The Rhodes Journal of Biological Sciences

Volume XVII Spring 2000

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Statement of Purpose:
The Rhodes Journal of Biological Sciences is a student-edited, annual publication which recognizes the scientific achievements of Rhodes students. Founded seventeen years ago as a scholarly forum for student research and scientific ideas, the journal aims to maintain and stimulate the tradition of independent study. We hope that in reading the journal, other students will be encouraged to pursue scientific investigations and research.

Acknowledgements: The editors would like to thank Dr. Bobby Jones for his invaluable support and assistance.

Cover: Giant Cloudless Sulphur Butterfly

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Biology Department Faculty

Rhodes Biology Faculty: bottom row, left to right, Dr. Stinemetz, Kate O Leary, Dr. Jones, Dr. Olsen Top row, left to right, Dr. Carolyn Jaslow, Dr. Alan Jaslow, Dr. Kesler, Dr. Blundon, and Margaret Sowles *not pictured- Dr. Hill*

Dr. Jay Blundon- For the past 15 years I have been involved in studying biological phenomena associated with communication between neurons. The implications of my research have often been relevant to such diverse areas of neurobiology as neuronal degeneration and regeneration, effects of alcohol on behavior and neuronal function, and mechanisms of learning and memory. My past research has frequently utilized non-mammalian animal models to study synaptic transmission because invertebrate neurons are frequently much larger in size but still show physiological properties similar to mammalian neurons. Research that I am now beginning in my sabbatical year away from Rhodes, in collaboration with molecular biologists in the Department of Developmental Neurobiology at St. Jude Children s Hospital, focuses on the role that cellular proteins play in organizing neuronal synapses into functional units.

Dr. Connie Kurschner at SJCH has recently characterized a neuronal protein (termed NIL-16 for neuronal interleukin-16), found in the mouse cerebellum and hippocampus (both structures involved in learning and memory) that may serve to cluster together several membrane proteins into functional aggregates. Some proteins believed to be grouped together by NIL-16 include potassium and calcium ion channels and glutamate receptors. In addition, NIL-16 contains a protein region which is identical to the immune interleukin IL-16. Further, neurons that contain NIL-16 also contain IL-16 receptors and the enzyme that has the capability to cleave IL-16 from NIL-16. For at least the next two years, I will be working with Dr. Kurschner to characterize the role of NIL-16 in organizing neuronal membrane proteins, and I will also test whether IL-16 has any influence on synaptic transmission, and perhaps memory formation, in mouse brains.

Dr. Alan Jaslow- I was an undergraduate Zoology Major at the University of Wisconsin in Madison. I earned my MS in Zoology and Ph.D. in Biological Sciences from the University of Michigan. After completing my doctorate I was a postdoctoral fellow at the University of Chicago Department of Anatomy for two years. My research has been focused on the structure and function of vertebrates as well as animal communication. Past research had dealt with the evolution and function of vertebrate middle ears, evolution of male acoustical signals, and the anatomy of mammal leg bones. Students working in my lab have worked with leg bone shape, flamingo social behavior, prey selection in lizards related to mimicry, spider shape change during development, and sexual differences in tarantula growth energetics.

Dr. Carolyn Jaslow- I was an undergraduate Biology Major at Mount Holyoke College. Subsequently, I earned an M.S. in Zoology from Ohio University and my Ph.D. from the Anatomy Department at the University of Chicago. Over the past several years, my research has focused on skeletal biomechanics, specifically, how the morphological designs of mammalian skulls and teeth allow them to accommodate the forces generated during specialized activities of these animals.
Recently, I have studied the biomechanics of two features: the cranial sutures and the incisor teeth of rodents. Students working in my lab have carried out experiments investigating how the cranial suture morphology of rats changes during development, and whether those changes are related to bite forces acting on the skull.

Dr. Bob Jones- I joined Southwestern at Memphis as an assistant professor of biology in 1968. It was obvious from the onset that I could not continue the research I had started at the University of Missouri because Southwestern did not have the facilities to do genetic research on *Chlamydomas eugametos*. I spent a few summers in the Biochemistry Department at St. Jude Children s Research Hospital before I turned my attention to the genetics of the cotton bowl weevil.

The biology department at the University of Memphis gave me an adjunct appointment around 1975 and I have published papers with three of their faculty. The last paper I published with Dr. Charles Biggers was a chapter in a book and was a summary of 20 years of work. This work was presented at an international meeting in Cardiff, Wales, in 1996.

Dr. David Kesler- My research has ranged widely in topics from dealing with freshwater ecology. A long-term research project of mine has been determining the species composition and distribution of freshwater mussels in west Tennessee rivers, especially the Wolf River, where to date we have found 25 species and recorded, using GPS, the locations of over 4,200 individuals. These data are critical for management decisions and formulating regulations to support the river s biota.

Next semester I will work with students looking at glycogen concentration in freshwater mussels of the Wolf River in west Tennessee. Concentration of this storage carbohydrate is an indicator of stress the animals are experiencing. We are testing the prediction that animals in a reach of the river receiving wastewater effluent will have lower glycogen concentrations than animals living up river from these effluents. Coincident with this research is the investigation of survivorship and growth in the Asian Clam (*Corbicula fluminea)* that we will set out in enclosures. another student research project for next semester will be cataloguing the fishes in a section of Wolf River.

Dr. John Olsen, Department Chair- My research interests are connected to the field of Systematics and questions of evolutionary relationships between plants. I have an interest in the ultrastructure of plants, utilizing the scanning electron microscope (SEM) to examine variations in features of plant structures such as pollen grains and hairs. This has generated a number of student projects since the SEM is a relatively easy instrument to use and generates very interesting images. The most recent project done in this regard has compared SEM and TEM images of cotton fibers.

I am also interested in examining chromosomes, both from the straightforward determination of chromosome number and from the analysis of meiotic chromosome behavior. Our imaging lab provides us with an easy way to capture microscopic images of cells in mitosis and meiosis for evaluation.

Finally, I am interested in secondary plant products, particularly flavoniod compounds. Flavonoid fingerprints are useful systematic tools and we can utilize the HPLC to separate individual compounds from the complex mixture found in the methanolic extracts of leaf or flower tissues.

Dr. Charles Stinemetz- Dr. Stinemetz is a former resident of the Buckeye state (Ohio) where he received two BA degrees in Botany & Bacteriology and Chemistry at Ohio Wesleyan University. He was awarded his MS and Ph.D. degrees from The Ohio State University. While in graduate school he became interested in the physiological responses of plants to changes in their environment. His research principally dealt with the role of calmodulin and calcium in root gravitropism and was sponsored by NASA.

Dr. Stinemetz joined the faculty of Rhodes College in 1989 where he has continued to work on response of plants to their environment including both gravitropism and hydrotropism. His work with hydrotropic responses of plants began during a one year sabbatical in Japan which was sponsored by the Japanese governmental agency MONBUSHO. Each year, Dr. Stinemetz teaches Botany and Mechanism of Developmental Biology. As permitted, he also offers courses in Plant Physiology, Art and Science of Wine, Plants, Genes, and Agriculture, and Space Biology.

