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IN SCHOOL ARTICLE

A Comparison of Laboratory-Reared Stock and Captured Fruit Flies (*Drosophila melanogaster*) using Upward Movement, Phototaxic, and Starvation Assays Reveals Significant Behavioral Differences

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Abstract

Fruit flies (Drosophila melanogaster) are model research organisms and are frequently reared in research institutions. Research specimens domesticated in a laboratory-reared setting may have different behavioral phenotypes as compared to their wild counterpart. Additionally, it has been determined that the absence of "key" stimuli in the physical environment of captive animals may result in altered behavioral patterns[1]. The purpose of this investigation was to test for differences in the behavioral phenotype of outdoor captured fruit flies as compared to laboratory-reared fruit flies with the use of three tests: an Upward Movement Assay, a Starvation Assay, and a Phototaxic Assay. Results from the Upward Movement Assay demonstrated statistically significant differences in the vertical moving speed of laboratory-reared flies and outdoor-caught flies. Results from the Phototaxic Assay revealed outdoor captured fruit flies exhibited a natural phototaxic behavior while laboratory-reared flies exhibited an inverse phototaxic behavior. The Starvation Assay proved that flies recently descended from outdoor caught fruit flies were able to withstand starvation twice as long as laboratory-reared flies. These results indicate a strong behavioral difference between flies that are descended from laboratory stock and flies that are caught from the outdoors. Research regarding the differences in domesticated organisms is an imperative topic for study because domestication and genetic drift have the potential to alter the behavioral phenotype. Changes in the behavioral phenotype may jeopardize the results of research experiments. Thus it is crucial to have a thorough comprehension of the behavior of outdoor caught fruit flies as compared to the behavior of their wild counterpart. It is suggested scientists change their fruit fly stock every few hundred generations in an effort to protect the natural gene pools of organisms which are bred in captivity for extended periods of time.

Introduction

Domestication is the evolutionary genetic change arising from the transition of a population from nature to deliberate human cultivation[2]. Animals have been domesticated both unconsciously and methodically since the end of the Pleistocene Era. Due to cultivation and routine human interactions with species, selection pressures are created and the effected species is forced to adapt to a new environment. The transition of a free-living culture to captive status is often accompanied by changes in availability and/or accessibility of shelter, space, food, water, predation, and the social environment[1]. It is implied that the phenotype of the domesticated species will differ from its counterpart once it has undergone domestication. As natural selection has the ability to change the frequency of traits within a population, it has been discovered that the brain of an organism has the tendency to shrink when bred in captivity for extended periods of time[3]. Two approaches have been developed to gain a better understanding of the domestication process: a comparison of wild and domestic stocks (of a species), and the study of wild and domestic hybrids[4].

This experiment utilized the first approach, a comparison of the wild and domestic stocks of a species, to understanding the domestication process. The wild stock (outdoor captured fruit flies) is used as a representative and ancestor of the domesticated population (laboratory-reared fruit flies). A comparative approach between specific populations of wild versus domesticated animals at a single point in time is applied to this study. This experiment and other similar research tests suggest altered behaviors are a result of altered genes. Specific alleles have a relatively large impact on the development of behavioral characteristics specific to domesticated animals. For example, a study was conducted to identify the genetic variation between cultivated rice and its wild progenitor. This study assessed the genetic basis of the changes associated with the process of rice domestication. A total of 19 traits related to domestication in cultivated rice were discovered[5].

Another study demonstrated that laboratory-reared flies and wild fruit flies exhibit differences in an ovipositor choice test[6]. In the choice tests, using white and black artificial ovipositor domes, the wild flies' selected black domes almost exclusively, but the laboratory-reared flies failed to display any preference[6]. Additionally, a study compared the behavior of wild and domestic stocks of Brook Trout. Results proved the domesticated group was much more vulnerable to trap-netting than the wild groups. After a week of trapping fish, 84% of the captured fish were from the domesticated group[7].

