moves through the channel from left to right, first triggering emitter/detector diode 1 and then 2. (C, D) Corresponding signals from a typical crossing event passing into the state machine. A specific pair of signals may pass into the state machine independently (as shown in C) or together (as shown in D), depending on the size and angle of the fly and the intensity/sensitivity of the emitter/detector diodes. The state machine registered crossings of flies from the opposite direction, from 2 to 1, similarly. (E) Illustration of the shortest movement of a fly along the channel that registers a crossing event. A fly moves through the detector far enough to completely pass through and trigger 1, but only transiently triggers 2 before reversing its direction and returning to where it originated. (F) Corresponding signals from 'in and out' that register a crossing event. (G) Illustration of the farthest movement of a fly along the channel that does not register a crossing event. A fly moves through the detector far enough to trigger a continuous signal in 1, but triggers only a transient signal in 2. (H) Corresponding signals from 'nose poke' that do not register a crossing event. (I) Illustration of two flies coming from opposite sides, both flies triggering the emitter/detector diode that they pass through coming from their respective directions before they reverse their direction and return to where they originated. (J) Corresponding signals from the combination of two flies approaching from adjacent chambers.

Chapter 3

Hunger regulates the dispersal of *Drosophila* from food

3.1 Summary

Assessing the quality of a feeding site and deciding whether to move to a site of possibly higher quality food is an important and constant decision in an animal's life. Cues from both an animal's physiology and the environment influence the decision to disperse from identified sites containing food. Hunger has been implicated as a important factor influencing the search behavior of most motile animals (Barton Browne, 1993) and has been frequently studied in insects (Bell, 1990).

We have used a system of environmental chambers to carry out laboratory experiments from which we suggest that hunger regulates the dispersal of the fruit fly, *Drosophila melanogaster*, independent of sensory cues arising from food. As expected, food inhibited the dispersal of hungry flies; however, hungry flies dispersed from detectible, yet inaccessible food at a similar elevated rate as they dispersed from a chamber containing only water. Further, sated flies dispersed at a low and similar rate irrespective of the presence or accessibility of food. Flies homozygous for different alleles of the *forag-*

ing gene, noted for differences in their locomotor behavior on and around food, were comparable in their dispersal from food. Results from experiments measuring a fly's general locomotor activity indicate that a change in the intensity of activity was insufficient for explaining the hunger-induced dispersal. From these experiments we suggest that the hunger state of flies can override the visual and olfactory cues from food; we hypothesize that the observed increase in dispersal resulting from hunger was due to a qualitative change in locomotor behavior related to food search.

3.2 Introduction

Hungry flies, like various other insects deprived of food, behave in ways to increase their probability of finding and consuming food (Bell, 1985; Barton Browne, 1993). Detailed studies indicate that the movement before and after feeding is similar among blow flies, *Phormia sp.* (Dethier, 1957, 1976; Nelson, 1977), house flies, *Musca sp.* (Mourier, 1964; White et al., 1984), and fruit flies, *Drosophila* (Bell et al., 1985; Mayor et al., 1987). *Phormia*, *Musca*, and *Drosophila* have been described to move in relatively straight paths at moderate speeds until they come upon a patch of food (Dethier, 1957; Mourier, 1964; Bell et al., 1985). Upon finding food, it has been reported that their locomotor rate decreases and their turning rate increases (Bell et al., 1985), that eventually they stop, and if the food is acceptable, they feed (Nelson, 1977; White et al., 1984). Upon finding a sufficiently large patch of food, several studies report that flies stop and eat until satiety, afterwards moving very little (Green, 1964a; Dethier, 1976; Bell et al., 1985). After feeding, as flies become hungry, their movement has been characterized as speeding up and straightening out (Dethier, 1957; White et al., 1984; Bell et al., 1985), thereby displacing them from the site of food (White et al., 1984). Many

studies have shown that hungry flies search longer, farther, and more intensely on and around patches of food (Dethier, 1957; Mourier, 1964; Nelson, 1977; Bell et al., 1985; Mayor et al., 1987). In particular it has been observed that as *Drosophila* become hungrier, they return to food in greater numbers and also stay closer to food (Mayor et al., 1987). We have no prior knowledge of how flies respond to detectable, yet inaccessible food.

The primary goal of this work was to investigate to what extent hunger influenced the dispersal of flies from a patch of food, independently from sensory cues from food. Additionally, we considered whether a change in the intensity of a fly's general locomotor activity was sufficient to explain the observed effects of hunger. We carried out these studies within the laboratory using a custom-built system of connected chambers. This technology allowed us to systematically manipulate features of simplified environments while automatically quantifying the movement of *Drosophila*.

3.3 Results

3.3.1 Hunger regulates dispersal

To examine and characterize to what extent hunger influences the dispersal of flies from patches of food, we carried out a series of experiments using environmental test chambers that we have described previously. As expected, hungry flies introduced to a chamber containing food dispersed to a second chamber at an inhibited rate compared to when they left only water (Fig. 3.1). Their emigration rate was significantly different whether dispersing from water, 65 μL food, or 100 μL food (Kruskal-Wallis, p = 0.0001), with the 100 μL patch inhibiting dispersal more than the 65 μL patch (0.7 $\pm$ 0.7 exit h^{-6} vs.

2.4 ± 1.1 exit h^{-6}, T-test, $p < 0.0001$). However, sated flies that were given food *ad libitum* for 12 hours prior to the start of the experiment dispersed at a low and comparable rate whether or not food was present in the first chamber (Fig. 3.1). This dispersal was significantly lower than that of hungry flies from water (Mann-Whitney U, $p < 0.0001$). Taken together, these results suggest that dispersal is triggered by hunger, and not by the limited availability of food.

To test more directly whether odor or visual cues associated with food might inhibit dispersal, we placed food in chambers beneath a mesh so that the flies could see and smell the food but could not touch or consume it. Hungry flies dispersed from the inaccessible food at a similar elevated rate (2.2 ± 0.4 exit h^{-6}) as they dispersed from a source of water (accessible, 2.6 ± 0.7 exit h^{-6}, covered, 2.1 ± 0.3 exit h^{-6}) (Fig. 3.2A, B, D-F, and H; ANOVA, $p = 0.691$). This rate was significantly greater than that of flies dispersing from accessible food (Fig. 3.2; ANOVA, $p = 0.002$; Tukeys HSD, significance level of 0.05). As observed before, sated flies dispersed at a rate (Fig. 3.2A-H; ANOVA, $p = 0.280$) that was significantly lower than hungry flies, irrespective of the presence or accessibility of food (Fig. 3.2A-H; Collective dispersal for hungry and sated flies from all resources, Mann-Whitney U, $p < 0.0001$). These results suggest that hunger drives dispersal despite the attractive sensory stimuli associated with food.

The elevated dispersal observed for flies in the presence of inaccessible food might be explained by the flies' inability to detect the food. To verify that flies could detect the presence of the inaccessible food, we introduced flies into a single chamber with food placed beneath a mesh cover and observed their behavior directly from recorded digital video. To help visualize the behavior of these flies, we modified our basic design in two ways. First, we blocked the exit from the chamber. This prevented the flies from dispersing and therefore allowed us to observe their behavior over long periods

of time. Second, we excluded accessible water. We found that the majority of the flies would congregate near water, diminishing our ability to determine whether or not they could detect the inaccessible food (data not shown). Our results show that a significantly greater number of flies loitered over water and food than they did over water alone (Fig. 3.3). We observed this difference whether we examined the full-length experiment or just the first two hours. (Full experiment, Mann-Whitney U, $p < 0.0001$; First two hours, Mann-Whitney U, $p = 0.04$, 1-tailed.) The number of flies loitering over water and food and water alone were both much greater than the number of flies loitering over empty cuvettes (Fig. 3.3G-J), as expected for flies deprived of both food and water. These results suggest that flies could detect the amount of food placed beneath the mesh that was used during the dispersal experiments, and that the food remained attractive to hungry flies if flies were confined to an area and not permitted to disperse.

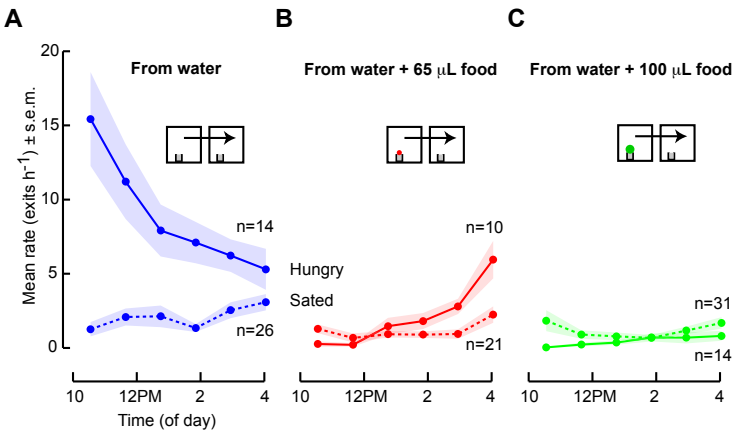

Figure 3.1: Hunger inhibits dispersal from food. Food in a first chamber inhibits the dispersal of hungry flies (solid) to a second chamber containing only water. (A) Mean $\pm$ s.e.m. exit h^{-1} dispersal rates from chambers containing only water (solid blue, 2 mL 0.5% agar). (B, C) The mean dispersal rate was significantly greater from chambers containing 65 μL of food on 2 mL 0.5% agar (solid red) than 100 μL of food on 2 mL 0.5% agar (solid green). (A-C) Sated flies (dashed) dispersed at a significantly lower rate than hungry flies (solid). This rate was comparable whether or not food was present in the first chamber.

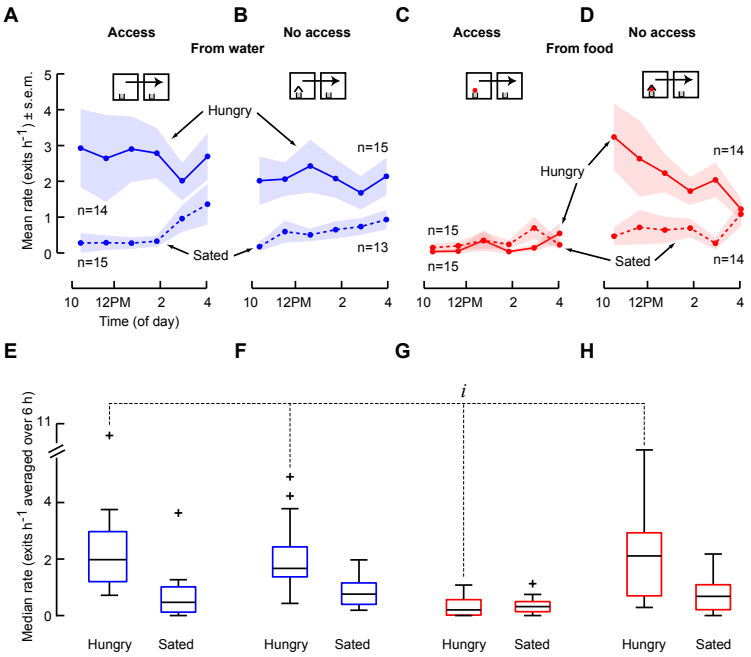

Figure 3.2: Hunger and not cues from food inhibits dispersal. Hungry flies dispersed at a similar rate from chambers in which food cues are present, but access to food was inhibited (D, solid red, H, 65 μL on 2 mL 0.5% agar beneath a mesh) and from chambers in which food was absent (A, solid blue, E, 2 mL 0.5% agar; B, solid blue, F, 2 mL 0.5% agar beneath a mesh). This rate was significantly greater than the dispersal of hungry flies from chambers in which food was accessible (C, solid red; G, indicated by *i*). (A-H) Sated flies (dashed) dispersed at a similar rate irrespective of the presence or accessibility of food in the first chamber, and this rate was significantly lower than hungry flies. (E-H) Median hourly rates averaged over 6 hours. The top and bottom edges of the boxes represent 75^{th} and 25^{th} percentiles; the whiskers extend to the most extreme point not considered outliers, and outliers are plotted individually (+).

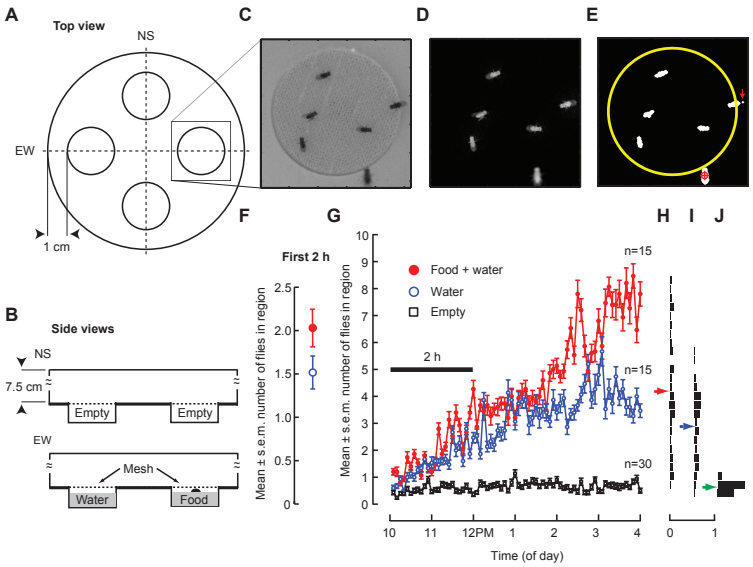

Figure 3.3: Hungry flies can detect the presence of food embedded in the floor covered beneath a mesh. (A) Schematic showing the possible locations of cuvettes containing food. (A, B) North-South (NS) and East-West (EW) cross-sections illustrate a possible configuration of the cuvettes containing either water and a patch of food, only water, or nothing. (C) Cropped region from a digital video sequence showing the typical number of flies observed loitering over a mesh covering a cuvette containing water and a patch of food. (D) Image from C minus a background image made from the average of 10 frames of video from the same region. (E) Thresholded image from D used to determine the location of flies within this region. We tallied the number of flies loitering within the specified region (yellow circle). To avoid false counts, we excluded objects that were larger and smaller than the range of pixel areas that could reasonably include flies. For example, a fly sitting upside down on the lid of the chamber had a pixel area too large and was not counted, as shown by the fly at the bottom of the image (red crosshairs). Small objects due to digital noise or processing errors, as shown by the fly's wings in the right of the image, were also not counted (red arrowhead). (F) Flies loitered over water and a patch of food (65 μL on 2 mL 0.5% agar, red closed circle) to a significantly greater extent than over only water (2 mL 0.5% agar, blue open circle) during the first 2 hours of the experiment. (G) Mean $\pm$ s.e.m. number of flies loitering over water and a patch of food (65 μL on 2 mL 0.5% agar, red closed circles), only water (2 mL 0.5% agar, blue open circles), and nothing (black squares) over the length of the experiment. Evidence that a greater number of flies loitered over water and food than water alone became stronger if we compared loitering during the full experiment. (H) Histograms showing the mean frequency of flies loitering over water and food, (I) only water, (J) and empty containers during the full experiment. We show the mean number of flies loitering over water and food (red arrowhead), only water (blue arrowhead), and empty containers (green arrowhead).

3.3.2 Activity does not explain dispersal

One possible explanation is that the increased rates of dispersal observed in hungry flies could be explained by a change in the level of their general activity. To test this hypothesis, we introduced individual hungry and sated flies into activity monitors and measured their activity for 6 hours. We repeatedly did not observe an increase in the level of their activity. Females showed a similar level of activity whether they were hungry or sated (hungry, 5.4 $\pm$ 0.7 beam crosses h^{-6}, n = 22; sated, 4.8 $\pm$ 0.5 beam crosses h^{-6}, n = 19; T-test, p = 0.503) and males were less active when deprived of food (hungry, 4.9 $\pm$ 0.7 beam crosses h^{-6}, n = 24; sated, 8.3 $\pm$ 1.0 beam crosses h^{-6}, n = 26; Mann-Whitney U, p = 0.010). Results from these experiments suggest that the greater level of dispersal observed for hungry flies has a more complicated explanation than a change in the level of general activity, as measured with the widely utilized *Drosophila* activity monitors. Rather, the activity arises from an increase in search behavior that is not detectable in the simple geometry of an activity monitor.

3.4 Discussion and conclusions

Using a system of connected environmental chambers, we have shown that hunger alone, and not cues emanating from food, regulates the dispersal of *Drosophila*. Hungry flies rapidly left chambers containing only water, whereas the presence of accessible food inhibited their dispersal (Fig. 3.1, 3.2). The rate of dispersal varied according to the amount of food present; the greater the amount of food within a chamber, the slower the flies dispersed from it (Fig. 3.1). A key observation in this study was that hungry flies dispersed from detectible, though *inaccessible* food at a similar elevated rate as if they were dispersing from only water (Fig. 3.2). This implies that the sensory stimuli

originating from food do not inhibit dispersal. The importance of hunger, and not stimuli related to food in controlling dispersal, is further supported by the behavior of sated flies, which dispersed between connected chambers at a low and similar rate irrespective of the presence, amount, or accessibility of food (Fig. 3.1, 3.2). Collectively, these experiments suggest that to initiate dispersal the hunger state of flies can override the visual and olfactory cues from food.

To the best of our knowledge, a hungry fly's response to detectible but inaccessible food is unknown. Previous studies report that flies presented with and consuming only a small amount of food searched afterward in loops and spirals (Dethier, 1957) within a 5≈6 cm diameter region (Nelson, 1977; Bell et al., 1985) around the area that contained the food patch (Mourier, 1964; Mayor et al., 1987). This convoluted movement has been reported to be remarkably similar in *Phormia*, *Musca*, and *Drosophila* (Murdie and Hassell, 1973; White et al., 1984). It is unlikely, however, that such a response could explain the elevated dispersal we have observed of hungry flies from inaccessible food (Fig. 3.2). It has been reported that hungry flies foraging without finding food stop less often (Dethier, 1957) and forage for relatively greater amounts of time than sated flies (White et al., 1984). It is therefore possible that a change in the level of a fly's general locomotor activity might explain its regulated dispersal. However, blood-borne factors associated with hunger and saity that have been shown to regulate the general locomotor activity of blow flies (Green, 1964b) did not affect their food searching response. Unfed parabiotic blow flies, pairs of flies that have been surgically connected so they share haemolymph, continued the searching response after their partners had fed and stopped searching, suggesting that this behavior is not simply a by-product arising from hormonally controlled changes in general locomotor activity (Nelson, 1977).

An important issue for this study is whether the regulated dispersal due to hunger

results from a general increase in locomotor activity or, alternatively, is due to a transition to a specific locomotor mode related to food search. Although our experiments cannot test these alternatives, our results do suggest that a change in general locomotor activity alone was insufficient to explain dispersal. In our studies, during the same 6 hour time window as we had run the dispersal experiments, we observed a steady and similar rate of locomotor activity for sated and hungry females and a decreasing rate of locomotor activity in hungry males, presumably as they began to dehydrate (see Results section on activity). Previous studies have reported that locomotor activity increases with food deprivation, but these observations are difficult to directly compare with our results (Connolly, 1966a; Bell et al., 1985). In several of these studies, the authors sampled short, less than 5 minute periods of movement. The recent handling of the flies possibly affected the results of such experiments. Knoppien and colleagues (Knoppien et al., 2000) measured the locomotor activity of food-deprived flies over a longer period of time and reported a steady level of higher activity instead of a graded, increasing level of locomotor activity. Martin (Martin, 2004) continually measured the locomotor activity of flies and found that as sated flies become hungry, they spend more time moving and move greater distances, but their activity reaches a maximum steady level after 2 hours. During our studies, we allowed flies 1 hour to settle down in the chambers before recording their activity. We assume that this time, plus the additional time taken to introduce each fly individually into the activity monitors (0$\approx$45 min), explains why we observed a steady, elevated level of activity in sated flies as opposed to an increasing level of activity as has been reported previously (Martin, 2004). From these results, we hypothesize that a change in a fly's general level of locomotor activity, as assayed in the *Drosophila* activity monitors, cannot directly explain the increased rates of dispersal that we have observed in hungry flies. One possible explanation is that the behavior

recorded in the small activity monitor represents an escape response to the confined space, which supersedes the locomotor response due to hunger.

3.4.1 Genetic contribution

Flies that posses *rover* or *sitter* (Osborne et al., 1997), allelic forms of the *foraging* gene that have been shown to exhibit significant differences in the flies' movement on and around food (Pereira and Sokolowski, 1993), dispersed at comparable rates from food. We report that groups of *rover* flies exhibited a sometimes similar, but overall lower rate of dispersal from food (1.5 $\pm$ 0.4 exit h^{-6}, n = 15) than sitters (3.1 $\pm$ 0.4 exit h^{-6}, n = 16)(T-test, p = 0.003). In contrast, however, we observed that groups of *rover* flies moved at a greater rate (forward, 7.9 $\pm$ 0.9 exit h^{-6}, return, 4.9 $\pm$ 0.8 exit h^{-6}, n = 20) between chambers containing only water than *sitters* (forward, 4.9 $\pm$ 0.5 exit h^{-6}, return, 2.9 $\pm$ 0.4 exit h^{-6}, n = 20)(Forward, T-test, p = 0.005; reverse, T-test, p = 0.038). This finding was consistent with a non-significant trend observed over a shorter time period reported previously (Pereira and Sokolowski, 1993).

3.4.2 Concluding remarks

We have designed and built a flexible system of hardware and software able to regulate and monitor the movement of groups of flies between controlled sensory environments in the laboratory. Through a series of experiments, we provide evidence suggesting that hunger regulates the dispersal of *Drosophila* independently of stimuli arising from food. Furthermore, a change in the level of the flies' general locomotor activity cannot directly explain hunger-induced dispersal. We require a richer description of dispersal before making conclusions regarding the mechanisms underlying the various factors

contributing to this complex behavior. It would be both informative to directly observe the movement of individual sated flies as they become hungry and to monitor in greater detail the response of flies deprived of food as they disperse from patches of accessible and inaccessible food. This is a direction of research that we are currently pursuing.

3.5 Materials and Methods

3.5.1 Animal stocks and handling

We performed experiments on 3- to 4-day-old adults from three laboratory colonies of the fruit fly, *Drosophila melanogaster* (Meigen). The first colony descended from a wild-caught population of 200 females. The second and third colonies came from *rover* and *sitter* stocks of the *foraging* gene isolated from natural populations provided by Marla Sokolowski. We reared, entrained, and tested all flies on a 16 h: 8 h light: dark photoperiod. Transitions between light and dark were immediate. The light-on phase started at 7AM PST. We maintained fly stocks at $25\,^{\circ}\mathrm{C}$ and at a relative humidity of either 30% or 60% on Lewis food medium in standard 250 mL bottles (Lewis, 1960).

Unless otherwise noted, we housed groups of 50 flies in vials (AS-515; Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA), on a 2 mL aliquot of food from a food medium (Ralph Greenspan, personal communication) consisting of 30 mL Karo dark corn syrup, 15 g sucrose, 15 g Torula yeast (Lake States, Wisconsin, USA), 10 g agar, and 1.0 L distilled water. To help with counting and sorting, we immobilized flies by cooling them to $4\,^{\circ}\mathrm{C}$ on a Peltier stage (Marlow Industries, Inc., Dallas, Texas, USA).

3.5.2 Environmental test chambers

We used a system of hardware and software developed to help automate studying the movement of flies between controlled sensory environments described previously.

3.5.3 Dispersal assay protocol

We introduced groups of 50 flies into the first of two connected chambers. Unless otherwise stated, in all experiments we deprived flies of food, but not water, by transferring 50 flies into single vials containing 2 mL of 0.5% agar for 12 hours preceding a given trial. If an experiment included food, we used the same recipe as we had for rearing. This food was introduced as a small dollop on the top surface and the center region of a 2 mL plug of 0.5% agar. Unless noted, in all chambers we provided access to a 2 mL plug of 0.5% agar to prevent dehydration. We introduced flies into chambers at 9AM and waited 1 hour for them to settle down before starting experiments. We opened gates leading into connected chambers at 10AM and monitored the movements of flies until 4PM. In these and all subsequent experiments, we ran trials during this midday, 6-hour time window to avoid confounding interactions with crepuscular morning and evening peaks in activity.

For all experiments, we ran simultaneous trials in 16 pairs of connected chambers. Within a given experiment, we pooled results from trials run over several days. Unless otherwise indicated, all data within this report were reported as mean $\pm$ s.e.m. exit rates per hour and were averaged over 6 hours for statistical analyses (SPSS, SPSS, Inc., Chicago, Illinois, USA).

3.5.4 Detection assay protocol

We designed this experiment to assess whether flies could perceive the amount of inaccessible food used for the studies of dispersal. We introduced groups of 50 flies into a single chamber and recorded their locations using a camera mounted above each chamber. These flies had been deprived of food, but not water, as described for the dispersal experiments. In each chamber, we placed a quartet of containers, each embedded within holes in a false floor. Each cuvette contained water and food (65 μL of food on top of a 2 mL plug of 0.5% agar), only water (2 mL plug of 0.5% agar), or was empty (Fig. 3.3A, B). We covered all containers with mesh so their contents were inaccessible to flies. In each trial we positioned a cuvette containing water and food opposite to one containing only water, and the two other cuvettes were empty (Fig. 3.3A, B). Between trials we used fresh cuvettes, switched the mesh covers, and rotated the location of the cuvettes to control for the build up of olfactory cues or effects that might bias movement, such as asymmetric geometry or lighting. After allowing the flies to settle for 1 hour, we recorded their position every 5 minutes throughout each experiment, using custom software written in Python (Straw and Dickinson, 2009). We determined the number of flies positioned within specified regions using custom software written in Matlab (Mathworks Natick, MA, USA). We normalized loitering frequencies to take into account that during each trial, chambers contained one cuvette with food and water, one with only water, and two that were empty.

3.5.5 Activity experiments

To test whether a change in the intensity of a fly's general locomotor activity might have contributed to their differences in dispersal, we measured the effects of hunger on

their general locomotor activity using commercially available *Drosophila* Activity Monitors (TriKinetics, Inc., Waltham, Massachusetts, USA). Unless otherwise specified, we reared, housed, entrained, and handled flies, as well as ran experiments, over the same midday, 6-hour time window, as we had in the dispersal and detection experiments. During trials all flies had access only to water. When the channel from the monitor for a particular fly stopped registering events, and continued not registering events throughout the rest of the experiment, we assumed that this marked the death of the fly. We adjusted the calculation for mean activity for each 5 minute period throughout the experiment, taking into account the death of the individuals making up the mean.

3.5.6 Supplementary Table

Table 3.1: Ambient environmental conditions from experiments within this study and from a representative sample of studies published from the 1970s until present on the behavior of *Drosophila melanogaster*

Experiment	Figure	Year	Duration (days)	Temperature (°C)*	% Relative Humidity*
Hunger	3.1	2004	10	25.9±0.2	28.9±8.6
Accessibility of food	3.2	2005	9	23.7±0.7	30.3±4.4
Detection of food	3.2	2008	11	21.3±0.3	41.5±9.4
Foraging gene	Discussion	2005	10	25.7±0.3	43.3±2.7
Literature‡	>1970			24.1±1.8	62.8±9.4

*Mean±s.t.d., n.r.Not recorded, ‡From 62 articles.

Chapter 4

Characteristic exploratory behavior persists as individual *Drosophila* become hungry

4.1 Summary

Here we show early attempts to demonstrate the individuality of adult *Drosophila* reared and observed in homogeneous conditions. Using a simple machine vision strategy to track the movements of single flies within model environments, we describe a characteristic structure in the movements of individuals making up their exploration and dispersal. The characteristic structure persists over the period of hours and is robust to systematic shifts in the movement of these flies over this time that are presumably due to entrained crepuscular activity and changes in their hunger state.