Dr. Stinemetz is married and has two children (Nichole-8, and Alex-7). Besides working in the lab and on the computer, he also enjoys movies, Sunday drives, hiking, sports (NBA and college football), wine and gourmet dinning.

Science Journal Editors

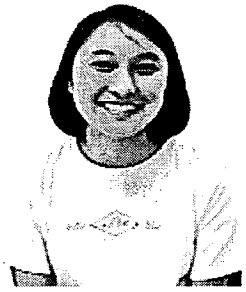
Ellen Barton, a native of Birmingham, Alabama, is currently a sophomore at Rhodes College. Although she has special interests in biology and research, she is pursuing a French major and hopes to do research abroad after graduation. She also enjoys running, riding horses, traveling and being with her friends.

An Huynh is a junior biology major. She is originally from Benton, Arkansas.

Margo Kamel is sophomore biology and political science majors. She is originally from Egypt, and plan to go to medical school. As a Bonner scholar, she volunteers her time at St. Jude's Children s' hospital, Evergreen After-School program, and LeBonheur's Children's Hospital.

Andrea Master, a native of Shreveport, Louisiana, is currently a senior biology major at Rhodes, College, and plans to attend veterinary school of medicine upon graduation.

Ann Young, a native of Shreveport, Louisiana, is currently a freshman at Rhodes College. She will be a biology major, and plans to attend a veterinary school of medicine upon graduation. She is a certified CASA volunteer, and is involved in several campus organizations. Other interests include reading, horseback riding, and road trips.



Pictures: left, An, group photo, l-to-r, Ann, Margo, & Ellen, right, Andrea

Analyzing Drosphila Eye Pigments Using Adobe PhotoShop

Linda Lasselle, Andrea Master, and Kasey Sweeney

Rhodes College, 2000 North Parkway, Memphis, TN, 38112

Abstract: *Drosophila* eye pigments obtain darker coloration over a duration of time. The objective of this experiment is to determine the effect of duration of time on eye pigment coloration of *Drosophila* using Photoshop. The eye pigment change was recorded by the use of the computer program Adobe PhotoShop 5.0. The fruit flies were etherized and decapitated. Their eyes were smeared onto a piece of filter paper. The smeared pigment images were then scanned and seen in PhotoShop. Initially, we worked on determining consistency and accuracy. The smearing and scanning technique was modified in order to see more consistent results. From our results, we concluded that with accurate decapitating, smearing, and scanning techniques the developmental process and darkening of *Drosophila* eye pigments can be accurately recognized through the use of the Adobe Photoshop computer program.

Introduction:

The *Drosophila* compound eyes are relatively very large and widely separated and are of the same size and shape in the two sexes. Their eyes also have stiff setae, which arise from each angle of the intersecting facets. An ocular sclerite is also found to surround each eye. In many insects the ocular foramen is very small, and the eye bulges out around the foramen much like the head of a mushroom on a very short stalk, usually being very closely appressed to the head. The derm immediately beneath the bulging eye is commonly deeply pigmented and forms an ocular diaphragm, but it is definitely not a distinct sclerite. In *Drosophila*, the ocular foramen is practically as large as the eye itself, and there is no indication of the ocular diaphragm. However, the ocular foramen provides for a large amount of pigmentation area and coloration differences within the eye pigment

Eye color in *Drosophila* is principally determined by the physiological interaction of types of compounds, the ommochromes (producing brown pigmentation, derived from trypthophan) and the pteridines (producing red pigmentation). These two types are formed in separate biochemical pathways and both must be present to produce wild type eye color. Different mutant strains of flies can produce a different pattern of pigment spots characteristic for each strain, depending on which step in the pathway is blocked in that mutant strain. Not all of the pigments are easily visualized in normal lighting.

.In addition, *Drosophila* eye pigments have been known to be affected by many genes, such as *vermilion, cinnabar, rosy, scarlet, white, etc.* Some of these cause the absence of the normal brown pigment and appear bright red (e.g., vermilion, cinnabar); others lacked the bright red pigment and appeared ruby or brown (e.g., apricot, sepia); while others lacked both

pigments and were devoid of color (i.e., *white*). However, at present only two main types of pigments are known to be carried in Drosophila eyes, the pterins and the ommochromes, each affected by a different set of genes.

The objective of this research was to examine the effects of time on *Drosophila* eye pigments using the Adobe PhotoShop program to determine the coloration. Since the eye pigments changed regularly within a period of nine days, we hypothesized that as flies develop their eye pigments darken. We also focused on determining each mutation type of pigment (pterin or ommochrome) and the changes in genetic make up, as evident by the change of amount of pigment and by the pattern of how our ten mutations darkened each day.

Methods:

As part of a multigenerational study, virgin fruit flies of all samples (wild type, scarlet, vermilion, cinnabar, rosy, sepia, brown, apricot, and eosin) were obtained. The research began by attempting to gain consistent results from *Drosophila* eye pigment using Adobe PhotoShop 5.0. The fruit flies were etherized and their heads were cut off using a scalpel. The heads were then smeared on the filter paper using the glass rod. The eye pigment stuck to the filter paper so it could easily be scanned and seen in PhotoShop. We read the histogram readings of the RGB spectrum in PhotoShop and determined the mean average for each color of the spectrum. For each fly mutant, we determined the results over a span of nine days, recording the spectrum on days one, three, six, and nine. This allowed amply time for the eye pigments to mature.

Simple averaging from random samples of eye pigment was first used, but this method yielded inconsistent results. Problems were also encountered with the type of scanning technique used. Silverfast was inadequate because the colors often timed were displayed as black. Thus, Twain Acquire was chosen because it yielded the most accurate color. Differences were also seen between eye pigments depending on the tolerance or zoom used. We finally decided to use histograms instead of random sampling to find the color of the eye pigment. Another complication to obtaining consistent eye color was the fact that the images were scanned using 256 colors. In order to obtain only the dominant colors, the colors were indexed at 50 colors. No difference in the quality of the smear could be detected by the human eye. Also, a tolerance of 32 was chosen to be used consistently since it was the default tolerance. Zoom was set at 100%.

Results:

We found that in all *Drosophila* mutations there was a consistent pattern of color maturation over a span of nine days.. *Figures* 1-10, demonstrate how each primary color (red, green, and blue) changed over nine days for each mutant. From this change, the pattern of color maturation for each mutant was determined. Wild type, rosy, vermilion, scarlet, cinnabar, and sepia had the 1-3, 6-9 pattern. Where as, apricot, white eosin, and brown had the 3-6 pattern. The patterns were formulated by indicating the days where the greatest amount of change in color value had occurred. *Figures* 11-18, illustrate the change in color including the standard deviations, in order to measure accuracy.