Drosophila's favorable characteristics make it an ideal research specimen. They require minimal care, space, and equipment. In addition, fruit flies are easily cultivated in the laboratory; they have a high fecundity, and a short generation time. Thus, they are highly susceptible to genetic drift and domestication. Drosophila is presently one of the most commonly used model organisms in biological research. They serve as a genetic model for numerous diseases. They are also used to study aging, oxidative stress, immunity, diabetes, cancer, obesity and drug abuse[8].

Laboratory-reared fruit flies are essential in biological research and it is crucial to have a thorough understanding of their

behavior compared to the behavior of a fly originating from a natural environment. Laboratory-reared fruit flies which are domesticated have the ability to jeopardize the validity of significant experimental results. Although this is known, domestication of fruit flies within a laboratory is hardly taken into consideration.

The laboratory-reared fruit flies were reared in the laboratory for approximately 1,500 generations which made them an ideal specimen for a study of domestication. Outdoor captured and laboratory reared fruit flies were both genetically variable. However, low genetic variation due to a consistent environment, laboratory conditions such as small culture vials, and genetic drift, accounts for predictably less genetic variation among the laboratory reared population[9]. Neither of the fly populations were isogenic. Descendants of flies caught outdoors (wild) and descendants of laboratory-reared fruit flies (domesticated) were tested for differences in behavioral phenotypes with the use of three assays: an Upward Movement Assay, a Phototaxic Assay, and a Starvation Assay.

The Upward Movement Assay was used to test the flies' vertical movement. The Phototaxic Assay was used to test fruit flies locomotive movement in response to the stimulus of light (phototaxic behavior). The Starvation Assay was constructed to test the flies' abilities to withstand starvation.

Materials and Methods

Outdoor captured flies were caught from locations on Long Island, New York: Commack, East Northport, and Mount Sinai. Strain Oregon R laboratory reared fruit flies were obtained from Carolina Biological. These flies are known to have descended from flies cultivated in the laboratory for 54 years. Carolina Biological's fruit fly culture has remained unchanged without the addition of fruit flies from external sources. Both outdoor captured and laboratory-reared fruit flies were cultivated in the school laboratory for no more than 20 generations. Fruit flies used for this experiment were descendants of either outdoor captured or laboratory-reared locations, and they were all cultivated at the same ambient conditions. The fruit fly life cycle consist of four stages: egg, larva, pupa and adult. At a standard classroom temperature of 21°C, the fruit fly life cycle lasts two weeks. Flies were re-cultivated every two weeks. The Upward Movement Assay was used to determine the flies' upward velocity toward a light source (figure 1). This assay consisted of a laboratory clamp, a 35cm glass tube with a 0.5 cm diameter, a bright light, a fly aspirator, syringe, and a stopwatch. This experiment was conducted in a dark room. The glass tube was held vertically by the laboratory clamp and the bright light was attached to the top of the glass tube. Flies were individually aspirated and syringed into the glass tube. The 25cm flight time was recorded. Data was averaged and recorded as time per centimeter. Each fly location consisted of 5 blocks and every block had 10 trials. In total, the Upward Movement Assay was comprised of 200 trials (Supplementary tables 1-4). The Phototaxic Assay was used to determine the strength of the flies' phototaxic behavior. This assay consisted of Plexiglas, a syringe, a fruit fly aspirator, a bright light, and two flight arenas (light and dark). Plexiglas was cut into 3 fragments and glued together to create narrow corridors (2mm diameter) for the flies to travel (figure 2). One narrow canal branched off into two canals. At the end of each canal was either a light or dark flight arena. The light flight arena was illuminated with an incandescent light and the dark flight arena was fully covered in black construction paper. The remainder of the apparatus was enclosed in red translucent paper. Flies are unable to distinguish red light and this prevented the flies from getting distracted by excess light. Fruit flies were injected into the device and they showed an attraction for either the light or dark flight arena. The data from this assay was recorded in 5 blocks: each block contained 5 trials totaling 100 trials (Supplementary tables 5-8). The Starvation Assay was used to test the flies' ability to withstand starvation. This assay consisted of vials with moist cotton. Ten flies from each location were anesthetized and placed into vials with moist cotton. At the end of each day the number of flies alive were recorded. Each fly location had 5 blocks of 10 flies, totaling 200 trials (Supplementary tables 9-12).