4.2 Introduction

We have previously suggested that hunger overrides the visual and olfactory cues from food, driving the fruit fly, *Drosophila melanogaster*, to disperse from inaccessible food

patches. To describe this movement, which seems largely driven by the animal's changing internal physiological state, in terms of behavioral algorithms, we mounted single cameras above the same environmental chambers previously used and developed a simple machine vision strategy to reconstruct the 3D trajectory of single, isolated flies moving within these chambers. We started recording the movements of the flies just after they had been removed from food, and therefore we captured onto digital video the change in the behavior as the flies became hungry. Upon analyzing the search movements of flies near a water resource as they shift to exploring and then to exiting from the chamber to an adjoining chamber, we noticed a surprising non-uniformity in their movement (see Figure 4.1).

As a starting point to determine whether flies exhibit *individualistic* exploratory movement over the period of hours, we learn a function that inputs a quantitative description of the behavior of a fly during one time period and predicts this description during another period. We then show for a number of behavioral statistics describing the movement of exploring flies, that the error in this prediction is significantly lower than for a control experiment, in which we try to predict the behavior of a fly given the behavior of a different fly.

Within this terse introduction of the project, we analyze 1 of 4 collected data sets: (1) 34 males and 34 females over 6 hours that may freely move between the chambers. We are also working with, but largely do not mention, results from the three other data sets: (2) 10 females over 6 hours blocked from moving between chambers by a visually transparent window allowing the flies to see out of the chamber, (3) 16 males and 14 females over 12 hours that are shut within the first chamber with a plug made to appear as near a possible as more of the chamber wall, effectively acting as a *single* chamber, and (4) 12 hours of behavior for 9 females that have been food deprived for 3 hours

and 9 females that have been food deprived for 12 hours that may pass back and forth between the connected chambers.

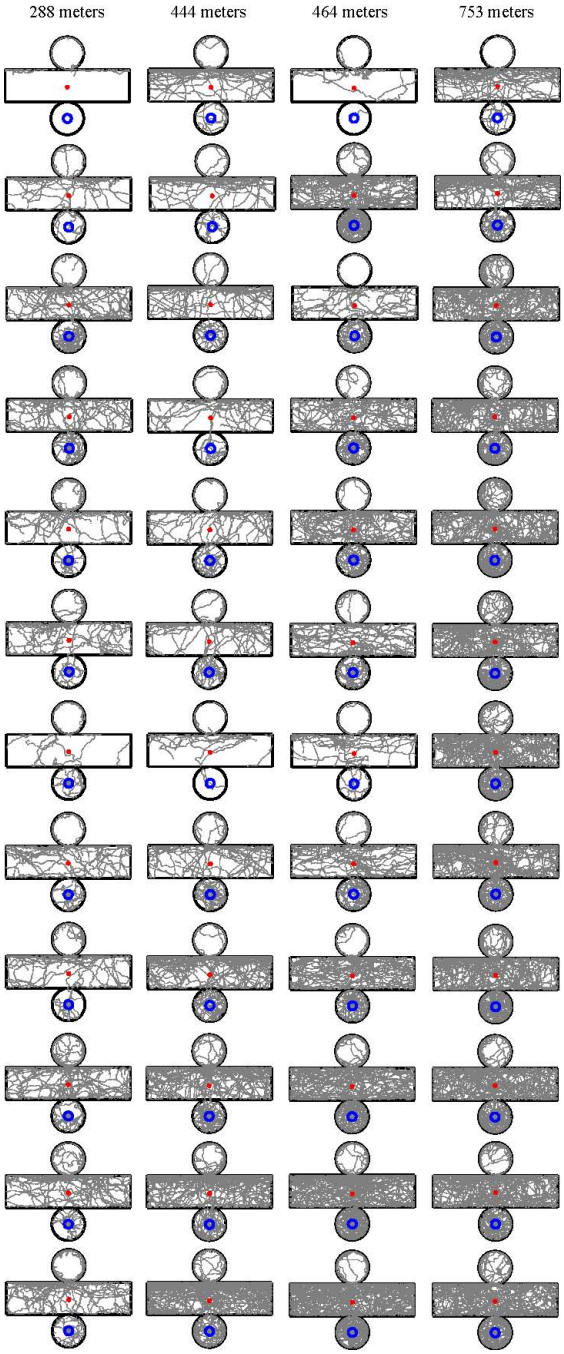

Figure 4.1: Individual trajectories of isolated, single flies moving within a single cylindrical chamber for 12 hours. Shown are the hourly movements of four individual flies throughout the 12 hour trials (top to bottom). The trajectories from two flies come from trials where flies walked for a medium total distance (444 and 464 meters), whereas the other two come from flies that had walked two standard deviations shorter (288 meters) or further (753 meters) than the medium distance. To help illustrate the trajectory of a fly, we unwrapped its 3D positions within the experimental chambers and report its movement in a flattened representation. A patch of agar (blue circle) embedded within the center of floor prevented flies from dehydrating. The exit (red dot) leading to a connected second chamber was blocked during these particular trials.

4.3 Materials and Methods

4.3.1 Animals and their handling

We performed experiments on 4-day-old adults from a laboratory colony of the fruit fly, *Drosophila melanogaster* (Meigen), descended from a wild-caught population of 200 females. We reared, entrained, and tested all flies on a 16 h: 8 h light: dark photoperiod. Transitions between light and dark were immediate. The light-on phase started at 7AM PST. We maintained fly stocks at $\approx 25\,^{\circ}\mathrm{C}$ and at a relative humidity of $\approx 30\%$ on Lewis food medium in standard 250 mL bottles (Lewis, 1960). We introduced individual flies from stock vials directly into the experimental chambers with a mouth pipette. The stock vials were kept within a controlled density of flies containing an equal mixture of males and females of comparable age, which were provided *ad libitum* access food.

4.3.2 Long-duration recordings of movement within environmental chambers

To test the exploratory behavior of individual flies, we introduced single flies fed *ad labitum* into the first of a pair of connected environmental chambers where flies could move freely between the chambers through narrow tubes, as described previously. Each day we ran two trials, simultaneously observing a single male and a single female. We switched back and forth each day which pair of chambers contained the male or the female, and after every experiment, we washed down the chambers with water and dilute ethanol. All chambers provided access to a 2 mL plug of 0.5% agar that was embedded into the center of the floor to prevent dehydration. We introduced flies into chambers at 9AM and started observing their movements immediately, until 4PM or 10PM. Flies

were free to move back and forth between the adjacent chambers, unless in the particular trial the exit to the second chamber was blocked. To record the change in behavior as a fly became hungry, we mounted digital cameras above the first chambers and recorded the fly's movement at a rate of 15 s^{-1} (Fig. 4.2A). To avoid large video files, instead of saving a full record of their movement, we extracted and saved only the x,y coordinates of the fly as observed within the 2D image plane (Fig. 4.2B), a corresponding cropped image containing just the region surrounding the fly for each of these coordinates (Fig. 4.2C), and a single median background image calculated from XXX frames from the video containing a view of the entire chamber (Straw and Dickinson, 2009). With this data we have developed software capable of reconstructing a high spatial and temporal 3D representation of the fly's movement that is cross-indexed to each original video. Data in this form allow us to confirm the quality of tracking and also provide an efficient means to extract movie clips of interesting behaviors, or over specified time windows, for further analysis.

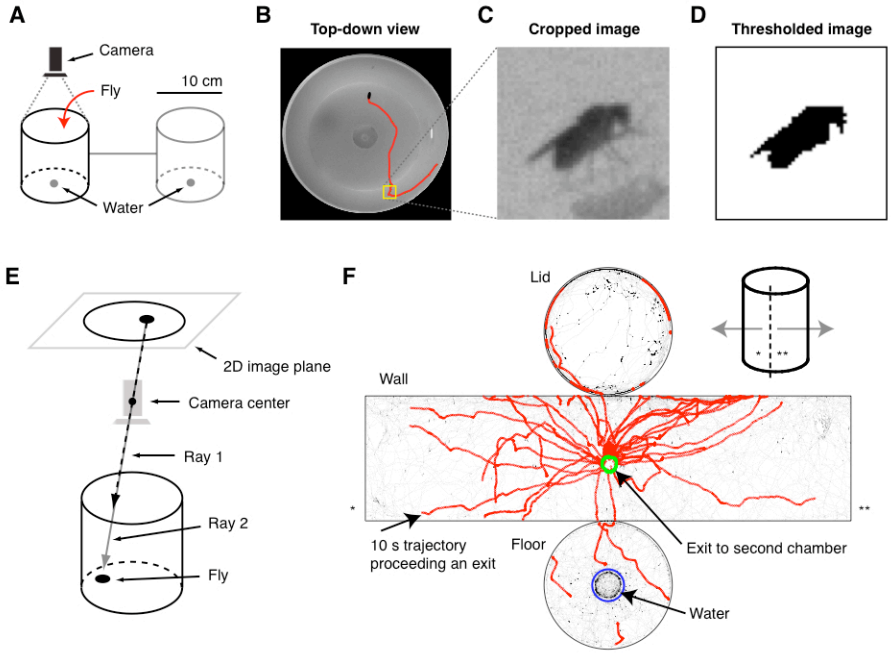

Figure 4.2: Technology devised to study the movement of *Drosophila* within a controlled sensory environment. (A) An individual fly is introduced to the first of two connected chambers, both containing only water. A single camera is mounted above the first chamber. (B) An example of the 2D position of a fly obtained from digital video using custom software from the laboratory. (C) Cropped image of the fly from this video. (D) Binerized threshold image of the fly in C. (E) Cartoon illustrating the two possible locations for a fly from the perspective of the camera. *Ray 1* represents the possible location of a fly on the underside of the chamber lid (dashed; black arrow); *Ray 2* indicates the true location of the fly in this illustration (solid; gray arrow), which sits on the chamber floor. (F) Reconstructed trajectory of a fly filmed for 6 hours as it became hungry (black). We highlighted 10 second segments for all trajectories proceeding exits (red) into the second chamber (green circle). To help visualize and compare the variability in movement between individual flies, we rotated and unwrapped their trajectories so that the exit hole is aligned directly opposite from the readers. We made a vertical slice down the front section of the chamber (dotted line) and then folded the lid up, the floor down, and walls apart as shown. Note * and ** denote corresponding sections of wall; see inset and reconstructed trajectory.

4.3.3 Single-camera strategy for three-dimensional video-tracking

To reconstruct the 3D positions of a fly as it moved throughout a chamber, we calculated the total pixel area representing a fly from a thresholded image (Fig. 4.2D) for each cropped image. We used this information together with 2D coordinates to deduce the location of the fly throughout the length of a video. If the pixel area representing a fly was greater than a specific computed amount, we would assume that the fly at this point in time was closer to the camera and therefore on the underside of the chamber lid; conversely, if the pixel area was less than this amount, we would assume that the fly was farther from the camera, either on the wall or floor of the chamber (Fig. 4.2E). Our strategy assumes in accordance with our observations that single flies introduced to our experimental chambers spent the majority of their time on the surface of the chamber, rather than flying within its volume. From over 1200 hours of video, isolated flies remained on the surface of the chamber for more than 99% of the time. Using this strategy, we could build up a fly's trajectory frame-by-frame over 6 or 12 hours. We calibrated the projection between the 2D coordinates of a fly and its 3D positions using known anchor points. The points were assigned within an image of the experimental chamber corresponding with known positions within the chamber, using a direct linear transformation (standard DLT). Finally, we estimated the most likely sequence of positions (lid vs. wall or floor) for the fly between each video image and used this estimate to reconstruct the trajectory for a fly (Viterbi optimization). For example, the probability of a fly transitioning between a location on the lid to a location on the wall is quite low if the fly is in the center of the chamber; this transition is more probable if the fly is on the lid near the wall. We wrote custom code in Matlab (Mathworks Natick, MA, USA) for transforming, optimizing, and analyzing all data.

4.3.4 Quantitative descriptors of exploratory behavior

Each behavioral statistic describes the behavior of a fly during a one-hour interval. There are 4 types of statistics: those describing the behavior of flies while walking, flying, searching near water, and dispersing from the chamber through the exit into the second chamber. In Figures 4.5–4.21, we plot the interval number versus one of various statistics for each of the 20 selected flies, and that were sorted based on the total distance traveled during the 6 hour trial. For each statistic and selected fly, we plot the interval number versus the statistic value. We plot the raw statistic in part (A) of each figure. In many of the statistics, we can see temporal dependencies throughout the day. As the flies grow hungrier with time since last feeding, various measurements of their locomotor movements increase. Their behaviors then decrease during the middle of the day, and for the 12 hour trials, the flies' behavior then increases again around dusk near the end of the experiment (for example see 4.3). In part (B) of each figure, we plot the z-scored statistic. That is, we compute the mean and standard deviation for each interval and statistic over all flies, then plot the number of standard deviations from the mean the statistic is for a given fly. This manipulation largely removes the temporal dependencies of the statistic. For some of the statistics analyzed, the statistic is only computed from a few observed values, and thus will be noisy. For example, Figure 4.4 shows values on which the noise in a particular statistic depends in (A), and the standard error of the median-based estimates in (B).

4.3.4.1 Walking statistics

We segment a sequence of a trajectory in which the fly is both (1) in the chamber and (2) not flying into subsequences in which the fly is walking or stopped using a variant

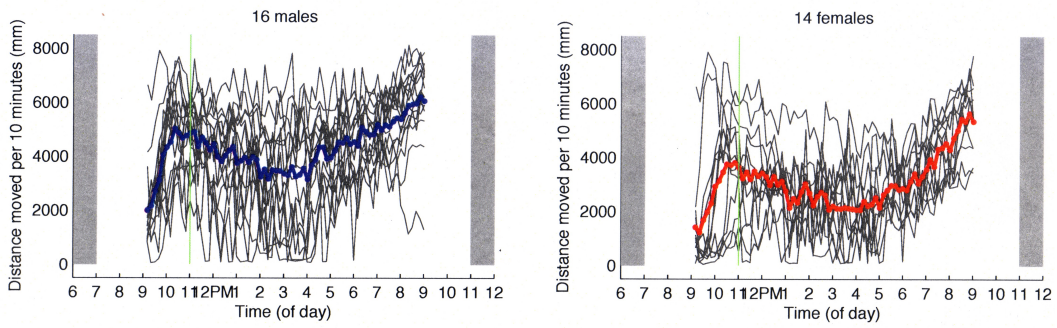

Figure 4.3: Movement duration for individuals as they become hungry. Total distance traversed for isolated, single flies for each successive 10 minute interval over the period of 12 hours. Collective means for male (blue) and female (red) flies are noted. The transition from dark (gray boxes) to light are indicated. The green vertical line denotes 2 hours into experiment.

of the Viterbi algorithm (Cormen et al., 2001). We model the probability of a sequence of walking/stopped states as a first-order, binary hidden Markov model. The intuition behind the chosen model is as follows. First, there is a higher probability that the fly will remain in the same state, i.e., either remain walking or stopped, than switch to the other state. Second, if the fly's speed in the current frame is small, there is a higher probability that the fly is stopped than walking. Conversely, if the fly's speed is high, there is a higher probability that the fly is walking. We use dynamic programming to find the sequence of states with globally maximal probability.

More formally, let $s_t = 1$ represent the classification of frame t as walking and $s_t = 0$ the classification of frame t as stopped. Using a first-order Markov assumption, we can write the probability of a sequence of hidden states $\mathbf{s_{1:t}}$ for frames 1 through t given the observed speeds $\mathbf{v_{1:t}}$ recursively as

$$P(\mathbf{s_{1:t}}|\mathbf{v_{1:t}}) \propto \mathbf{P}(\mathbf{s_t}|\mathbf{s_{t-1}})\mathbf{P}(\mathbf{v_t}|\mathbf{s_t})\mathbf{P}(\mathbf{s_{1:t-1}}|\mathbf{v_{1:t-1}}).$$

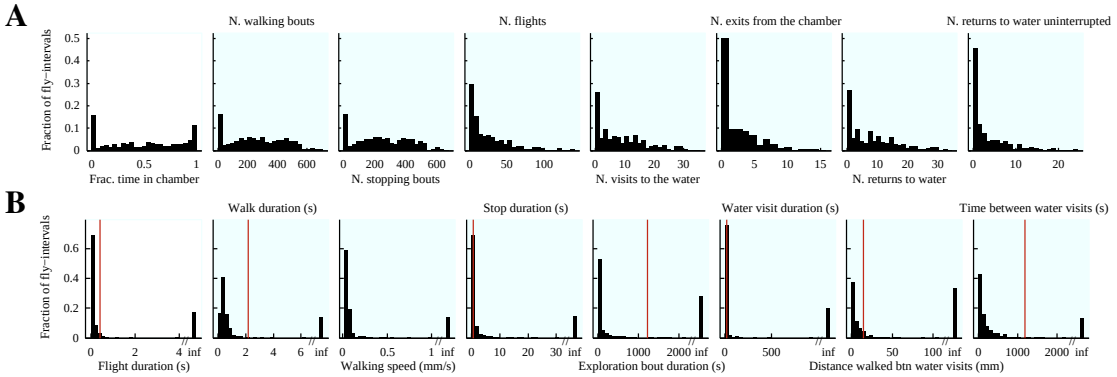

Figure 4.4: Uncertainty in estimates of per-interval statistics. (A) We histogram the value on which the noise of various statistics depends. (B) We show the standard error of the median estimate, assuming the data is normally distributed. In both, infinity corresponds to intervals with no data available. (B) for comparison we plot the mean value of the statistic in red (for walking speed, the mean value is 8.5, which is off the plot). We see that for the rarer events – water visits, return trips to the water – the noise is high. "inf" stands for infinity and corresponds to n = 0, complete uncertainty.

The transition probability $P(s_t|s_{t-1})$ is set to 0.98 if the state remains the same, $s_t = s_{t-1}$, and 0.02 if the state changes $s_t \neq s_{t-1}$. The likelihood of observing speed v_t (in mm/s), given that the fly is walking, is assumed to be proportional to

$$P(v_t|s_t = 1) \propto \exp(-(v_t - 1)^2/(2 \cdot .15)),$$

that is, proportional to a Gaussian distribution with center 1 mm/s and variance .15 (cm/s)^2. The likelihood of observing speed v_t (in mm/s), given that the fly stopped, is assumed to be proportional to

$$P(v_t|s_t = 0) \propto \exp(-(v_t - 0)^2/(2 \cdot .005)),$$

that is, proportional to a Gaussian distribution with center 0 mm/s and variance .05 (cm/s)^2.

The fraction of time walking (*fractimewalking*) statistic reports the fraction of time the fly is in the observed chamber that it is classified as walking (Figure 4.5). It is a unitless quantity. The less time the fly spends in the chamber during the interval, the noisier this statistic is. If the fly is not in the observed chamber at all during the interval, then this statistic is completely unknown.

The distance traveled (*disttraveled*) statistic reports the total distance in centimeters the fly travels while it is classified as walking in the observed chamber (Figure 4.6). This value is normalized by the number of seconds the fly is in the observed chamber, thus the units reported are $\text{cm} \cdot \text{s}^{-1}$. The noise in this statistic also depends on the amount of time the fly spends in the chamber during the interval.

The frequency of walk onsets (*freqwalk*) is the number of times the fly begins a bout of walking during the interval, normalized by the number of seconds the fly spends in the observed chamber (Figure 4.7). The units reported are therefore onsets per second. If a walking bout crosses the division between two intervals, we choose the interval in which the middle frame of the bout falls. We follow this policy with all other bout-related properties. The noise in this statistic also depends on the amount of time the fly spends in the chamber during the interval.

The mean walking speed (*meanwalkspeed*) is the mean speed of the fly over all frames in which the fly is classified as walking in the first chamber (Figure 4.8). The units reported are centimeters per second. The noise in this statistic depends on the amount of time the fly spends walking in the observed chamber during the interval.

The median duration of walking bouts (*walkdur*) is the median duration of sequences during which the fly is classified as walking while in the chamber (Figure 4.9). This statistic is reported in seconds. The noise in this statistic depends on the number of bouts of walking the fly performs in the observed chamber during the interval.

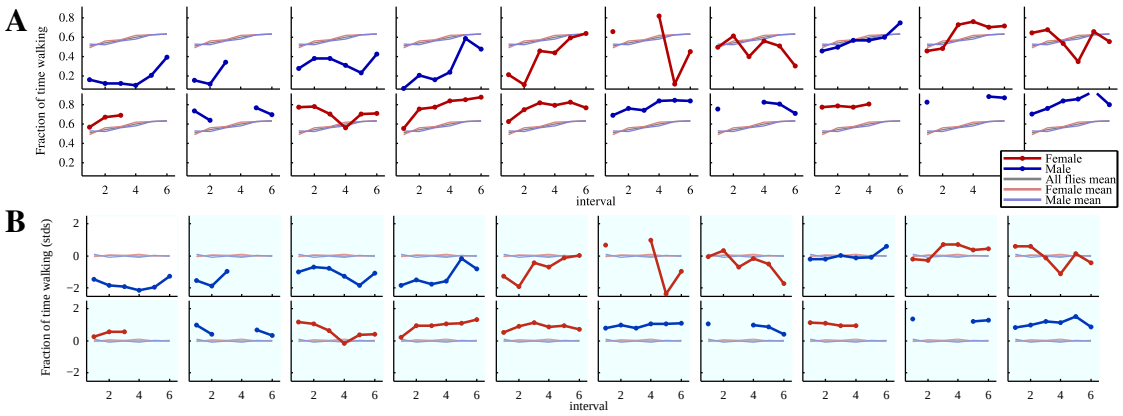

Figure 4.5: Interval vs. fraction of time walking for selected individuals. We select 20 flies whose ranks based on total distance travelled are evenly spaced. The top left fly walks the least of all flies and the bottom right fly walks the most of all flies. We plot the fraction of time walking for each interval, where the interval length is 1 hours. Missing points indicate intervals for which the statistic cannot be computed. (A) We plot the interval number vs. the raw statistic, while in (B) we plot the interval number vs. the number of standard deviations from the mean statistic value for the given interval (i.e. the data in each interval has been z-scored. In gray, we plot the mean value over all flies. In light red, we plot the mean value over all female flies. In light blue, we plot the mean value over all male flies. We plot the value for the selected fly in dark red if it is female and dark blue if it is male.

The median duration of stop bouts (*stopdur*) is the median duration of sequences during which the fly is classified as stopped while in the chamber (Figure 4.10). The statistic is reported in seconds. The noise in this statistic depends on the number of bouts of stopping the fly performs in the observed chamber during the interval.

4.3.4.2 Flying statistics

The fraction of time flying (*fractimeflying*) is the fraction of time the fly is in the chamber that it is classified as flying, i.e., the fly's velocity was $>$0.6 cm/s (Figure 4.11). It is a unitless quantity. The less time the fly spends in the chamber during the interval, the noisier this statistic is.

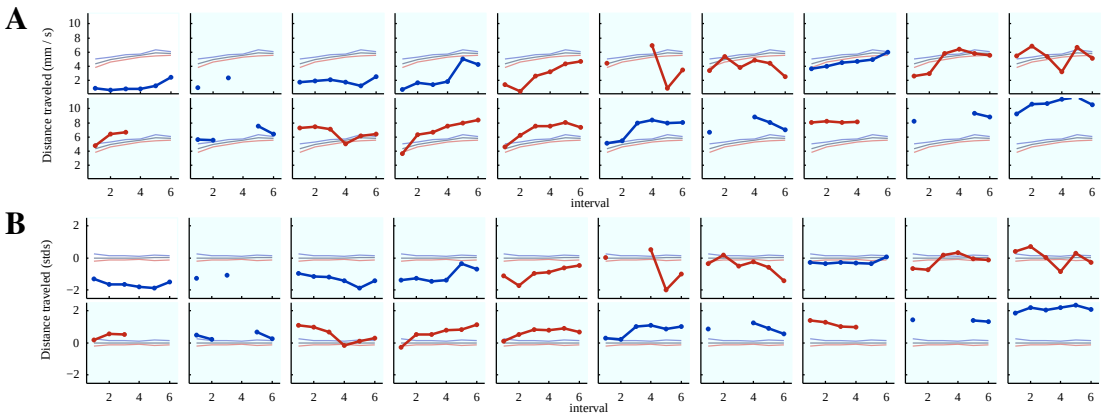

Figure 4.6: Interval vs. distance traveled for selected individuals. See Figure 4.5 for a more complete description.

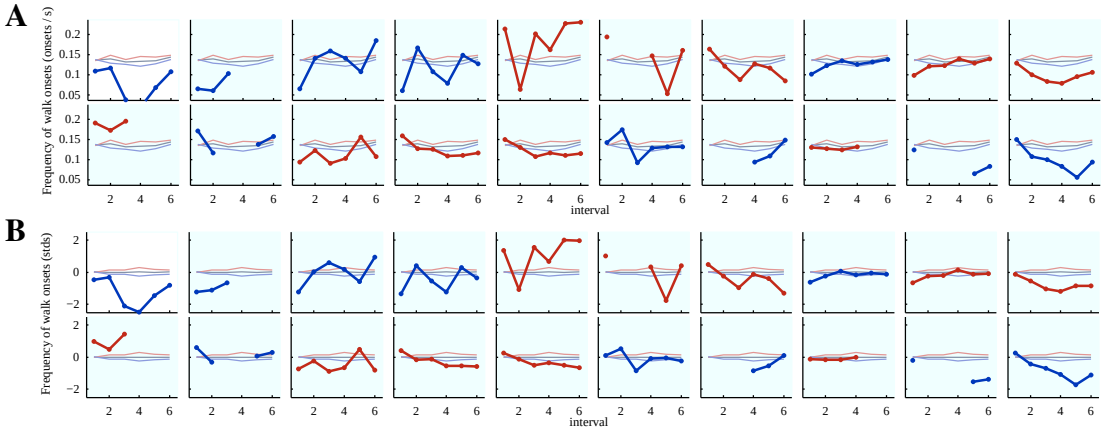

Figure 4.7: Interval vs. frequency of walk onsets for selected individuals. See Figure 4.5 for a more complete description.

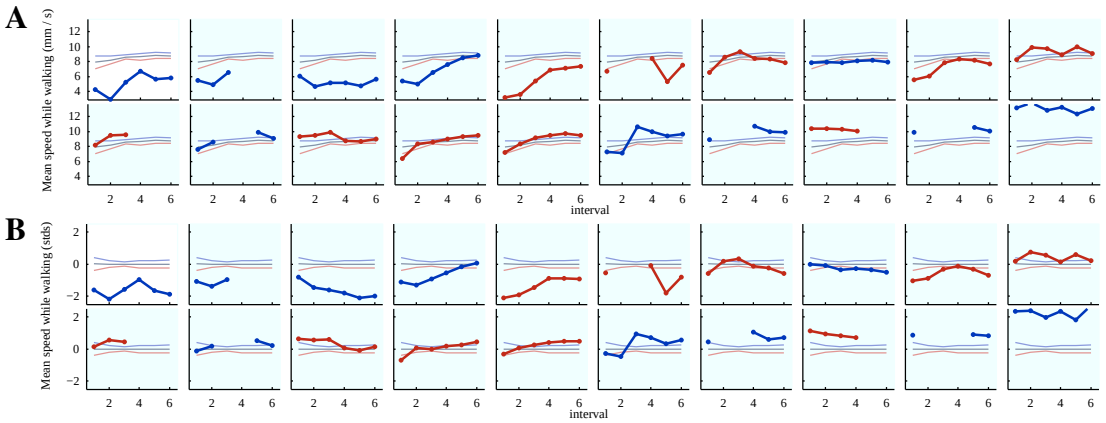

Figure 4.8: Interval vs. mean speed while walking for selected individuals. See Figure 4.5 for a more complete description.