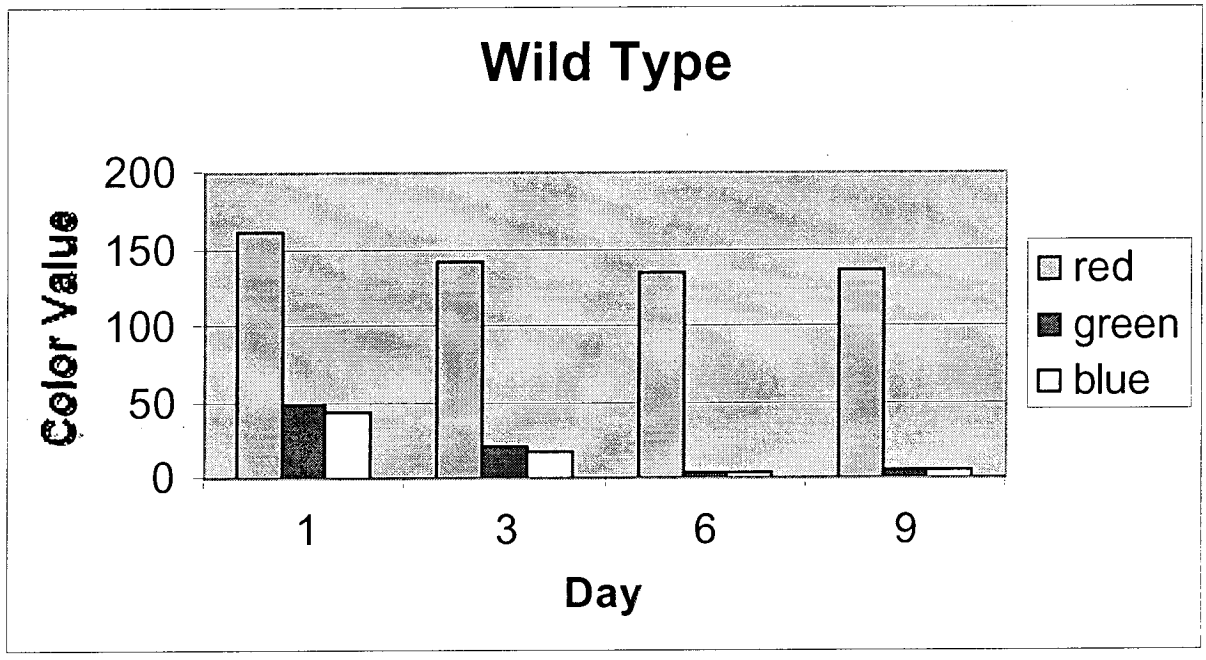


Figure 1: Change in color value over time for red, green, and blue for the ild type flies.

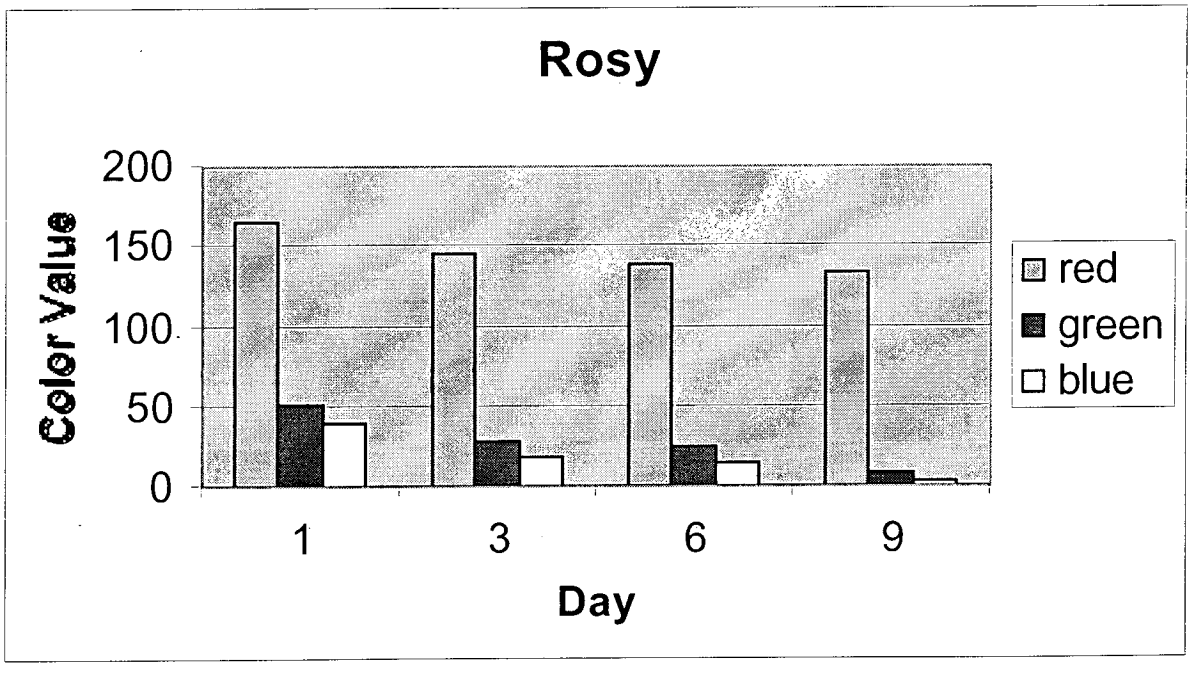


Figure 2: Change in color value over time for red, green, and blue for the rosy flies.

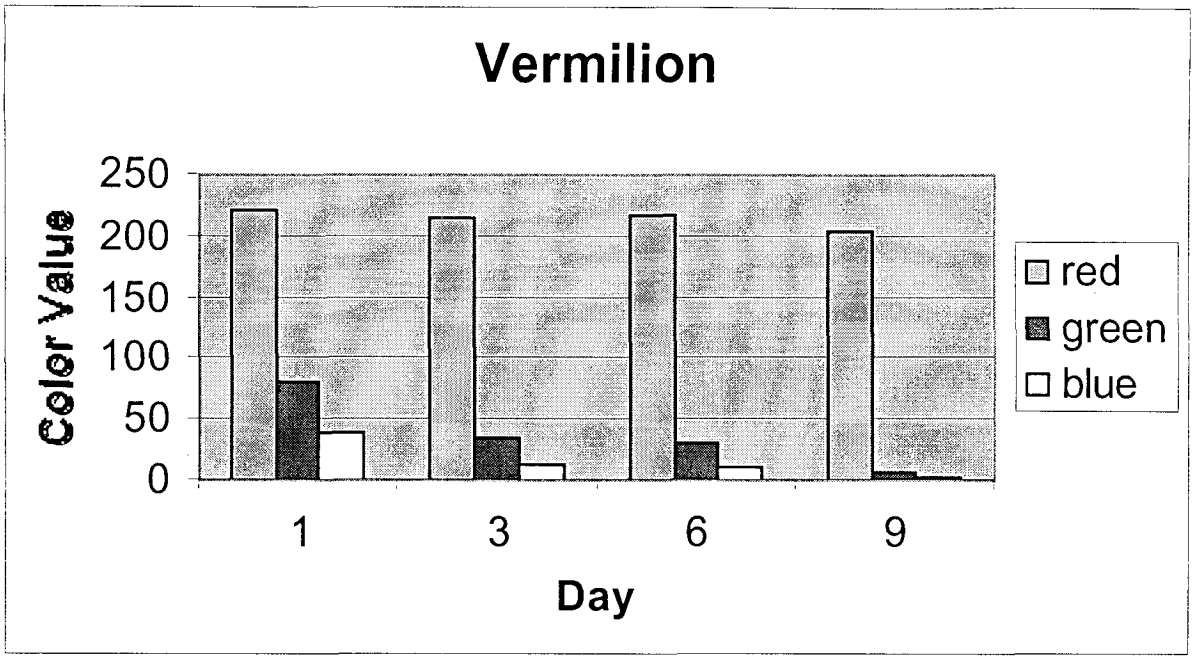


Figure 3: Change in color value over time for red, green, and blue of the vermilion flies.