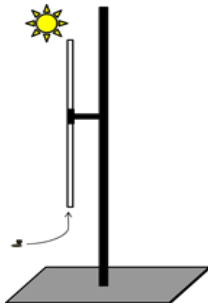

Figure 1. Upward Movement Assay Design. The glass tube was held vertically by the laboratory clamp and the bright light was attached to the top of the glass tube.

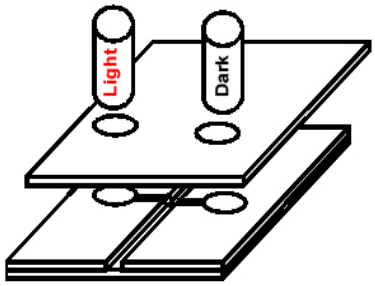

Figure 2. Phototaxic Assay Design. Plexiglas was glued together to create a narrow corridor for the flies to travel. One narrow canal diverged to either a light or dark flight arena. The light flight arena was illuminated with a bright light and the dark flight arena was covered in black paper. The apparatus was enclosed in red translucent paper.

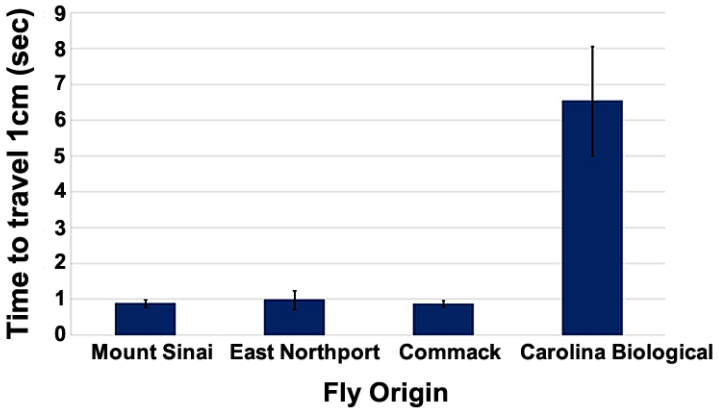

Figure 3. Upward Movement Assay. Flies from outdoor locations (Mount Sinai, East Northport, and Commack) traveled 1cm in an average of approximately 1 second. Flies from Carolina Biological (lab-reared) traveled 1cm in an average of 6.5 seconds. (Error bars = Standard error)

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Results

Laboratory-reared fruit flies exhibit behavior which was different from its wild counterpart. They exhibited a different locomotive behavior, a stronger phototaxic behavior, and they were able to survive longer when starved.

Results from the Upward Movement Assay demonstrate a statistical difference in vertical flight of lab reared and wild caught flies. Flies which were reared in laboratories took approximately six times longer to travel the same distance as flies which were captured from the outdoors. In addition, the behaviors of laboratory-reared flies were observed to be sporadic and inconsistent (figure 3). Results were assessed with an Analysis of Variance (ANOVA Microsoft Excel). A single factor 1- Way Analysis of Variance Test (ANOVA) was applied to the data. The upward movement probability was 1.12E-06 indicating that there was a statistical difference in the vertical flight speed of the flies (due mostly to the difference in lab-reared flies).

Results from the Phototaxic Assay demonstrate a statistical difference in phototaxic behavior of lab reared and wild caught flies ($p = 0.004$). Flies which were caught from the outdoors all exhibited a natural phototaxic behavior. Flies which were reared in laboratories exhibited an inverse phototaxic behavior (figure 4). A non-parametric test was applied to the ordinal data. The chi square probability was 0.004. This means that there is evidence to support the alternative hypothesis that the behavior/movement is dependent on the origin of the fly. The probability of accepting the alternative hypothesis when the null hypothesis should have been accepted is very small.