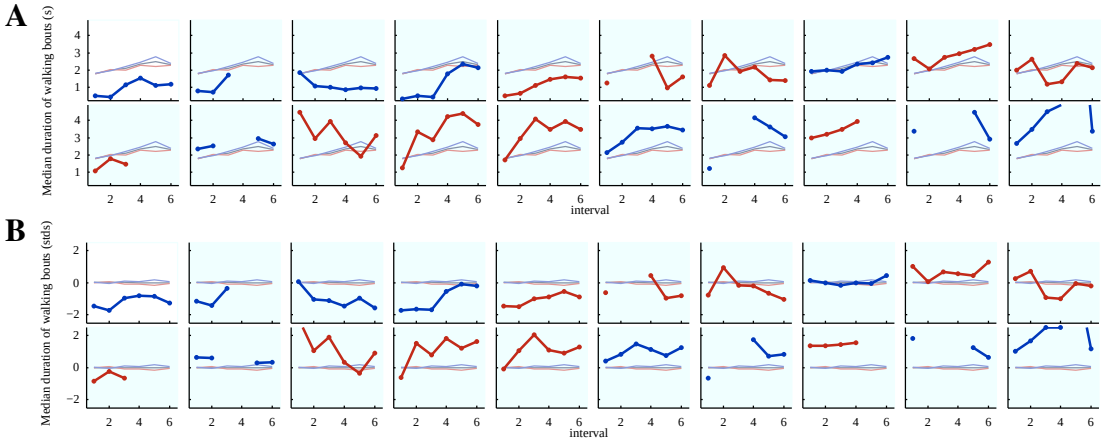

Figure 4.9: Interval vs. median duration of walking bouts for selected individuals. See Figure 4.5 for a more complete description.

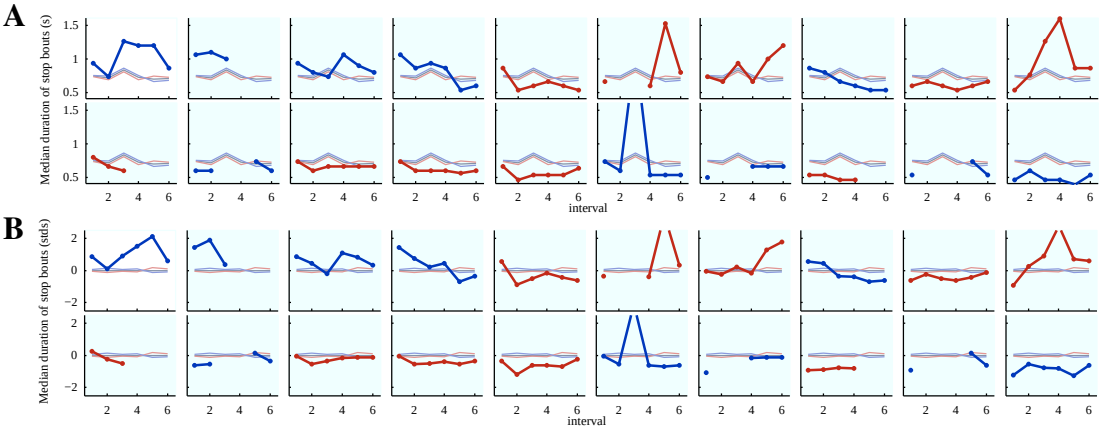

Figure 4.10: Interval vs. median duration of stop bouts for selected individuals. See Figure 4.5 for a more complete description.

The frequency of flights (*freqflight*) is the number of times the fly begins a bout of flying during the interval, normalized by the number of seconds the fly spends in the observed chamber (Figure 4.12). The units reported are therefore onsets per second. The noise in this statistic depends on the amount of time the fly spends in the chamber during the interval.

The median duration of flights (*flightdur*) is the median duration of sequences during which the fly is classified as flying while in the chamber (Figure 4.13). This statistic is reported in seconds. The noise in this statistic depends on the number of flights the fly performs in the observed chamber during the interval.

4.3.4.3 Local search near water statistics

We segment the trajectory of a fly into sequences in which it is either visiting or not visiting the patch of agar, a source of water. A fly is considered visiting the water if it is ≤ 0.1 cm from the water's edge, or if it is ≤ 0.3 cm from the edge and within 2 frames from a frame in which the fly is ≤ 0.1 cm from the edge. We based this classification

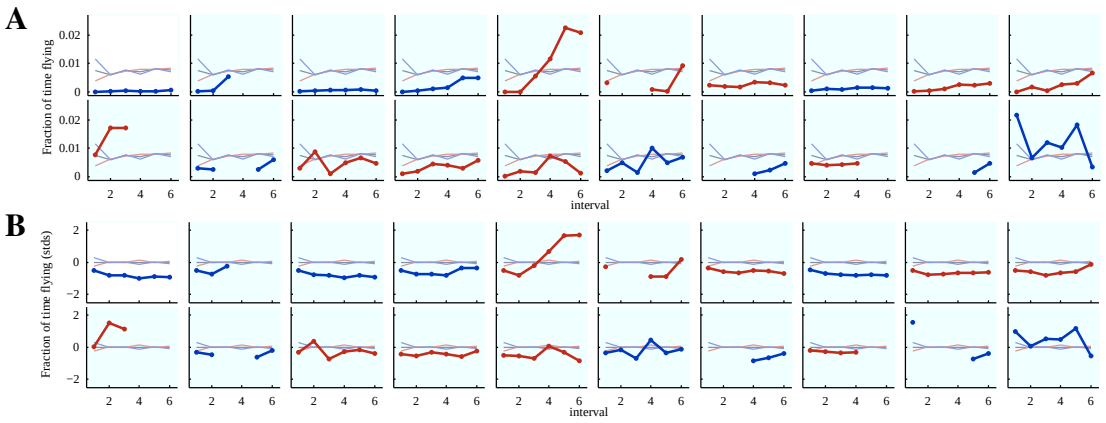

Figure 4.11: Interval vs. fraction of time flying for selected individuals. See Figure 4.5 for a more complete description.

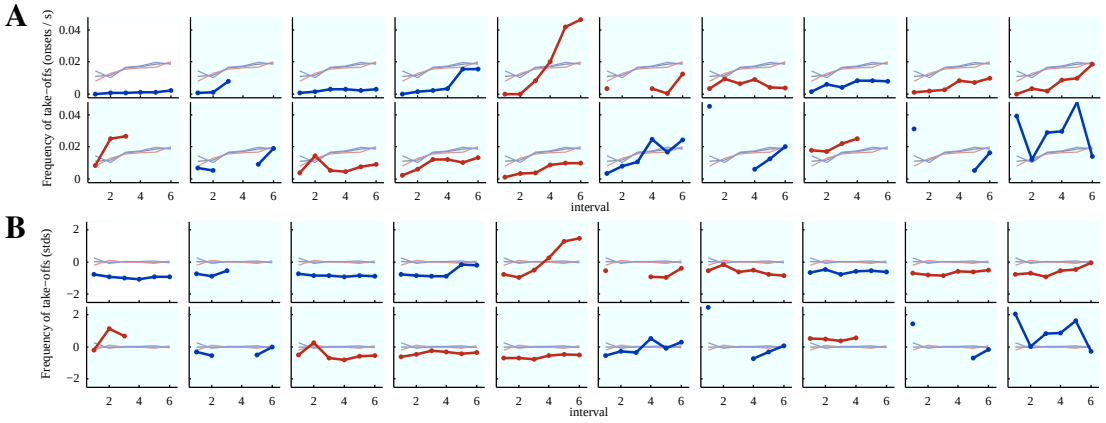

Figure 4.12: Interval vs. frequency of take-offs for selected individuals. See Figure 4.5 for a more complete description.

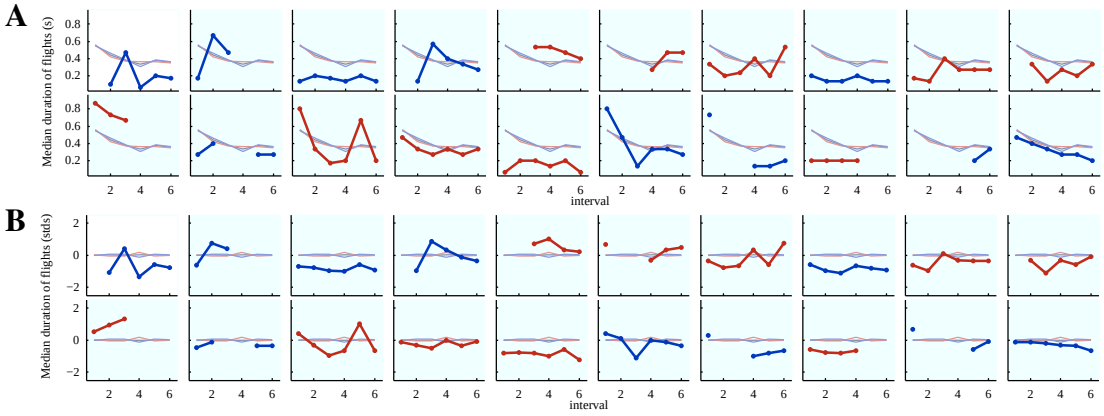

Figure 4.13: Interval vs. median duration of flights for selected individuals. See Figure 4.5 for a more complete description.

from average transit probabilities from 125 flies as they search near the agar (see Figure 4.30). This definition results in an average of 9 visits to the water resource per interval, with 20% of fly-intervals having no visits to the water.

The fraction of time near the water (*fractimenearwater*) is the fraction of frames the fly is in the chamber that it is classified as near the water resource (Figure 4.5). It is a unitless quantity. The noise in this statistic depends on the amount of time the fly spends in the chamber during the interval.

The frequency of visits to water (*freqvisitwater*) is the number of continuous sequences of frames in which the fly is classified as visiting the water, normalized by the number of seconds the fly spends in the observed chamber (Figure 4.6). The units reported are therefore sequences per second. The noise in this statistic depends on the amount of time the fly spends in the chamber during the interval.

The median duration of visits to water (*watervisitdur*) is the median duration of sequences during which the fly is classified as near the water resource (Figure 4.7). This statistic is reported in seconds. The noise in this statistic depends on the number of times the fly visits the water resource during the interval.

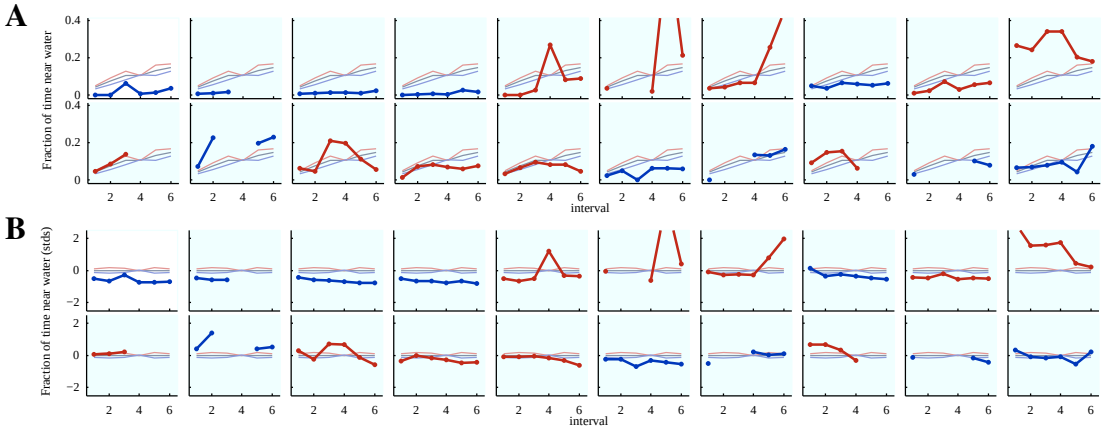

Figure 4.14: Interval vs. fraction of time near water for selected individuals. See Figure 4.5 for a more complete description.

The median time between visits to water (*timebtnwater*) is the median duration of sequences which begin when the fly leaves the water resource and end when the fly returns to the water resource (Figure 4.8). As with other types of sequences, if a return trip crosses the division between two intervals, we choose the interval in which the middle frame of the trip falls. This statistic is reported in seconds. The noise in this statistic depends on the number of return trips to the water resource during the interval, which of course is related to the number of times the fly visits the water resource.

The median length of the walking path between visits to water (*pathlengthbtnwater*) is the median distance traveled in *uninterrupted* sequences that begin when the fly leaves the water resource and end when the fly returns to the water resource (Figure 4.9). By uninterrupted, we mean that the fly does not leave the chamber or fly during the return trip, as we cannot measure the distance traveled during these periods. This statistic is reported in centimeters. The noise depends on the number of uninterrupted return trips.

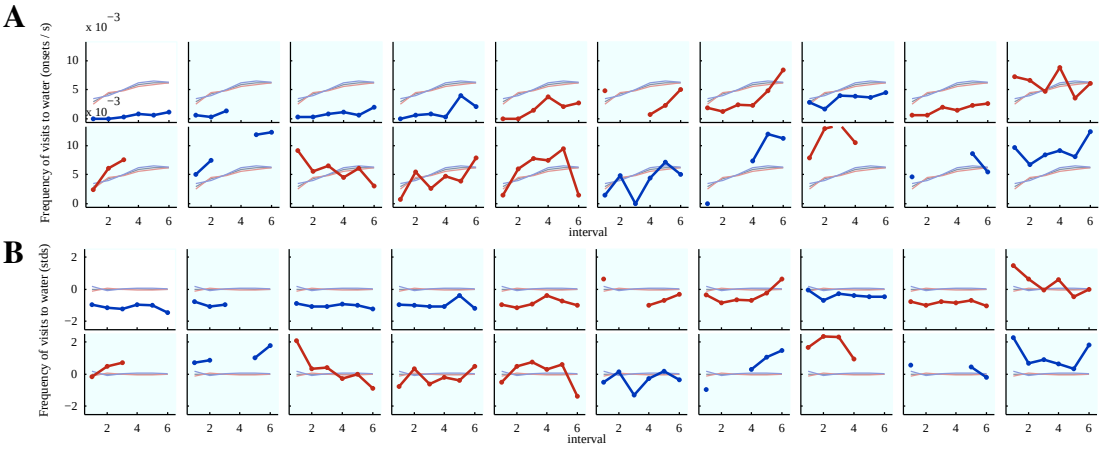

Figure 4.15: Interval vs. frequency of visits to water for selected individuals. See Figure 4.5 for a more complete description.

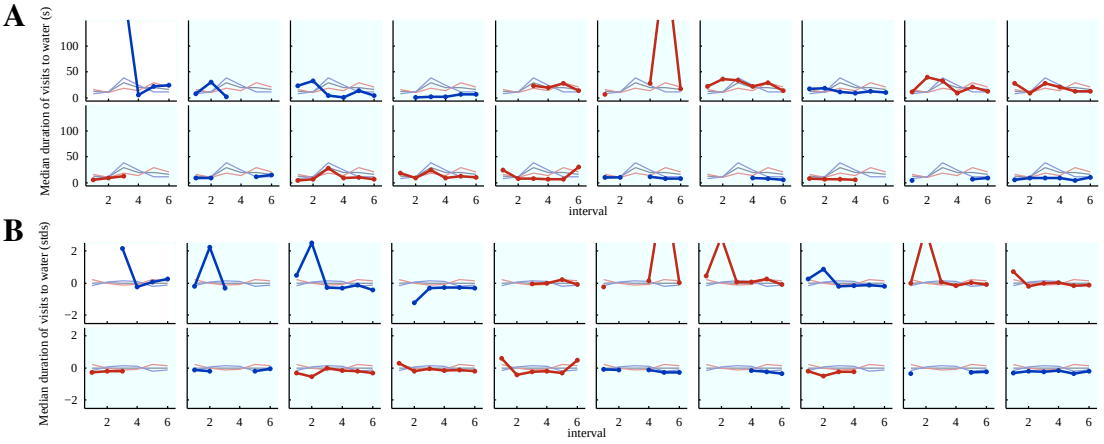

Figure 4.16: Interval vs. median duration of visits to water for selected individuals. See Figure 4.5 for a more complete description.

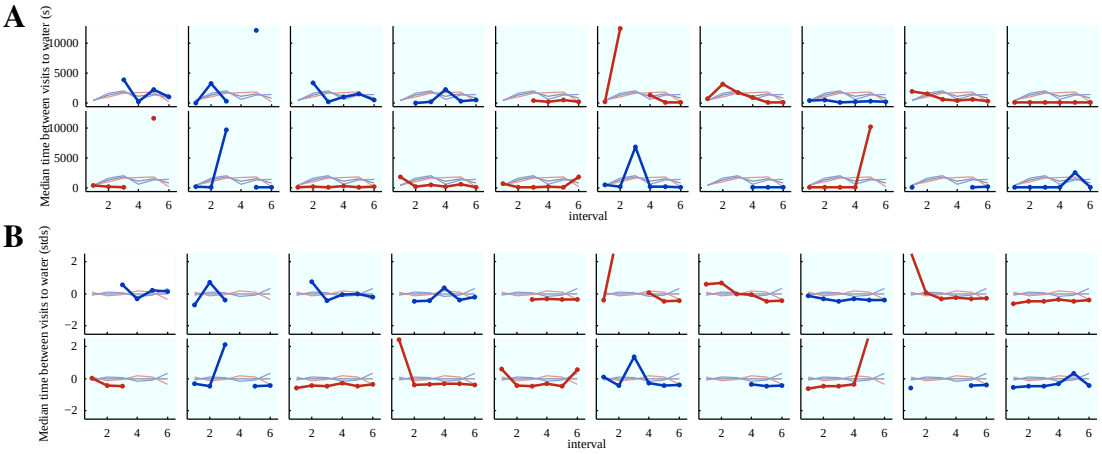

Figure 4.17: Interval vs. median time between visits to water for selected individuals. See Figure 4.5 for a more complete description.

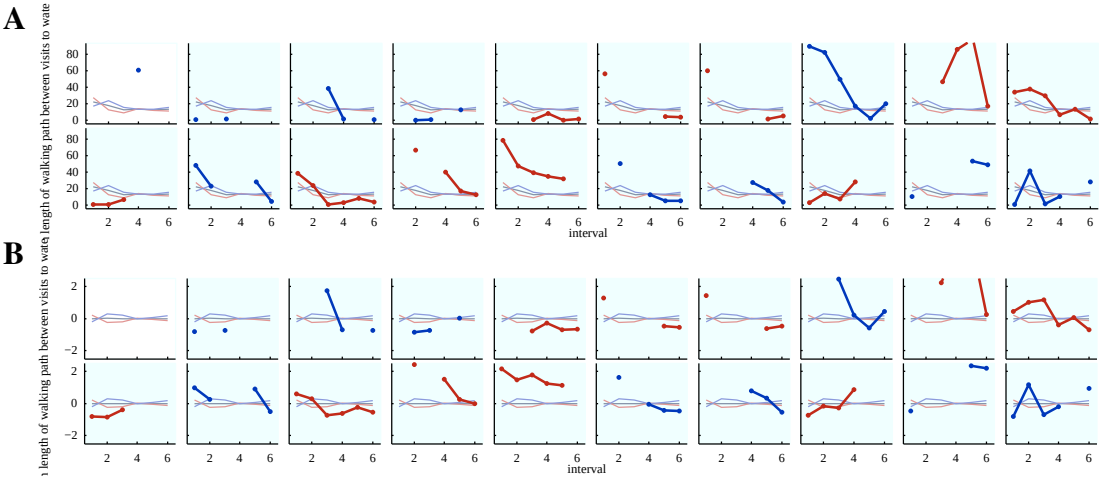

Figure 4.18: Interval vs. median length of walking path between visits to water for selected individuals. See Figure 4.5 for a more complete description.

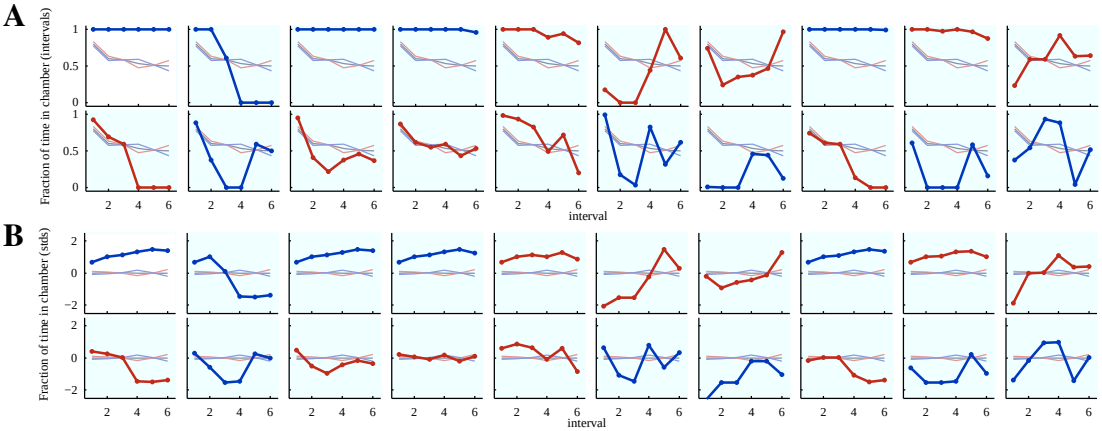

Figure 4.19: Interval vs. fraction of time in chamber for selected individuals. See Figure 4.5 for a more complete description.

4.3.4.4 Dispersal from chamber statistics

The fraction of time in the chamber (*fractimeinchamber*) is the fraction of the interval the fly spends in the main, observed chamber (Figure 4.19). This is a unitless quantity. The noise in this statistic depends only on the interval length. The classification of when flies left the chamber was primarily based on when the pixel area of a thresholded video image dropped to zero (see Figure 4.32), and (see Figure 4.33 for details).

The frequency of exits from the observed chamber (*freqexits*) is the number of times the fly exits the interval, normalized by the number of seconds the fly spends in the interval (Figure 4.20). The units reported are therefore onsets per second. The noise in this statistic depends on the amount of time the fly spends in the chamber during the interval.

The median duration of exploration bouts (*awaydur*) is the median duration of visits to the second, unobserved chamber. This statistic is reported in seconds. The noise in this statistic depends on the number of times the fly visits the second chamber.

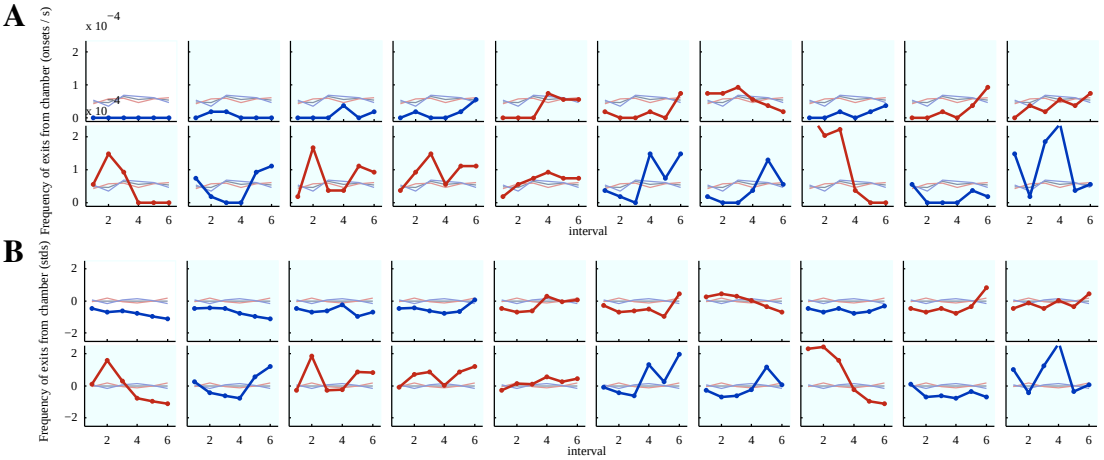

Figure 4.20: Interval vs. frequency of exits from chamber for selected individuals. See Figure 4.5 for a more complete description.

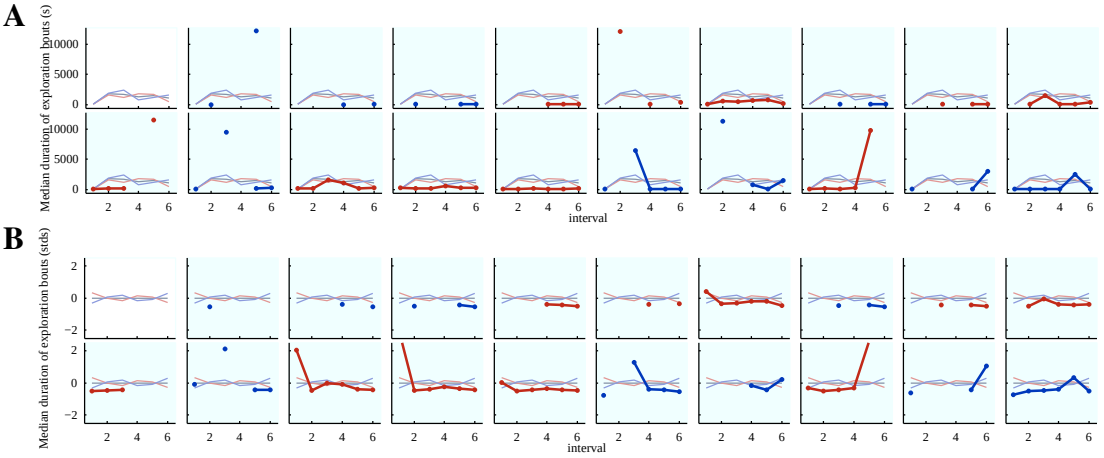

Figure 4.21: Interval vs. median duration of exploration bouts for selected individuals. See Figure 4.5 for a more complete description.

4.3.5 Training algorithms and strategy for predicting behaviors

For a given statistic and a given interval, we learn a function that inputs the values of this statistic for a subset of the remaining intervals and predicts the value of this statistic in the given interval. For example, we learn a function that inputs the 5-D vector consisting of the distance traveled in intervals 1–3 and 5–6, and outputs an estimate of the distance traveled in interval 4. We do this for each statistic and each of the 6 intervals in the 6-hour experiment. We also consider different lengths of buffers between the input and predicted intervals. A buffer length of 0 intervals corresponds to predicting the given interval using all the remaining intervals. A buffer length of 1 interval corresponds to predicting the given interval using all remaining intervals except for those adjacent to the given interval (e.g., predicting interval 4 from intervals 1–2 and 6). More generally, a buffer length of n intervals corresponds to predicting the given interval using all remaining intervals except those within $n+1$ intervals of the given interval. The larger the buffer, the less data we have to predict the given interval, thus we expect our estimates to be less accurate. However, we consider these larger buffers to demonstrate that the individuality effects can be seen over larger time frames.

We use a form of regularized linear regression to learn the predictor function. Let $\mathbf{x_{ij}}$ represent the input statistic vector for statistic i and fly j, and the predicted interval, and y_{ij} the true value of statistic i and fly j for the current predicted interval. Any linear predictor of y_{ij} given $\mathbf{x_{ij}}$ can then be represented as

$$f(\mathbf{x_{ij}}) = \mathbf{c_i}^\top \mathbf{x_{ij}} + \mathbf{c_{i0}},$$

where $\mathbf{c_i}$ is a constant vector of coefficients and c_{i0} is a constant offset for statistic i and all flies. For ease of notation, let us append the input data vector $\mathbf{x_{ij}}$ with an element

that is always 1, and fold the constant offset c_{i0} into the vector of coefficients $\mathbf{c_i}$.

In ordinary linear regression, we choose the coefficients $\mathbf{c_i}$ that minimize the mean-squared error:

$$J_o(\mathbf{c_i}) = \frac{\mathbf{1}}{\mathbf{n}} \sum_{\mathbf{j=1}}^{\mathbf{n}} \left(\mathbf{c_i^\top x_{ij}} - \mathbf{y_{ij}} \right)^{\mathbf{2}},$$

where the sum is over the data for the n flies in the training set.

