Chapter 2

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

2.1 Summary

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

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

2.2 Introduction

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

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

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

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

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

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

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

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

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

2.3 Materials and Methods

2.3.1 Animals

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

2.3.2 Animal handling

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

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

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

2.3.3 Experimental test chambers

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

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

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

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

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

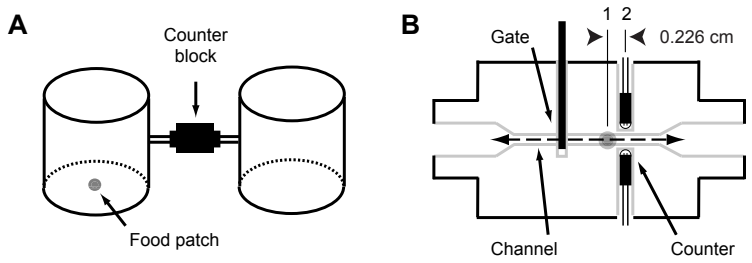

Figure 2.1: Instrument devised to study the movement of *Drosophila* between controlled sensory environments. (A) Illustration showing two experimental chambers connected by tubes feeding into the opposite sides of a counter block. For dispersal experiments, we either did or did not provide a patch of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

2.3.4 Dispersal assay protocol

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

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

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

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

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

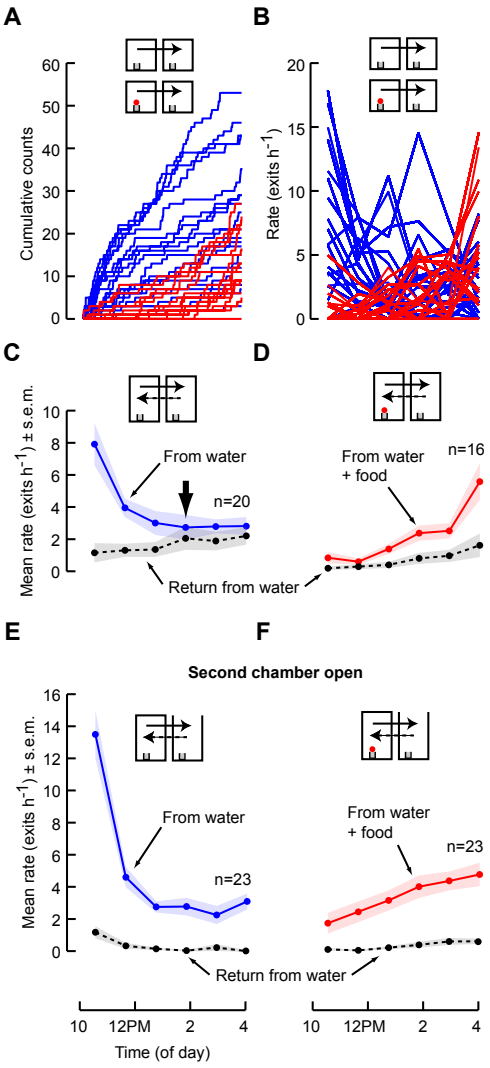

Figure 2.2: Sample data showing the movement of flies between two connected chambers containing either water and a patch of food or water alone. (A, B) Cumulative forward counts and corresponding forward rates from independent trials for transitions from only water (blue, 2 mL 0.5% agar) and water and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

2.3.5 Justification for using a natural isolate

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

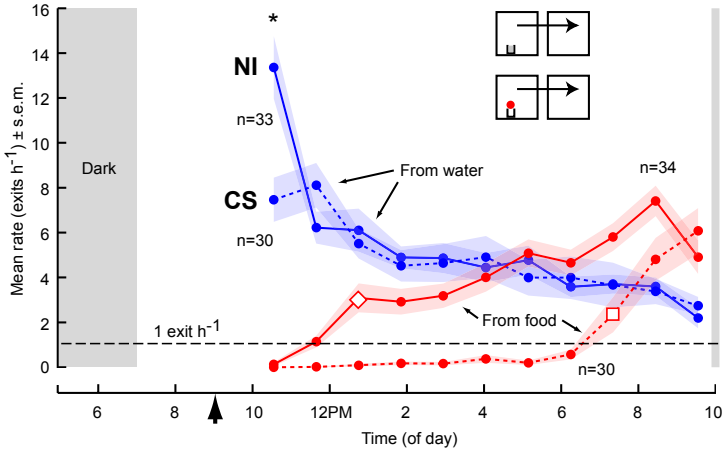

Figure 2.3: Genetic background is an important factor in the dispersal of *Drosophila* from food. The figure shows the dispersal rates of the natural isolate (NI, solid) used throughout this report and Canton-S (CS, dashed) from chambers that contained water and a patch of food (red, 65 μL on 2 mL of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