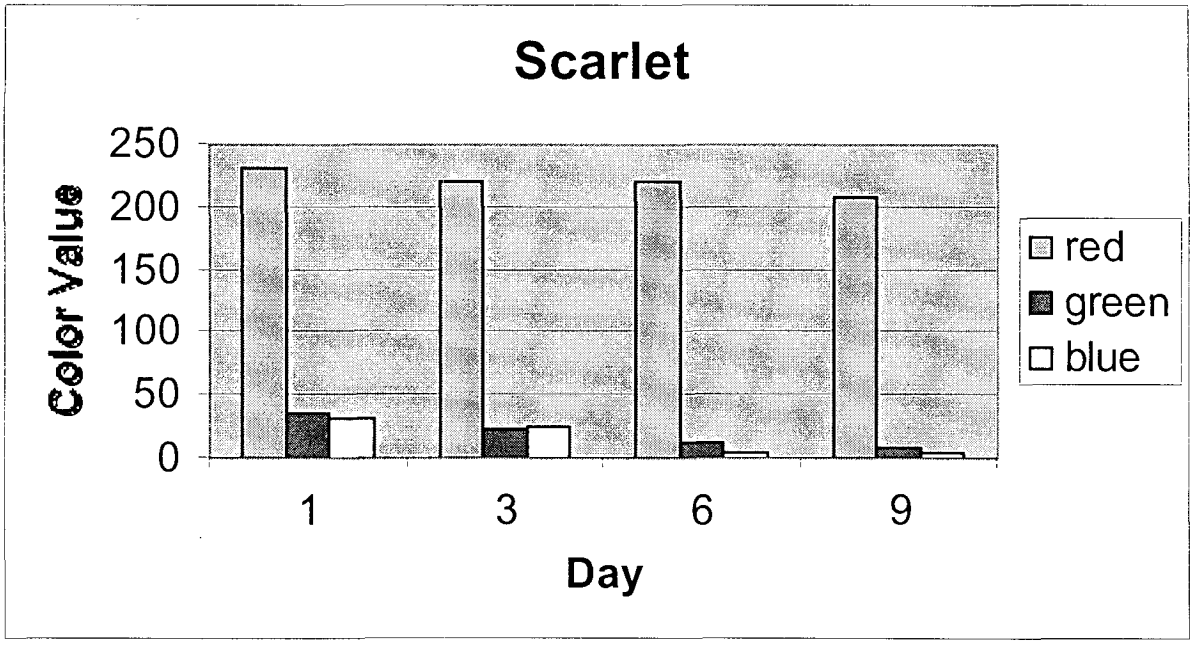


Figure 4: Change in color value over time of red, green, and blue over time for the scarlet flies.

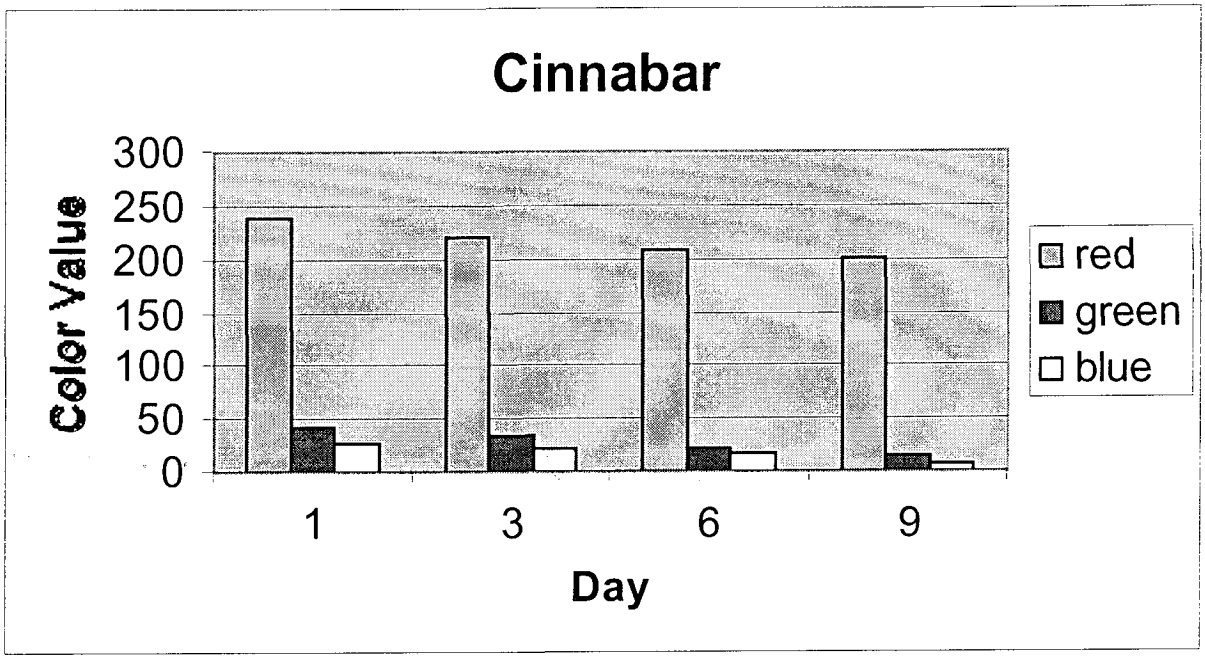


Figure 5: Change in color value over time for red, green, and blue for the cinnabar flies.