Because we had limited amounts of data compared to the amount of noise in the statistics, particularly for statistics such as the median water visit duration, we used a regularized form of linear regression. First, we z-score the inputs $\mathbf{x_{ij}}$ and outputs y_{ij} using the mean and standard deviation computed from the training set only. That is, we subtract the training set sample mean and divide by the training set sample standard deviation for each statistic and interval. This manipulation takes into account much of the temporal dependencies of the statistics. Note that z-scoring the data is itself a linear transformation, thus it would not affect the results of ordinary linear regression. Let $\mathbf{x_{ij}}$ and y_{ij} now represent the z-scored data. The linear regression can then be thought of as a weighted mean of the statistics for the given intervals. We will most likely want to give higher weights to intervals closer to the predicted interval, or perhaps to give lower weights to intervals that are less reliable. Based on the assumption that the coefficients for different statistics but the same interval will be somewhat similar, we consider the following regularized criterion:

$$J_r(\mathbf{c_1}, ..., \mathbf{c_m}) = \frac{\mathbf{1}}{\mathbf{m}} \sum_{\mathbf{i=1}}^{\mathbf{m}} \frac{\mathbf{1}}{\mathbf{n}} \sum_{\mathbf{j=1}}^{\mathbf{n}} \left(\mathbf{c_i^\top x_{ij}} - \mathbf{y_{ij}} \right)^{\mathbf{2}} + \lambda \frac{\mathbf{1}}{\mathbf{m}} \sum_{\mathbf{i=1}}^{\mathbf{m}} \left(\mathbf{c_i} - \frac{\mathbf{1}}{\mathbf{m}} \sum_{\mathbf{k}} \mathbf{c_{k=1}^m} \right)^{\mathbf{2}}.$$

There are two differences between this regularized criterion, J_r, and the ordinary least-squares criterion, J_o. First, this criterion is a function of the coefficients for all the

statistics, rather than just one statistic. Thus, we are solving for the coefficients for all statistics simultaneously. The first term in J_r is the same as the first term in J_o, except that we are summing over all the statistics. The second difference between J_r and J_o is the inclusion of the second term, the regularization term. This term penalizes differences between a coefficient vector for one statistic and the mean over all statistics. The constant λ weights the data term and the regularization term. We only experimented with setting $\lambda = 1$. To improve robustness to outliers, we threshold all inputs and training outputs at 3 standard deviations. Figure 4.34 shows the coefficients learned using ordinary linear regression in (A) and regularized linear regression in (B) for the z-scored data. Figure 4.35 shows a comparison of the mean-squared error for regularized linear regression to other regression algorithms. We compare to linear and quadratic regression with the ordinary least-squares criterion, quadratic regression for the proposed regularized criterion, and linear and quadratic regression using iteratively reweighted least-squares with the bisquare weighting function, implemented with the `robustfit` function in Matlab. The regularized linear regression was usually the best performing method, and the most reliable when training data was scarce, particularly in data sets with smaller numbers of flies, not reported here.

While J_o can be minimized by a simple matrix inversion for each statistic, J_r is slightly more difficult to minimize, as the coefficients for all statistics must be simultaneously selected. However, J_r is convex, so we can choose an arbitrary initialization, perform a gradient descent, and be guaranteed to find the global optimum. We initialize with the ordinary least-squares regression coefficients. At each iteration of our algorithm, we hold the coefficients for all statistics except one constant, then find the optimal values for the coefficient vector for this single statistic. For efficiency, we order the statistics whose coefficients we will optimize based on their sum-squared error (J_o).

The optimal $\mathbf{c_i}$ for fixed $\{\mathbf{c_k}, \mathbf{k} \neq \mathbf{i}\}$ can be found in closed form as

$$\mathbf{c_i} = \left(\frac{\mathbf{1}}{\mathbf{N}} \mathbf{X_i^\top X_i} + \lambda \left(\mathbf{1} - \frac{\mathbf{1}}{\mathbf{M}} \right) \mathbf{I_{P \times P}} \right)^{-\mathbf{1}} \left(\frac{\mathbf{1}}{\mathbf{N}} \mathbf{X_i^\top y_i} + \lambda \frac{\mathbf{1}}{\mathbf{M}} \sum_{\mathbf{k} \neq \mathbf{i}} \mathbf{c_k} \right).$$

Here P is the dimensionality of the input vector $\mathbf{x_{ij}}$, X_i is the $N \times P$ matrix in which row j is $\mathbf{x_{ij}^\top}$, $\mathbf{y_i}$ is the $N \times 1$ vector in which element j is y_{ij}, and $I_{P \times P}$ is the $P \times P$ identity matrix. Note that most of the quantities involved do not change from one iteration to another, thus the iterative optimization is efficient.

Many of the statistics are often undefined, e.g., if the fly does not spend any time in the observed chamber, or the fly does not perform a certain behavior. If we remove all flies for which the statistic for some interval is undefined, then we will lose a lot of data. Instead, we only remove flies for which the predicted interval and statistic is undefined, and set the undefined input statistics to the sample mean over the training data.

In all our experiments, we use hold-one-out cross validation. That is, we learn the regression coefficients from all flies except one, then compute the error on this held-out fly. We do this for each fly and therefore learn a different regressor for each fly. In this way, we keep the training and test data independent for all parts of the learning.

4.3.6 Control data

To see the effects of individuality on the regression error, we create semi-synthetic data sets which should not have any effects of individuality. Within each interval, we randomly permute the identities of the fly. For example, we may end up with statistics for fly 10 in interval 1, fly 29 in interval 2, fly 7 in interval 3, etc. To control for effects due to gender, we only permute identities within gender. Thus, the fly identities chosen for a given vector will all have the same sex as the identity in the first interval.

4.4 Results

4.4.1 Behavioral statistics of individual flies persist over time

Figures 4.22–4.25 graphically compare the true statistics with the predictions from the learned regressors. We plot the true versus predicted statistics for each fly for selected statistics, intervals, and buffer sizes. Each point on each plot corresponds to one fly. The x-axis corresponds to the true statistic, while the y-axis corresponds to the prediction from the learned regressor. Each figure corresponds to a different statistic. These statistics were chosen to span the range of normalized, mean-squared generalization error for buffer length = 0 intervals. Part (A) corresponds to the real data. Part (B) corresponds to the semi-synthetic control data; however, note that we generated 20 control sets for these plots, thus there are 20 times more points in the control plots. The left column (i) corresponds to buffer length = 0 intervals; the right column (2) corresponds to buffer length = 3 intervals. We plot the true versus predicted statistics for intervals 1, 2, 5, and 6, for these are the only intervals that can be predicted for buffer length = 3 intervals. So that we could use the same axes for each statistic and interval, we plot the number of standard deviations from the mean on each axis. If predictions were perfect, the data points would lie on the line of slope 1 through (0,0). We see that the real data does indeed look correlated for the well-predicted statistics, but less-so for the poorly predicted statistics. The buffer length = 0 data also looks more correlated than the buffer length = 3 data. In all the control plots, we see no correlation.

We can quantitatively compare the accuracy of the predictions for the real data to the accuracy for the control data. We measure error as the square-root of the mean-squared error (the square-root of J_o). So that errors on the different statistics can be directly compared, we normalize the error by the standard deviation of the statistic and

interval over all flies. In these normalized units, the standard deviation of the data is 1, hence the error of always predicting the mean statistic for an interval will be 1. The expected hold-one-out, cross-validation performance of the mean statistic for flies in the training set will be slightly more than 1. In Figure 4.26 (A), we plot the normalized square-root of the mean-squared error for the real and control data for each statistic. The x-axis corresponds to the statistic and the y-axis to the error. We plot the per-interval error (thin lines) as well as the mean error over all intervals (thick lines). Each plot corresponds to a different buffer length, with (i) corresponding to buffer length = 0 and (v) corresponding to buffer length 5. We generated 100 sets of control data. We observe that the error for the control data is indeed near 1 for all statistics and intervals – thus it is not performing better than just the sample mean – the statistics from other intervals are not useful for predicting the statistic for a given interval. For many statistics, the error for the real data is less than 1. The statistics on the x-axis are ordered by the mean error over all intervals for buffer length = 0 (i).

We can determine whether the error for the real data is significantly less than the error for the control data by computing the fraction of randomly generated control sets that have an error as good as the real data. This is an empirical measure of the probability of achieving the error computed for the real data regressors if there were no effect of individuality. This p-value is plotted in Figure 4.26 (B). The x-axis again corresponds to the statistic and the y-axis to the log p-value. The gray horizontal line corresponds to $p = .05$. We see that for many of the statistics, the effects of individuality are highly significant.

If the real data produces regressors with significantly less error than the control data, then we have observed the effects of individuality. That is, a positive result indicates an effect. Conversely, a negative result does not necessarily imply that there is no effect of

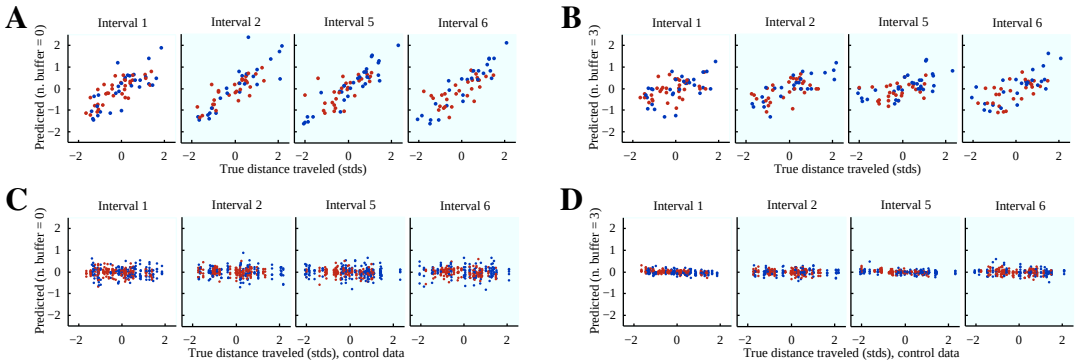

Figure 4.22: True vs. predicted distance traveled. Each axis corresponds to a different interval, where the interval length is 1 h. Each point corresponds to a fly. Male flies are plotted in blue, females in red. For each fly, we plot the true distance traveled versus the distance traveled predicted by the regression (measured in standard deviations from the mean). In the perfect regression, all points would be along the diagonal. In **A**–**B**, we create this plot for the real data. Each column corresponds to a different number of buffers maintained between the predicted and predicting intervals. In **C**–**D**, we create these plots for control data created by randomly permuting the identities independently in each interval (preserving sex). For each of the 5 control sets, we learn a regressor as with the real data, and plot the true control vs. the predicted control.

individuality for the statistic. No significant difference could result from three possible cases. First, there could be a true lack of an effect of individuality. Second, it could be that regularized linear regression does not fit the data well. Finally, it could be that there is too much noise and not enough data to accurately learn the regressor (or, a combination of the latter two). In particular, the errors greater than 1 correspond to overfitting the data, and are evidence that there is not enough data to overcome noise and learn a proper fit. To determine which of these cases apply, one would need to repeat the experiment with larger numbers of flies.

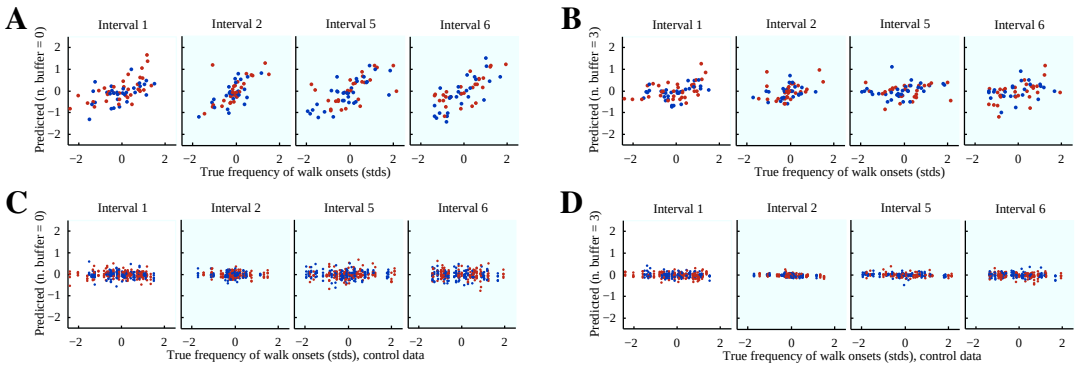

Figure 4.23: True vs. predicted frequency of walk onsets. See Figure 4.22 for a more complete description.

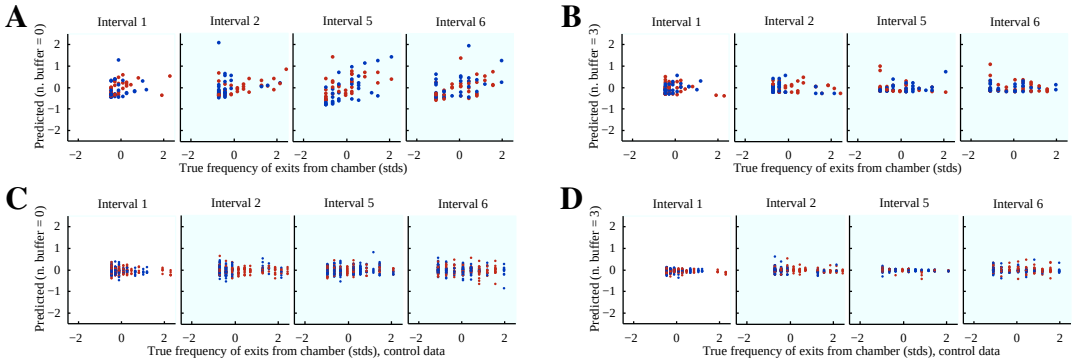

Figure 4.24: True vs. predicted frequency of exits from chamber. See Figure 4.22 for a more complete description.

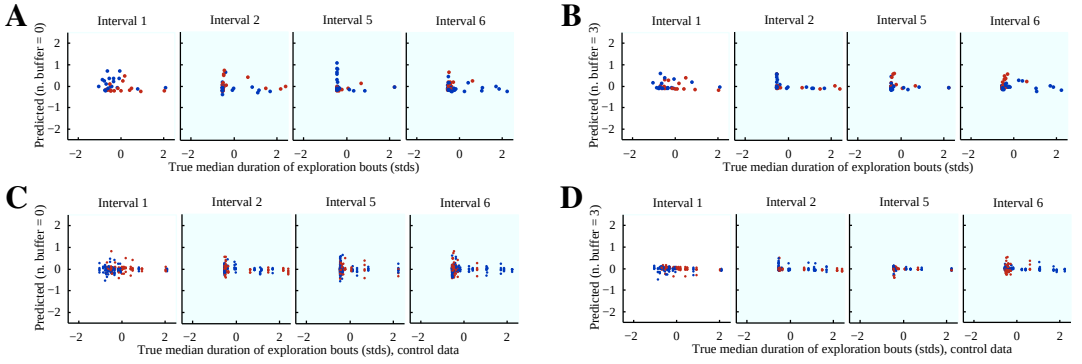

Figure 4.25: True vs. predicted median duration of exploration bouts. See Figure 4.22 for a more complete description.

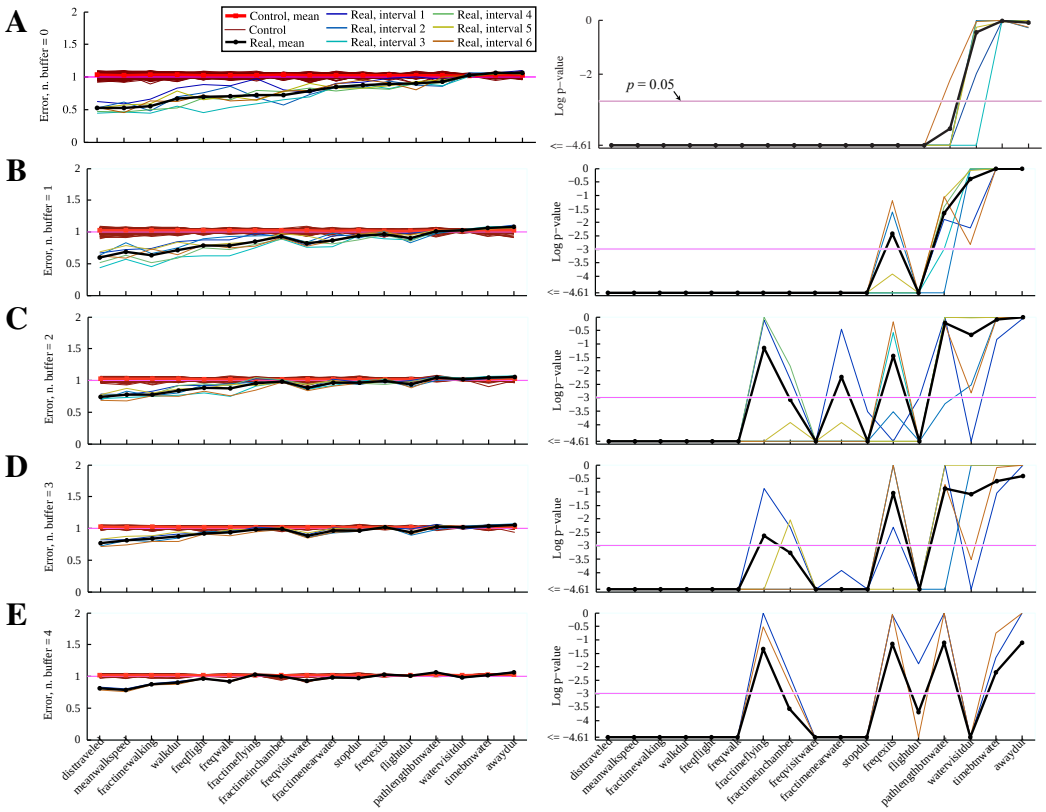

Figure 4.26: Mean squared-error for real and control data for interval length = 1 h. Each row of plots corresponds to a different numbers of buffers maintained between the predicted and predicting intervals. In the left column, we plot the square root of the mean squared error between the true and predicted behavioral statistic. For each statistic, we normalize the error by the standard deviation of the statistic so that errors are comparable between different statistics. Because of this normalization, a regressor that always predicts the mean statistic will have a mean normalized error of 1 (horizontal magenta line). The red lines correspond to the randomly permuted data, all other lines correspond to the real data. The thin lines correspond to the errors for single intervals, while the thick lines correspond to the mean over all intervals. Note that there are many thin red lines because we plot 100 control sets. In the right column, we plot the log of the fraction of the 100 control set errors that are less than the real errors. This is an empirical estimate of the probability that we would observe an error as low or lower than the real residual if the behavioral statistic was independent of identity. As in the left column, the thin lines correspond to per-interval p-values, while the thick line corresponds to the mean over all intervals. The magenta line corresponds to $p = 0.05$.

4.4.2 Dimensionality reduction analysis

We used principal component analysis (PCA) to examine what structures in the data may be used to predict a given behavioral statistic. For each of the 12-most predictable statistics (for buffer length = 0), we examine the 6-dimensional vector composed of the per-interval statistics (e.g. element 1 is the distance traveled in the first hour, element 2 is the distance traveled in the second hour, ...). We perform PCA on the z-scored data set consisting of these 6-D vectors for all flies (thresholding outliers at 3 standard deviations) to find the 6-D directions of greatest variance.

Figure 4.27 shows the results. For each statistic, there are three plots. In the top row, we plot the projection of the per-interval statistics on the first two principal components, the highest variance 2-D linear subspace. For no statistic do we see clearly clusterable data.

In the middle row, we plot the error of the projections onto increasing numbers of principal components. As emphasized in Figure 27, we see that the usefulness of the first principal component corresponds with predictability of behavior statistics based on individuality (Figure 4.26).

In the bottom row, we show the directions of the first and second principal components. For all the statistics plotted, the first principal component is flat across all intervals, implying that the first principal component represents the average value of the statistic across all intervals. Thus, for instance for the distance traveled statistic, the highest-variance direction corresponds with the average distance traveled in all intervals some flies walk far and some flies do not. In addition, for all the statistics, the second principal component increases nearly monotonically with time. To emphasize the similarities between statistics, we flip the sign of the component to be increasing it is the

monotonicity of the change that is relevant. At interval 3 and below, the coefficient is usually negative, while above it is positive. Thus, the second principal component corresponds to how the statistic increases or decreases with time, implying that flies differ in how their behaviors change over time. In addition, the coefficients seem to level off in the last 23 intervals, perhaps relating to the change in behavior due to hunger.

Based on the observation that the first and second principal components for different statistics are similar, we found the average first and second principal components (where signs are set as above). We project the data onto these 2 principal components for each statistic, resulting in 24-dimensional vectors. We repeated the analysis in Figure 4.27 on this new 24-dimensional data set. That is, we performed PCA on these statistics of all 12 plotted behavior statistics. The results are shown in Figure 4.29. As in Figure 4.27, the first plot shows the projection of the 24-dimensional data on the first two combined principal components, that is, the highest variance 2-D linear subspace of the combined data. Again, we see no clear clusters. Male flies appear to be more extreme in the first dimension, and female flies more extreme on the second dimension. In the second plot, we show the error of the projections onto increasing numbers of principal components. In the third and fourth plots, we show the directions of the first 3 principal components. The sign is chosen so that the mean element is positive. The third plot shows the elements of the combined principal component corresponding to the first per-statistic principal components, while the fourth plot shows the elements of the combined principal component corresponding to the second per-statistic principal components. We can attempt to interpret the first combined principal component; the second and third have no obvious interpretations. We see that the first combined principal component is close to 0 for all the second per-statistic components, and the absolute weight of the first per-statistic component decreases with the error of the regression. For most of the behavior

statistics, the weight is positive. The statistics with positive weight correspond to those we associate with an active fly (with the exception of the fraction of time near the water, which has a small but positive weight). The two negative weight statistics fraction of time in the observed chamber and median stop duration, would be associated with a more sedentary fly. Thus, we can interpret the first principal component as a measure of how active the fly is.

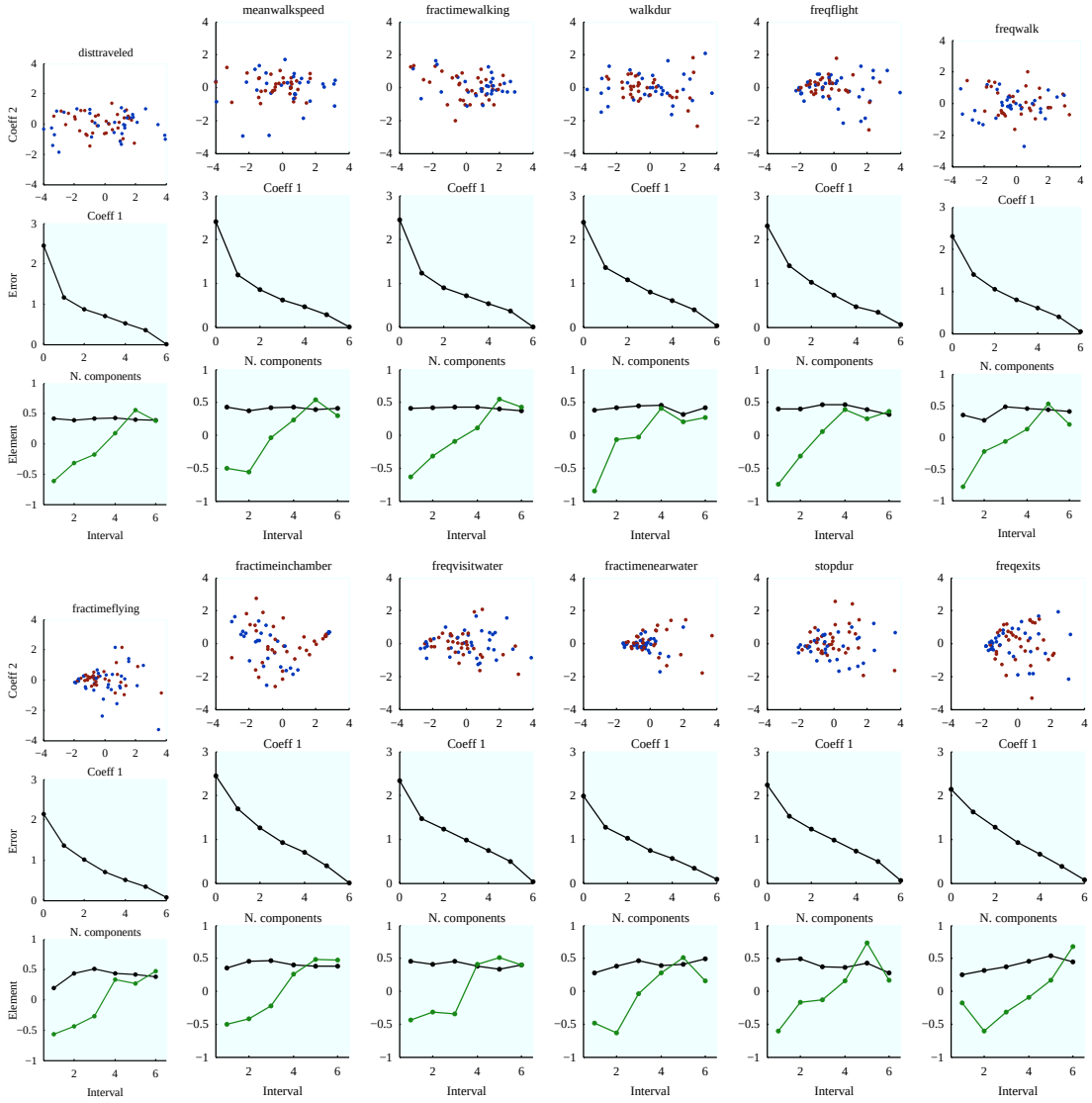

Figure 4.27: Principal component analysis per statistic. For each statistic, in the top row we plot the projection of the 6-D vector of per-interval statistics onto the first 2 principal components. There is a point for each fly; males are plotted in blue, females in red. In the middle row, we plot the error (square root of the average sum-squared error) of the reconstruction of the z-scored per-interval statistic vector with varying numbers of principal components. N. components = 0 corresponds to just using the mean, while N. components = 6 corresponds to using all principal components, and thus will always have error = 0. In the bottom, we plot the first (black) and second (green) principal components. For many of the statistics, the first component is an average over all intervals, while the second measures change in the statistic over the trial. We flip the sign of the first principal component so that its average element is positive, and the second principal component so that the last element is bigger than the first element to emphasize these trends.

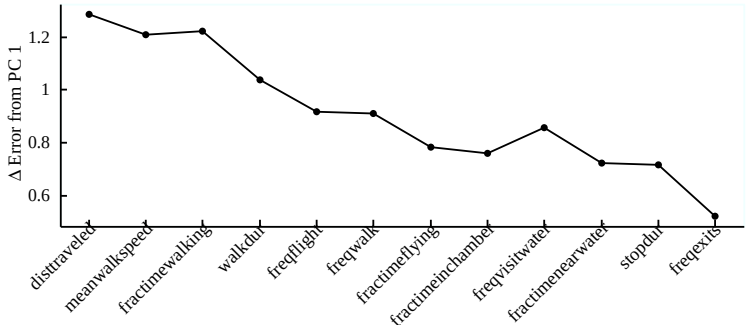

Figure 4.28: Decrease in reconstruction error by including the first principal component. For each statistic, we compute the decrease in error between the mean-based reconstruction and the one-dimensional principal component reconstruction. The statistics are sorted by error in the regression for buffer length = 1 (see Figure 4.26). Note that the decrease in error decreases monotonically as regression error increases.