2.3.6 Activity experiments

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

2.4 Results

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

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

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

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

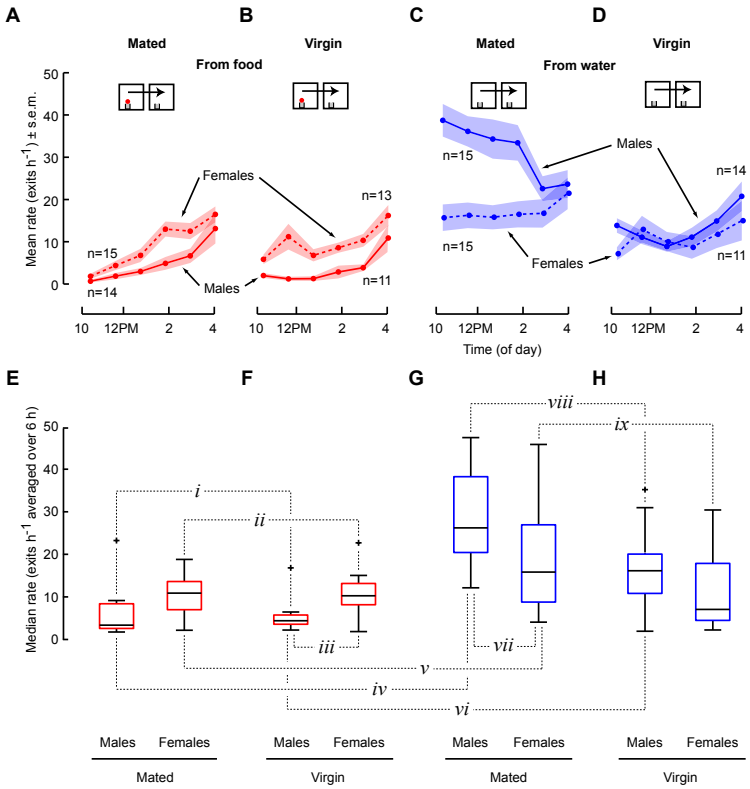

Figure 2.4: Mated flies are more sensitive than virgins to both the presence and absence of food. (A, B) In the presence of food (red, 65 μL on 2 mL 0.5% agar), (A) previously mated males (solid) and females (dashed) dispersed slower than (B) virgin males (solid) and virgin females (dashed). (C, D) In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

2.4.2 Males disperse at a higher rate than females.

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

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

2.4.3 Males are more active than females.

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

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

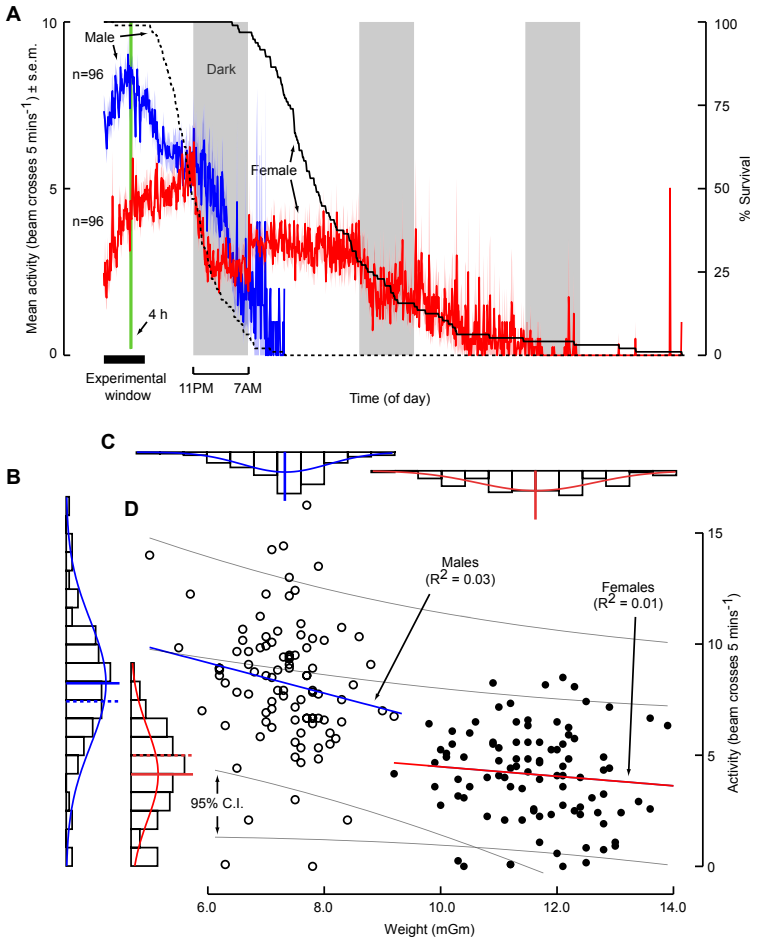

Figure 2.5: Gender and not weight explains why males are more active than females. We measured the activity of individual flies using the commercially available *Drosophila* Activity Monitor (DAM). (A) Male flies (blue) exhibited a significantly greater mean activity level than females (red) during the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

2.4.4 Weight does not explain the greater activity of males.

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

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

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

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

2.5 Discussion

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

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

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

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

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

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

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

perhaps any stock cultivated too long within the laboratory.

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

2.5.1 Supplementary Figure and Table

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

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

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

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

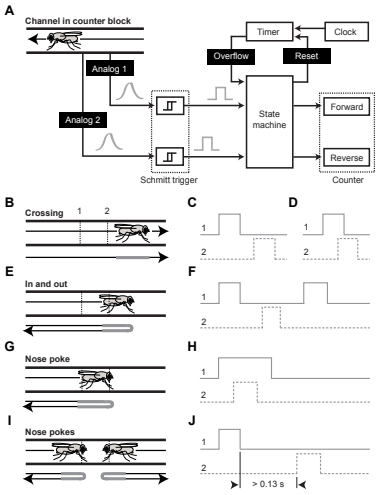

Figure 2.6: Diagram of the operational logic and examples of behavior near the counter. (A) Block diagram illustrating how the counter detects and assigns the bi-directional movement of flies. As a fly walks through the channel, it triggers two pairs of emitter/detector diodes, each pair producing an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