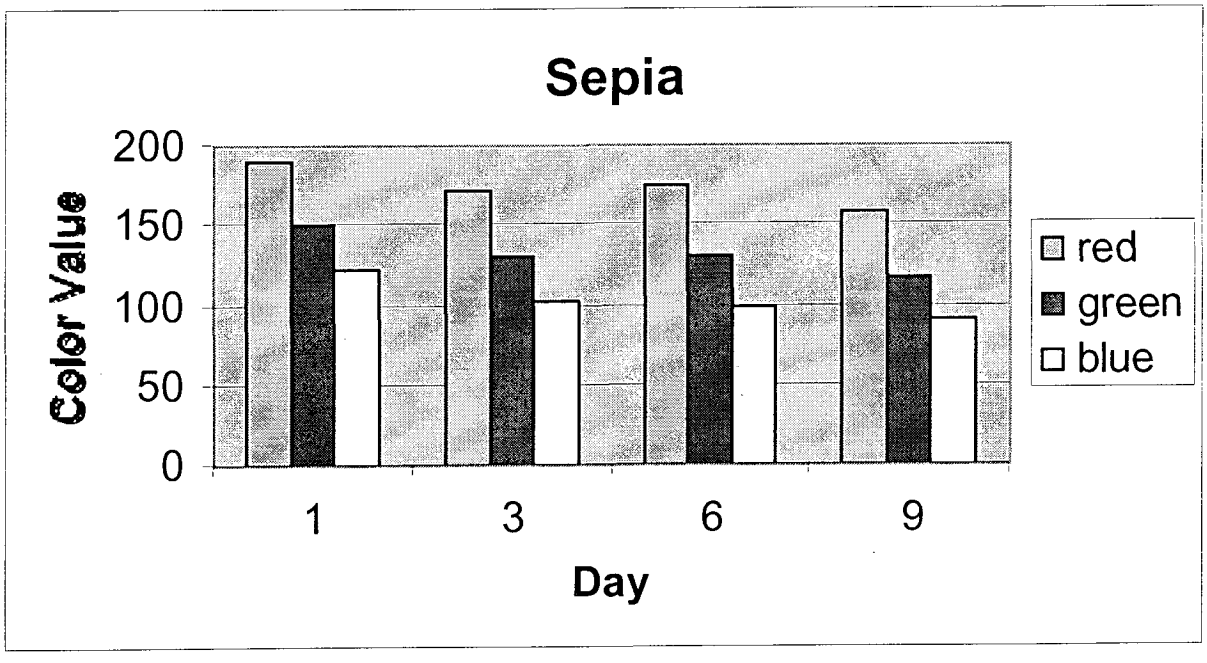


Figure 6: Change in color value over time for red, green, and blue for the sepia flies.

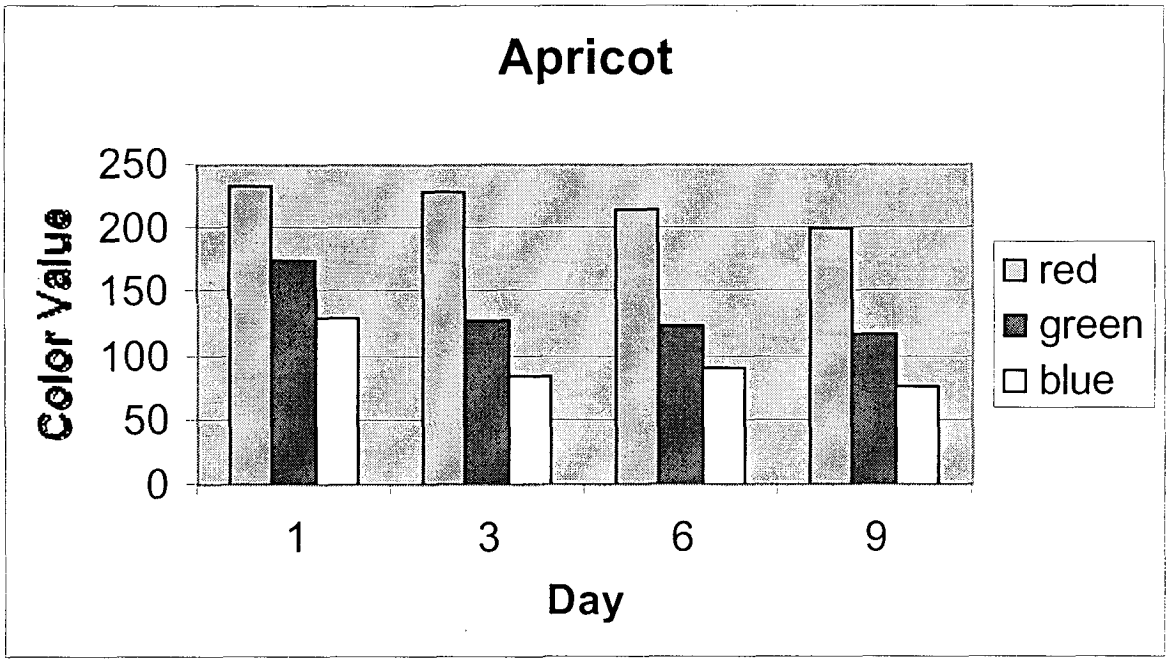


Figure 7: Change in color value over time for red, green, and blue for the apricot flies.

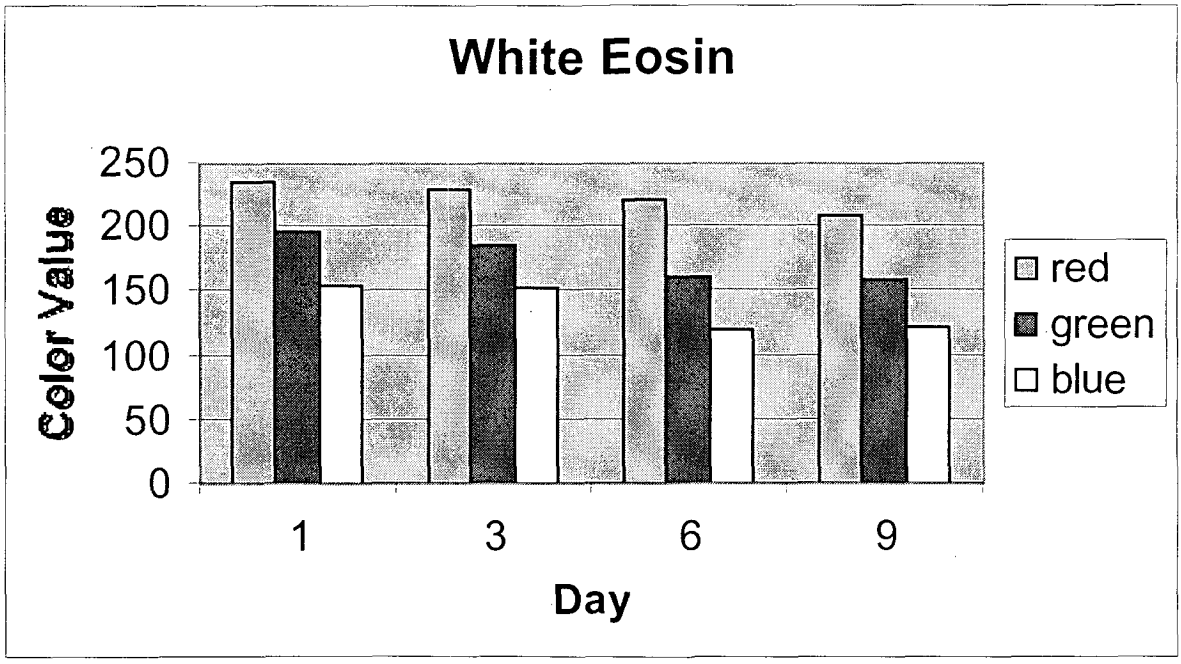


Figure 8: Change in color value over time for red, green, and blue for the white eosin flies.