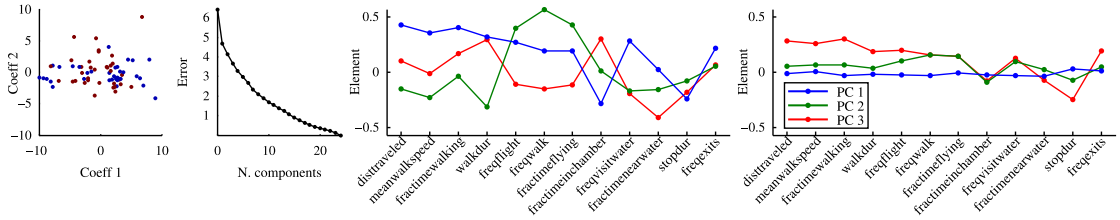

Figure 4.29: Principle component analysis of first 12 statistics combined. Following the observation that the first and second principal components for the first 12 statistics were similar, we found the average first and second principal component over all plotted statistics. We projected the per-interval vectors for each of these 12 statistics onto these first two mean principal components, resulting in 68 flies $\times$ 24-dimensional vectors. We then performed the same analysis as in Figure 4.27 on these new vectors. In the first plot, we show the projection onto the first two principal components. There is a point for each fly; male flies are blue, female are red. In the second plot, we show the error (square root of the average sum-squared error) of the reconstruction with varying numbers of principal components. In the third and fourth plot, we illustrate the first 3 principal components. The third plot shows the principal component elements for the elements derived from the first per-statistic principal component. The fourth plot shows the principal component elements for the elements derived from the second per-statistic principal component.

4.5 Discussion

Behavioral flexibility is often proposed as an adaptation that allows individuals to maximize their fitness within the multifaceted environments an animal may encounter over its lifetime (Dingemanse and Réale, 2005). However, it has been reported that the majority of the time individuals exhibit very limited behavioral plasticity (Sih et al., 2004a,b) and also that animals exhibit consistent differences in their reaction towards the same sensory stimuli in their local environment (See references within (Dingemanse and Réale, 2005)). In order to observe the degree of behavioral plasticity and the consistency of differences of an individual's behavior to the same environmental stimuli, we introduced single flies that were reared and handled in a similar manner into homogenous model environments, and observed their movement over the period of hours. We were particularly interested in the exploratory movement patterns of individuals in relation to a source of water and the exit into an adjacent chamber. We quantified various basic measures of walking and flying that contribute to exploration, and also several higher-order measures of the the flies' exploratory movements. We tested the following predictions: (1) would individuals show markedly different degrees of exploration?, (2) would the difference an individual's exploration persist over time?, and (3) would the various measures of exploration be independent and thus not merely a consequence of a more general phenomena such as activity level?

We report significant differences among individuals in their exploratory movements. The differences among individuals were apparent in our raw observations of their movement, e.g., the position of each fly throughout the time course of the experimental trial (see Figure 4.1), or the total distance traversed during each successive 10 minute internal (see Figure 4.3). The differences among individuals were also salient in simple

per-frame statistics describing their walking and flying movements (see Figures 4.5–4.13), and in higher-order quantitative descriptions of their exploratory and dispersal behaviors, e.g., search near water and movements that resulted in leaving the chamber (see Figures 4.14–4.21).

At the time of writing up this dissertation, we have only examined the 6-hour data set. Within this data set, the characteristic structure in exploratory movements of individuals that were observed was stable for greater than over 5 hours in all behavioral descriptors observed except in the following measurements: (1) the fraction of time flies spent flying (*fractimeflying*), two related measurements of local search near water: (2) the time flies spend between water visits (*timebtnwater*) and (3) the travel length of movement between water visits (*pathlengthbtnwater*), and also two measurements related to dispersal from the chamber: (4) the number of exits from the chamber (*freqexits*) and (5) the total time spent in outside the chamber (awaydur). It is likely, however, that these measurements would exhibit persist characteristic structure if we had a larger sample size, for these five descriptors of exploratory behavior are made up of the other descriptors that *did* exhibit persistent structure, and also there was a significant amount of error observed in the measurements of these descriptors due to some movements quantified by descriptors never or very infrequently occurring. Finally, from our the dimensionality reduction, we suggest that many of differences seemed related and were due to activity and a component related to time of day (see Figures 4.27,4.28,4.29).

4.6 Supplementary materials

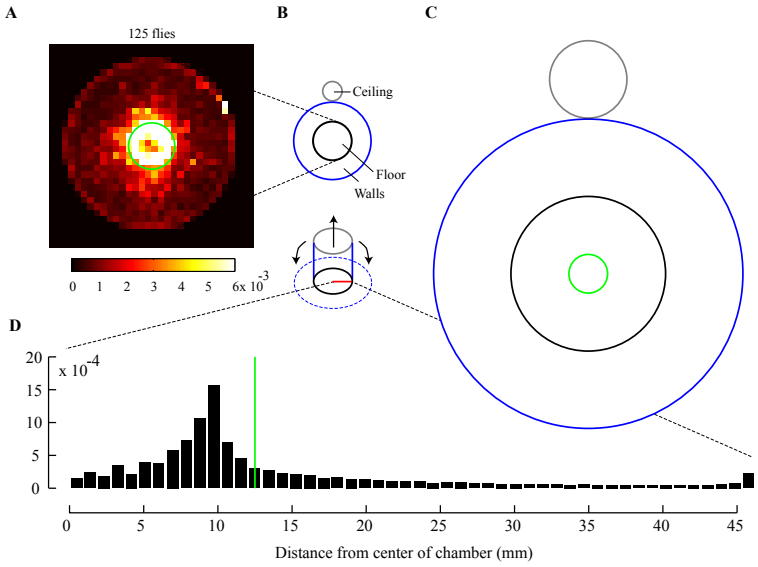

Figure 4.30: Collective transit probabilities and individual local searching movements near a source of water. (A) Collective transit probabilities for 125 flies on the floor of chambers near a source of water (green circle). (B) Illustrations to help visualize the flattened projection for displaying the individual movements of flies near water shown in C and also to show the cross-section transect (red) for the probability histogram in D. (C) To help illustrate the trajectory of a fly, we unfold its 3D positions within the experimental chambers and report its movement in a flattened representation. A patch of agar (green circle) embedded within the center of floor prevented flies from dehydrating.

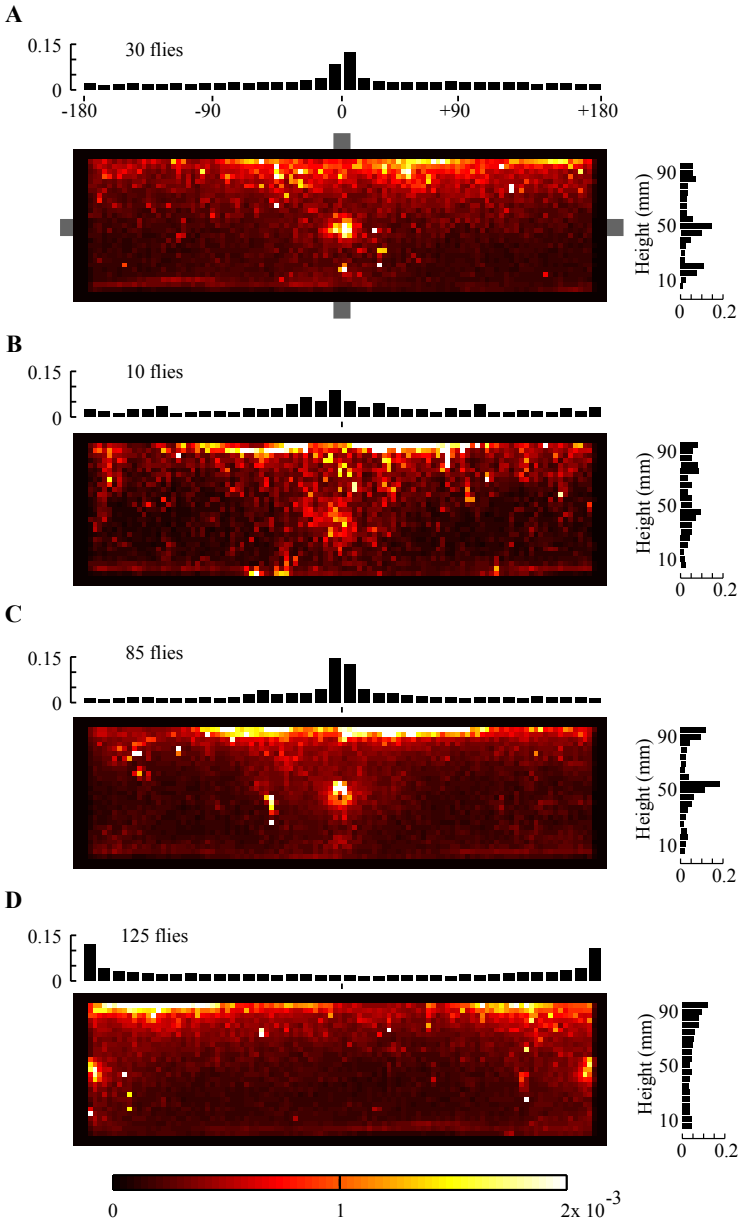

Figure 4.31: Tactile and visual cues are salient features of the exit leading between chambers. Collective transit probabilities for flies on the wall of chambers when the exit is (A) blocked, (B) covered with transparent material allowing light to pass through, (C) open to a second connected chamber, and (D) rotated 180 degrees, for all trials from A-C. Histograms of the transit probabilities calculated from one centimeter horizontal and vertical strips (gray bars, as denoted in A) are shown above and on the side of each panel.

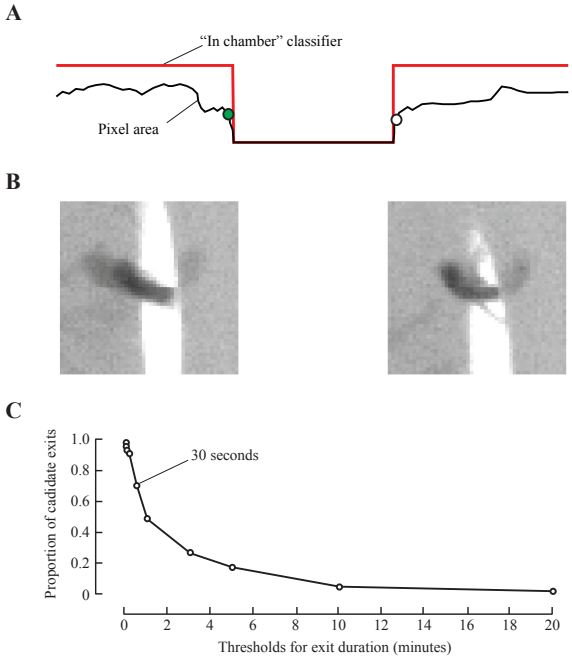

Figure 4.32: Graphic illustrating the classification of exits from a chamber. (A) Flies were *nominated* as exiting the chamber at a particular image frame when at that frame the pixel area of the binerized difference between the image and its corresponding background image dropped to zero. (B) Example image frames for a fly just proceeding to an exit (filled green circle in A) and just after returning from the second chamber (open circle in A). (C) Proportion of classified exits from total number of candidate exits as a function of the shortest time away that constitutes an exit. A fly was required to have left the chamber for at least 30 second to be considered an exit; this criteria, in one particular data set, restricted the number of leaving events that were classified as exits to 860/1222.

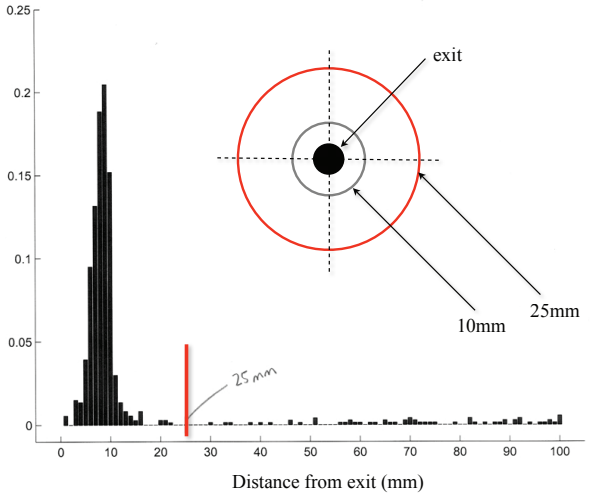

Figure 4.33: Frequency histogram of distances from known 3D exit locations for candidate exits events. Candidate exits initially classified by pixel area were excluded if their distance was greater than 25 mm from the center of the known exit location (red line; red circle within inset). False exits were rare, e.g., 66/1222 for a particular set of trials, and generally due to an adaptive thresholding error when a fly had exited and was currently outside the chamber.

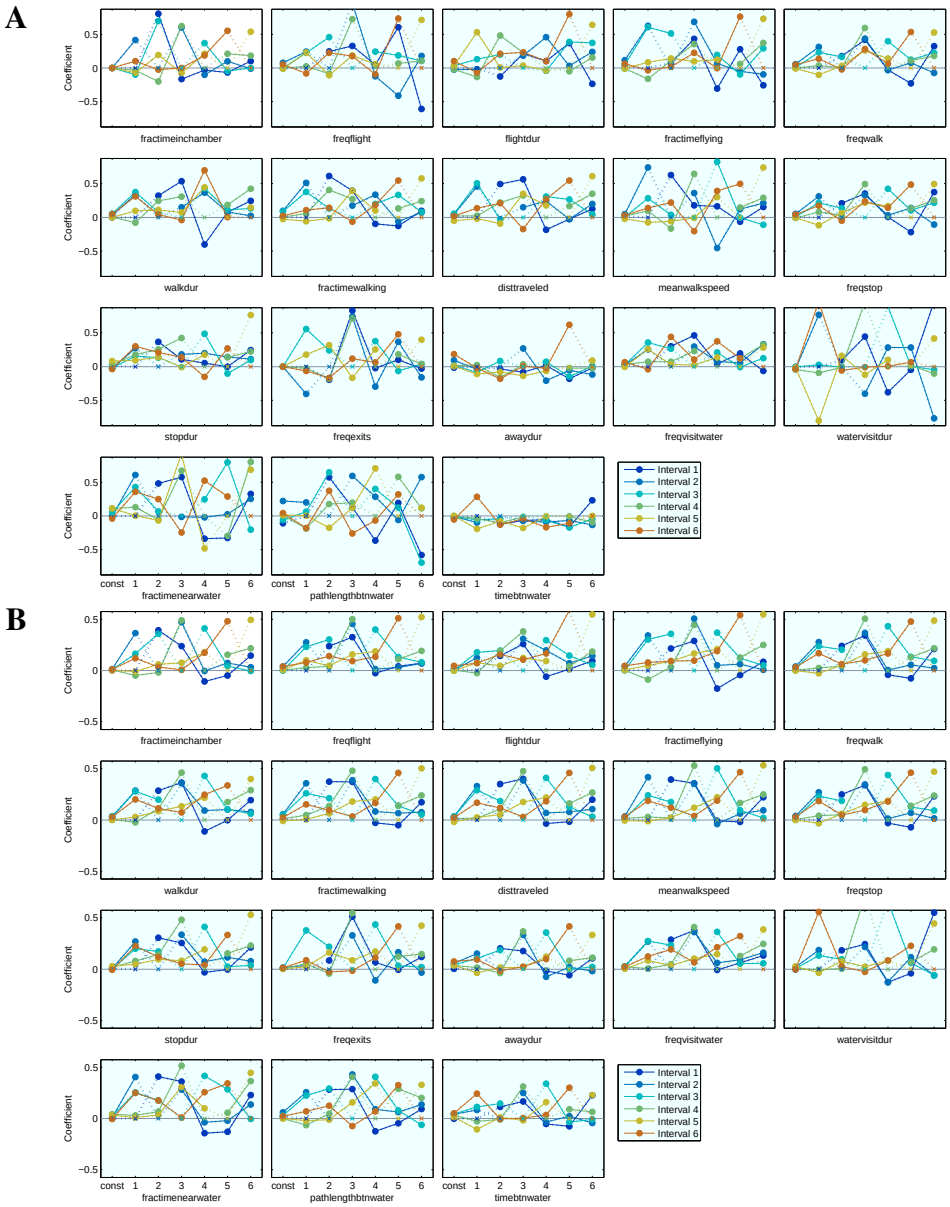

Figure 4.34: Coefficients of regressors learned. Each plot corresponds to a different statistic. The x-axis describes to which input vector element the coefficient corresponds, either the constant offset 1 or the statistic for one of the input intervals. The y-axis corresponds to the value of the coefficient for that input element. There is a line for each of the intervals predicted. The dashed lines and X's indicate intervals that are not input. The buffer length for these regressions is 0. (A) shows the coefficients learned with ordinary linear regression, while (B) shows the coefficients learned with regularized linear regression.

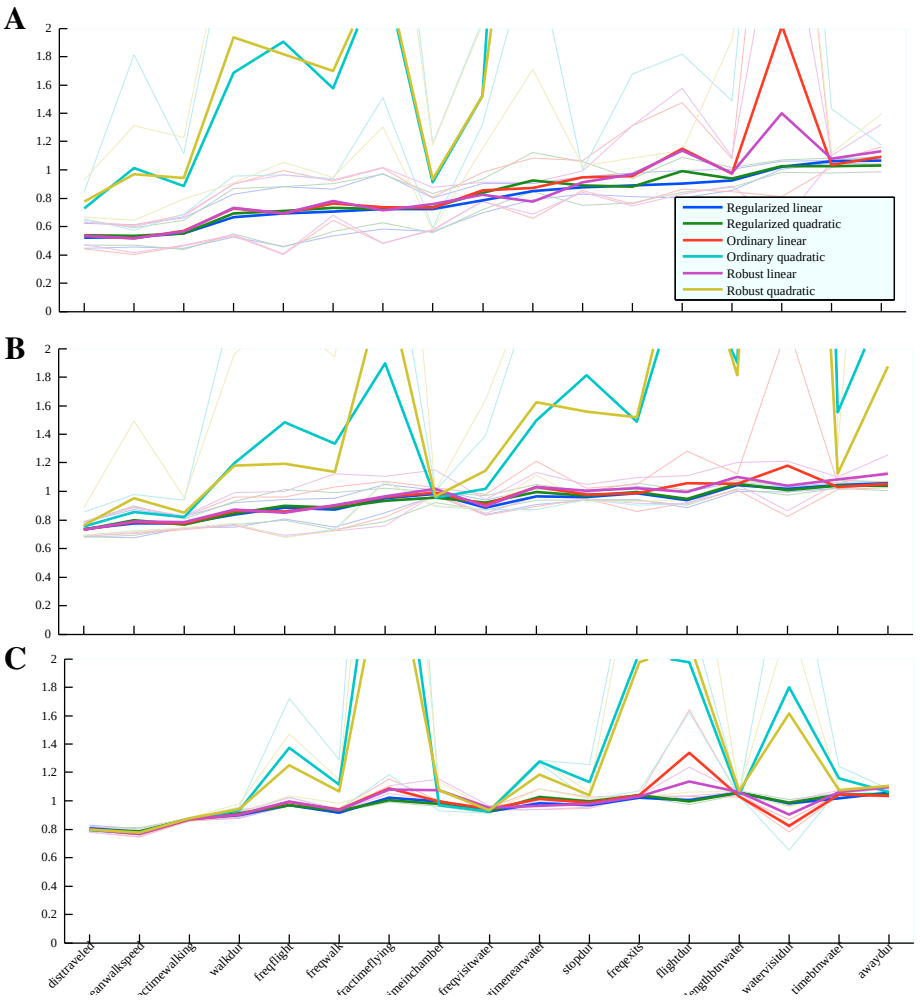

Figure 4.35: Comparison of different learning algorithms. Each plot shows the square root of the mean squared error between the true and predicted behavioral statistic, as in Figure 4.26. Each color corresponds to a different type of learning algorithm/regression criterion optimized. The thick lines show the mean error over all intervals for a particular regression type, and the thin lines show the minimum and maximum over all intervals for a particular regression type. Each plot corresponds to a different buffer length: (A) buffer length = 1 intervals, (B) buffer length = 3 intervals, (C) buffer length = 5 intervals.

Chapter 5

A new experimental chamber for studying the social behaviors of *Drosophila*

5.1 Summary

Methods available for quickly and objectively quantifying the behavioral phenotypes of the fruit fly, *Drosophila melanogaster*, lag behind in sophistication the tools developed for manipulating their genotypes. We have developed a simple, easy-to-replicate, general-purpose experimental chamber for studying the ground-based behaviors of fruit flies. The major innovative feature of our design is that it restricts flies to a shallow volume of space, forcing all behavioral interactions to take place within a monolayer of individuals. The design lessens the frequency that flies occlude or obscure each other, limits the variability in their appearance, and promotes a greater number of flies to move throughout the center of the chamber, thereby increasing the frequency of their interactions. The new chamber design improves the quality of data collected by digital video and was conceived and designed to complement automated machine vision methodologies for studying behavior. Novel and improved methodologies for better quantifying

the complex behavioral phenotypes of *Drosophila* will facilitate studies related to human disease and fundamental questions of behavioral neuroscience.

5.2 Introduction

Due to the development of sophisticated genetic tools, *Drosophila* has emerged as a powerful model system for studying the causal relationships between genes, neurons, and behavior (Callaway, 2005; Zhang et al., 2007; Luo et al., 2008). Progress in identifying such relationships is inhibited by the fact that the methods available for quantifying behavior lag in sophistication behind the tools available for manipulating gene or neuron function. Machine vision offers a promising strategy for automatically tracking and measuring the behavioral phenotypes of flies (Martin, 2004; Valente et al., 2007; Grover et al., 2008; Wolf et al., 2002; Ramazani et al., 2007; Hoyer et al., 2008; Katsov and Clandinin, 2008; Dankert et al., 2009; Branson et al., 2009). However, the robustness of these automatic methodologies is highly dependent on the quality of the raw data contained within the digital movies of the flies' behavior. Conventional chambers used for studying the behaviors of flies possess several features that make the measurement and analysis of behavior difficult. For example, high ceilings permit flight, which is difficult to track using a single low-temporal resolution camera. Vertical walls in a chamber allow flies to walk up and onto the ceiling, creating a situation in which flies may overlap and obscure each other. Vertical walls also lead to significant changes in the appearance of flies as they move among the different surfaces of the floor, wall, and ceiling. These deviations in appearance can obscure identifiable features that might have been useful for detecting specific behaviors, such as the position of the fly's wings and limbs. Furthermore, cracks, corners, and vertical surfaces are attractive to flies and

promote their clustering on the wall or in the periphery of the chamber. These features all result in problematic scenarios for automatic tracking methods based on a digital video stream (Fig. 5.1).

Here we present a new strategy for constructing experimental chambers that restrict the behavior of flies to within a monolayer. Low ceilings prevent flies from hopping or flying over each other, an acute angle formed between sloped walls and the ceiling reduces the number of flies walking onto the ceiling, and a slippery ceiling limits the duration flies may cling to the ceiling before falling to the floor. Previous methods to keep flies within a monolayer have required elaborate designs, such as water moats (Götz and Wenking, 1973) or thermal barriers (Branson et al., 2009), and the limitation and tedium of clipping off the flies' wings. Our design lessens the probability that flies obscure each other, limits the variability in their appearance due to moving among various regions of the chamber, and promotes a greater number of flies to move throughout the center region of the arena. Flies within the new chamber can exhibit all of the behaviors normally observed in a laboratory setting, with the exception of flight. The new design helps in generating improved-quality raw data and therefore complements machine vision methodologies for automated studies of complex behavioral phenotypes of fruit flies.

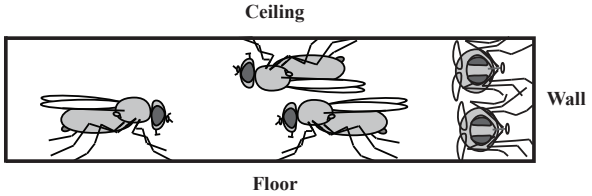

Figure 5.1: **Side-view illustration of typical arrangements of flies in chambers with vertical walls.** Problematic conjunctions occur when a fly clings to the ceiling, partially occluding a fly standing on the floor, and when two flies stand one above the other on the wall.

5.3 Results

5.3.1 Fewer problematic conjunctions

To compare the number of flies that have a high probability of overlapping, we introduced groups of 50 flies into chambers with our new sloped-wall design and conventional chambers with vertical walls. Aside from the shape of the wall, the chambers had comparable heights and diameters. After allowing flies to settle for 1 hour, we counted the number of problematic flies, i.e., flies residing on the walls and ceiling of each chamber (Fig. 5.2). For 14 days, we observed groups of flies introduced into 2 chambers with sloped walls and 2 chambers with vertical walls. As expected, chambers with sloped walls contained negligible numbers of problematic flies, whereas the percent of problematic flies for chambers with vertical walls ranged from 30% to 70% (Fig. 5.2B).

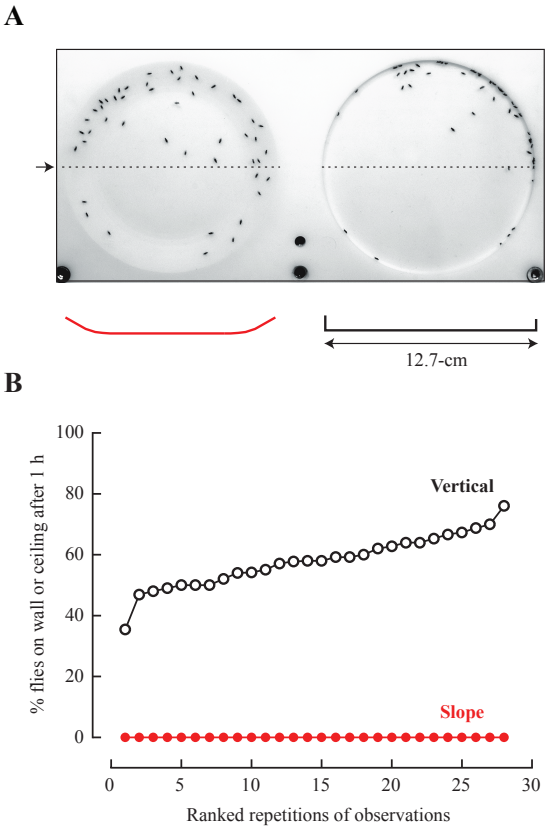

Figure 5.2: **Sloped walls lessened the probability of problematic conjunctions between flies.** (A) Photograph showing a typical distribution of 50 flies observed in chambers with sloped and vertical walls with associated drawings that depict the contour of the floors of the chambers along the cross section shown (arrow). (B) Comparison of the percent of problematic flies from groups of 50 individuals observed after 1 hour in chambers with sloped (red closed circles) and vertical walls (black open circles).

5.3.2 Behavior restricted to monolayer

To illustrate how chambers with the new design complement automatic methodologies for studying behavior, we used Ctrax software designed to track and retain the identity of individuals within large groups of flies (Branson et al., 2009). For these observations, we introduced 25 male and 25 females flies into a 12.7-cm diameter chamber with sloped walls and recorded their movements for 30 min (Fig. 5.3; See supplementary movie: MS1). Ctrax requires that the flies remain within a planar arena and not overlap. As described above, chambers designed with sloped walls prevented flies from obscuring each other by moving up the wall or onto the ceiling of the chamber. The glass ceiling on these chambers prevented flies from leaving and also allowed an unobstructed view for recording their behavior. By design, the entire chamber was uniformly backlit, creating high contrast silhouettes of the flies to facilitate the tracking of their movements and classifying their identity and gender. As indicated in Fig. 5.3, the Ctrax software was particularly robust when analyzing data collected in our sloped-wall chambers.