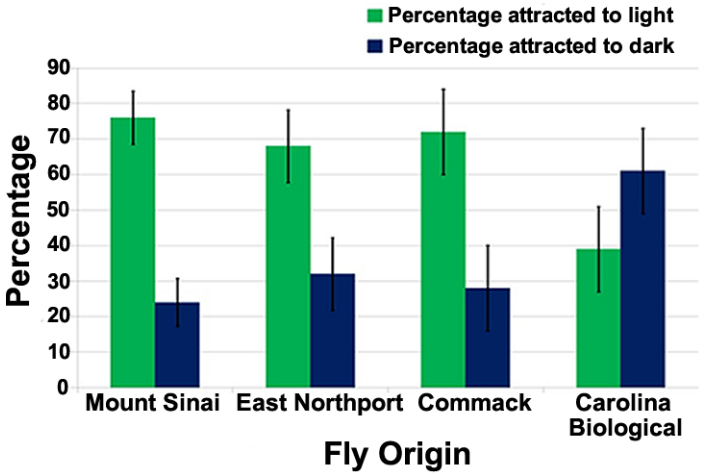

Figure 4. Phototaxic Assay Data. Wild caught flies were mostly attracted to the light flight arena and laboratory reared flies were mostly attracted to the dark flight arena. Wild caught fruit flies exhibited a natural phototaxic behavior. Laboratory reared flies (Carolina biological) exhibited an inverse phototaxic behavior. (Error bars = Standard Error)

Results from the Starvation Assay show that fruit flies which are reared in laboratories survived a maximum of three days. Fruit flies which are captured from the outdoors survived a maximum of four to five days. Fruit flies which were reared in laboratories died more rapidly than flies which were captured from the outdoors (figure 5).

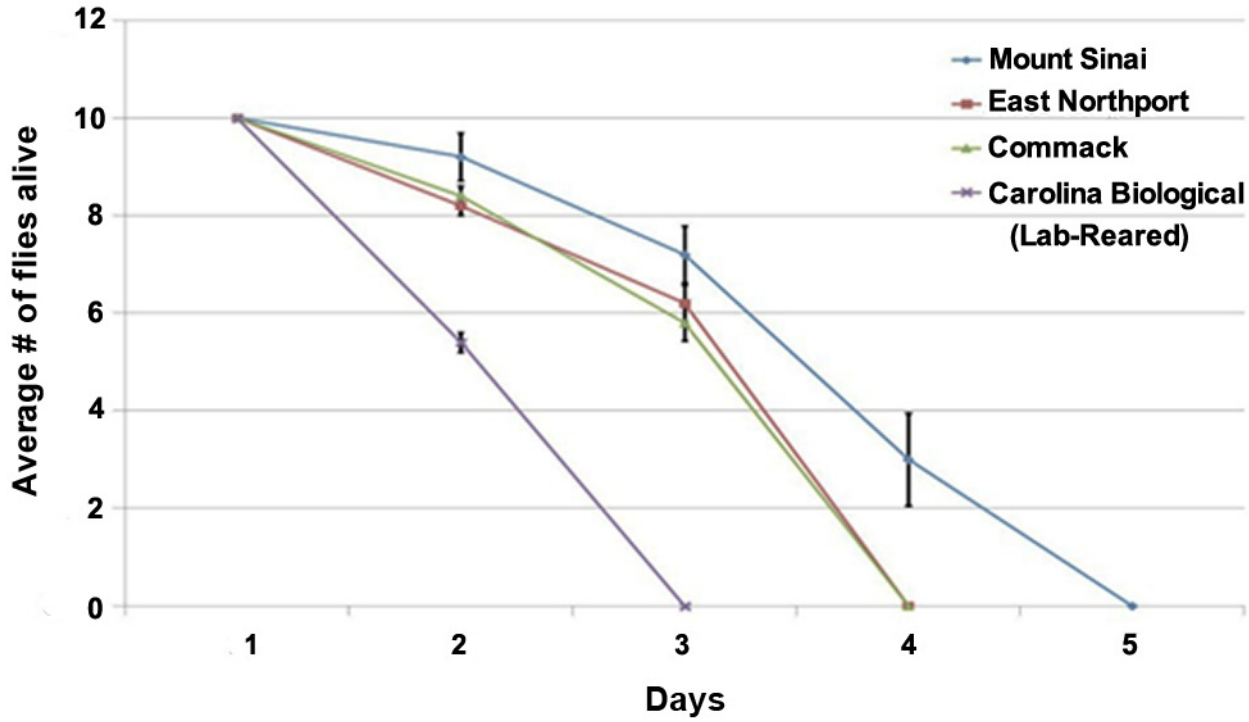

Figure 5. Starvation Assay Data. Flies from outdoor captured locations (Mount Sinai, East Northport, and Commack) survived a maximum of four to five days when starved. Flies from Carolina Biological were all dead by the third day of starvation. (Error bars = Standard Error)

