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Gender-Specific Differences in Spatial Behavior of the Flesh Fly,

*Sarcophaga crassipalpis*

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A thesis

presented to

the faculty of the Department of Biological Sciences

East Tennessee State University

In partial fulfillment

of the requirements for the degree

Masters of Science in Biology

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by

Caleb Paquette

May 2008

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Dr. Darrell Moore, Chair

Dr. Karl Joplin

Dr. Istvan Karsai

Keywords: flesh flies, *Sarcophaga crassipalpis*, spacing behavior, territoriality

## ABSTRACT

Gender-Specific Differences in Spatial Behavior of the Flesh Fly,

*Sarcophaga crassipalpis*

by

Caleb Paquette

Territoriality in the flesh fly, *Sarcophaga crassipalpis* (Diptera: Sarcophagidae) was studied in the laboratory. In rectangular enclosures, male flies exhibited a lower tolerance (occupation of the same physical space) of same-sex conspecifics than did female flies. In circular arenas, male flies showed significantly higher levels of spatial separation among themselves (as determined from nearest neighbor analyses) than did females: males were distributed uniformly whereas females were nearly random. The male spatial behavior occurred during the photophase but not the scotophase of light-dark cycles, suggesting that visual cues are required for maintenance of inter-individual spacing. No significant differences in male spacing behavior occurred between subjective day and subjective night in either constant dark or constant light conditions, suggesting that spatial patterning is not driven by a circadian rhythm.

## ACKNOWLEDGMENTS

First, I would like to thank my committee members for their help in the development and completion of this project. I also would like to thank Dr. Edith Seier for her help with statistical analysis. I would like to extend a special thanks to Karl Joplin and my committee chair, Darrell Moore. Without their help, the completion of this project would not have been possible.

Second, I would like to thank Josh Coakley for his help with time consuming data recording and data management. The analysis of behavioral interactions would still be in the beginning stages without his help.

Third, this project greatly benefited from the Dr. Denise Pav Graduate Research Award. This funding helped purchase rewritable mini DVD discs that were essential for analysis of behavioral interactions.

Finally, I would like to thank my mom, dad, sister, and especially my girlfriend Ashley Strickland. I cannot thank them enough for their love and support. I love you all.

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## CHAPTER 1

### INTRODUCTION

An important decision that all animals must make is where to live. The area in which an animal chooses to live and carry out all necessary activities critical to its survival is defined as the home range (Brown and Orians 1970; Baker 1983). Within the home range, animals often establish a territory. For an area to be recognized as a territory, the area must be defended from intruders so that the owner has exclusive rights to the resources found in the area, and the area must be one that the owner repeatedly returns to, though the time spent at and faithfulness to a specific area differs among different species (Fitzpatrick and Wellington 1983). Some animals defend their entire home range. For example, workers of the ant species, *Oecophylla longinoda*, patrol their entire home range, defending hundreds of nests distributed among as many as 17 trees as well as hunting on the ground on which the trees stand (Holldobler and Lumsden 1980). The workers aggressively defend their territories from inter- and intraspecific ant intruders. Other animals defend areas used for reproductive purposes while some defend only their mate. Male dung flies (*Scatophaga stercoraria*), for instance, remain in physical contact with a female after copulation during a “passive phase” in which the female oviposits (Parker 1970). The original male will defend his possession of the female when “searching males” attack. In another example, male dragonflies (*Plathemis Lydia*), after copulation, take a female to an oviposition site where they will guard her from intruding males while she oviposits (Jacobs 1960). The male does not remain in contact with the female during oviposition enabling him to mate with other females while he guards. In all cases, territoriality is used to sequester a resource (e.g., food, mating space, oviposition site, etc.) that confers some selective advantage to the territorial individual.

Territorial defense consists of behaviors such as vocalization, physical conspicuousness or display, and scent marking which serve to identify the owner of the territory to prospective rivals (Brown and Orians 1970; Baker 1983). For example, the songs of birds often play a role in the establishment and maintenance of a territory. Pairs of great tits (*Parus major*), for instance, are more likely to settle in an area where great tit song recordings are not played as opposed to areas in which the songs are played (Krebs and others 1978). Acoustic signaling can also play a similar role in invertebrates. The spatial distribution of male katydids (*Panacanthus pallicornis*) is dependant upon their calling song (Chamorro-R and others 2007). Deafened males aggregate whereas control males will distribute themselves randomly throughout an experimental environment.

Physical display by males is common in species that aggregate at leks, such as black grouse (*Lyrurus tetrrix*), ruff (*Philomachus pugnax*), the Uganda kob (*Adenota kob thomasi*), and the hammer-headed bat (*Hypsignathus monstrosus*) (Baker 1983). At lekking sites, males will display and fight with each other for the opportunity to mate with visiting females. Lek behavior is not exclusively exhibited by vertebrates as some insects are known to gather at leks as well. For example, certain Hawaiian species of *Drosophila* aggregate at leks where the males will visually advertise their presence by adopting a ritualized posture while extruding and withdrawing a bubble of fluid from their anal papillae (Spieth 1968, 1974).

Scent marking identifies the owner of a territory to intruders in many different species, vertebrates and invertebrates alike. Adult members of an Ethiopian wolf pack will scent mark near their territory boundaries with the dominant pair marking the most frequently (Sillero-Zubiri and Macdonald 1998). The marking is thought to signal to neighboring packs the composition and status of the marking packs, thereby reducing aggressive encounters between them. For

invertebrates, workers of the ant species, *O. longinoda*, mark their territory with colony specific pheromones that deter ants from foreign colonies from entering the territory (Holldobler and Wilson 1977). In a second example of invertebrate scent marking, males of the endemic Hawaiian species of *Drosophila* (*D. crucigera*, *D. grimshawi*, and *D. engyochracea*) identify their presence on a territory by depositing a film of liquid on the substrate (Spieth 1968, 1974).

When advertisement by the territory owner does not dissuade a rival from intruding into the territory, the owner must resort to agonistic behavior such as chasing or physically attacking the intruder. The resulting aggressive interactions are often costly for both of the individuals involved, often leading to injury or death. For example, male damselflies (*Mnais pruinosa costalis* Selys) are dimorphic with both non-territorial and territorial individuals present in a population (Tsubaki and others 1997). Due to the costs of aggressive interactions with other individuals, the territorial males of this species have a shorter lifespan than do the non-territorial individuals.

Not only can aggression lead directly to exhaustion, injury, or even death, but indirect mechanisms related to aggressive behavior can also have harmful consequences on an individual. Male mountain spiny lizards (*Sceloporus jarrovi*) that are given testosterone implants expend more energy due to an increased activity period and increased territorial displays than do control males (Marler and Moore 1989). The males with increased testosterone spend less time foraging and have lower survivorship than control males. The lower survivorship is possibly due to decreased growth resulting from less foraging or to greater conspicuousness from the increased number of territorial displays and higher activity levels.

Because territorial defense is costly, the benefits of territorial defense should outweigh the costs if an animal is to employ a territorial strategy successfully. Golden-winged Sunbirds

(*Nectarinia reichenowi*), for instance, defend territories that contain nectar producing *Leonotis nepetifolia* (Labiatae) flowers (Gill and Wolf 1975). Flowers within a territory usually produce more nectar than the flowers found outside of a territory. Territorial defense is thus beneficial when the flowers found outside of the territorial boundaries have low nectar levels. The calories saved from decreased foraging time outweigh the calories lost during territorial defense. The birds choose not to be territorial when nectar production is high in undefended flowers because more energy is spent defending the territory than would be spent foraging on flowers outside of a territory.

Reproductive benefits can be gained from territorial defense as well. Territorial *Dryomyza anilis* males defend carcasses that serve as oviposition sites and defend females from other males during copulation and oviposition (Otronen 1984). The territorial males have greater success capturing females for copulation than do non-territorial males. Because the territorial males are often the last to mate with a female before she oviposits, the sperm of the territorial male often has a competitive advantage over existing sperm from previous copulations with different males (Baker 1983). Using radioactively labeled sperm, Parker (1970) showed that the last *S. stercoraria* male to mate with a female fertilizes about 80% of the next batch of eggs that she oviposits.

Territorial disputes can be resolved by asymmetries in resource holding power between two opponents, or by asymmetries in the value of the resource to either the owner or the intruder (Baker 1983). For example, asymmetries in size often influence the outcome of conflicts with larger individuals having a higher resource holding power than smaller individuals. In *D. melanogaster* and *D. pseudoobscura* under field conditions for instance, larger males win more aggressive interactions with conspecifics and have a mating advantage with both virgin and

inseminated females (Partridge and others 1987). Similar results were obtained in the laboratory for *D. melanogaster* and *D. simulans* (Hoffman 1987). Though differences in size can affect the outcome of conflicts, these differences do not always determine the winner of territorial disputes. For example, male damselflies (*Calopteryx macculata*) engage in territorial disputes in which the winners had a higher fat content than did the losers in 88% of the contests, whereas other factors such as size or flight ability did not influence the outcome of the contests (Marden and Waage 1990). Furthermore, the males do not fight until their energy reserves are completely depleted, but rather the males seem able to assess the fat reserves of their opponent as fatter males win 95% of long contests as opposed to 68% of short contests (Marden and Rollins 1994).

Territoriality is widespread among the insects with no shortage of examples illustrated by the Dipterans (for reviews, see Baker 1983; Fitzpatrick and Wellington 1983). Territoriality has been extensively studied under laboratory conditions in *D. melanogaster*. Males will defend food cups against intruding males (Jacobs 1960). Males that successfully defend these food resources have a higher mating success than males that do not (Dow and von Schilcher 1975; Hoffman 1987). Artificial selection studies indicate substantial genetic variation for territorial success within wild-type populations (Hoffman 1988). Males selected for territorial success escalate encounters more frequently against territory owners and win more of these escalated encounters than do unselected control males. Males from these selected lines are more successful in mating than males from unselected lines except under conditions where male density is low and therefore not all territories are defended, and in matings with virgin females (Hoffman and Cacoyianni 1989). Evidence suggests that territoriality is thus a conditional strategy that males exhibit when it leads to higher mating success. For example, male *D. melanogaster* typically are more likely to establish territories when females are present and to

defend territories that are viewed as good oviposition sites by females. Thus, the males are less likely to defend very large or very small food areas or to establish territories when the density of male flies is high (Hoffmann and Cacoyianni 1990). Males that are 3 days or more posteclosion are more likely to establish and successfully defend a territory than are younger males (Hoffman 1990). Isolated males are more aggressive and will establish territories more quickly than males with prior social experience.