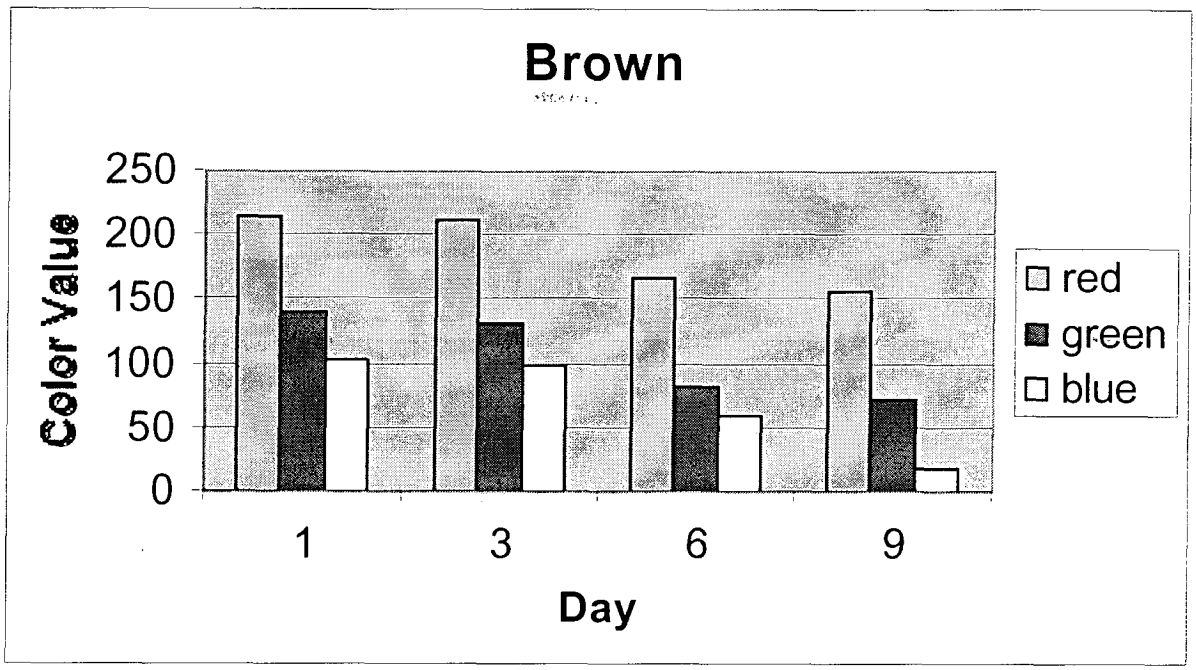
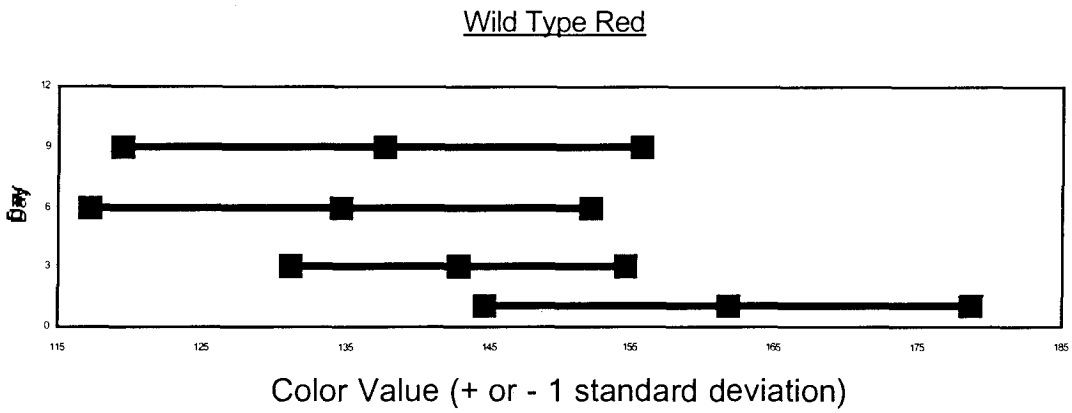
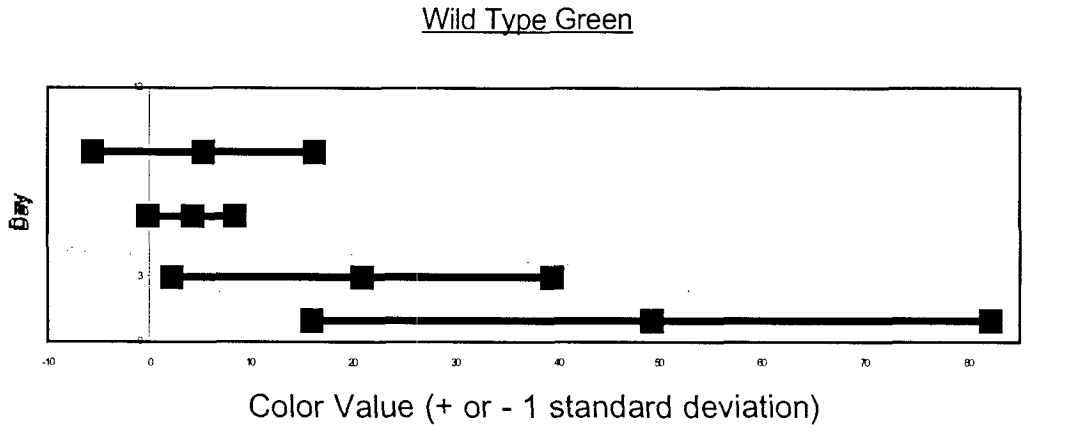


Figure 9: Change in color value over time for red, green, and blue for the brown flies.





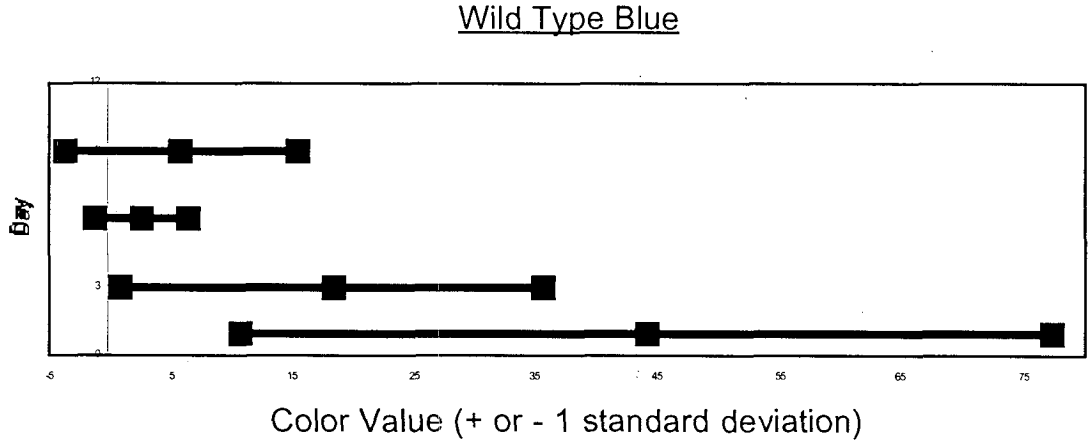


Figure 10: Change in color including standard deviations for wild type flies.