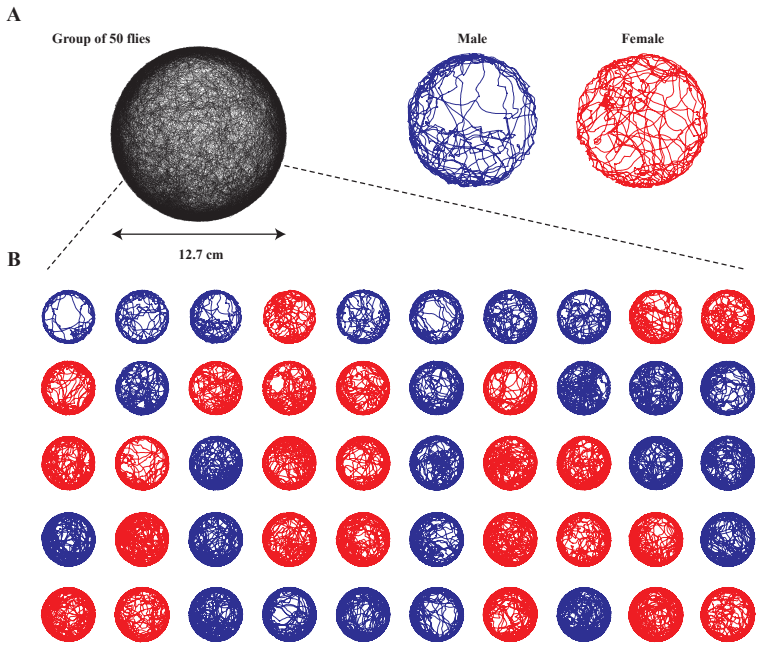

Figure 5.3: **Trajectories of 50 flies moving for 10 minutes within a chamber designed with sloped walls.** (A) Superposition of the individual trajectories from the group of 50 flies. (B) Individual trajectories of the 50 flies making up the group shown in **A** with individual males (blue) and females (red) sorted along rows from the shortest to the longest distance traveled (Top left to bottom right).

5.3.3 Reduced variability in appearance

To compare the variability of a fly's appearance between chambers with sloped and vertical walls, we used movies recorded of single flies moving for the first hour after introducing them into the chambers (Straw and Dickinson, 2009). After subtracting the corresponding background image, we determined the number of pixels making up a thresholded representation of the fly from each frame for each movie (Fig. 5.4A). For each fly we determined its median pixel value during the entire length of each 6-hour movie. We used the number of pixels from each frame over the median number of pixels from the entire 6-hour movie as a proxy measure for the deviation in a fly's appearance (Fig. 5.4B, C). From direct observation of movies, we observed that much of the deviation in appearance in chambers with sloped walls was due to changes in the fly's behavior, including short flights, hops, grooming, various wing movements, and changes in typical walking posture. In addition to the deviation due to these changes in behavior, large deviations resulted from changes in the fly's profile when it moved among the floor, wall, and ceiling in chambers with vertical walls. To illustrate that there was less variability in a fly's appearance in chambers with sloped walls, we compared the deviation in appearance for 26 flies, introducing 13 flies into each chamber (Fig. 5.4D). The results indicate that variation in pixel area is much lower in the sloped-wall chambers.

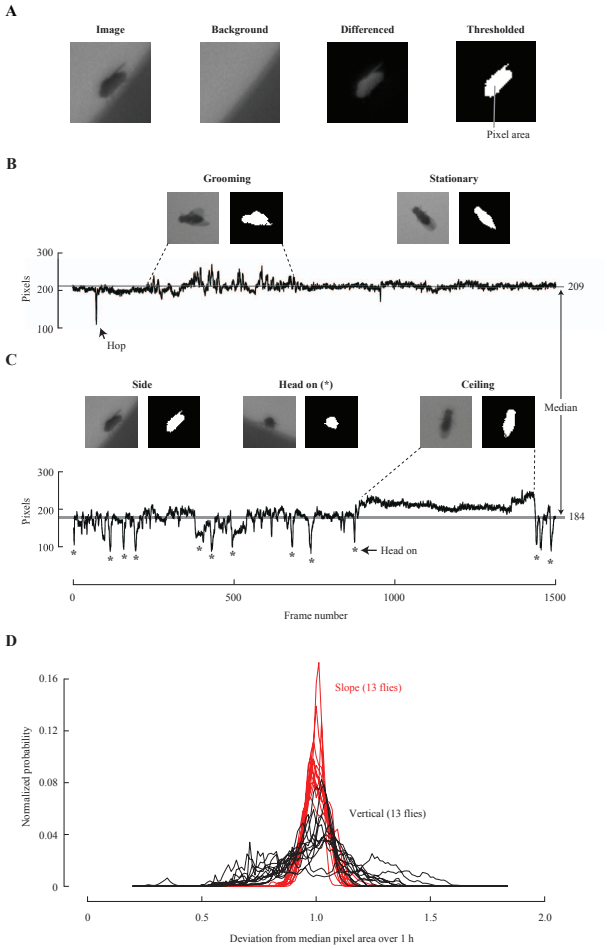

Figure 5.4: **Sloped walls reduced the variability in a fly's appearance.** (A) Cropped image taken from a movie that included only a small region surrounding the fly, a cropped background image from the same region of the chamber excluding the fly, a differenced image between the cropped image and the cropped background image, and a binerized representation of the difference between the images determined by a threshold. The total number of pixels from the binerized representation of the flies was calculated for each frame. (B, C) Examples using 100-second windows of movie illustrating the lower variability in the total number of pixels extracted from movies of flies recorded within the chambers with sloped walls, as compared to those with vertical walls. The median pixel area was calculated from the entire movie (gray line) and was approximately equal to when the fly was on the floor and stationary in the chambers with sloped walls. (B) Significant deviations from the median pixel area in the chamber with sloped walls corresponded to a hop (arrow) and a period when the fly was grooming (region between dashed lines). (C) Deviations in pixel area in the chamber with vertical walls were due to changes in the fly's appearance as it rotated on the wall between side and head on (astrisk) or moved from the wall onto the ceiling (region between dashed lines). (D) Normalized histogram of deviation in pixel area over the first hour of movie from flies observed in the chambers with sloped (red) and vertical walls (black). Numbers on the x-axis represent the deviation from the median pixel area, where 1 is no deviation and 0.5 and 1.5 are $\pm$ 50% deviation from the median pixel number.

5.3.4 Decreased measurement errors

CADABRA, a recently developed method for automatically measuring social interactions, bases its classifications for specific behaviors on changes in the relative position between flies (Dankert et al., 2009). This method then fine-tunes the classifications and determines the *detection* of specific behaviors by correlating the measured positions to changes in the flies' appearance, i.e., patterns of wing postures or measures of relative body length and width. For such a strategy to work, it is critical that measurements of body orientation and the identity of flies are correct. Here we used the outputs from CADABRA to illustrate that the vertical walls found in conventional chambers increase the number of measurement errors for body orientation and fly identity, undoubtedly contributing to missed and mischaracterized social interactions. We analyzed 36 movies of single males courting single females up to the onset of copulation or up to 20 minutes, whichever came first. Half of these movies were recorded in a new sloped-wall chamber and half were recorded in a conventional chamber with vertical walls. We measured the number of erroneous flips in body orientation by comparing the output of CADABRA to an estimate of body orientation based on a global optimization from all frames of a fly's trajectory from each movie sequence, part of the error-correcting capacities of Ctrax software (Branson et al., 2009). This optimization simultaneously finds the head-tail assignment for all frames such that (1) the change in the orientation between consecutive frames is small and (2) the velocity direction and orientation of a fly match the frames in which the fly is walking. We used the difference between the automatic measurement from CADABRA and the corrected estimate as a metric for the number of erroneous flips in orientation. We also estimated the number of frames containing erroneous swaps in identities between flies, by setting a classification threshold

in which both flies had an identical change in the distance of their positions that was greater than 1.5 mm within a single frame. We based this estimation on the changes in their positions between consecutive frames that were also measured automatically with CADABRA. Using these metrics, we compared the number of erroneous flips in body orientation per second for individual flies and the number of frames with identities swapped per second between pairs of flies. We observed that the rate of erroneous orientation and frames containing erroneous identities were significantly less in sloped chambers than in conventional chambers (Orientation: Mann-Whitney U, $p<0.0001^{\dagger}$; Identity: Mann-Whitney U, $p<0.0001^{\dagger\dagger}$; Fig. 5.5; See supplementary movies: MS2, MS3).

To further illustrate that it was the *vertical* walls in the conventional chambers that increased the number of measurement errors, we compared body orientations and swaps in identity when the flies were either both on the wall, both on the floor, split with one fly on the wall and the second fly on the floor, or measured for both flies irrespective of where they were throughout the chamber. The rate of erroneous orientation calculated when both flies were on the floor of conventional chambers was intermediate between the lesser rate observed for flies found throughout the sloped-wall chambers and the greater rate when both flies were on the vertical walls of conventional chambers (^{i}Mann-Whitney U, $p=0.012^{\dagger}$; ^{ii}Mann-Whitney U, $p<0.0001^{\dagger}$; Fig. 5.5B). The higher rate of erroneous orientation observed when both flies were on the the vertical wall was comparable to the higher rate observed when flies were found throughout the vertical chamber (Mann-Whitney U, $p=0.312^{\dagger}$, Bonferroni correction for multiple comparisons, †n=36 flies for each comparison). Similarly, the rate of erroneous identities for flies on the floor of conventional chambers was comparable to the rates in sloped chambers (Mann-Whitney U, $p=0.153^{\dagger\dagger}$). These rates were also significantly less than the rate

of erroneous identities whether both or just one of the flies were on the wall of conventional chambers, and if flies were found through the chamber ([iii]Mann-Whitney U, $p<0.0001$[††]; Fig. 5.5D). The rate of frames with erroneous identity was similar for all comparisons that included at least one fly on the vertical wall[††](Results from statistical analyses are within the figure legend;[††]n=18 pairs of flies for each comparison; Fig. 5.5D). The classification of a fly's location between the wall and floor was made based on its x, y position. Flies equal to or less than 2 mm from the periphery of the chamber were considered to be residing on the wall. From these results, it is clear that the presence of a vertical wall introduces additional variability in the appearance of flies, increasing the frequency of error in basic measures such as body orientation and identity, and undoubtedly would lead to poorer classifications and detections of behavior observed among interacting flies.

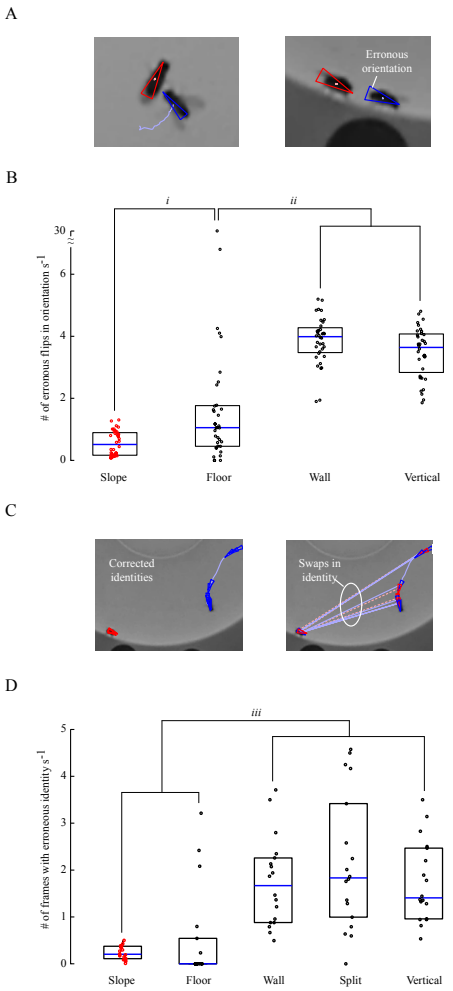

Figure 5.5: **Example of movie images of males courting females, including corresponding errors in automatic classifications by body orientation and identity.** (A) Examples of movie images with body orientations annotated (triangle apex denotes the position of the fly's head) that were extracted with the CADABRA software system. (B) Error rates of body orientations calculated for individual flies from movies recorded for males courting females within the chambers with sloped (red) and vertical (black) walls. Error rates from the chambers with vertical walls were decomposed into erroneous flips in orientation during periods when individual flies were either on the floor or on the wall. Medians (blue) and 25th and 75th percentiles are shown (black box). (C) Examples of movie images with identity annotated while males (blue) courted females (red). Trajectories represent the location of flies for the past 30 frames ($\approx$1 s). Swaps in identity are denoted by the discontinuities in the trajectories and changes in color between triangles representing past locations of flies, and therefore can be compared to a movie image that has been corrected (left). (D) Error rates for the classification of identity between pairs of male and females from movies recorded within the chambers with sloped (red) and vertical (black) walls. Error rates from the chambers with vertical walls were decomposed into swaps in identity during periods when both flies were on the floor, both on the wall, and split with one fly on the floor and the second on the wall. Medians (blue) and 25th and 75th percentiles are shown (black open box). The rate of errors were similar between *Wall* vs. *Split*, Mann-Whitney U, p=0.628; *Wall* vs. *Vertical*, Mann-Whitney U, p=0.864; and *Split* vs. *Vertical*, Mann-Whitney U, p=0.521.

5.3.5 Flies spend less time in periphery

To quantify and compare the amount of time flies loitered in various regions in the chambers and also to observe if sloped walls might increase the interaction between flies, we introduced pairs of virgin males and virgin females into 7.0-cm diameter chambers. We then monitored their courtship until the onset of copulation or for 20 minutes, whichever came first. Flies introduced into chambers with sloped walls spent less time near the walls than in chambers with vertical walls. This difference was apparent immediately and could be seen in the trajectories of individuals making up the male-female pairs (Fig. 5.6A-D). This difference was also apparent in the trajectories from individuals making up large groups (Fig. S1A-C) and in the trajectories of single flies (Fig. S1E-J). We observed that flies moving near the extreme periphery of the chamber, less than or equal to 2 mm from the vertical wall, were nearly always walking on the wall. Moreover, flies moving toward vertical walls nearly always moved onto the walls and were also less often observed returning back to the chamber floor. This resulted in the flies spending the majority of their time on the wall (Fig. 5.6E).

The specifics of how and when the flies moved onto the wall contributed to the variability in their courtship. When females moved onto the wall first, males did not always immediately follow them, but instead sometimes spent a significant amount of time searching for the females on the chamber floor. Other times both flies moved up and onto the wall, and if this happened, usually the female would slow and stop. Once the female became stationary, typically the male would then find her, court quickly, and copulate. Alternatively, the male might move along the wall in the opposite direction and then spend a significant amount of time moving back and forth on the wall until he found her again. In several of the trials, the male would then not find the female

within the observation period. In contrast, courtship was fairly uniform in chambers with sloped walls. Upon locating a potential mate, the male would court her without distraction from the geometry of the chamber until successful copulation (see supplementary movie: SM4). Consequently, courting pairs of flies in chambers with sloped walls were, on average, closer to each other as compared to flies in chambers with vertical walls (Fig. 5.6F; *Mann-Whitney U, p=0.038, 1-tailed). However, mean $\pm$ s.e.m. courtship latency, i.e., the time measured from when we released flies into the chamber until the onset of copulation, was comparable between chambers (Fig. 5.6G; Sloped walls, 425.5s $\pm$ 73.1s; Vertical walls, 369.3s $\pm$ 98.9s; T-test, p=0.629). Finally, in addition to partitioning the space used by courting pairs, vertical walls also significantly affected the quality of the flies' behavior, increasing the frequency of erratic hops and movements among the floor, wall, and ceiling (see supplementary movies: SV5, SV6).

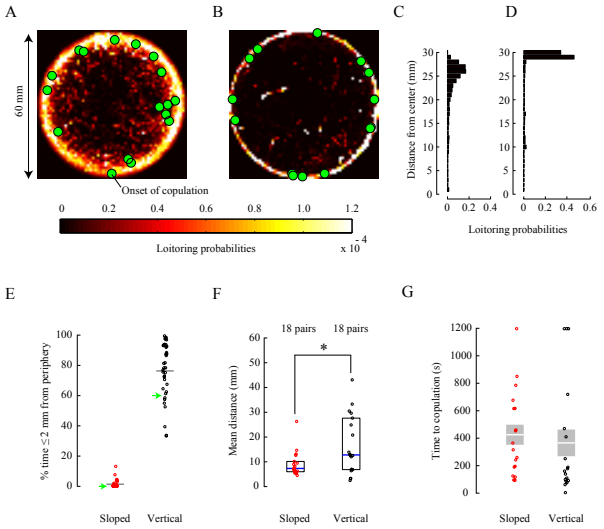

Figure 5.6: **Pairs of males and females in the chambers with sloped walls spend less time near the periphery of the chamber and spend more time near each other.** Normalized loitering probability prior to copulation and the locations when courting flies began copulating for (A) 18 pairs of flies in the chambers with sloped walls and (B) 18 pairs of flies in the chambers with vertical walls. Collective loitering probability normalized by area in 30 concentric regions for flies in the chambers with (C) sloped and (D) vertical walls. Concentric annuli making up the regions were 1 mm thick. (E) Collective mean percent total time (gray lines) and percent total time for individuals from pairs of flies spent near and on the wall in the chambers with sloped (red) and vertical (black) walls. Percentage of pairs of flies beginning to copulate on and near walls is also denoted (green arrowheads). (F) Collective medians (blue lines) of the average distance between pairs of flies prior to copulation in the chambers with sloped (red) and vertical (black) walls. The top and bottom of the boxes represent 25th and 75th percentiles (black open box). (G) Collective means (white lines) and average copulation latencies for pairs of flies in the chambers with sloped (red) and vertical (black) walls. The top and bottom of the boxes represent $\pm$ s.e.m. from collective means (gray filled box).

5.4 Discussion

We have developed a general-purpose experimental chamber that can be used for studying the locomotor behavior of single flies, interactions between pairs of flies, and the complex social interaction of individual flies behaving within large groups. The new chamber design restricts the movement of flies to a planar arena and limits variability in their appearance, without inhibiting the behaviors they typically display within a laboratory setting. The new design does not require the use of a thermal barrier (Branson et al., 2009) nor a water moat (Götz and Wenking, 1973). More importantly, the new design does not require clipping off the flies' wings, a manipulation that consequently inhibits a significant mode of communication during courtship or bouts of aggression. We believe the new chamber design should be complementary to a variety of methodologies designed to analyze movies from an overhead viewing angle. Moreover, the design provides a simple alternative to the more complicated machine vision methodologies that are required if cameras can view flies from difference poses.

The height of the chamber must be within critical range, but within this range, height may be tailored to fit the needs of a particular study. We have tracked the movement of flies in chambers with heights ranging from 1.8 mm to 4.5 mm. The advantage of the shorter chambers was a decrease in the frequency of overlapping flies, thereby limiting the effort required for correcting tracking errors. The trade off was that the shorter chambers restricted the repertoire of behaviors displayed by flies. For example, low chamber heights inhibit copulation (Hotta and Benzer, 1976). In prior studies, the range of chamber heights that have been used has varied from 3 mm to 6.35 mm for studies of courtship (Kyriacou and Hall, 1980; Joiner and Griffith, 1999; Demir and Dickson, 2005; Hotta and Benzer, 1976) and from 11 mm to 120 mm for studies of

aggression (Dankert et al., 2009; Dierick and Greenspan, 2006; Dow and von Schilcher, 1975; Hoyer et al., 2008). We found chambers with a height of 3.5 mm allowed most, if not all, of the behaviors carried out between flies. (See supplementary movies illustrating various courtship and aggressive behaviors recorded from the new chamber design that may be automatically monitored with current machine vision methodologies: SM7-SM24.) The 3.5-mm height of the chambers used within this report was optimized for *Drosophila melanogaster*, but may be easily adjusted for studying smaller and larger species of fruit flies, or even other insects.

The slope of the chamber wall was more critical than its height, but might also be adjusted. Specifically, chamber walls made more shallow than the 11° slope used here should further decrease the distance between flies. However, chambers developed with more shallow slopes will also restrict the useable space near the periphery of the chamber. It is worth noting that chambers designed with linear-sloped walls worked as well as the sigmoid-linear sloped walls described here (see supplementary figure: S2). Finally, we have tested chambers possessing diameters ranging from 30 mm to 300 mm. There does not seem to be an upper bound on the diameter of the chamber; eventually the size of the chamber will be bounded by the resolution of the camera system used.

The rapidly increasing development of new molecular tools for dissecting the genes and neural circuits regulating the behaviors of these flies has led to a recent surge in the machine vision tools that automatically monitor their complex behavioral phenotypes (Wolf et al., 2002; Ramazani et al., 2007; Hoyer et al., 2008; Katsov and Clandinin, 2008; Dankert et al., 2009; Branson et al., 2009). To make progress on the difficult task of automatically quantifying the complex social behavior of these flies, the developers of these new methodologies have focused on tracking, classifying, and quantifying behaviors. Next-generation methodologies that build upon these strategies will bundle

together the key components of these methodological advances to provide a powerful tool for quantitative descriptions of the phenotypes of this genetic model organism. By restricting the movements of flies to a planar arena, limiting their profiles to a single viewing pose, and keeping flies from clustering in the periphery of the arena, we believe that the chamber design we have described within this report will make the task of automatically quantifying the complex behavior of flies significantly easier.

5.5 Materials and Methods

5.5.1 Animal rearing, housing, and handling

We performed experiments on 4- to 6-day-old adult fruit flies, *Drosophila melanogaster* (Meigen), from two laboratory colonies. The first colony descended from a wild-caught population of 200 isofemales and has been used in our laboratory for approximately 15 years. The second colony was from a laboratory stock of Canton-S (CS) from the laboratory of Martin Heisenberg. We used flies from the CS colony for the observations of courtship and aggression and used the natural isolate for all other observations. We maintained fly stocks at $25\,^{\circ}\mathrm{C}$ and at 40% relative humidity on Lewis food medium in standard 250 mL bottles (Lewis, 1960), on a 16 h: 8 h light: dark photoperiod. The light-on phase started at 7AM PST. Transitions between light and dark were immediate. Replicate observations were run at the same time each day over several days, and we ran trials during either the morning or evening activity peak. We collected flies from culture bottles and housed them at a density of 50 flies per vial overnight in standard 10 mL *Drosophila* vials on food, and observed their behavior the next day. For the observation of individuals from a group of 50 flies, the morning of the day that we were

to monitor their behavior, we housed 25 male and 25 female together in standard 10 mL *Drosophila* vials containing only agar in order to deprive them of food, but not water, for 7 hours prior to their observation. For our observations of courting and fighting pairs, we collected virgins $<$7 hours post-eclosion. We isolated males individually and housed 15 females collectively in vials containing food for 4$\sim$5 days before monitoring their behavior. Each day we wiped down chambers with ethanol and allowed chambers to dry for $\geq$15 min. To help with counting and sorting, we immobilized flies by cooling them to $4\,^{\circ}\mathrm{C}$ on a Peltier stage (Marlow Industries, Inc., Dallas, Texas, USA). We used a mouth pipette to introduce flies into chambers.

5.5.2 Chamber design with sloped walls

The key feature of the new experimental chamber design is that its walls are not vertical with square corners, as has been typical in past studies, but they were gently sloped (Fig. 5.7). The gently sloping walls intersected with the ceiling forming an acute interior angle that effectively deterred flies from moving onto the ceiling. Occasionally flies did move onto the ceiling, mostly as a result of jumps and flights. We have found that a ceiling made from glass coated with Sigmacote (Sigma-Aldrich), a silicone paint, provided a clear, but slippery surface that flies had difficultly clinging to. In chambers with a coated ceiling, most flies that did move onto the ceiling slipped off and fell back to floor. We have determined that a gradual slope with an angle of $11\,^{\circ}$, as measured from the horizontal floor, worked well in chambers with a 3.5 mm high ceiling for studying many behaviors (Fig. 5.7B). To remove the obtuse edge between the floor and the base of the walls, we designed the walls to have a smooth profile. The cross-sectional profile of the walls was made up of two segments, the first half a sigmoid and the second a

straight line (Fig. 5.7C). The piecewise continuous function that specifes the height of the wall as a function of horizontal distance, x, is:

$$y(x) = \begin{cases} h\dfrac{1-\cos\left(\frac{\pi x}{2I}\right)}{2} & \text{if} \quad 0 \leq x \leq i, \\ \tan\theta(x-I)+\frac{h}{2} & \text{if} \quad i < x \leq X_{\max}, \end{cases} \tag{5.1}$$

where h is the full height of the chamber; θ is the angle of slope; I is the distance from the end of where the floor was horizontal to where the sigmoid and the straight segments join, at i, and is halfway up the height of the chamber:

$$I = \frac{h\pi}{4\tan\theta,} \tag{5.2}$$

and $X_{\max}$ is the width of the slope from its base at the floor to where it meets the ceiling of the chamber:

$$X_{\max} = \frac{h}{2\tan\theta + I.} \tag{5.3}$$

The design of the sloped walls removed the discontinuity between the floor and walls, and also eliminated the unused space that was too shallow for flies to enter if the profile of the wall followed the sigmoid to the ceiling. To keep a constant thickness for ideal backlighting, we removed material from the underside of the chamber (Fig. 5.7A, D).

The shape of this undercut followed the curvature of the chamber floor. For the chambers discussed within this study, we machined floors to have a constant thickness of 5 mm. We observed the movement of groups and single flies within 12.7-cm diameter chambers. For the observations of courtship and aggression, we used chambers with a 7.0 cm diameter. Holes providing access to food and water were machined into the floors of chambers and used as needed (see Supplementary Figure S3). To compare the behavior of flies in chambers with sloped walls to conventional chambers, we machined "control" chambers of comparable height and with comparable diameters that had vertical walls. We manufactured chambers from opaque, white Delrin (McMaster-Carr), which is easily machined, has good chemical resistance, and diffuses light, making it ideal for backlighting. We mounted chambers on base plates made from thick aluminum to insure that the chambers maintained their shape.

5.5.3 Experimental setup

To provide a visual stimulus, we surrounded the chamber described above with a paper cylinder (Fig. 5.7D). This paper was printed with a random checkerboard pattern with 50% black squares and 50% white squares. We capped the cylinder with an annulus cut from plain white paper so the camera lens could peer through. The cylinder and lid were backlit by an array of fluorescent lights (GE helical 26W 120 VAC 60 Hz 370 mA) and standard fluorescent room lights with a 120 Hz flicker, both shining through a projection screen (Gerriets International OPERA creamy white). The result was diffuse light creating a luminance of 75 lux at the center of the chamber. We used the visual stimulus only for observing the groups of 50 flies. Without the visual stimulus, the luminance was 500 lux. We used a 12x12-inch array of 850 nm LEDs (12x12 850

nm IR lighting unit, Illumination Control, Inc., Quincy, MA) mounted underneath the chambers for backlighting. We recorded the movements of the flies with a camera mounted from above (Straw and Dickinson, 2009). The movements of the group of 50 flies were recorded at 20 frames per second (fps) using a 1280x1024-pixel firewire camera (Basler A622f), equipped with an 8 mm lens (Pentax). The single flies were recorded at 15 fps using a 1280x1024-pixel firewire camera (PointGray IEEE-1394), equipped with a 12 mm lens (Pentax). An infrared pass filter was placed in front of the camera to block stray light. For observing pairs of courting and aggressive flies, we used a (Sony DCR-HC38) camcorder and recorded the behavior at 30 fps as was done previously (Dankert et al., 2009). In these recordings, we backlit the chamber with visible light (Cold-cathode fluorescent backlight J58-332 8 x 12, Edmund Optics, Barrington, NJ).

5.5.4 Machine vision methodologies

For the analysis of the behavior of individuals from groups of flies, we used Ctrx (Branson et al., 2009), a general-purpose system designed for tracking the individual positions and orientations of a large number of flies simultaneously. This system can be adapted to different laboratory setups and comes with software for detecting a suite of typical behavior exhibited by flies. For tracking and measuring the changes in the appearance of single flies over long durations, we used additional software developed in our laboratory. Flytrax (Straw and Dickinson, 2009) records a spatially cropped image that includes only a small region surrounding a single fly, its x,y position, and orientation from each frame. With this software, we reconstructed a high spatial and temporal representation of a fly's locomotor movement that cross-indexed each frame to its original

movie. From the cropped images, we extracted the measures of the fly's appearance with custom code written in Matlab (Mathworks, Natick, MA). For monitoring and analyzing the interactions between male-female pairs, we used measurements of x, y locations, change in position, and body heading using the CADABRA system (Dankert et al., 2009).

