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

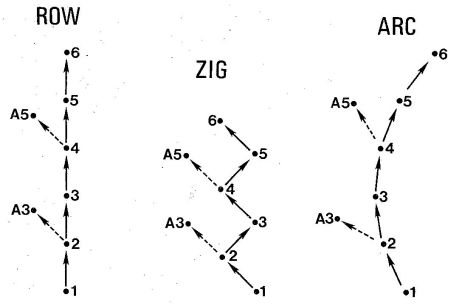

Figure 1.1: Various patterns of sucrose drops used to demonstrate that *Drosophila* possess short-term retention of the spatial patterns of resources making their search for food more efficient. As flies moved along the various patterns, they located the alternative drop, "A5," significantly fewer times 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

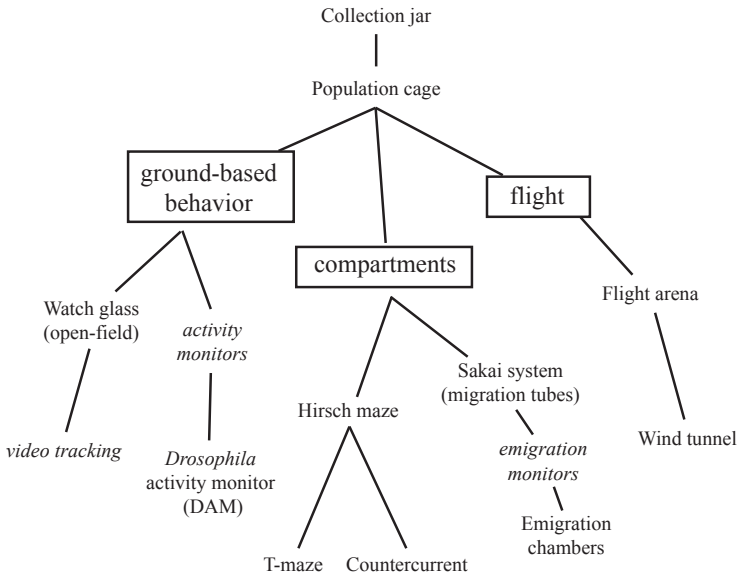

Figure 1.2: Experimental chambers for studying freely moving *Drosophila*. Chambers are categorized into types that have been developed for studying flight, ground-based behaviors, and networks of compartments allowing users to partition flies. The Sakai system was instrumental in shaping the type of 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-

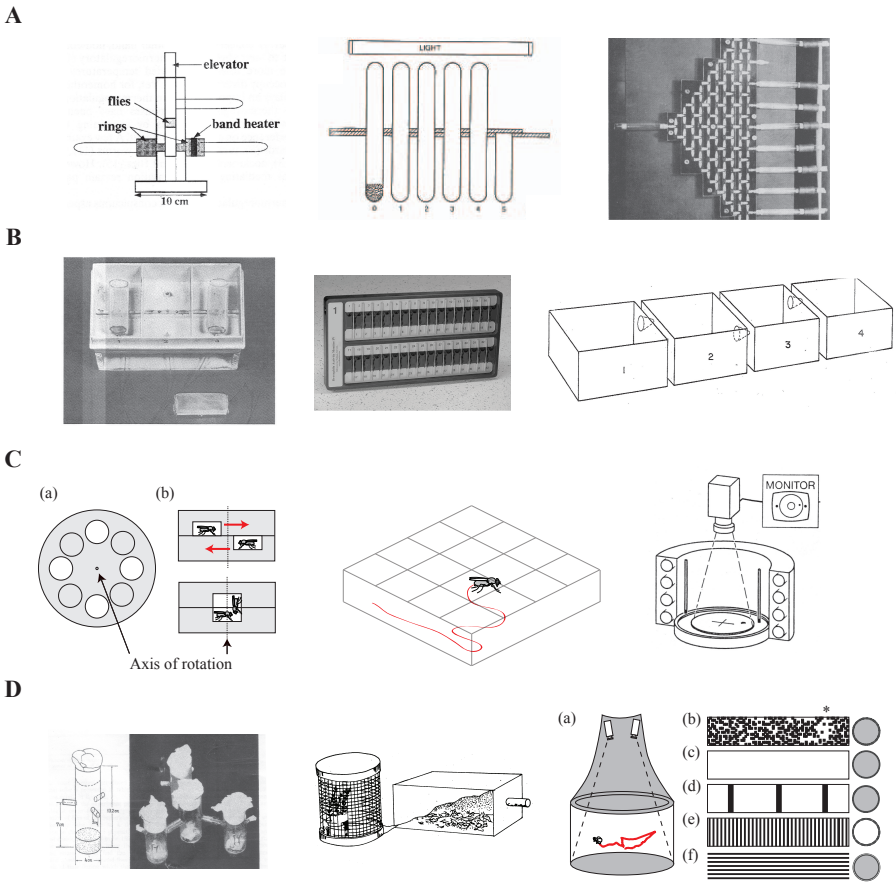

Figure 1.3: Images and illustrations for various experimental apparatuses used for studying the behaviors of freely moving *Drosophila*. (A) Apparatuses designed for assessing forced choices: "T-maze" (taken from (Sayeed and Benzer, 1996)), "Countercurrent" (taken from (Benzer, 1973)), and "Hirsch maze" (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).