Territorial defense requires the ability to display aggressive behavior. In *D. melanogaster*, males and females exhibit gender-selective differences in staged aggressive encounters. Males fight with other males, especially in the presence of a potential mate (Chen and others 2002). Female fights may be elicited in the presence of yeast paste, a desirable food (Ueda and Kidokoro, 2002). Although some agonistic behaviors are common to both sexes, males perform higher intensity behaviors that are not seen in females. Furthermore, males establish lasting dominance relationships based upon fighting outcomes whereas females do not (Nilsen and others 2004). Detailed knowledge of behavioral expression patterns derived from studies of paired male and paired female agonistic encounters conducted under controlled, laboratory conditions (Chen and others 2002; Nilsen and others 2004) substantiates the use of *Drosophila* as a model system to probe the physiological, genetic, and molecular control of aggressive behavior (Baier and others 2002; Dierick and Greenspan 2006; Edwards and others 2006; Vrontou and others 2006).

Territorial behavior among Dipterans is known for a number of species in nature as well as the laboratory. Males of the endemic Hawaiian species of *Drosophila* exhibit courtship behavior that, contrary to many other *Drosophila* species, does not take place on feeding and oviposition sites. Instead, males fly to the surrounding vegetation after feeding and establish

territories that they defend against other males and unreceptive females (Spieth 1968, 1974). Many flies exhibit aggressive station-keeping behavior. Male hoverflies (*Eristalis*) establish a station in the environment where they hover and wait for passing insects that they then chase on the chance of encountering a potential mate (Collett and Land 1975). After the chase, the hoverflies return to the same area from which they started the chase. Similar behavior is seen in male blow flies (*Calliphora*) (Collett and Land 1975) and house flies (*Musca* and *Fannia*) (Land and Collett 1974), except that these flies establish a sitting position from which they chase passing insects or objects. Males of the Sarcophagidae also establish a territorial position from which they fly out and attempt to capture females (Arnett 2000; KHJ personal communication). Similar perching territorial behavior is observed in butterflies (Baker 1972; Davies 1978; Bitzer and Shaw 1979; Jones and others 1998). Male butterflies will fly toward any intruder that enters his territory and if the intruder is a conspecific male, the two butterflies will engage in a climbing, spiral flight where each male attempts to establish a position above and behind his opponent (Baker 1972). When the conflict is resolved and the males separate, the previous owner of the territory will often return to his original perching spot.

In the present study, we examine the phenomenon of territoriality in the flesh fly *Sarcophaga crassipalpis* under artificial, laboratory conditions. In nature, males establish perches from which they observe and often chase other insects that appear in the vicinity. No territories have been observed for females (Arnett 2000). If the male perching behavior is indeed an expression of territoriality, then it is expected that there should be significant disparities between territorial (male) and non-territorial (female) flesh flies with respect to how the nervous system is programmed to respond to same-sex conspecifics. The working hypothesis is that the tendency of male flesh flies to distribute themselves into spatially separate waiting perches in

nature is a consequence of a fundamental neural program for maintaining inter-individual separation. If true, then there should be a significant difference in the spatial distribution patterns between males and females that will be expressed in virtually any environment, including simplified laboratory settings.

## CHAPTER 2

### MATERIALS AND METHODS

#### Insects

Flesh flies, *Sarcophaga crassipalpis*, were maintained under non-diapause conditions (LD 15:9 h, lights-on 07:20 h; 25°C for pupae and adults, 20°C for larvae) in a colony at East Tennessee State University. The flies used in this study were taken from generations 102 through 129. Newly emerged flies (1 or 2 days post-emergence) were collected for the experiments.

#### Data Collection

Three different enclosures were used to investigate the spatial patterning of adult flies. The first type of enclosure was a rectangular, transparent Plexiglas chamber (2.3 x 2 x 25.5 cm with screen at both ends) placed over a grid pattern of 8 equal-sized spaces (length 3.2 cm, width 2 cm) labeled A through H (Figure 1). Food (sugar cube) was located in section A and plastic tubing provided access to water in section H.

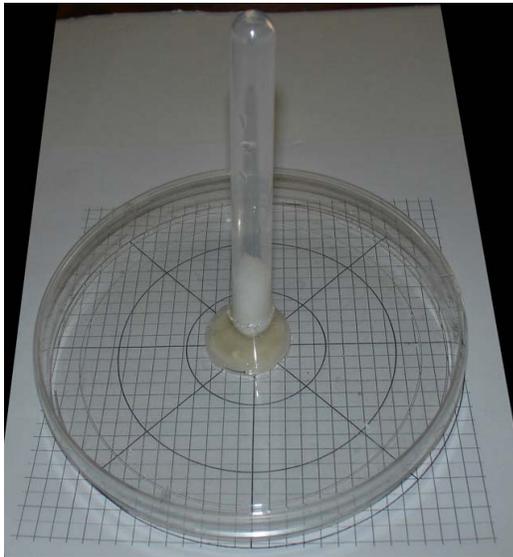


Figure 1 Rectangular Enclosure

The second and third types of enclosure were made from circular Petri dishes (diameter 14.2 cm, height 1.6 cm, area 158.4 cm<sup>2</sup>) (Fig 2). One type of circular enclosure contained food

(a dough-like mixture of powdered confectionary sugar and honey) in the center of the dish (Figure 2a), and the other type contained the same food evenly distributed around the peripheral edge of the dish (Figure 2b). A plastic ring (diameter 2.8 cm, height 0.4 cm) held the food in the center (occupying an area of  $6.2 \text{ cm}^2$ ), while a plastic-coated wire barrier (height 0.3 cm) held the food in the periphery (occupying an area of  $45.3 \text{ cm}^2$ ). In both types of circular enclosures, water was provided via a 13 x 100 mm polypropylene test tube inserted vertically through the center of the Petri dish lid. A cotton plug prevented water leakage from the inverted test tube. The transparent circular enclosures were placed over a grid pattern of concentric circles and 8 “pie-slice” sectors.

(a)



(b)

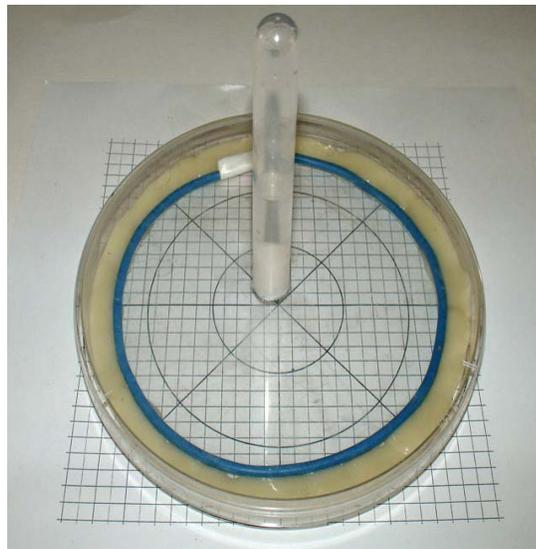


Figure 2 Circular Enclosures. (a) Food located in the center. (b) Food located in the periphery.

All of the experiments were conducted inside an aluminum shed, constructed within the laboratory proper. Temperature ( $24^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ) was regulated by a thermostat-controlled space heater. Illumination was provided by a pair of 40 watt, cool white, fluorescent tubes suspended 1 m above the enclosures which were placed on a table top. To minimize vibrations from the

observer, the table legs were placed in buckets of sand. The table surface was surrounded by a black cloth suspended from the ceiling. Because there was no illumination outside of the cloth, the observer could monitor the flies through viewing slits in the cloth and be invisible to the flies. Black cardboard partitions ensured that flies in any one enclosure were visually isolated from flies in all other enclosures (Figure 3).

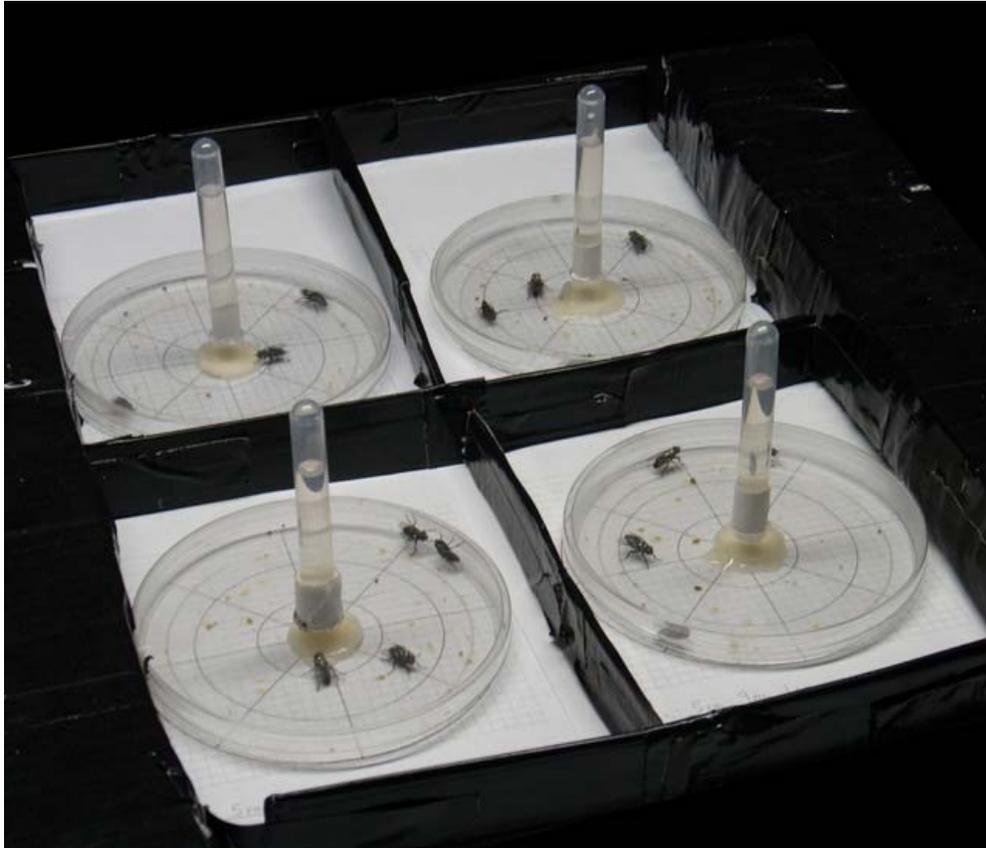


Figure 3 Enclosure Setup within the Shed. Groups of 4 females or groups of 4 males were housed in circular enclosures separated by black cardboard partitions.