Discussion

Humans can play a role as a buffer between an animal and its environment. Fruit flies that are reared in laboratories have reduced sensitivity to their environment[2]. It is speculated that the laboratory-reared fruit flies' impaired vertical movement may be caused by a lack of predation. Laboratory-reared fruit flies may have not honed mechanisms for escaping predators. Without the need to hone flight skills, laboratory-reared fruit flies may have developed flight mechanisms that are different from their wild counterpart. Fruit flies which are reared in laboratories may not be exposed to a natural 12 hour light 12 hour dark photoperiod. It is speculated that an inconsistent photoperiod may cause a lab reared fruit fly to develop an inverse attraction to light. It has also been discovered that wild caught flies had evolved better mechanisms for storing sugars and fats as compared to laboratory-reared flies[10]. Higher extrinsic adult mortality rates leads to an early reproductive effort, eclosion at an earlier age and a smaller sized fruit fly[11]. Therefore, the inability to properly store fats and sugars, ultimately affect laboratory-reared offspring. A recent study which tests starvation in Oregon R fruit flies suggests there is a correlation between the sex of a fruit fly and its ability to withstand starvation. Results suggest that wild female fruit flies survive longer than wild male flies. In addition, wild female fruit flies have been discovered to survive longer than both male and female laboratory reared flies. The sex of a fruit fly is a possible source of variability within the results of the three behavioral assays[9].

Laboratory reared, and outdoor captured fruit flies were both genetically variable. The impact of genetic diversity and its effect on the results is unknown due to the inability to determine the level of variability for each fly location. While it may be expected for the wild population to display a greater behavioral variance, compared to the domesticated stock, their behavior was surprisingly similar. The results of the three assays depicted the laboratory reared population to have a greater variation in behavior. The results allude to the assumption that laboratory reared fruit flies have a greater level of variability. A comparative study of genetic variation between outdoor captured and laboratory reared fruit flies would be a fruitful avenue for additional research. Furthermore, domestication may possibly be a result of chromosomal and clustered blocks of genes. This clustering of genes may provide explanations for the genetic basis of domestication[5].

It has been shown that domestication has an important effect on the development of the domestic phenotype. Scientists who use fruit flies for experimental purposes are transferring free living insects to captive status. This domestic phenotype results in genetic drift, artificial selection, and relaxed selection[1]. Many organisms are domesticated in laboratory-reared settings and these organisms may have a different behavioral phenotype from their wild counterpart. Changes in behavioral phenotype can alter the outcome of experiments and invalidate scientific results. Scientists frequently use laboratory-reared fruit flies as experimental specimens and the effects domestication has on the behavior of a fly should be considered with respect to the results. It is suggested scientists change their fruit fly stock every few hundred generations. "Natural" gene pools should be protected when breeding animals in captivity for extended periods of time.

In the future, additional outdoor captured fruit flies from Long Island will be compared to laboratory-reared fruit flies from Long Island. Flies from various states and various parts of the world will be tested to further validate the results. A test will be conducted to determine the rate of evolution for a fruit fly. With this data, scientists will be able to create a more precise time period for changing and restoring their fruit fly stock. A study will also be conducted to determine the specific genes responsible for the domestication of a fruit fly.

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Supplementary Data

Table 1. Mount Sinai Upward Movement Data (wild caught, time in sec. to travel 1 cm).