5.5.5 Data and statistical analysis

All output measurements from the various machine vision methodologies were analyzed with custom software in Matlab. Statistical analyses were carried out using SPSS (SPSS, Inc., Chicago, IL).

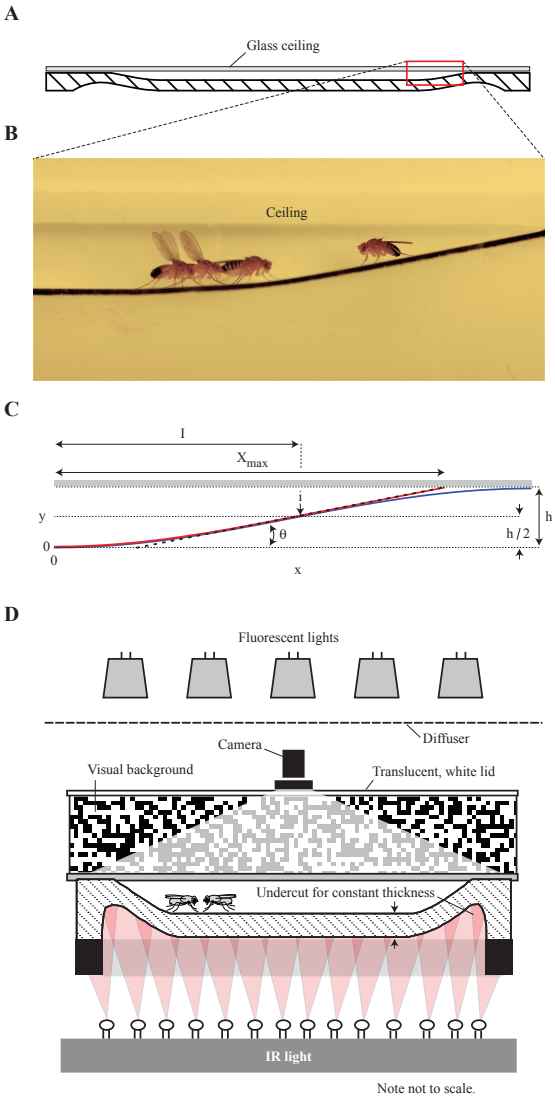

Figure 5.7: **Drawings and photograph illustrating the new experimental chamber design and setup.** (A) Drawing from the side of the experimental chambers, highlighting the sloped wall of the new chamber design (red box) possessing a severely acute interior angle that prevents flies from moving up the wall and onto the ceiling. The ceiling of the chambers was made of glass coated with silicone paint to limit the frequency and duration flies could cling to its surface. (B) Photograph from a cross section wedge of the chamber showing that the height of the chambers provided sufficient room for flies to carry out their normal range of locomotor behaviors. (C) Technical drawing for the profile of the slope that is described within the text. The red line depicts the slope, the blue line represents the profile of the sigmoid curve near the ceiling that was not used in making the slope, and the dashed line denotes the line tangent to the sigmoid that was matched to linear segment of the slope. (D) Drawing from the side illustrating the experimental setup. Chambers were illuminated with standard fluorescent lights projecting through a screen and a cylinder with a lid made of translucent paper. The behavior of the flies was recorded with a camera mounted above the chambers. Chambers were mounted on an aluminum base to help prevent warping and to hold the chamber above lights used for backlighting.

5.5.6 Supplementary movies

We have included short digital movies of typical behaviors displayed by males during courtship and aggression that we believe could readily be classified from a single, top down viewing angle. All of these movies were recorded at 30 fps from chambers designed with sloped walls. The examples of aggression come from five 30-minute movies recording the behaviors displayed by pairs of males around a patch of food, as in (Dankert et al., 2009). The height of the ceiling for these chambers was 3.5 mm and the diameter was 7.0 cm. Frame numbers (red) are shown for all movies and can be used to determine if and when the movie was slowed down or sped up by a factor of 4, a step we took to help illustrate the behaviors.

5.5.7 Supplementary Figures

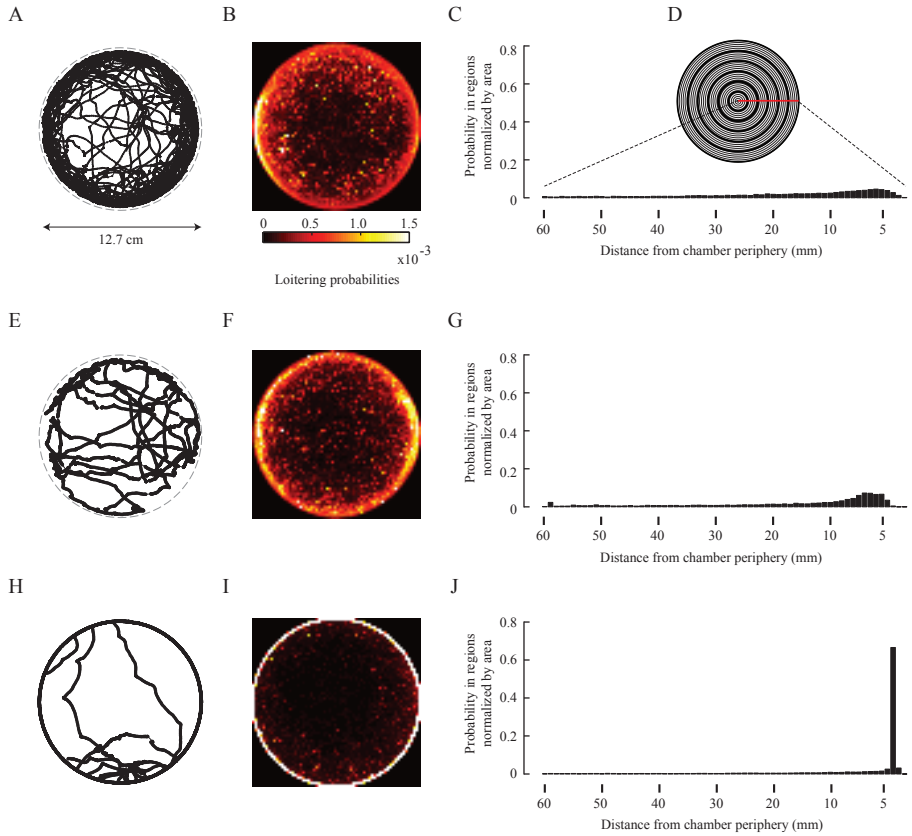

Figure 5.8: **Even without an attractive vertical wall, flies spend a significant amount of their time near the periphery of a chamber.** Representative 0.5 hour trajectories from (A) individual flies in sloped and single flies in chambers with (E) sloped and (H) vertical walls. (B) Normalized, collective transit probability over 0.5 hour for 50 individual flies moving within a group in a chamber with sloped walls. Normalized, collective transit probability over 6 hours for 13 single flies moving in chambers with (F) sloped or (I) vertical walls. (C, G, and J) Collective transit probability normalized by area in 63 concentric regions for individual and single flies. (D) Concentric annuli making up the regions were 1 mm thick.

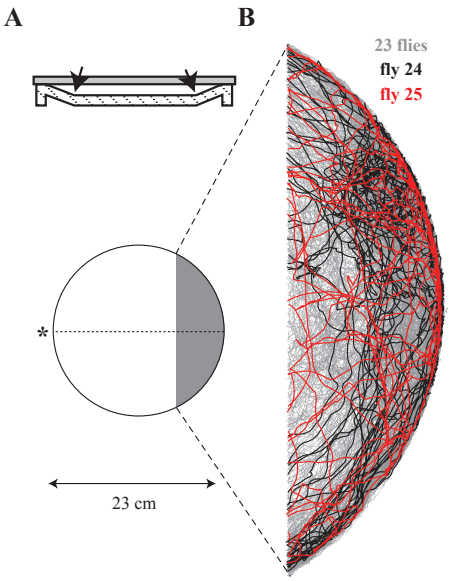

Figure 5.9: **Chambers designed with linear sloped walls are comparable to the chambers designed with sigmoid-linear walls.** (A) Side profile of linear slope along denoted cross section (asterisk). Obtuse corners between the wall and floor (arrowheads). (B) Superposition of the individual trajectories for 25 flies (gray) with the trajectories of two flies chosen randomly and highlighted (red and black lines).

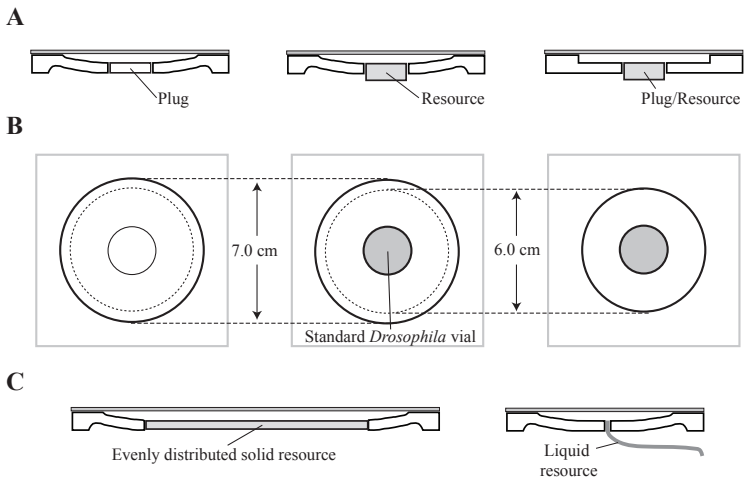

Figure 5.10: **Drawings of various chambers designed for studying social behavior.** (A) Side view drawings of chambers designed with a plug used for courtship assays and solid resource used for observations of aggression and the conventional chamber with vertical walls possessing comparable dimensions to chambers with sloped walls. (B) Corresponding top view drawings of chambers shown in **A**. (C) Alternative chamber designs that could be used for providing an evenly distributed solid resource or a liquid resource from a localized spot.

Chapter 6

Concluding Remarks

The previous four chapters document the results of experimental work carried out within a laboratory setting in order to understand various causal factors that inhibit and drive *Drosophila* from food. I have described in detail how the exploratory behavior and dispersal of flies are (Chapter 2) modulated by differences in a fly's previous mating status, (Chapter 3) regulated by its hunger state, and (Chapter 4) influenced by the persistence of a fly's characteristic individual exploration. I also described (Chapter 5) a new experimental chamber that I am currently using for studying how the movements of flies around food are modified by social context. I wrote each of the chapters of my dissertation thesis, excluding the introduction and conclusion, in the form of a manuscript so that each may be read independently from the rest. This is to assist readers in finding and re-finding particular sections, and also to serve as working drafts for the manuscript to come.

6.1 Overview of scientific contributions

With the set of experiments described within the second chapter, I studied the influence of mating experience on the movement priorities of *Drosophila*. I reported that hungry

males free from food cues in their local environment were both more active and dispersed between distinct model environments at a greater level than hungry females; the superior level of movement by males seems unrelated to their lesser weight. Adding a small patch of food within the environment decreased the dispersal of both genders and more dramatically inhibited that of males. Preventing flies from acquiring previous mating experience appeared to diminish their requirement for food – in general, food played a lesser role in inhibiting the dispersal of unmated flies, and flies left chambers that were empty of food at a lesser rate. This is consistent with a behavioral state in which flies would prioritize movements other than those in search of food. I also showed that general activity, as measured by widely utilized *Drosophila* activity monitors (DAM), could not directly explain the increase that I have observed in dispersal due to prior mating experience. From these experiments, I suggest that prior mating experience is a significant and likely an important factor modulating the dispersal of *Drosophila*, and that the change in dispersal results from a change in the fly's priorities rather than simply a change in the general levels of activity.

In chapter three, using methods similar to those used to assess the modulatory effects of mating, I explored how the amount and accessibility of food affects the dispersal of hungry *Drosophila*. Using similar densities of flies, I showed that with larger amounts of food – a simple dimension of food quality – I could inhibit the dispersal of flies to a greater extent. I reported that sated flies dispersed at a low and similar rate irrespective of the presence, amount, or accessibility of food. Moreover, I showed that hungry flies dispersed from detectible, yet inaccessible food, at a similar elevated rate as they dispersed from a chamber containing only water. From these results, I suggest that hunger regulates the dispersal of these flies independently of sensory cues arising from food. Flies homozygous for different alleles of the *foraging* gene, noted for differences in

their locomotor behavior on and around food, were comparable in their dispersal from food. Again, as with previous mating experience, results from assaying a fly's general locomotor activity indicated that a change in the intensity of activity was insufficient for explaining the hunger-induced dispersal. From these experiments, I suggest that the hunger state of flies can override the visual and olfactory cues from food, and I hypothesize that the observed increase in dispersal resulting from hunger is due to a qualitative change in locomotor behavior related to food search.

With a new machine-vision tracking strategy discussed within the fourth chapter, I studied the exploratory behaviors of individual flies as they searched near and far from a water source within the environmental chambers discussed in Chapters 2 and 3. I introduced single, isolated flies that had recently consumed food into chambers and tracked their walking and monitored their flying movements over the course of 6 or 12 hours. In some trials, each chamber was connected to a second chamber, as described within the two earlier chapters. This set up allowed me to study the transitions of the search of flies near the water source to the flies' movements that resulted in dispersing into a second, distinct environmental chamber. The motivation of this work was two-fold: (1) to quantitatively describe the exploratory movements of flies transitioning from the local, restricted search near a resource through their exploratory dispersal to distinct new environments, and (2) to statistically analyze the persistence of characteristic individual exploration-related behaviors over the several hours that I had recorded their movements. In collaboration, I have attempted to use learning algorithms based on the statistics of each fly's behavior during short windows of time [I report the analysis of 1 hour epochs within this dissertation] to predict the fly's behavior during the rest of their experimental trial. I report the findings from these studies – in an early form – on a subset of my data.

I conclude with chapter five by describing a new experimental chamber that I have conceived and developed to complement machine-vision methods for tracking individuals within large groups. The motivation behind developing these chambers was to study the changes of social interaction, e.g., courtship and aggressive posturing, as flies become hungry near detectible, although ultimately, never accessible food.

To make possible the studies discussed within this dissertation, I spent a significant amount of my time during my graduate studies developing three new, general-purpose methodologies for studying the behavioral phenotypes for flies from the *Drosophila* genus. I have provided some specifics for each methodology within the various chapters as required. In the remaining sections of this final chapter I include a general, more complete description of these new methodologies, and also I briefly describe how I am using these methodologies for my current and future experimental activities.

6.2 Creating and improving tools for quantifying complex behaviors in a genetic model organism

Researchers in thousands of laboratories around the world use the powerful molecular genetic tools developed for the fruit fly, *Drosophila melanogaster*, with hopes of better understanding the biological underpinnings driving the behavioral pathologies inflicting humans. Nearly a century of study has revealed that fruit flies possess versions of genes that are remarkably similar to those regulating human development and that these genes, when altered, contribute to disease. Discoveries in these flies have laid the foundation for placing many disease-causing genes in the context of known gene networks. It is likely that the rapid pace of fruit fly research will provide an abundant source of new

data and hypotheses to further investigate in humans and other mammalian systems. Novel and improved methodologies for better characterizing the normal behavior of fruit flies should also accelerate the discovery of the biological underpinnings contributing to human disease. During my graduate tenure, I have created and helped spearhead three general-purpose tools to better quantify the complex behavior of fruit flies.

6.2.1 Experimental biospheres for behavioral ecology

Chief among the tools that I have developed is 'Flyworld,' a flexible system that enables users to regulate and monitor the movement of flies between controlled sensory environments, thereby facilitating studies that focus on the movement preferences and behavioral priority of *Drosophila*. The building blocks of this system are cylindrical chambers 10 cm high and 10 cm in diameter that may be configured to set up model environments. Chambers may be arranged in a variety of networks connected by thin tubes that allow flies to move between environments (See Fig. 6.1). Each tube is equipped with a gate and detectors, enabling a user to regulate and count flies moving between chambers. Each sensory environment has a dedicated circuit board containing programmable microcontrollers and a handful of input/output ports. Input ports allow a user to place sensors within chambers to measure properties such as light level, sound, vibration, humidity and temperature, and also to place sensors to monitor properties such as pH and osmolarity in items positioned with chambers. Output ports enable users to drive actuators to turn on lights, play sounds, pump in gasses or liquids, drive heaters, and drive motors. This experimental platform allows a user to set up a variety of behavioral assays to test specific hypotheses.

Strengths of this system are that it is scaleable, can be used to monitor groups of

more than 1000 flies, allows users to run many trials in parallel, and is controlled using a single computer. Previous studies on the movement of flies have been carried out by hand or restricted to inflexible, small chambers with minimal sensory features. Available commercial devices are limited to measuring the level of activity for single flies within short, narrow tubes or, the intensity of aggregate activity of fewer than 100 flies in 8-cm high and 2.5-cm in diameter vials.

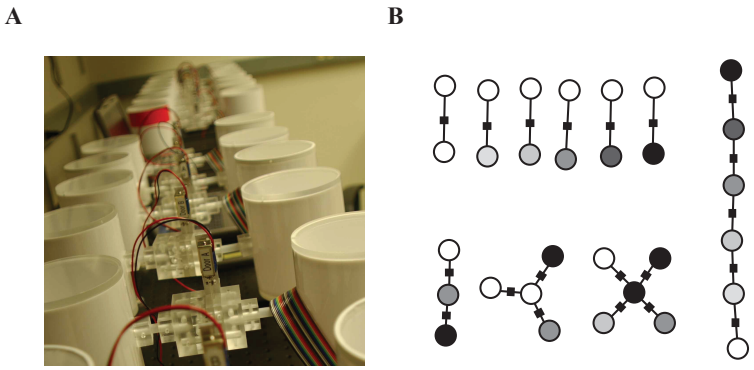

Figure 6.1: FlyWorld: a new technology devised to study the movement preferences of *Drosophila* between controlled sensory environments. (A) Photograph of an array of connected chambers. The general modular design of this system allows users the flexibility for a variety of quantitative behavioral studies. (B) Schematics of possible configurations of modeled habitats: an array of connected pairs for parallel trials (as shown within A), a linear concatenation to assay subtle preference between habitats, and various bifurcating setups that allow experiments testing the flies' choices for particular habitats. The top-down views show units denoted as black rectangles that possess doors and counters that can regulate and monitor the movement of flies between connected chambers. The shade of gray denotes different modeled habitats. Flies may be introduced into any chamber.

6.2.2 Single-camera strategy for tracking isolated individuals in three-dimensional space

Many of the behaviors exhibited by *Drosophila*, and all of the behaviors among individuals, occur while flies are on a substrate, e.g., on the ground within the leaf litter, on the trunk of a tree, or on the lip of a trash bin. To monitor the behavior and movement of individual flies, I conceived and developed a computer-tracking scheme, "FlyCam," that uses a single camera to extract the 3D trajectory of single flies walking for long durations of time, from hours to days (see Fig. 6.2). From over 1200 hours of footage of flies moving within the sensory environments described previously, flies were observed walking or standing more than 99% of the time. Video analysis of behavior traditionally requires extensive memory stores, even for short video clips that last merely seconds to minutes. Tracking strategies that do not save video data limit the characterization of specific behaviors. My strategies capitalize on custom software that requires minimal computer memory (Straw and Dickinson, 2009). This software saves a background image, a stack of cropped images that include only the small region surrounding the fly from each frame, and the 2D coordinates of a fly's image within the complete image frame. With this data I have developed software capable of reconstructing a high spatial and temporal 3D representation of the fly's movement that is cross-indexed to each original video. Data in this form allow a researcher to easily measure the quality of tracking and also provide an efficient means to extract video clips of interesting behaviors for further analysis. This software can be adapted to various chamber sizes and geometries.

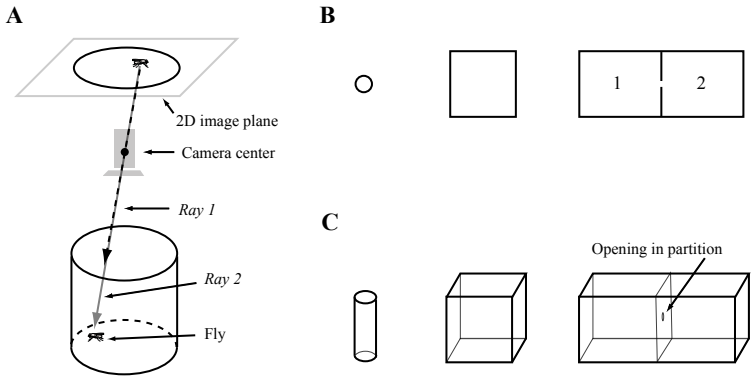

Figure 6.2: FlyCam: single camera, machine vision strategy developed to study the movement of *Drosophila* within a controlled sensory environment. (A) Illustration of two possible locations for a fly from the perspective of the single camera mounted above a cylindrical experimental arena. *Ray 1* represents the possible location of a fly on the underside of the chamber lid (dashed; black arrow); *Ray 2* indicates the true location of the fly in this illustration (solid; gray arrow), which sits on the chamber floor. (B) Top views for various experimental arenas: a standard *Drosophila* vial, a rectangular arena, and an arena partitioned by a thin wall into two regions, 1 and 2. (C) Corresponding side views for the various experimental arenas in B.

6.2.3 Sloped-walled chamber for studies of social behavior

My third contribution is "Flybowl," a multi-purpose observational chamber. I conceived, designed, and built this chamber to complement computer-vision tracking algorithms for studying the behavior of individual flies within a group. My innovation is relatively simple – a shallow chamber with sloped walls (see Fig. 6.3). The sloped wall has three functions. First, sloped walls restrict flies from moving to the ceiling and thus passing over flies on the floor. Preventing foreground objects from overlapping is paramount for computer vision-based tracking systems attempting to retain the identity of individuals. In the past, researchers have restricted the movement of flies to flat surfaces by clipping their wings and designing experimental chambers with barriers that flies find difficult to cross, e.g., water moats (Götz and Wenking, 1973) and heated walls (Branson et al., 2009). These past chamber designs required significant development and maintenance. More importantly, the removal of a fly's wings has effects beyond abolishing flight. Flies use their wings to communicate, such as during courtship and bouts of aggression. Second, by restricting flies from moving among the floor, wall, and ceiling, movement that is possible within conventional chambers with vertical walls, chambers designed with sloped walls limit the deviation in the flies' appearance. This reduces the frequency of erroneous classification and detection of behaviors. A frustration in many behavioral assays is that flies spend a majority of their time clustered in the crack between the wall and the floor of a chamber. The third function of the sloped wall is that it eliminates the attractive crack from the chamber, promoting a more uniform spacing between flies. This makes tracking easier and also keeps flies in the center of a chamber engaging with a specific experimental set up.

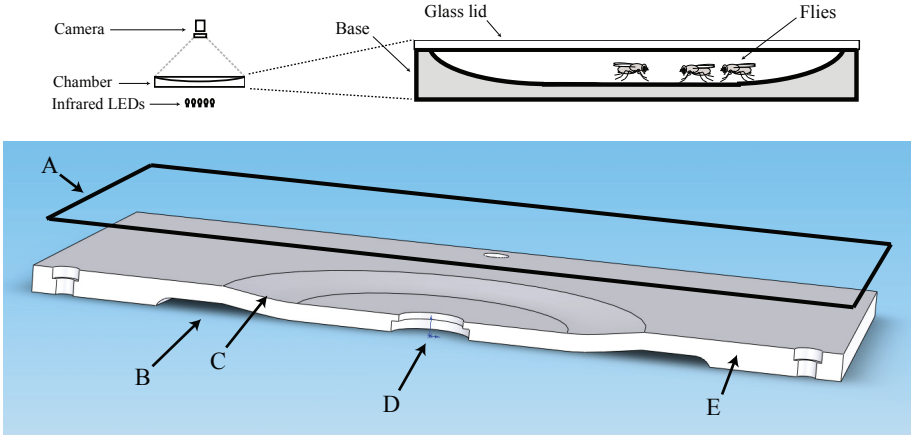

Figure 6.3: Illustrations and CAD drawing of the basic features of FlyBowl. (A) Glass lid is coated with a slippery, but clear, silicone paint preventing flies from hanging from the lid while allowing visible light to penetrate through to the chamber. (B) Base is undercut to keep constant thickness for uniform backlighting. (C) Sloped wall of chamber has dual functions: preventing flies from walking on to the lid of the chamber and also keeping flies from clustering in the crack between the wall and the chamber floor. (D) Base can be designed with hole(s) in the floor to embed standard fly husbandry vial(s). (E) Base is made from diffuse material allowing for IR backlighting.

Using the tools described above, or tools inspired by these devices, I hope researchers may create an experimental research program consisting of rapid, quantitative, high-resolution behavioral studies to better describe the rich repertoire of normal behaviors displayed by fruit flies as well as abnormal behaviors that might have disease correlates in humans.

6.3 Future directions: the effects of hunger on social behavior near food

Using the system of environmental chambers described within this dissertation, I have carried out experiments from which I suggested that the hunger state of *Drosophila* can override the visual and olfactory cues from food. I further hypothesized that the observed increase in dispersal resulting from hunger was due to a qualitative change in locomotor behavior related to food search, rather than simply resulting from a change in the level of the fly's general locomotor activity. The specific findings that support my conclusion are as follows: (1) Hungry flies did not disperse from chambers containing a patch of food at the level they would have if the chamber contained only water, but rather at a greatly inhibited rate (see Fig. 3.1). (2) Sated flies dispersed at a similar rate irrespective of the amount or accessibility of food contained within a chamber; this rate was much lower than that of hungry flies from chambers that contained only water, and was slightly higher than the rate hungry flies left chambers containing food (see Fig. 3.1). (3) Hungry flies left inaccessible food that was covered by a mesh – not at an intermediate rate, as might be expected – but as if no signs of food were present within the chamber (see Fig. 3.2). (4) The food covered by a mesh *was* in fact

detectible by the flies (see Fig. 3.3). (5) Experiments using a commercially available *Drosophila* activity monitor to measure a fly's general locomotor activity (i) during the same period of time and (ii) attempting to match the level of hunger of the flies run in my dispersal experiments indicate that a change in the intensity of activity was insufficient for explaining the hunger-induced dispersal (see results within text in Chapter 4). (6) Using the machine vision tracking strategy described within this dissertation, I showed that the locomotor activity of flies increased for the first 1≈3 hours in the morning and then leveled off, dipped in the middle of the day, and remained steady until increasing again in the evening (see Fig. 4.3). The level of this activity was comparable with that observed by Martin (Martin, 2004) over the matched window of time since the flies last consumed food. My two independent measures of general activity, consistent with Martin's study using a different strain and independent experimental practices, strengthens the hypothesis that hunger is not increasing the general activity of the flies during the period I observed their dispersal. Using a recently developed multiple-fly tracking methodology (Branson et al., 2009) and the new experimental chamber I have most recently developed, I am continuing to carry out experiments to directly observe the movement of individual sated flies within groups as they become hungry, and also I am monitoring the response of hungry flies as they disperse from patches of accessible and inaccessible food (see Fig. 6.4). In conjunction with this direction of investigation, I am also studying the changes in social behavior, i.e., courtship and aggressive posturing, of flies as they become hungry but cannot access – yet can detect – food in their local environment.