Each experimental enclosure housed a group of 4 females or a group of 4 males. To distinguish among individuals within these groups of 4, the flies were cooled on ice and marked on the dorsal thorax with a spot of colored enamel. The flies were then placed in an enclosure within the shed and allowed to entrain to the LD 12:12 h cycle (lights-on: 08:00 h, lights-off

20:00 h) for two days. After this entrainment period, observations of spatial patterning were made. For the rectangular enclosures, the position of each fly simply was recorded according to which section (A through H) the fly situated itself. For the circular enclosures, the exact locations of the flies were recorded on a circular map that was identical to the circular grid underneath the enclosures.

#### Experiment 1: Observations of Spatial Patterning in Rectangular Enclosures

Immediately following two days of entrainment to the LD 12:12 h cycle, observations were made for groups of 4 males and groups of 4 females for 3 consecutive days under the same LD 12:12 h cycle. The location of each fly within the enclosure (section A through H) was recorded hourly during the photophase, beginning at 09:00 h (1 hour after lights-on) and ending at 19:00 h (1 hour before lights-off), thus yielding 11 observations per enclosure per day (Figure 4). To quantify the spatial distribution within the rectangular enclosures, we used a measure of tolerance: the proportion of the total number of observations in which 2 or more flies occupied the same section of the enclosure. A statistical comparison of tolerance between males and females was made using a chi-square contingency table analysis.

#### Experiment 2: Observations of Spatial Patterning in Circular Enclosures

After two days of entrainment to LD 12:12 h, hourly observations of spatial patterning were made during the photophase for 3 consecutive days, as described for the rectangular enclosures (Figure 4). There were 2 different sets of experiments with circular enclosures: one with food in the center and the other with food in the periphery. To quantify the spatial patterning within the circular enclosures, we used nearest neighbor statistics. Once the exact

location of each individual fly was determined, the distance between each fly and its nearest neighbor was measured. These distances were then used to calculate an R value, a test statistic of the degree of clustering (Clark and Evans 1954), for each enclosure at each observation time. A random distribution is indicated if  $R=1$ ; for maximal aggregation,  $R=0$ ; R values greater than 1 indicate a tendency toward a uniform distribution with the maximum value of  $R=2.1491$ . Comparisons of R values were made for the food location, the time of day within the photophase, the day of observation, and gender using a mixed procedure ANOVA model with repeated measures (SAS Institute).

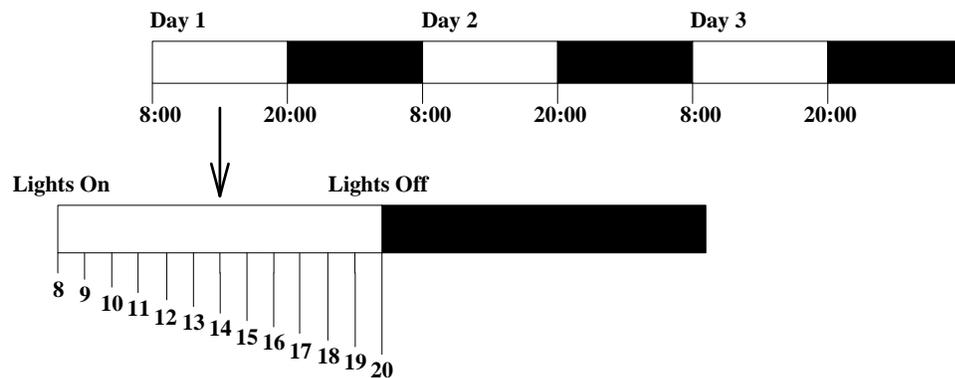


Figure 4 Light Cycle and Observation Schedule for Rectangular and Circular Enclosure Experiments. Figure depicts the 3 observation days following 2 days of entrainment to LD 12:12 h. Observations were made hourly beginning an hour after lights on (09:00) and ending an hour before lights off (19:00) for 3 consecutive days.

### Experiment 3: Observations of Spatial Patterning in Circular Enclosures under Constant Dark Conditions

Groups of 4 male flies were placed in circular enclosures with food in the center and then entrained to LD 12:12 h for 2 days. Observations of spatial patterning then were made under the same LD 12:12 h conditions for 3 consecutive days: fly positions were recorded at 11 hourly observation times during the photophase and 3 times during the scotophase (21:00, 02:00, 07:00

h) (Figure 5). Then, during the next 3 consecutive days, the light cycle was switched to constant darkness (DD) and position measurements were taken at 3 observation times during the subjective day (09:00, 14:00, 19:00 h) and 3 times during the subjective night (21:00, 02:00, 07:00 h). R value comparisons for observation times, day of observation, subjective day vs. subjective night, and the presence of light vs. dark were accomplished using mixed procedure ANOVA with repeated measures.

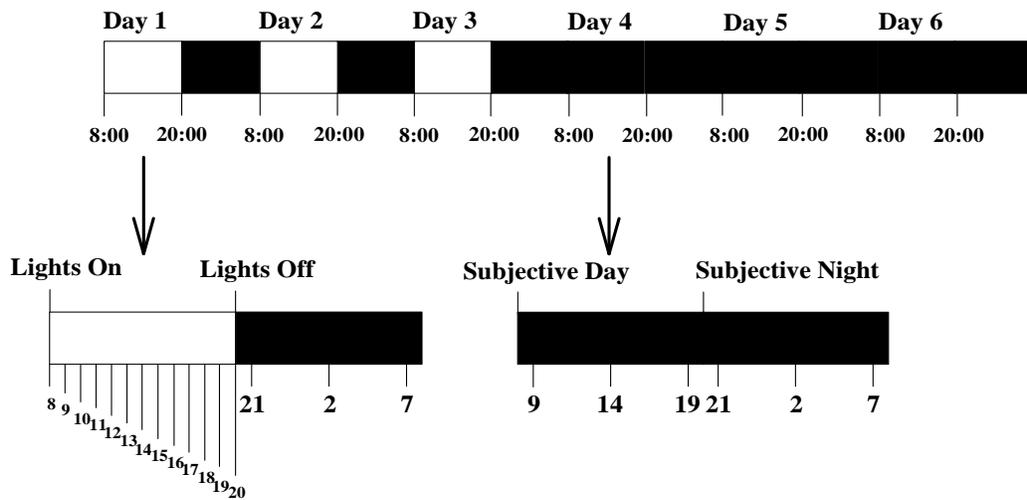


Figure 5 Light Cycle and Observation Schedule for Circular Enclosure Experiments under Constant Dark Conditions. Figure depicts the 6 observation days following 2 days of entrainment to LD 12:12 h. Observations were made hourly during the photophase beginning an hour after lights on (09:00) and ending an hour before lights off (19:00) and 3 times during the scotophase (21:00, 02:00, and 07:00) on days 1 through 3. On day 4 the light cycle was switched from LD 12:12 h to DD 12:12 h and 3 observations were made during subjective day (09:00, 14:00, and 19:00) and 3 during subjective night (21:00, 02:00, and 07:00).

Experiment 4: Observations of Spatial Patterning in Circular Enclosures under Constant Light Conditions

As in the previous experiment, groups of 4 male flies were placed in circular enclosures with food in the center and adapted to LD 12:12 h for 2 days. During the next 3 consecutive days, observations of spatial patterning were made under the same LD 12:12 h conditions. Fly positions were recorded at 3 observation times during the photophase (09:00, 14:00, 19:00 h) and 3 times during the scotophase (21:00, 02:00, 07:00 h) (Figure 6). For the next 3 consecutive days, the flies were subjected to constant light (LL): position measurements were continued at the same times of day. As in Experiment 3, R value comparisons for observation times, day of observation, subjective day vs. subjective night, and the presence of light vs. dark were accomplished using mixed procedure ANOVA with repeated measures.

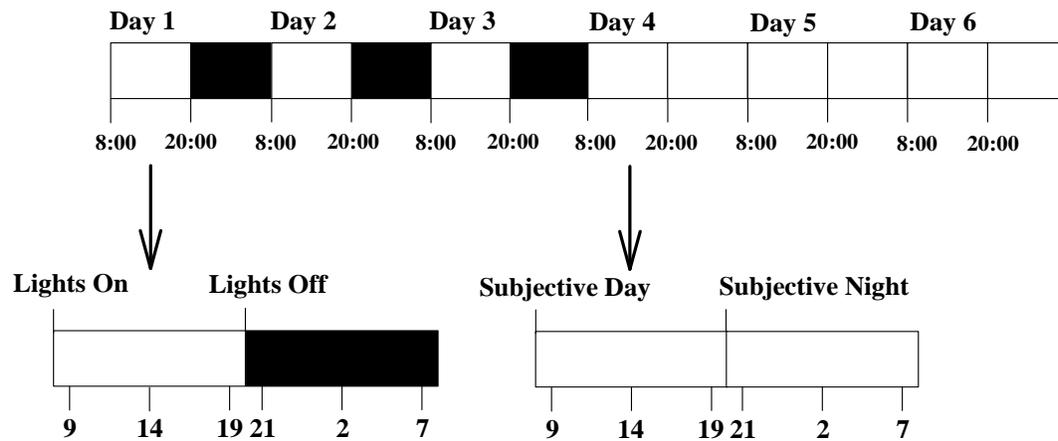


Figure 6 Light Cycle and Observation Schedule for Circular Enclosure Experiments under Constant Light Conditions. Figure depicts the 6 observation days following 2 days entrainment to LD 12:12 h. Three observations were made during the photophase (09:00, 14:00, and 19:00) and 3 were made during the scotophase (21:00, 02:00, and 07:00) on days 1 through 3. On day 4 the light cycle was switched from LD 12:12 h to LL 12:12 h and 3 observations were made during subjective day (09:00, 14:00, and 19:00) and 3 during subjective night (21:00, 02:00, and 07:00).

### Experiment 5: Observations of Interactions between Same-Aged, Socially Naïve Male Flies

Because the spatial patterns exhibited by flies are an emergent property of the interactions that occur between flies, the interactions resulting in the greater spacing seen between male flies were investigated. Male flies were collected at emergence and placed in isolation chambers (Petri dish with an area 50 cm<sup>2</sup>) with food and water. The isolation chambers were housed within the aluminum shed in LD 12:12 h and 24°C ± 2°C conditions. The isolation chambers were separated from one another by black cardboard partitions.