Fly Group A (Mount Sinai)- Second/centimeter						
Trial	Block 1	Block2	Block 3	Block 4	Block 5	Grouped Avg.
1	0.8	1.2	0.6	0.8	0.9	0.8
2	1.5	0.5	0.8	0.7	0.5	0.8
3	1.0	0.6	0.5	1.0	1.1	0.8
4	0.5	1.3	1.5	0.5	0.6	0.9
5	0.7	1.2	0.7	1.2	1.1	1.0
6	0.5	1.2	1.2	0.5	1.5	1.0
7	0.8	1.0	1.1	0.7	0.9	0.9
8	0.6	1.0	0.9	1.3	1.0	1.0
9	0.8	1.1	0.0	0.7	0.6	0.6
10	0.6	1.1	1.4	1.2	0.5	0.9
Average	0.8	1.0	0.9	0.9	0.9	0.9
Std. Dev.	0.3	0.2	0.4	0.3	0.3	0.3
Std. Error	0.1	0.1	0.1	0.1	0.1	0.1

Table 2. East Northport Upward Movement Data (wild caught, time in sec. to travel 1 cm).

Fly Group B (East Northport)-Second/centimeter						
Trial	Block 1	Block 2	Block 3	Block 4	Block 5	Grouped Avg.
1	1.0	1.2	0.7	1.2	0.6	1.0
2	1.2	1.3	1.1	1.2	0.6	1.4
3	0.7	1.2	1.6	1.3	0.9	1.6
4	1.7	0.8	1.5	1.0	1.2	1.8
5	0.9	0.7	0.6	1.4	0.6	1.7
6	1.2	0.6	0.7	0.7	1.2	1.8
7	0.9	1.6	0.6	1.2	0.6	2.3
8	1.1	0.6	1.1	1.0	0.9	2.4
9	1.0	1.2	1.0	0.4	1.2	2.5
10	0.7	0.6	1.1	0.4	1.3	2.6
Average	1.0	1.0	1.0	1.0	0.9	1.0
Std. Dev.	0.3	0.4	0.3	0.4	0.3	0.3
Std. Error	0.1	0.1	0.1	0.1	0.9	0.3

Table 3. Commack Upward Movement Data (wild caught, time in sec. to travel 1 cm).

Fly Group C (Commack)-Second/ centimeter						
Trial	Block 1	Block 2	Block 3	Block 4	Block 5	Grouped Avg.
1	0.8	0.7	1.3	0.7	0.6	0.8
2	0.6	0.6	0.7	0.8	0.6	0.6
3	1.4	0.8	1.2	1.0	0.6	1.0
4	0.5	1.3	0.8	1.2	0.8	0.9
5	0.8	1.2	0.6	0.4	1.4	0.9
6	0.5	0.5	0.8	0.6	0.7	0.6
7	1.5	1.0	1.2	1.1	0.6	1.1
8	1.2	1.2	1.3	0.6	0.8	1.0
9	0.6	0.8	0.5	0.8	1.3	0.8
10	1.3	0.6	0.6	1.3	0.7	0.9
Average	0.9	0.9	0.9	0.8	0.8	0.9
Std. Dev.	0.4	0.3	0.3	0.3	0.3	0.3
Std. Error	0.1	0.1	0.1	0.1	0.1	0.1

Table 4. Carolina Biological Upward Movement Data (laboratory- reared, time in sec. to travel 1 cm).

Fly Group D (Lab Reared)-Second/Centimeter						
Trial	Block 1	Block 2	Block 3	Block 4	Block 5	Grouped Avg.
1	1.7	0.6	2.1	1.3	1.7	1.5
2	2.5	1.7	2.6	2.3	13.3	4.5
3	13.3	13.3	1.2	1.6	0.5	6.0
4	0.9	8.0	1.0	40.0	0.6	10.1
5	1.7	1.7	1.7	4.0	1.0	2.0
6	0.9	0.9	40.0	1.6	2.3	9.1
7	1.6	1.7	3.3	1.0	40.0	9.5
8	40.0	40.0	0.8	2.5	0.7	16.8
9	1.1	1.4	3.0	13.3	0.9	3.9
10	3.3	0.7	0.8	1.3	3.3	1.9
Average	6.7	7.0	5.7	6.9	6.4	6.5
Std. Dev.	12.3	12.3	12.1	12.2	12.4	4.9
Std. Error	3.9	3.9	3.8	3.8	3.9	1.5

Table 5. Mount Sinai Phototaxic Data (wild caught, Light versus Dark).