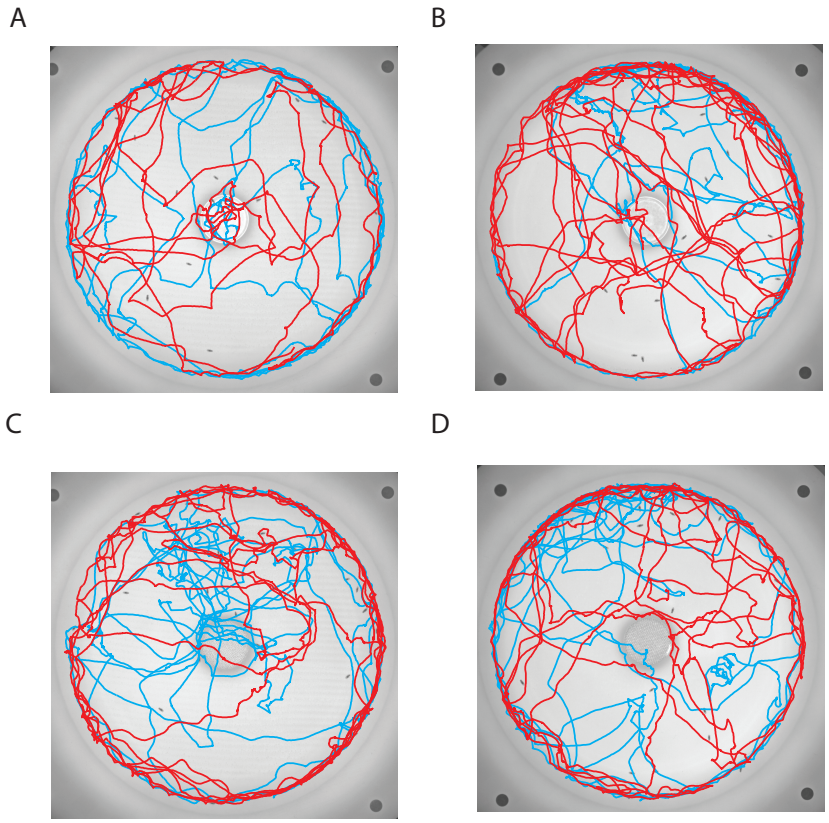

Figure 6.4: Preliminary results from observations for groups of hungry *Drosophila* near inaccessible food. Superposition of the individual trajectories of 2 selected from 25 flies moving for 10 minutes within a chamber containing (A) food, (B) agar, (C) food covered by a mesh, and (D) agar covered by a mesh. Trajectories for the two flies (blue and red) were randomly selected.

Appendix A

Annual Report 2004: FlyWorld

A.1 Introduction of 'FlyWorld'

Observation rather than experimentation dominates the study of animal behavior, limiting our understanding. We require the ability to study behavior in which aspects of an animals environment can be controlled. To meet this goal, we built a multi-chambered, biosphere in which we can control parameters to replicate and examine pertinent aspects of an animals natural environment, while precisely quantifying its behavior. 'FlyWorld' allows both controlled input manipulation and precise behavioral quantification, while capable of parallel, high-throughput analysis essential for neuro-genetic studies in the fruit fly *Drosophila melanogaster*. Whereas we built this tool for studies of the elementary decision processes in *Drosophila*, its design being both general and modular anticipates a variety of future behavioral studies.

A.2 Modular, experimental chambers

'FlyWorld' is designed to give a researcher the control and quantitative means to compare the behavior of *Drosophila* both within parallel experimental runs on a single day

as well between trials run over separate days. This tool incorporates commercially available regulated environmental incubators (capable of creating various ambient temperatures, photoperiods, and monitoring humidity) to house isolated chambers of our own design connected by tubing that may be combined in various conformations (see Fig. A.1). Automated, solenoid-driven gates and infrared sensors together control and quantify locomotor behavior between the chambers described above (see Fig. A.2). The general, modular design allows the experimental flexibility for use in a variety of behavioral studies that include parallel configurations necessary for high-throughput experimentation for anticipated neuro-genetics studies.

A.3 Dedicated circuit boards

Dedicated, programmable microprocessors incorporated in circuit boards of our own design keep computation local and thus allow a single computer the capacity to run over 900 modules (see Fig. A.3 and Fig. A.4). The Atmel ATMega8 microprocessors we use are easily programmed with any compatible Atmel programmer. Activation of the gates between chambers is controlled through H-bridges (L293DNE). And finally, additional connections are incorporated into our circuit design to anticipate future experiments. Some examples of possible inputs are surface temperature readings through a thermocouple and humidity, gas, or vibration sensors. Examples of outputs are solenoid-driven doors to release animals, uncover food, or power to drive countless other devises.

A.4 User-friendly software

The final component of FlyWorld is a home-born computer software package to query and analyze the bi-directional counts from the microprocessors as well as drive gates between chambers to a variety of control networks. For example, we can specify that either all gates or a subset may be opened or closed by a specified time, or a directional count in a specified counter block. A user-friendly GUI (graphical user interface) aids in the setup of experiments (see Fig. A.5).

A.5 High-throughput, quantitative behavioral studies

Abstract: Ethological studies on resource-emigration in the fruit fly, *Drosophila melanogaster*

A new direction for the Dickinson laboratory is to understand how the simple nervous system of the fruit fly *Drosophila melanogaster* supports sophisticated behaviors such as decision making. Specifically, we aim to study decisions involved in resource assessment, an element of habitat selection. We propose a quantitative analysis of specific external, internal, and intrinsic factors that appear to influence emigration behavior from an established food resource. The objective is to establish a research program to assist in the defining of genes and neural hierarchies involved in elementary decision processes (see Fig. A.6 for an example of preliminary results).

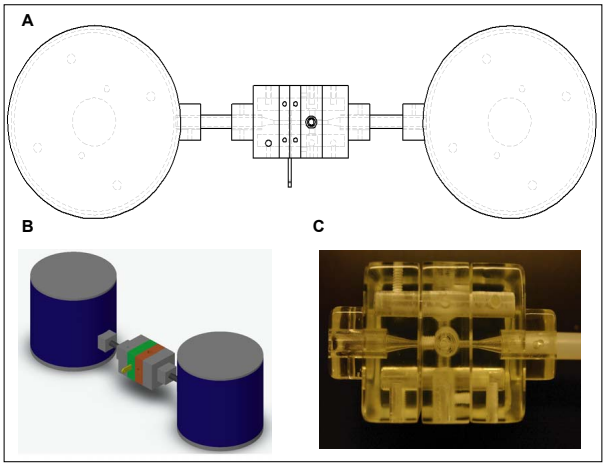

Figure A.1: Prototype components of the 'FlyWorld' apparatus. (A) Top schematic view of simple configuration consisting of two canisters, connector tubing, detector, and gate. (B) CAD-rendered view of configuration drawn in A. (C) Photograph of prototype detector block fabrication from stereo lithography.

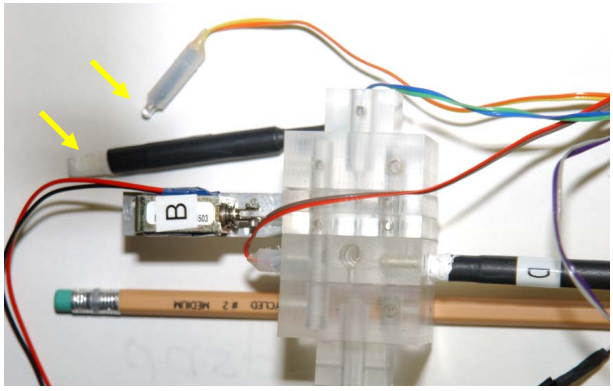

Figure A.2: Photograph of detector block and solenoid-driven gate. One of two infrared LED emitter and infrared photodiode detector pairs (arrows) that make up the bi-directional counter has been removed and displayed along the detector and gate unit.

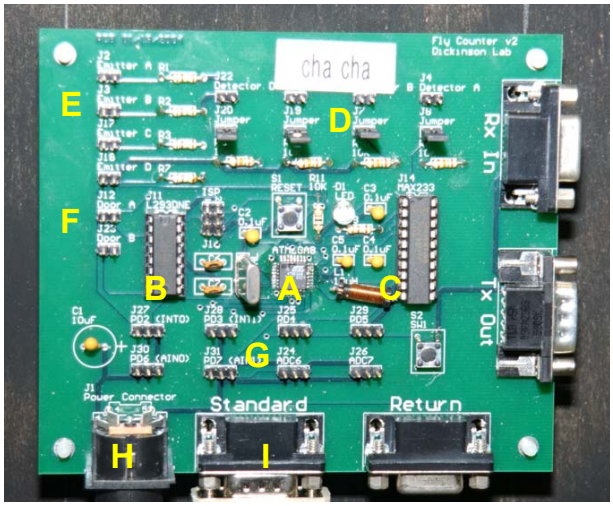

Figure A.3: Photograph of the circuit board with key components identified. (A) Atmel microchip, (B) H-bridge to drive gates, (C) inductor/capacitor act to filter oscillations in power required for the analog to digital converter needed to read signals from infrared detectors, (D) detector jumpers, (E) emitter lines, (F) gate activation lines, (G) extra connections for future experiments, (H) 12V and 5V power input, and (I) serial ports to connect circuit board to computer.

Figure A.4: Photograph of the FlyWorlds in a room with temperature and photoperiod-control. The parallel, two chamber configuration is used for experiments described within this report.

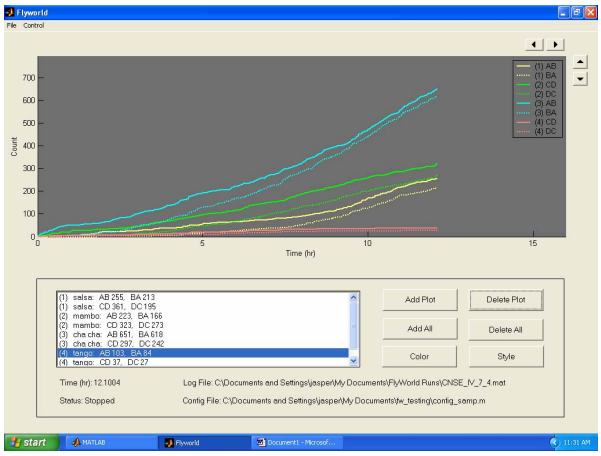

Figure A.5: A computer "screen shot" of our software's application window. A reader can see pull-down menus and toggle buttons that enable the experimenter to modify experimental parameters. Here we test the influence of food deprivation and genetic variation on the baseline locomotor behavior of 50 flies per experiment between two chambers ("blue" and "red" traces are a natural fly isolate collected from Chicago, IL deprived of food for 12 or 36 hours respectively; the "green" and "yellow" traces are two distinct isolates collected by others from Toronto, Canada and deprived of food for 12 hours).

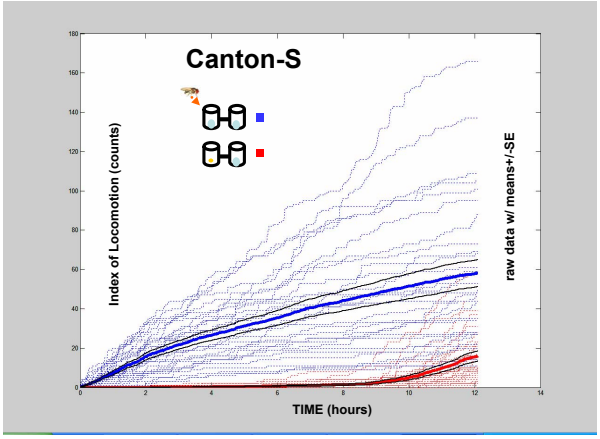

Figure A.6: An example of the results where we establish emigration baselines by manipulating various experimental parameters. Shown above is the baseline locomotor behavior from a first to a second chamber ≈50 12-hour food-deprived flies (blue) as compared to the locomotor behavior of ≈50 12-hour food-deprived flies introduced to a first chamber that contains a small food resource (red). Here we examine Canton-S the widely-used fly stock from which many molecular-genetic tools have been derived. We manipulate the amount of food resource to be consumed over the time course of the experiment. While initially the flies stay in the first chamber, eventually they become food-deprived and emigrate to the second chamber presumably in search of food.

Appendix B

Supplementary Materials

B.1 Density affects dispersal

B.2 Chamber geometry affects dispersal

B.3 Numbers of drosophilid flies emerging from different breeding sites

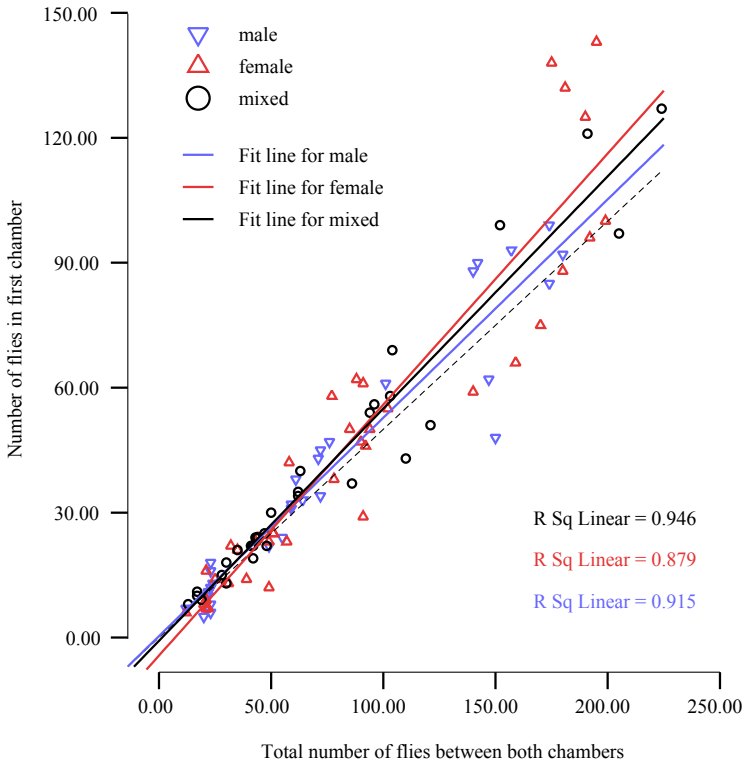

Figure B.1: Dispersal movement of flies scales with group density. Groups of flies consisting of <10 individuals to 250 individuals dispersed comparably between two connected chambers of the type described within this dissertation, which were empty of food or water, irrespective of whether groups of flies were comprised of a single gender or were mixed gender. Comparable effects of density were observed for larger groups, and if observed over a longer period of time when food was present in both chambers.

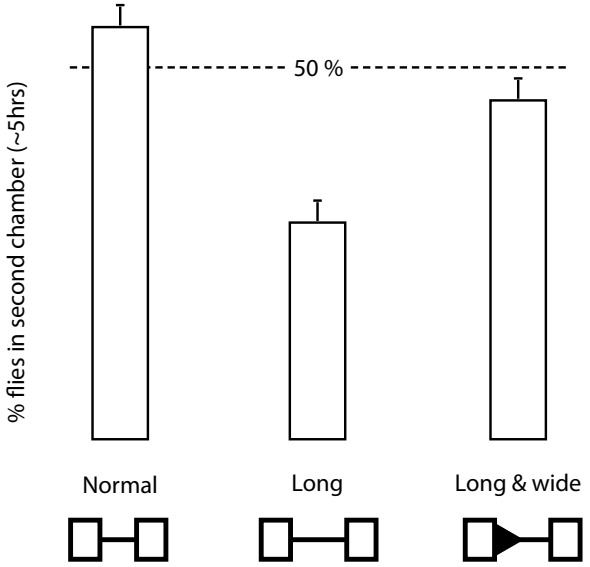

Figure B.2: Arena geometry affects the dispersal of flies between two connected chambers. After 5 hours, flies from a group introduced to the first of two connected chambers were equally distributed between the chambers (Normal). The number of flies moving into the second chamber was less if the channel connecting the chambers was lengthened (Long). The original equal distribution could be restored if the opening to the channel connecting the chambers was widened (Long and Wide). The chambers used for these experiments were the same as those described within this dissertation apart from the changes mentioned.

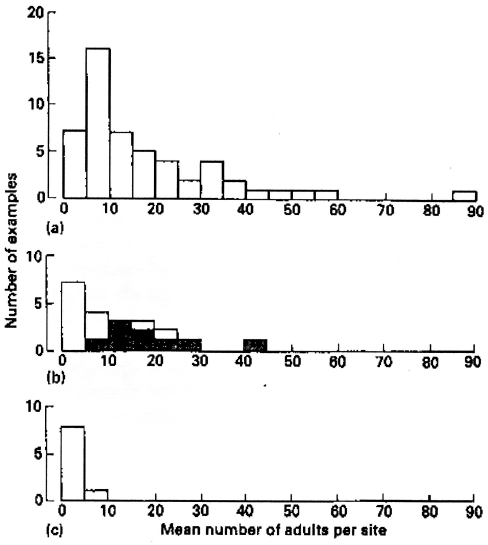

Figure B.3: Frequency histogram of mean numbers of drosophilid flies emerging from different breeding sites. (A) Fungi, (B) fruit, and (C) flowers (After (Shorrocks and Rosewell, 1986)).

Appendix C

Animal Behavior Interest Group (ABIG)

Figure C.1: Graphic from **ABIG website**

During the first year after joining my thesis laboratory, I decided that the folks interested in animal behavior were spread too thinly throughout the Caltech community. I decided to start the focus group, ABIG. I invited and hosted speakers from within and off campus for three years, until I began preparing for fatherhood. The laboratories involved and the speakers and the titles of their talks are documented below.

C.1 Laboratories affiliated with ABIG (behavior; animal; technique)

Adolphs (social cognition, emotion; human; neuropsychology, neuropsychiatry, and neuroimaging)

Andersen (sensory guided behavior, decision making; primates; multi unit recording, brain imaging, electrophysiology, neural prosthetics)

Anderson (fear, anxiety, emotion; fly, mouse; molecular genetics)

Allman (primate brain evolution, functional imaging, stereology)

Benzer (fly; molecular genetics)

Dickinson (flight; fly, bee, moth, humming bird; electrophysiology, robotics, behavioral analysis)

Koch (visual attention and awareness, associative conditioning; mouse, human)

Konishi (song bird, owl; electrophysiology)

Laurent (olfactory learning; fly, bee, locust; electrophysiology, two-photon imaging, modeling)

Lester (nicotine addiction, movement disorder, and epilepsy; mouse; electrophysiology, optical probes, reward)

O" Doherty (human fMRI, conditioning, reward, decision making)

Patterson (acoustic startle, social interaction, ultrasonic vocalization, learning & memory, anxiety, motor coordination; mouse; human disease model)

Schuman (learning & memory; rat; electrophysiology)

Siapas (learning & memory; rat; multi-electrode recordings)

Sternberg (mating behavior, locomotion, mechanosensation; nematode; genetics, evolution, machine vision analysis)

C.2 ABIG speaker schedule

C.2.1 Spring 2006

April 10th **Larry Swanson**, Biological Sciences, Psychology, Neurology, USC, "Structural organization of neural networks underlying mammalian eating and drinking behaviors."

April 17th **David Lentink** [van Leeuwen Lab], Wageningen University, "How swifts control their glide performance with morphing wings."

April 24th **Walter Lerchner** [Anderson Lab], "Neurons turning silent - Mice turning silently."

May 1st **Yan Zhu** [Zipursky & Frye Labs], UCLA, "The Fly Stampede: dynamics of visually forced walking behavior in fruit flies."

May 8th **Elissa Hallem** [Sternberg Lab], "The molecular and cellular basis of infectivity in the insect parasitic nematode Heterorhabditis bacteriophora." (Noyes 153).

May 15th **Allyson Whittaker** [Sternberg Lab], "Turning inside out: Control of locomotory behavior during *C. elegans* male mating."

May 22nd **Michelle Arbeitman**, Biological Sciences, USC, "Genomic and molecular analyses of *Drosophila* courtship behaviors."

C.2.2 Winter 2006

Jan. 23rd **Gaby Maimon** [Dickinson Lab], "Monkey parietal cortex and the internal timing of action."

Jan. 30th **Mike Reiser** [Dickinson Lab], "Visually-mediated control of translatory flight in *Drosophila*."

Feb. 6th **Jagan Srinivasan** [Sternberg Lab], "Conservation of sensory neuron polymodality during evolution of free-living nematodes."

Feb. 13th **Genes & Behavior Conference 2006**.

Feb. 27th **Joy Goto**, Division of Neurosciences, Beckman Research Institute, City of Hope, "From Fruit bats to Fruit flies: A Drosophila Model of ALS-PDC (amytrophic lateral sclerosis-Parkinsonian dementia complex)."

March 6th **Dick Zimmer**, Department of Ecology and Evolutionary Biology, UCLA, "Chemical communication and the language of sperm and egg."

March 13th **Amber Southwell** [Patterson Lab], "Anti-Huntingtin antibodies as a therapeutic for Huntington's Disease."

C.2.3 Fall 2005

Oct. 17th **Paul Patterson**, "Neuroimmune interactions in schizophrenia and autism: an animal model."

Oct. 24th **Biology retreat weekend**.

Oct. 31st **Peter Narins**, Department of Physiological Science and Biology, UCLA, "Communication at extreme frequencies: pushing the vertebrate limits."

Nov. 7th **David Chang** [Anderson Lab], "Rapid, systematic enhancer element screen using brain slice electroporation."

Nov. 14th **SFN: Society of Neuroscience 2005**.

Nov. 21st **Glenn Turner** [Laurent Lab], "Olfactory representations in the *Drosophila* mushroom body."

Nov. 28th **Marla Sokolowski**, Biology Department, U. of Toronto, Mississauga, Invited Graduate Student Chalk Talk: "The rover/sitter story: a personal account."

[Tuesday Nov. 29th **Marla Sokolowski**, 4PM Department Talk: "Fine Dining: A role for cGMP protein kinase in behaviour."]

Dec. 5th **David Chang** [Anderson Lab], "Strategies to improve the functional manipulation of brain region-specific expression."

Allyson Whittaker [Sternberg Lab], "Turning inside out: Control of locomotory behavior during *C. elegans* male mating." (**postponed**).

C.2.4 Spring 2005

April 4th **John O'Doherty**, "The neural correlates of behavioral preference."

April 11th **Stephanie White**, Physiology Department, UCLA. "A fox in the finch coop, or, candidate molecules in birdsong."

April 18th **Jagan Srinivasan** [Sternberg Lab], "Evolution of the polymodal neuron: Comparative analyses of behavior in nematodes."

April 25th **Ben Rubin** [Laurent Lab], "The honey bee dance language."

May 2nd **Curtis Loer**, Biology Department, UCSD, "Evolution of behavior and neuroanatomy in free-living nematode relatives of *C. elegans*."

May 9th **David Glanzman**, Physiology, Neurobiology Department, UCLA, "The Role of Modulation of AMPA Receptor Function in Behavioral Sensitization in Aplysia."

May 16th* **Marla Sokolowski**, Biology Department, University of Toronto, Mississauga, "Invited Graduate Student Chalk Talk." [*note specific date not final.]

May 23rd **Jasper Simon** [Dickinson Lab], "FlyWorld: a high-throughput instrument kit to study the genetics, neural circuits that underlie complex behavioral traits of *Drosophila*."

C.2.5 Winter 2005

January 31st **Bruno van Swinderen**, NSI, San Diego "Behavioral and electrophysiological measures of selective attention in *Drosophila*."

February 7th **Joanna Jankowsky** [Lester Lab], "Tetracycline-controlled APP transgenics: new mouse models for Alzheimer's disease."

February 14th **Carlos Fonck** [Lester Lab], "Spatial-temporal separation of seizure circuits in knock-in mice with hypersensitive nicotinic receptors."

February 28th* **Liz Phelps**, Department of Psychology, NYU, "Discussion Topic: Emotion, Cognition and the Human Amygdala." [*note BBB24 from 1-2PM.]

March 7th **Titus Neuman** [Dickinson Lab], "Towards the virtual fly: A computational approach to insect behavior."

March 14th **James Tong** [Wallace Lab], MAMMAG, UCI, "Dial "M" for Mitochondria–Mitochondrial Dynamics in Longevity and Memory."

C.2.6 Fall 2004

Oct. 4th **Henry Lester**, "A mouse genetic model for some aspects of nicotine dependence."

Oct. 11th **Michael Fanselow**, Department of Psychology, UCLA, "The Hippocampus and Pavlovian Fear Conditioning: A Rodent Model of Episodic Memory."

Oct. 18th **Biology Department retreat**.

Oct. 25th **Edward B. Lewis Memorial** (Beckman Auditorium, 2 to 4 PM; Reception in Dabney Gardens, 4 to 5 PM)

Nov. 1st **Joy Goto** Division of Neurosciences, Beckman Research Institute, City of Hope, "From Fruitbats to Fruitflies: A Model of ALS-PDC (amytrophic lateral sclerosis-Parkinsonian dementia complex)."

Nov. 8th **Jan Karbowski** [Sternberg Lab], "Robust sinusoidal locomotion of *Caenorhabditis* worms: Integrating theory with genetics."

Nov. 15th **Seth Budick** [Dickinson Lab], "Free flight responses of *Drosophila melanogaster* to attractive odorants."

Nov. 22nd **Karli Watson** [Allman Lab], "Neuroanatomy, functional imaging of Von-Economo's spindle cell regions: evidence of a recent event in primate brain evolution."

Nov. 29th **Break for Thanksgiving**

Dec. 6th **Gary Schindelman** [Sternberg Lab], "Characterization of the sperm transfer step of male mating behavior of *C. elegans*."

C.2.7 Spring 2004

April 5th **David Krantz**, Department of Psychiatry and Behavioral Science, UCLA, "The Making of a Well-Groomed Fly."

April 12th **Ralph Adolphs**, "How can we measure human social behavior? Studies in lesion patients, autism, and Williams syndrome."

April 19th **Bader Al-Anzi** [Benzer Lab], "The isolation and characterization of X chromosome mutations that cause obesity in the fruit fly *Drosophila melanogaster*."

April 26th **Allyson Whittaker** [Sternberg Lab], "Genes and circuits controlling *C. elegans* male mating behavior."

May 3rd **Doug Altshuler** [Dickinson Lab], "Of hummingbirds and helicopters: foraging, competition, and flight behavior."

May 10th **Chris Cronin** [Sternberg Lab], "Quantifying worm behavior using the Tracker."

May 17th **Mckell Carter** [Koch Lab], "Explicit and Implicit Learning in Humans and Rodents."

May 24th **Greg Suh** [Anderson Lab], "Feeding and defending fruit flies, genetic and circuit analyses of these innate behaviors."

C.2.8 Winter 2004

Jan. 12th **Paul Sternberg**, "Genetic control of *C. elegans* mating behavior."

Jan. 18th **Institute holiday**

Jan. 26th Organizational meeting/coffee

Feb. 2nd **Erin Schuman**, "Plasticity and memory consolidation in hippocampal circuits."

Feb. 9th **David Anderson**, "Genetic analysis of circuits for innate defensive behaviors in flies."

Feb. 16th **Institute holiday**

Feb. 23rd **Dai Watanabe** [Konishi Lab], "Transgenic approach to interneurons in cerebellar and retinal circuits."

March 1st **Jose Pena** [Konishi Lab], "Neural computation for sound localization in the owl."

March 8th **Tim Lebestky** [Anderson Lab], "Panicking Flies: modeling emotional responses in *Drosophila*."

March 15th **Rachel Wilson** [Laurent Lab], "Olfactory Representations in the *Drosophila* Brain: Electrophysiology, Anatomy, Genetics, and Behavior."

C.2.9 Fall 2003

Oct. 20th **Michael Dickinson**, "Flight behavior in *Drosophila*."

Oct. 27th **Mark Konishi**, "Current issues in birdsong research."

Nov. 3rd **David Anderson**, "Neural correlates of fear and anxiety in mice."

Nov. 10th none (**Neuroscience Meeting**)

Nov. 17th **Malcom Gordon** Department of Biology, UCLA, "Fish out of water: behavioral adaptations of amphibious fishes."

Nov. 24th **Teresa Nick** [Konishi Lab], "Neural correlates of the template: Song memory and learning in the zebra finch."

Dec. 1st **Mark Frye** [Dickinson Lab], "Edge orientation for visual course control in *Drosophila*."

Dec. 8th **Mark Zylka** [Anderson Lab], "Molecular and genetic analysis of mammalian pain circuitry and behavior."

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