Pairs of 1-, 2-, 3-, 4-, and 6- day old, socially naïve flies were marked on the dorsal thorax with a dot of colored enamel and released into a circular arena (Petri dish with an area 50 cm<sup>2</sup>) with a barrier dividing the arena in half. The arena did not contain food or water. Ten minutes after the flies were released into the arena, the barrier was pulled from the arena and the pair of flies was videotaped for 1 hour using a Sony Digital HD Handycam, HDR-UX1. Video recordings were analyzed on a Macintosh Powerbook G4 computer with Final Cut Pro HD software. An ethogram of fly behavior was constructed from the video recordings and used for basic analyses. Event recording and basic analyses were performed using JWatcher v.1.0, free behavioral analysis software available for public use at <http://www.jwatcher.ucla.edu>.

### Experiment 6: Determination of the Onset of Mating.

To determine the onset of mating, flies were collected at emergence in 2-hour intervals and same-aged pairs of males and females were grouped together in cages (volume 1 ft<sup>3</sup> with screen on all sides) with food, water, and liver. The cages were housed within the aluminum shed in LD 14:10 h and 24°C ± 2°C conditions. Three cages containing 8, 17, and 20 pairs of flies were observed every hour of the photophase for 6 consecutive days beginning the day of emergence. The number of pairs observed mating was recorded at each observation time.

## CHAPTER 3

### RESULTS

#### Spatial Patterning in Rectangular Enclosures

A simple measure of tolerance was used to quantify the spatial patterning exhibited by groups of 4 males and groups of 4 females of *S. crassipalpis* in rectangular enclosures. Females were significantly more tolerant of same-sex conspecifics than were male flies on days 1 and 2, but not day 3 of the experiment (chi-square contingency table,  $\chi^2 = 6.6$ , d.f. = 1,  $P < 0.02$  for day 1;  $\chi^2 = 9.9$ , d.f. = 1,  $P < 0.01$  for day 2;  $\chi^2 = 1.4$ , d.f. = 1,  $P = 0.23$  for day 3). When the data were pooled for all 3 days, females had a significantly higher tolerance for same-sex conspecifics than did male flies ( $\chi^2 = 18.7$ , d.f. = 1,  $P < 0.0001$ ). For 8 groups of male flies, 2 or more individuals were found occupying the same section of the enclosure in 58.0% of 88 observations on day 1, 58.0% of 88 observations on day 2, and 63.6% of 88 observations on day 3, and 59.8% of 264 total observations. For 7 groups of females, 2 or more individuals were found in the same section of the enclosure in 77.9% of 77 observations on day 1, 81.8% of 77 observations on day 2, 75.3% of 77 observations on day 3, and 78.4% of 231 total observations (Figure 7).

To examine if the distribution of flies within the rectangular enclosures differed between sections, the mean number of flies observed in each section was determined for days 1, 2, and 3. The data were pooled for all 3 days and a one-way ANOVA was used for statistical analysis. The number of times females and males were observed in each section of the rectangular enclosures (A through H) was significantly different between the 8 sections (ANOVA,  $F = 57.57$ ,  $P < 0.0001$  for females; ANOVA,  $F = 42.27$ ,  $P < 0.0001$  for males). Females were observed significantly more times in section A (location of food) than in sections B through H

(Figure 8, Pooled Data). Females were observed in section H (location of water) significantly more times than in sections C through G (Figure 8, Pooled Data). Like females, males were observed significantly more times in section A than in sections B through H (Figure 9, Pooled Data).

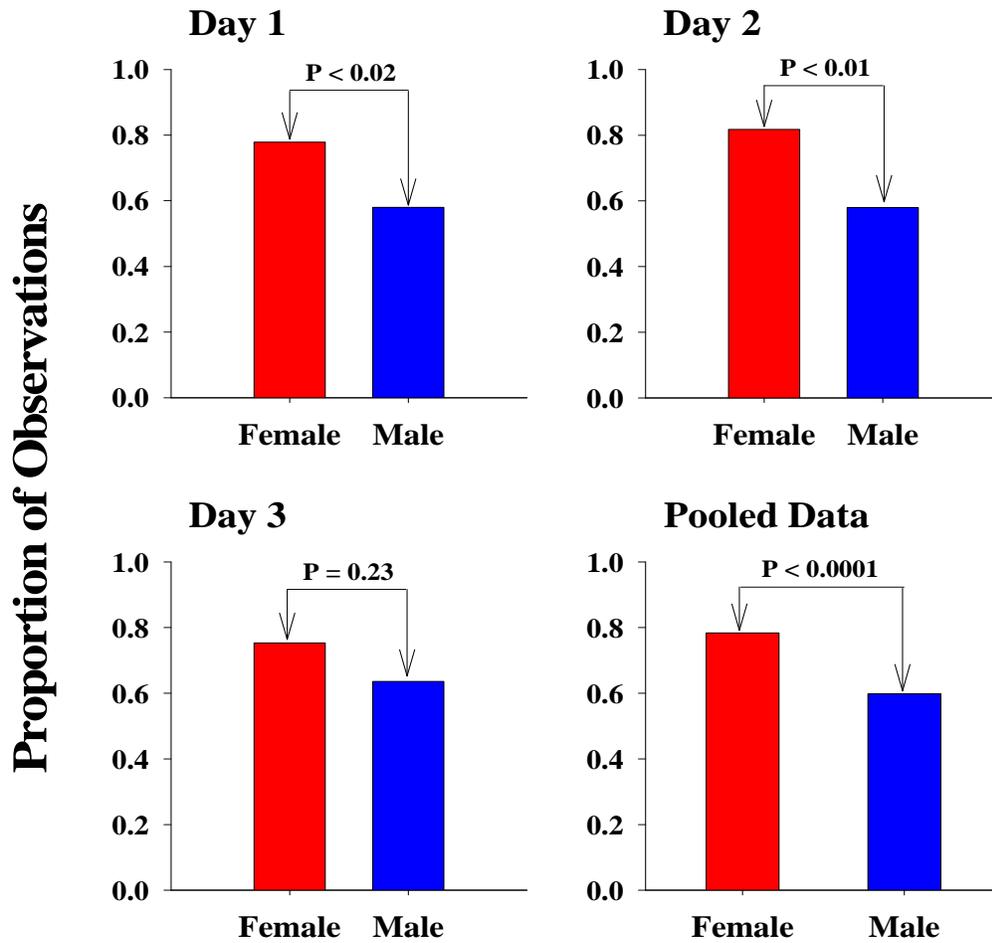


Figure 7 Tolerance of Conspecifics. In rectangular enclosures, female flies have a significantly higher tolerance of same-sex conspecifics than do male flies on days 1 and 2, but not day 3. Depicted is the proportion of observations in which 2 or more individuals are found in the same section of the enclosure for groups of 4 females (red bars) and groups of 4 males (blue bars). The observations were made every hour of the photophase in LD 12:12 h beginning an hour after lights on and ending an hour before lights off for 3 consecutive days.

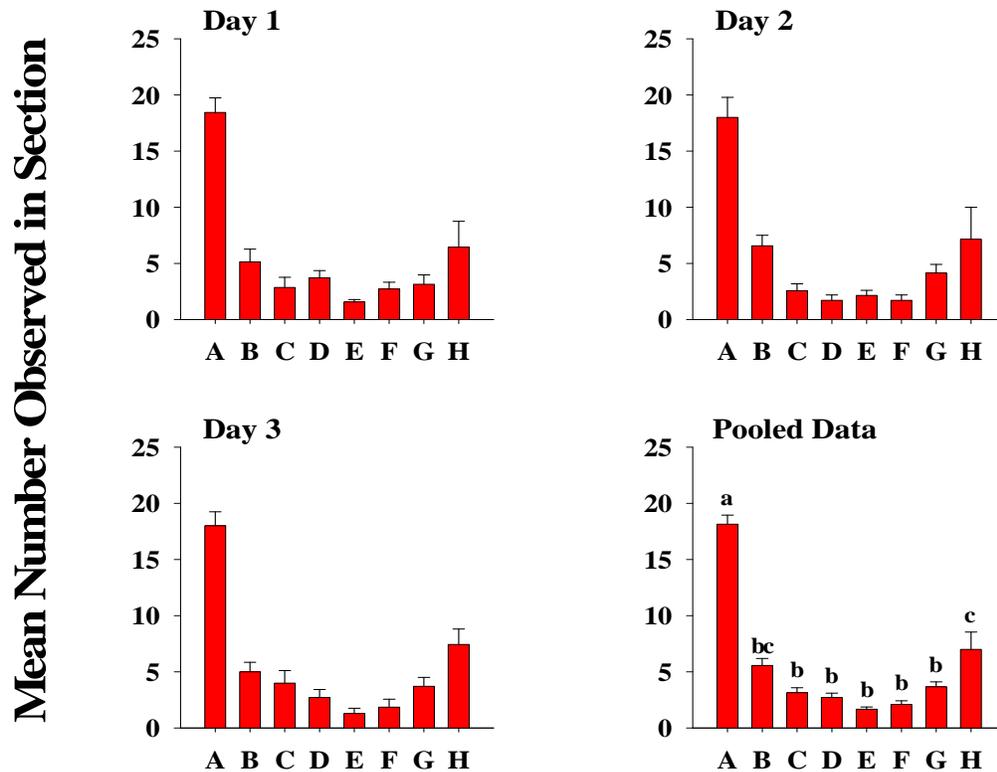


Figure 8 Distribution of Female Flies in Rectangular Enclosures. Figure depicts the mean (+ SEM) number of times flies were observed in each section of the enclosure for days 1, 2, and 3. The data for all 3 days were pooled for statistical comparisons between sections. Females were observed in section A significantly more times than in sections B through H. Females were observed in section H significantly more times than in sections C through G. Within the pooled data graph, bars that do not share letters are significantly different from each other ( $P < 0.05$ ).