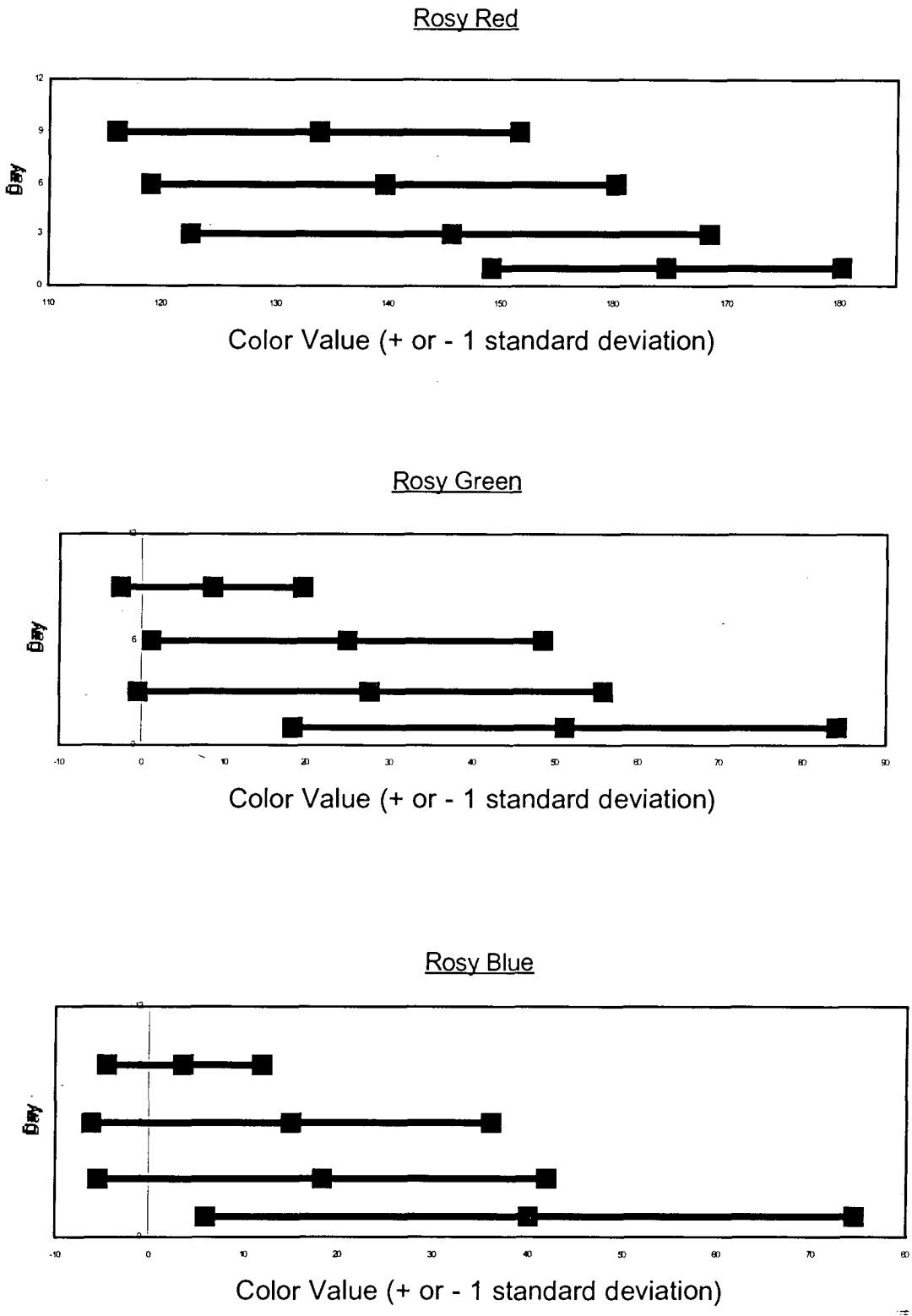


Figure 11: Change in color including standard deviations for rosy flies.

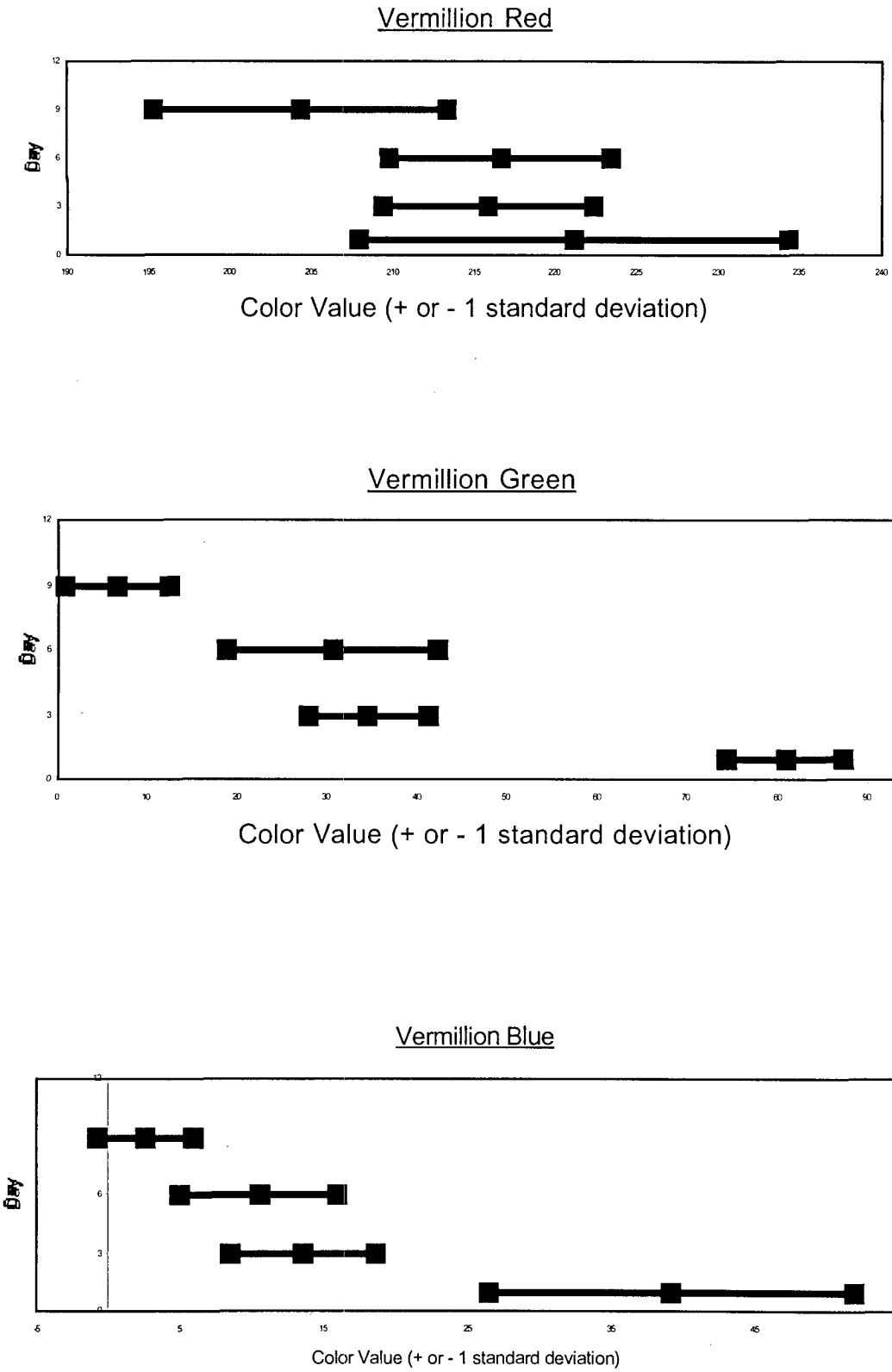
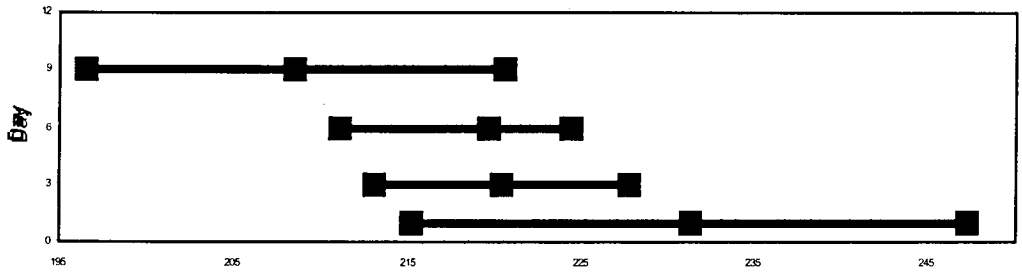


