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.
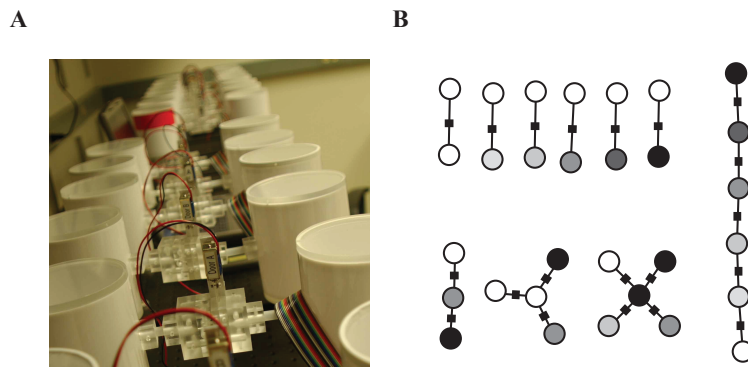

Figure 6.1: FlyWorld: a new technology devised to study the movement preferences of *Drosophila* between controlled sensory environments. (A) Photograph of an array of connected chambers. The general modular design of this system allows users the flexibility for a variety of quantitative behavioral studies. (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.
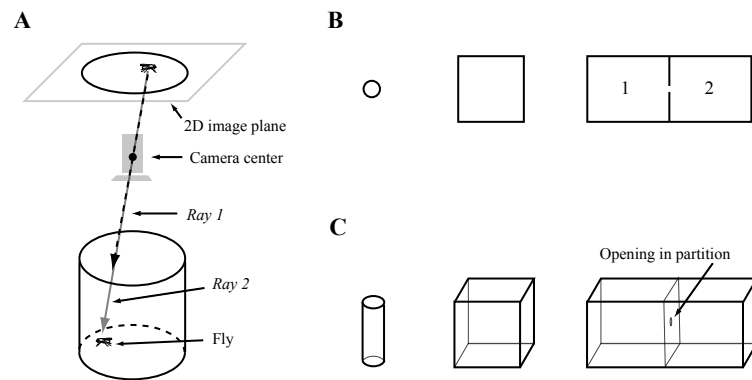
Figure 6.2: FlyCam: single camera, machine vision strategy developed to study the movement of *Drosophila* within a controlled sensory environment. (A) Illustration of two possible locations for a fly from the perspective of the single camera mounted above a cylindrical experimental arena. *Ray 1* represents 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.
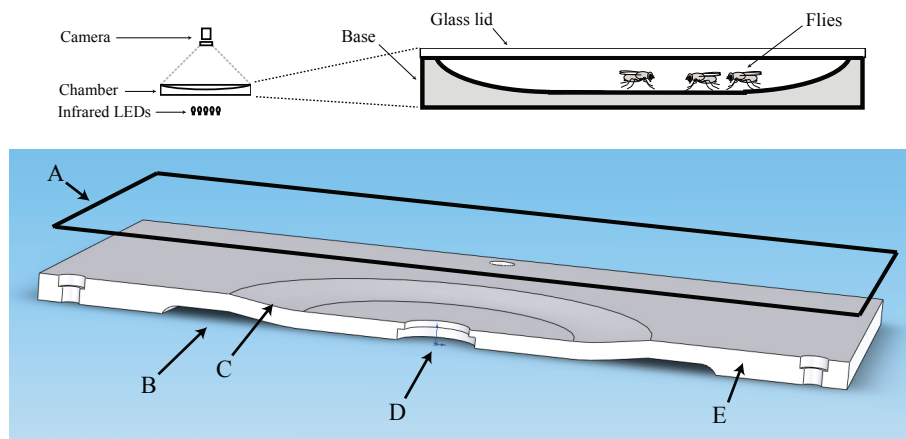
Figure 6.3: Illustrations and CAD drawing of the basic features of FlyBowl. (A) Glass lid is coated with a slippery, but clear, silicone paint preventing flies from hanging from the lid while allowing visible light to penetrate through to the chamber. (B) Base is undercut to keep constant thickness for 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 $1 \approx 3$ 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.
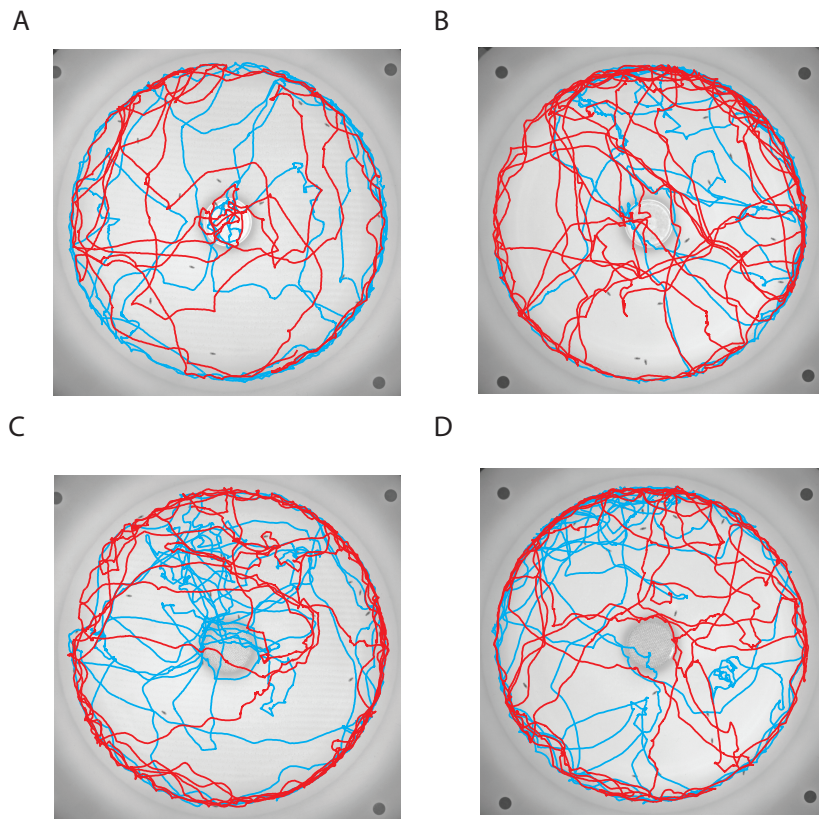
Figure 6.4: Preliminary results from observations for groups of hungry *Drosophila* near inaccessible food. Superposition of the individual trajectories of 2 selected from 25 flies moving for 10 minutes within a chamber containing (A) food, (B) agar, (C) food covered by a mesh, and (D) agar covered by a mesh. Trajectories for the two flies (blue and red) were randomly selected.