To compare distributions between males and females, a chi-square contingency table was used for analysis. The number of females observed in section A was significantly higher than the number of males observed in section A ( $\chi^2 = 28.43$ , d.f. = 1,  $P < 0.000001$ ) (Table 1). Females were observed in section A in 41.23% of 924 total observations whereas males were observed in section A in 29.67% of 1055 total observations (Figure 10). Significantly more females were observed in sections B and H as well ( $\chi^2 = 8.54$ , d.f. = 1,  $P < 0.01$  for section B;  $\chi^2 = 3.90$ , d.f. = 1,  $P < 0.05$  for section H) (Table 1). Males were observed significantly more times

in sections E, F, and G than were females ( $\chi^2 = 15.02$ , d.f. = 1,  $P < 0.001$  for section E;  $\chi^2 = 19.48$ , d.f. = 1,  $P < 0.0001$  for section F;  $\chi^2 = 16.70$ , d.f. = 1,  $P < 0.0001$  for section G) (Table 1).

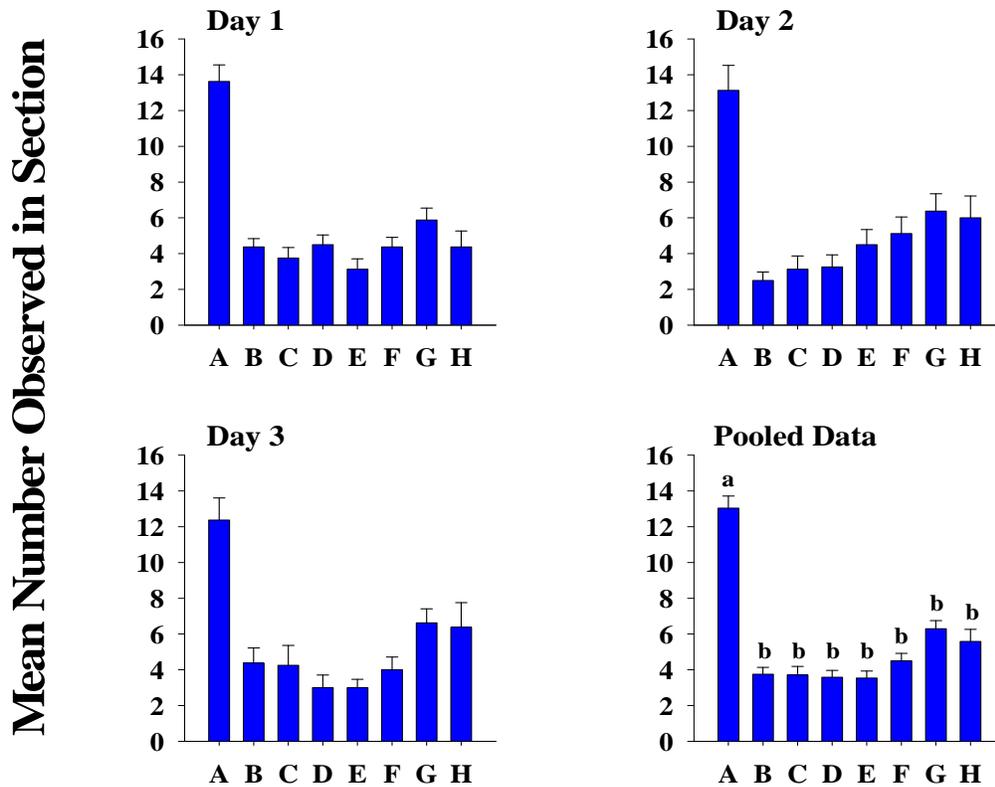


Figure 9 Distribution of Male Flies in Rectangular Enclosures. Figure depicts the mean (+ SEM) number of times flies were observed in each section of the enclosure for days 1, 2, and 3. The data for all 3 days were pooled for statistical comparisons between sections. Males were observed in section A significantly more times than in sections B through H. Within the pooled data graph, bars that do not share letters are significantly different from each other ( $P < 0.05$ ).

### Spatial Patterning in Circular Enclosures

Spatial patterning behavior was quantified using R values calculated from classical nearest neighbor statistics (Clark and Evans 1954). These measures of dispersal were determined hourly for each enclosure throughout the photophase in LD 12:12 h cycles for 3 consecutive days. For both types of circular enclosures, the R values for male flies were significantly higher than those for female flies (ANOVA with repeated measurements,  $F_{1,33} =$

85.79,  $P < 0.0001$ ), indicating a significantly greater departure from a random distribution. The elevation in R values for the males relative to the females was maintained for all 3 days of observations (Figure 11). There was no significant effect of time of day ( $F_{1,1180} = 0.63$ ,  $P = 0.429$ ) or day of the experiment ( $F_{1,1180} = 3.63$ ,  $P = 0.057$ ) on the R values for either male or female flies.

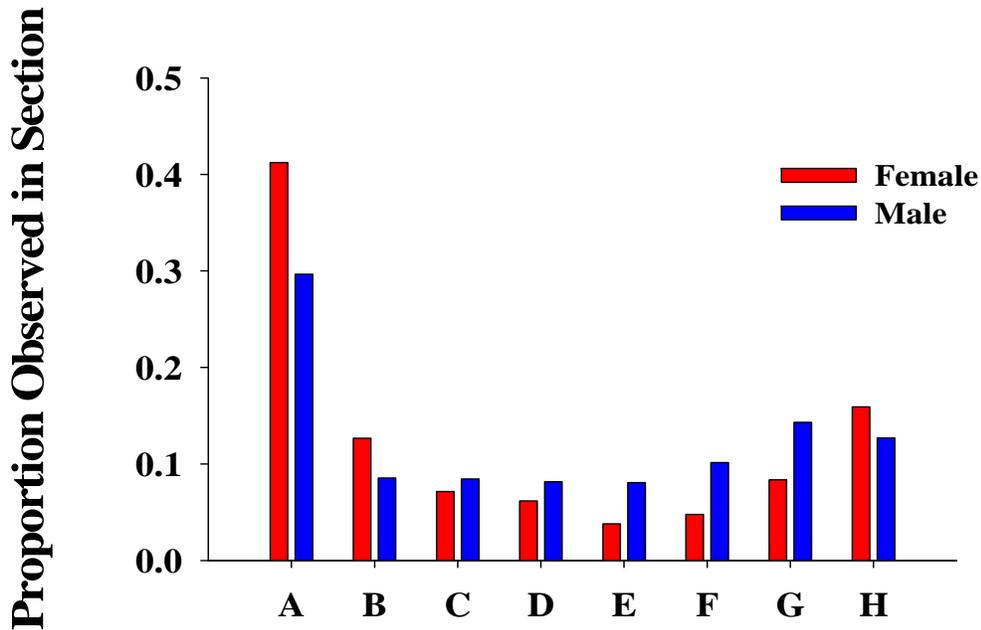
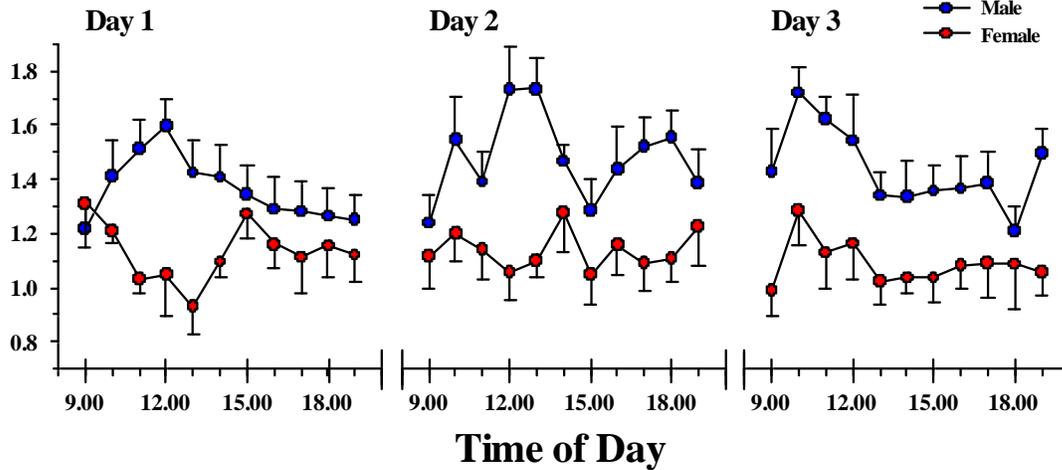


Figure 10 Proportion of Times Flies Found in Each Section of the Rectangular Enclosure. Red bars represent females and blue bars represent males. Data were pooled for all 3 days of the experiment. Significant differences between females and males are shown in Table 1.

Table 1 Chi-square analysis: Distribution of Females vs. Distribution of Males

| <i>Section of Enclosure</i> | $\chi^2$ | <i>P</i>   |
|-----------------------------|----------|------------|
| <i>A</i>                    | 28.43    | < 0.000001 |
| <i>B</i>                    | 8.54     | < 0.01     |
| <i>C</i>                    | 0.97     | = 0.32     |
| <i>D</i>                    | 2.60     | = 0.11     |
| <i>E</i>                    | 15.02    | < 0.001    |
| <i>F</i>                    | 19.48    | < 0.0001   |
| <i>G</i>                    | 16.70    | < 0.0001   |
| <i>H</i>                    | 3.90     | < 0.05     |

(a) Food in Center



(b) Food in Periphery

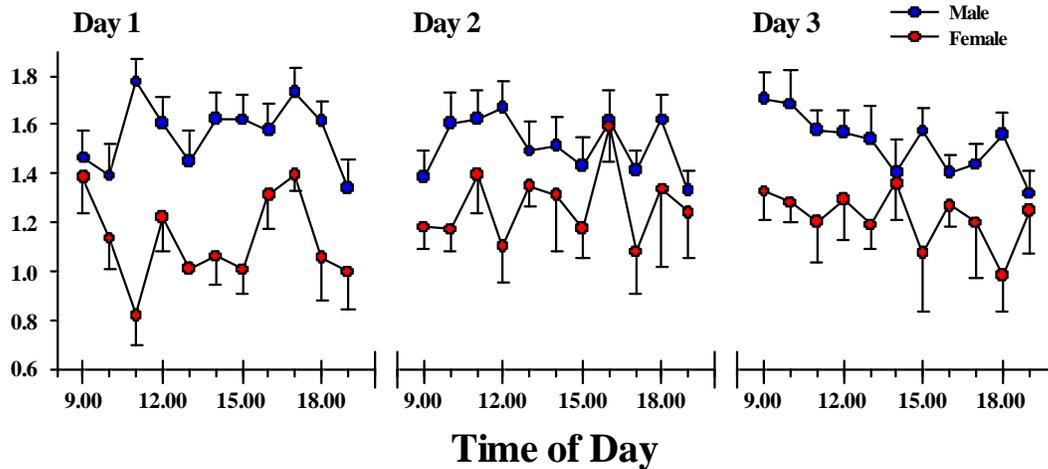


Figure 11 Mean R Values for Male and Female Flies. Male and female flesh flies exhibit significantly different spatial distributions within circular enclosures. Depicted are mean ( $\pm$  SEM) R values (an index of the degree of clustering, derived from nearest neighbor measures) for groups of 4 males (blue circles) and 4 females (red circles) in circular enclosures with food located in the center (a) and with food located in the periphery (b). Observations were made hourly throughout the photophase within LD 12:12 h cycles for 3 consecutive days. The R values for males are consistently and significantly higher than those for females, indicating greater spatial separation among male flies: males tend toward a uniform distribution ( $R > 1$ ), whereas females tend toward a random distribution ( $R = 1$ ).