Fly Group A (Mount Sinai)								
Trial	Block 1	Block 2	Block 3	Block 4	Block 5	Trial	Avg. Light	Avg. Dark
1	L	D	L	L	D	1	60	40
2	L	L	L	D	L	2	80	20
3	L	L	L	L	L	3	100	0
4	D	L	L	L	D	4	60	40
5	L	L	D	L	L	5	80	20
Attracted to light				76%		Average	76	24
Attracted to dark				24%		Std. Dev.	16.7	16.7
						Std. Error	7.5	7.5

Table 6. East Northport Phototaxic Data (wild caught, Light versus Dark).

Fly Group B (East Northport)								
Trial	Block 1	Block 2	Block 3	Block 4	Block 5	Trial	Avg. Light	Avg. Dark
1	L	L	D	L	D	1	60	40
2	D	L	L	L	L	2	80	20
3	L	L	L	L	L	3	100	0
4	L	D	L	D	L	4	60	40
5	L	D	L	D	D	5	40	60
Attraction toward light				68%		Average	68	32
Attraction toward dark				32%		Std. Dev.	22.8	22.8
						Std. Error	10.2	10.2

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Table 7. Commack Phototaxic Data (wild caught, Light versus Dark).

Fly Group C (Commack)								
Trial	Block 1	Block 2	Block 3	Block 4	Block 5	Trial	Avg. Light	Avg. Dark
1	L	L	L	L	L	1	100	0
2	L	L	L	D	D	2	60	40
3	D	L	D	L	L	3	60	40
4	D	L	D	D	L	4	40	60
5	L	L	L	L	L	5	100	0
Attracted to light				72%		Average	72	28
Attracted to dark				28%		Std. Dev.	26.8	26.8
						Std. Error	12	12

Table 8. Laboratory-reared Phototaxic Data (wild caught, Light versus Dark).

Fly Group D (Carolina Biological)								
Trial	Block 1	Block 2	Block 3	Block 4	Block 5	Trial	Avg. Light	Avg. Dark
1	L	L	D	D	L	1	60	40
2	D	D	L	L	L	2	60	40
3	D	D	L	D	D	3	20	80
4	D	D	D	D	D	4	0	100
5	L	D	D	D	D	5	20	80
Attracted to light				39%		Average	32	68
Attracted to dark				61%		Std. Dev.	26.8	26.8
						Std. Error	12	12

Table 9. Mount Sinai Starvation Data (wild caught, number of flies alive at the end of each day).

Fly Group A (Mount Sinai)								
Day	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	Std. Dev.	Std. Error
1	10	10	10	10	10	10	0	0
2	10	8	8	10	10	9.2	1.1	0.5
3	9	6	7	6	8	7.2	1.3	0.6
4	5	0	3	2	5	3	2.1	0.9
5	0	0	0	0	0	0	0	0

Table 10. East Northport Starvation Data (wild caught, number of flies alive at the end of each day).

Fly Group B (East Northport)								
Day	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	Std. Dev.	Std. Error
1	10	10	10	10	10	10	0	0
2	8	9	8	8	8	8.2	0.4	0.2
3	6	7	5	6	7	6.2	0.7	0.4
4	0	0	0	0	0	0	0	0

Table 11. Commack Starvation Data (wild caught, number of flies alive at the end of each day).

Fly Group C (Commack)								
Day	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	Std. Dev	Std. Error
1	10	10	10	10	10	10	0	0
2	8	9	8	9	8	8.4	0.5	0.2
3	6	7	5	5	6	5.8	0.7	0.4
4	0	0	0	0	0	0	0	0

Table 12. Mount Sinai Starvation Data (laboratory-reared, number of flies alive at the end of each day).

Fly Group D (Carolina Biological)								
Day	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	St. Dev	Std. Error
1	10	10	10	10	10	10	0	0
2	6	5	5	5	6	5.4	0.5	0.2
3	0	0	0	0	0	0	0	0