The location of the food (center vs. periphery) within the circular enclosures had a significant effect on the R values: for both males and females, food in the periphery yielded significantly higher R values (indicating greater spatial dispersal) than did food in the center ( $F_{1,33} = 18.56, P = 0.0001$ ) (Table 2). However, there was no significant interaction between the location of food and the gender of the flies ( $F_{1,33} = 0.021, d.f. = 33, P = 0.652$ ). Male flies maintained higher R values (indicating greater spatial separation) than did the female flies, regardless of the location of the food.

Table 2 Mean R values ( $\pm$  SEM) for each day of the experiment.

| <i>Day of Experiment</i> | <i>Food in Center</i> |              | <i>Food in Periphery</i> |              |
|--------------------------|-----------------------|--------------|--------------------------|--------------|
|                          | Male                  | Female       | Male                     | Female       |
| <i>Day 1</i>             | 1.37 (0.033)          | 1.13 (0.032) | 1.56 (0.033)             | 1.13 (0.043) |
| <i>Day 2</i>             | 1.48 (0.038)          | 1.14 (0.033) | 1.52 (0.033)             | 1.27 (0.051) |
| <i>Day 3</i>             | 1.44 (0.036)          | 1.09 (0.033) | 1.53 (0.032)             | 1.22 (0.046) |

#### LD Followed by DD

The previous experiments examined the spatial distribution of male and female flies throughout the photophase of LD cycles. To determine if the relatively high degree of dispersal of male flies could be attributed to visual cues, we conducted experiments in which the spatial locations of males in the circular enclosures (food in center) were recorded during both the photophase and scotophase for 3 consecutive days under LD 12:12 h cycles. To test for circadian rhythmicity in this phenomenon, we extended the experiments for an additional 3 days, during which the flies were exposed to DD conditions.

During the 3-day LD portion of the experiment, male flies showed significantly higher degrees of dispersal (higher R values) during the photophases than during the scotophases ( $F =$

24.85, d.f. = 16,  $P = 0.0001$ ). The obvious cycling between day and night, however, was absent during the following 3 days under DD (Fig 12). Consistent with dispersal behavior being driven by visual cues, the R values remained relatively low during DD, with no significant differences between subjective day and subjective night ( $F_{1,16} = 0.01$ ,  $P = 0.926$ ) and no effect of day of the experiment ( $F_{1,999} = 1.38$ ,  $P = 0.241$ ).

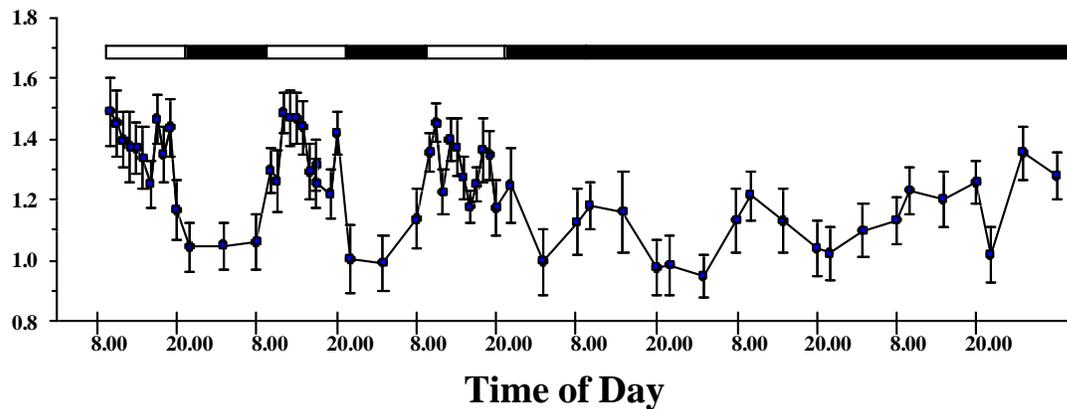


Figure 12 Mean R Values for the LD Followed by DD Experiment. R values are depicted for male flies for three days of LD 12:12 h followed by 3 days of constant darkness (DD); light conditions are indicated by a horizontal bar imbedded in the graph. Mean ( $\pm$  SEM) R values are illustrated at each observation time for all 6 days of the experiment. The R values are significantly lower during the scotophase than during the photophase during the LD 12:12h cycle (days 1 through 3). The R values remained relatively low (at typical scotophase levels) under DD conditions (days 4 through 6) with no significant differences between subjective days and subjective nights.

#### LD Followed by LL

The previous experiments indicated no circadian rhythmicity for male spacing behavior under constant dark conditions. However, if the behavior is predicated upon visual cues, then darkness presumably could override the expression of the behavior. Therefore, to further confirm that the male spacing behavior is not driven by an endogenous circadian rhythm, we provided (after 3 days of LD 12:12 h) groups of male flies with constant light (LL) for 3 consecutive days. As shown previously, the R values were significantly higher during the

photophase than during the scotophase under LD 12:12 h conditions ( $F_{1,8} = 30.08$ ,  $P = 0.0006$ ) (Figure 13). During the following three days of LL, the R values remained elevated at LD photophase levels with no significant differences between subjective day and subjective night ( $F_{1,8} = 0.00$ ,  $P = 0.9495$ ). There was an effect of day on the experiment ( $F_{1,311} = 6.58$ ,  $P = 0.0108$ ): this can be observed as a gradual decline in R values over the three days of LL.

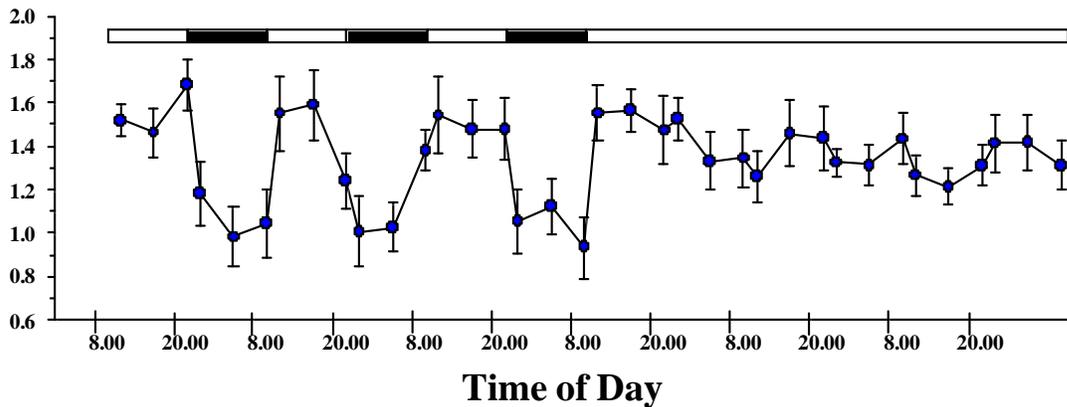


Figure 13 Mean R values for the LD Followed by LL Experiment. R values were calculated for male flies for 3 days of LD 12:12 h followed by 3 days of constant light (LL); light conditions are indicated by a horizontal bar imbedded in the graph. Mean ( $\pm$  SEM) R values are shown at each observation time for all 6 days of the experiment. The R values are significantly higher during the photophase than during the scotophase during the LD 12:12 h cycle (days 1 through 3). The R values remained relatively high (at typical photophase levels) under LL conditions (days 4 through 6) with no significant differences between subjective days and subjective nights.

### Dyadic Interactions Between Male Flies: Preliminary Results

Video recordings of interactions between same-age, socially naïve male flies were used to construct an ethogram of fly behavior (Table 3). Behaviors were divided into 2 groups: non-interactive and interactive behaviors. Non-interactive behaviors are behaviors that are exhibited by single flies independent of the actions of other flies while interactive behaviors occur in interactions between 2 flies.

Male flies spent the greatest proportion of time standing, walking, and grooming. One-, 2-, 3-, 4-, and 6-day old flies spent 96.01%, 92.98%, 94.47%, 85.93%, and 86.95% of the total observation time performing these 3 behaviors. Although all age groups spent the greatest proportion of time engaged in these 3 non-interactive behaviors, differences did exist in the proportion of time each age group spent performing the individual behaviors (Figure 14). Younger flies spent significantly more time standing and walking, while older flies spent significantly more time grooming. Three-day old flies spent significantly less time standing and walking and significantly more time grooming than did 1-day old flies. Three-day old flies also spent significantly less time standing and significantly more time grooming than did 2-day old flies although there was no significant difference in the time spent walking between 2- and 3-day old flies. Three-, 4-, and 6-day old flies did not significantly differ in the time spent walking or grooming but, 6-day old flies did spend significantly less time standing than did 3- and 4-day old flies.

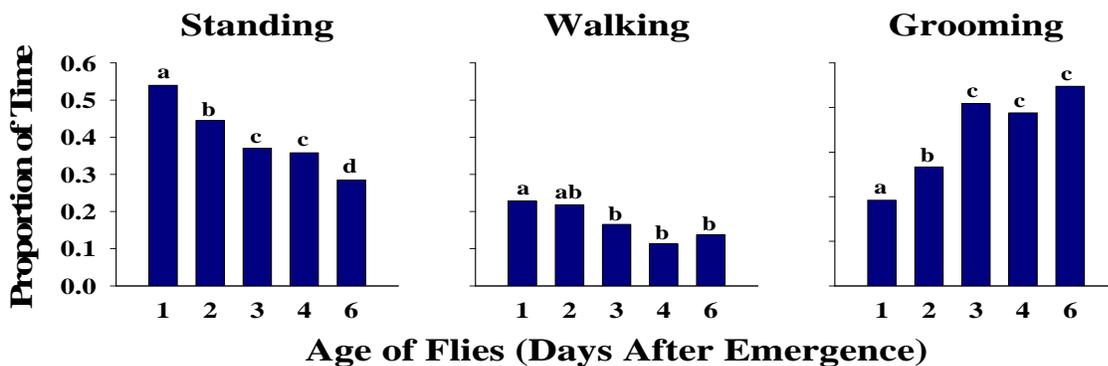


Figure 14 Proportion of Total Time that Flies Spent Standing, Walking, and Grooming Change with Age. Note the change in behavior from day 1 to day 3 with 3-day old flies always significantly different from 1-day old flies. Bars that do not share letters are significantly different from each other ( $P < 0.05$ , Tukey type non-parametric multiple comparisons of proportions).

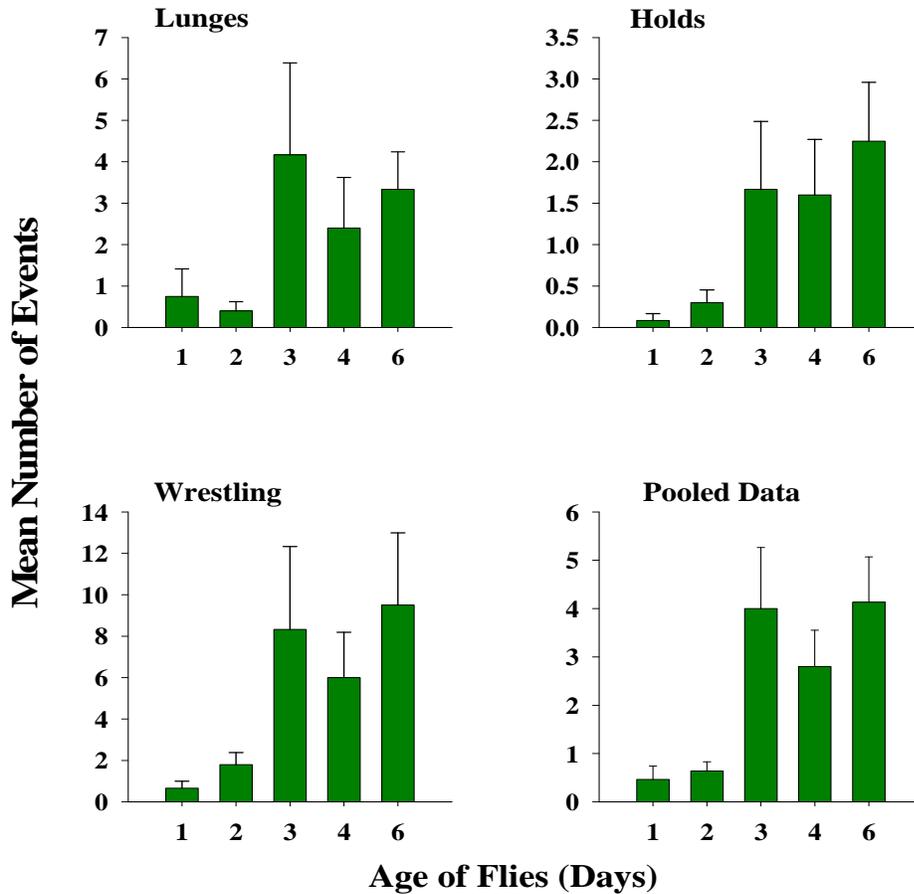


Figure 15 Mean (+ SEM) Number of High Intensity Aggressive Events. High intensity aggressive behaviors increase with age. Figure depicts the mean (+ SEM) number of high intensity aggressive events exhibited between pairs of socially naïve flies. Note the change in the mean number of events occurring from day 2 to day 3.

Interactive behaviors include behaviors in which a fly moves toward an opposing fly (approach and turn toward) and behaviors in which a fly moves away from an opposing fly (avoidance and retreat). Also included in the interactive behaviors are low intensity (chop, uppercut, back kick, head butt, fencing, and boxing) and high intensity aggressive events (lunge, holding, wrestling) that involve physical contact between flies. Preliminary data indicate that the number of high intensity aggressive events occurring in dyadic interactions increase as the flies age (Figure 15). Three-, 4-, and 6-day old flies lunge, hold, and wrestle more than do 1- and 2-

day old flies. Similar to standing, walking, and grooming, the noticeable change in high intensity aggressive events occurs at 3 days of age with 3-, 4-, and 6-day old flies exhibiting similar numbers of high intensity aggressive events. Conversely, whereas flies appear to gradually change the proportion of time spent standing, walking, and grooming as they age, high intensity aggressive events abruptly change from being rare in 1- and 2-day old flies to being a much more frequently observed behavior in 3-, 4-, and 6-day old flies.

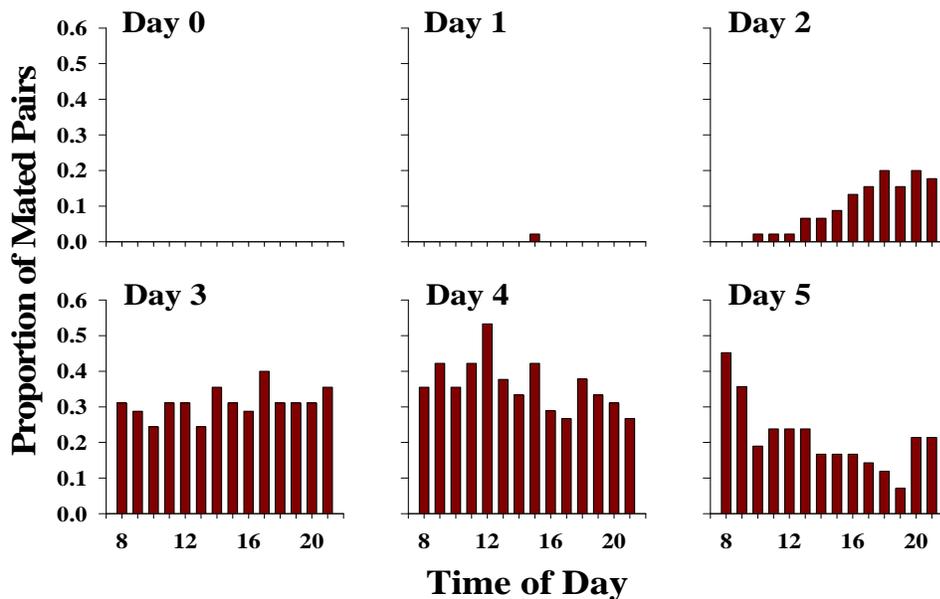


Figure 16 Proportion of Mated Pairs at Each Observation Time. Sexual activity increases 3 days after eclosion. Figure depicts the proportion of mated pairs at each observation time. Flies begin to mate on day 2 with a large increase in sexual activity occurring on day 3 and continuing through day 5.

#### Onset of Mating: Preliminary Results

To determine the onset of mating, pairs of male and female flies were observed every hour of the photophase (LD 14:10 h) for 6 consecutive days beginning on the day of emergence. No flies were observed mating the day of emergence and 1 pair was observed mating on day 1 (Figure 16). During day 2, the number of pairs mating increased throughout the day. Even so, the total proportion of pairs mating remained low, never rising above 20%. On day 3, the

number of pairs mating increased to above 20% for all observations with 40% of the pairs mating at 17:00 hours. The number of pairs mating continued to increase on day 4 with 53.3% of the pairs mating at 12:00 hours. A slight decrease in the number of pairs mating was seen throughout day 5. The data indicate that the onset of mating occurs on day 2 with a large increase in sexual activity occurring on days 3 and 4.

Table 3 Ethogram of Male Behavior

| <b>Component</b>                 | <b>Description</b>                                                     |
|----------------------------------|------------------------------------------------------------------------|
| <i>Non-interactive Behaviors</i> |                                                                        |
| Standing                         | Fly is still; no locomotion                                            |
| Walking                          | Fly walks throughout the arena                                         |
| Grooming                         | Fly grooms itself                                                      |
| Jump                             | Fly jumps into the air                                                 |
| Bobbing                          | Fly quickly raises and lowers body multiple times                      |
| Pop Up                           | Fly rises from and returns to standing position                        |
| <i>Interactive Behaviors</i>     |                                                                        |
| Approach                         | Fly advances toward opponent                                           |
| Turn Toward                      | Fly turns to face opponent                                             |
| Avoidance                        | Fly slowly moves away from advancing fly                               |
| Retreat                          | Fly quickly moves away from advancing fly to another area of the arena |
| <i>Low Intensity Aggression</i>  |                                                                        |
| Chop                             | Downward strike with foreleg                                           |
| Uppercut                         | Upward strike with foreleg                                             |
| Back Kick                        | Strikes with back leg                                                  |
| Head Butt*                       | Pushing opponent with head                                             |
| Fencing*                         | Both flies striking each other with one foreleg                        |
| Boxing*                          | Both flies rear up on back legs and strike each other with forelegs    |
| Lunge*                           | Fly rears up and jumps toward opponent                                 |
| Holding*                         | Fly grasps opponent with forelegs and attempts to immobilize           |

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| <b>Component</b> | <b>Description</b>                                                                                    |
|------------------|-------------------------------------------------------------------------------------------------------|
| Wrestling*       | Both flies grasp each other with forelegs and roll around the enclosure striking each other with legs |
| Falls on Back    | Fly falls on back and attempts to right itself                                                        |

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\* Similar behaviors have been described for *D. melanogaster* (Chen and others 2002; Nilsen and others 2004).

## CHAPTER 4

### DISCUSSION

In nature, male flesh flies appear to establish territories from which they fly out and attempt to capture females for mating (Arnett 2000). However, there is no evidence suggesting female territoriality. If males are indeed territorial and females are not, then males and females should differ fundamentally in the manner in which they react to same-gender conspecifics under a variety of conditions, not just in nature. Behavioral expression of territoriality in males, but not in females, should result from elemental differences in the manner in which males and females process and react to information in the environment. Our findings are consistent with this hypothesis.

Our results show that, in rectangular enclosures, male flesh flies (*S. crassipalpis*) have a significantly lower tolerance of each other than do female flesh flies. Males and females also differ in how they distribute themselves within the rectangular enclosures. Because male flies have a lower tolerance for other males, we would expect them to distribute themselves evenly throughout the enclosure in order to avoid one another. Our data support this prediction. The number of male flies observed in sections B through H does not significantly differ between sections. However, the presence of food attracts both males and females to section A of the enclosure. Male and female flies are observed significantly more times in section A than in sections B through H. Although both males and females are attracted to the food, significantly more females are observed with the food than are males. Perhaps the weaker attraction to the food exhibited by males is due to the lower tolerance of other male conspecifics. If so, male flies are more likely to avoid the food if another male is already positioned there. Female flies do not distribute themselves evenly but rather tend to position themselves in the sections with food and water. Females are observed in section H significantly more times than in sections C through G.

Females are also observed in sections B (section next to food source) and H (location of water) significantly more times than are the male flies.

In 2 different types of circular enclosures (i.e., one with food in the center and the other with food in the periphery), males maintain a significantly larger spatial separation among themselves than do females. As indicated by nearest neighbor analyses, the males tend to distribute themselves uniformly, whereas the females tend toward a random distribution. In our experiments, the territorial behavior displayed by the male flesh flies occurs in highly artificial conditions and is evoked in the absence of expected releasing cues (e.g., the presence of potential mates, restricted food caches, larviposition sites, etc.)

The mechanisms that lead to greater spatial separation and lower spatial tolerance among males relative to females remain to be determined. These gender-specific differences could be the result of innate avoidance behavior mechanisms. For example, if males establish territories in order to capture females for mating, then the males should be inclined to prevent other male flies from entering the territory, thus ensuring sole access to females entering the territory. Physical defense or behavior which identifies the owner to prospective rivals should function to keep intruders out (Brown and Orians 1970). Alternatively, the differences between male and female spatial patterns in our study may be the result of learned avoidance patterns derived from agonistic interactions between male, but not female, flies. Agonistic interactions between male *S. crassipalpis* do occur in the laboratory, with a distinct increase in intensity occurring 3 days after eclosion (Figure 15). Socially naïve, older flies are more likely to exhibit aggressive behaviors such as lunging, holding, and wrestling in interactions with same age conspecifics than are younger flies. Currently, we cannot discriminate between innate and learned behavioral mechanisms to explain the gender-specific differences in spatial patterning.

Gender-specific differences in behavioral responses to same-sex conspecifics are well known in other insects. For example, in the cockroach *Nauphoeta cinerea*, males will position themselves at regularly spaced, potential territory sites in an observation tank and perform guarding and patrolling behaviors (Ewing 1973). A dominance hierarchy develops among the males and not all males are able to guard and patrol a territory, even if potential sites are available (Ewing 1972, 1973). In contrast, female cockroaches do not exhibit territorial behavior, but instead form a group, usually on a male territory. The gregarious females will stay together except when they leave to give birth (Ewing 1973). In another example, when food is plentiful, female field crickets (*Gryllus bimaculatus*) are significantly less likely to attack, fight for significantly shorter times, and exhibit a more limited behavioral repertoire in encounters with same-sex conspecifics than male crickets. Also, females change their behavior when food becomes scarce, fighting more frequently and successfully than males (Adamo and Hoy 1995).

In the current study, the location of the food influenced the spacing behavior in the circular enclosures: for both male and female flesh flies, the R values were higher (indicating greater spatial dispersal) with food in the periphery than with food in the center of the enclosures. This result is not unexpected. In rectangular enclosures, males and females were attracted to the food as indicated by the greater number of flies located in section A. In circular enclosures with food located in the periphery, the food was positioned around the entire circumference of the circular arena and presumably provided a stimulus to attract flies toward the edges of the enclosure (encompassing a greater area). With food placed in the center, the attraction presumably was toward the center of the arena (a relatively smaller area). It is interesting to note that neither the male nor the female flesh flies were seen to congregate in the vicinity of the food nor did they appear to defend the food in the circular enclosures. The fact

that gender-specific differences in spatial distribution occurred irrespective of container geometry attests to the robustness of the phenomenon.

Our results show that, under light-dark (LD) cycles, the R values for male flies observed during the photophase are significantly higher (indicating higher levels of spatial dispersal) than those during the scotophase (Figure 12). The tendency toward a uniform distribution in the light apparently is relaxed in the dark, during which the R values approach levels indicative of a random distribution. These results suggest that the male spacing behavior is based upon the processing of visual rather than tactile, auditory, or olfactory inputs. The patrolling flights that male *S. crassipalpis* and other Dipterans make in their natural environment might serve to visually advertise their presence to conspecific intruders or to visually identify the individual entering the territory. Other insects use different methods to regulate spacing behavior. Male spacing in the katydid *Panacanthus pallicornis* appears to be based upon the calling song: deafened males tend to aggregate whereas control males distribute themselves randomly throughout an experimental environment (Chamorro-R and others 2007). In a second example, harvester ants (*Pogonomyrmex barbatus* and *Pogonomyrmex rugosus*) chemically establish foraging trails that never cross with trails from intraspecific neighboring colonies, effectively spacing themselves apart and reducing aggressive encounters (Holldobler and Lumsden 1980).

Providing further evidence supporting a visual basis for spacing behavior in male flesh flies are the results under constant dark (DD; Figure 12) and constant light conditions (LL; Figure 13). In both cases, there are no significant differences in R values between subjective day and subjective night observations. Furthermore, the R values measured during DD and LL are similar to those obtained during the scotophase and photophase, respectively, under LD 12:12 h conditions. The absence of significant variation between subjective day and subjective night

under constant conditions (DD or LL) suggests that the spacing behavior is not driven by a circadian rhythm. However, because our assay of spacing depends on the collective behavior within a group of 4 individuals, the possibility of circadian rhythmicity in 1 or more individuals cannot be rejected. Presumably, the expression of circadian rhythmicity in R values would require a high degree of behavioral synchrony among group members. Although not significant, the R values under DD show shallow, but detectable, daily oscillations for two cycles (Figure 12). It is important to note that other behaviors in *S. crassipalpis* (e.g., larval and adult locomotor activity, eclosion) are under circadian control (Joplin and Moore 1999). Further work is necessary to determine the relationship between endogenous circadian activity rhythms in individual flies and the expression of spacing behavior, the latter being an emergent property of multiple interactions within the group.

The fruit fly *D. melanogaster* is currently used as a model system for studying the physiological and genetic control of aggression (Baier and others 2002; Chen and others 2002; Nilsen and others 2004; Dierick and Greenspan 2006; Edwards and others 2006; Vrontou and others 2006). Male and female fruit flies exhibit different agonistic behaviors and these have been well described (Jacobs 1960; Dow and von Schilcher 1975; Hoffman 1987; Ueda and Kidokoro 2002; Nilsen and others 2004). Interestingly, dominance relationships form between males but not females (Nilsen and others 2004). Sex-specific behavioral patterns related to courtship and aggression may be attributed to male and female modes of splicing the *fruitless* gene in *D. melanogaster* (Demir and Dickson 2005; Vrontou and others 2006). Furthermore, it appears that expression of the male-specific fruitless protein establishes male-specific dendritic arborization patterns in certain interneurons, thus providing the substrate for sexually dimorphic neuronal circuits in the brain (Kimura and others 2005).

We propose that the flesh fly *S. crassipalpis* can be developed as a model system for investigating the physiological and behavioral bases for territoriality. The sit-and-wait territories established by male flesh flies provide an interesting contrast to *D. melanogaster* and other flies that set up and defend territories at food and oviposition sites (Jacobs 1960; Dow and von Schilcher 1975; Otronen 1984; Hoffman 1987). The flesh flies do not appear to defend any type of resource except for their perching position and the space surrounding it. Thus, the territorial and aggressive behavior exhibited by the flesh flies can be elicited in a minimal laboratory environment. Not only is the flesh fly system much simpler than the *D. melanogaster* system, there also appears to be an ontogeny to both aggressive and non-aggressive behavior exhibited by male flies in simplified dyadic interactions. The behavior exhibited by male flesh flies may have similarities to that exhibited by male butterflies that establish perching sites on vegetation and wait for potential mates to fly into the territory (Baker 1972; Davies 1978; Bitzer and Shaw 1979; Jones and others 1998). The ability to examine territorial behavior in the laboratory, such as in this study, enables detailed explorations of the proximal factors underlying the control and patterning of the behavior.

#### Further Research

Because the spatial behavior described in this study is an emergent property resulting from interactions among individuals within a group, the interactions that occur among flies need to be characterized. To characterize the interactions, the behavior must first be described. An ethogram of male behavior has been constructed from experiments in which dyadic interactions were observed between same-age, socially naïve flies (Table 3). Males spend the greatest proportion of time performing non-interactive behaviors such as walking, standing, and

grooming whereas interactions between flies are usually very brief. Preliminary results indicate that both the non-interactive (Figure 14) and high intensity aggressive behaviors (Fig 15) change as the flies age. An ethogram has not been constructed describing behavior in female-female interactions. Because gender specific differences exist in the spatial behavior, it is likely that differences between males and females also exist in behavior exhibited in dyadic interactions with same-sex conspecifics. Similarly, because the behavior observed in dyadic interactions changes as flies get older, it is likely that the spatial behavior will change with age as well, though no experiments investigating the ontogeny of the spatial behavior have been performed at this time.

Not only do changes in non-interactive and interactive behavior occur 3 days after eclosion, preliminary results indicate that an increase in sexual activity also occurs at this time (Figure 16). Flies begin mating 2 days after emergence with an increase in sexual activity seen 3 and 4 days after emergence. The results from this experiment are pooled from 3 cages with each cage containing a different number of pairs. Thus, density was not controlled for and further experiments need to be performed to see if density influences sexual activity and the onset of mating. However, the preliminary results from this experiment and from the dyadic interaction experiment do seem to suggest that the changes in behavior and sexual activity occur in parallel with each other. The nature of these parallel changes has yet to be determined. Furthermore, the nature of male-female interactions has not been investigated. It is possible that flies use different behaviors in encounters with conspecifics of the opposite sex than they do in encounters with same-sex conspecifics though no data exist to support or refute this idea.

By examining territorial behavior in the laboratory, *S. crassipalpis* are placed in simplified experimental situations where flies have to react only to conspecifics. Variables that

could possibly influence behavior in ways unknown to the investigators can be controlled for thus allowing easier interpretation of the results. Once the behavior has been completely described and quantified for *S. crassipalpis*, investigations into the physiological, molecular, and genetic mechanisms controlling the behavior can be examined.

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