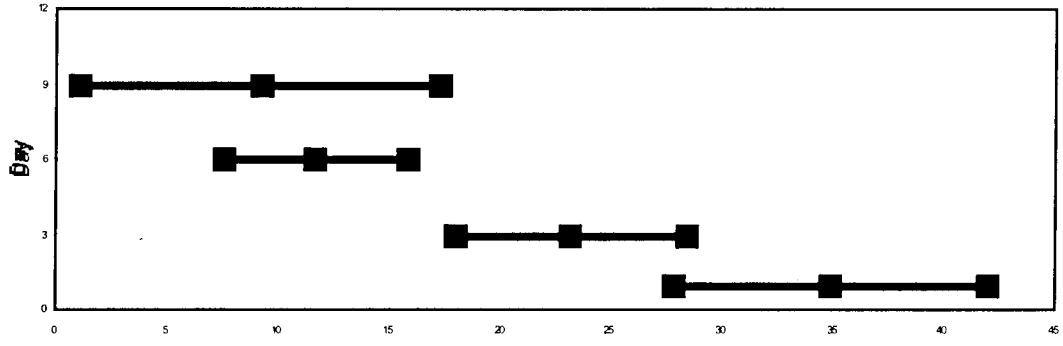
Figure 12: Change in color including standard deviations for vermilion flies.

Scarlet Red



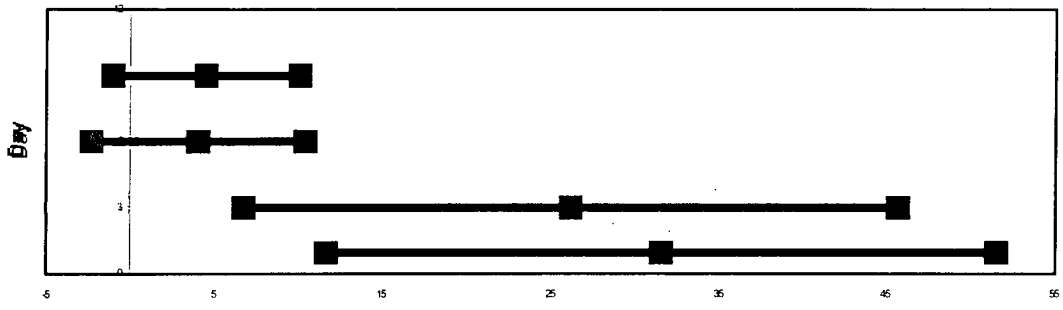
Color Value (+ or - 1 standard deviation)

Scarlet Green



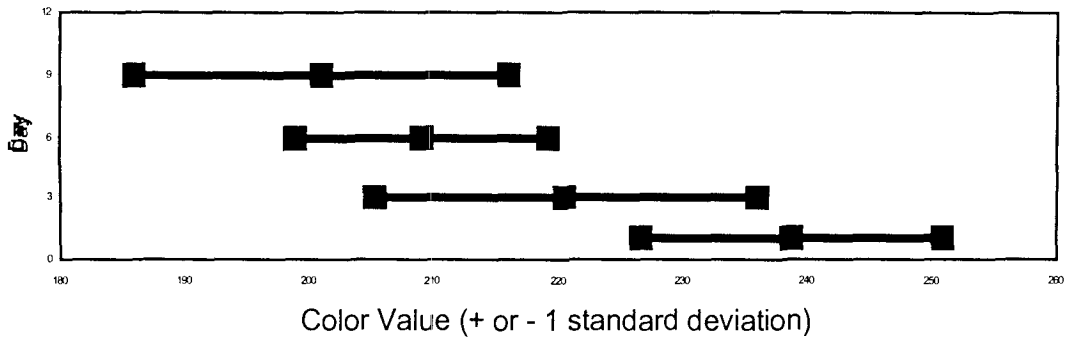
Color Value (+ or - 1 standard deviation)

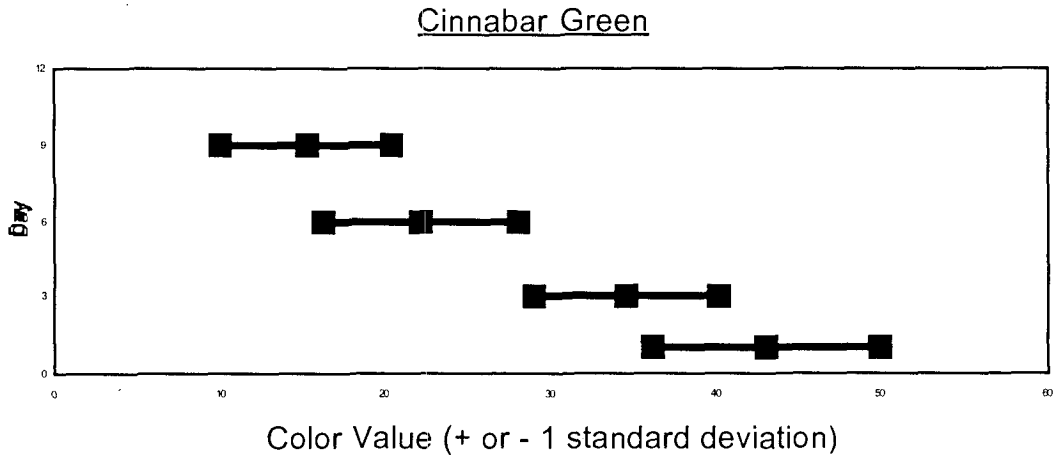
Scarlet Blue



Color Value (+ or - 1 standard deviation)

Figure 13: Change in color including standard deviations for scarlet flies.





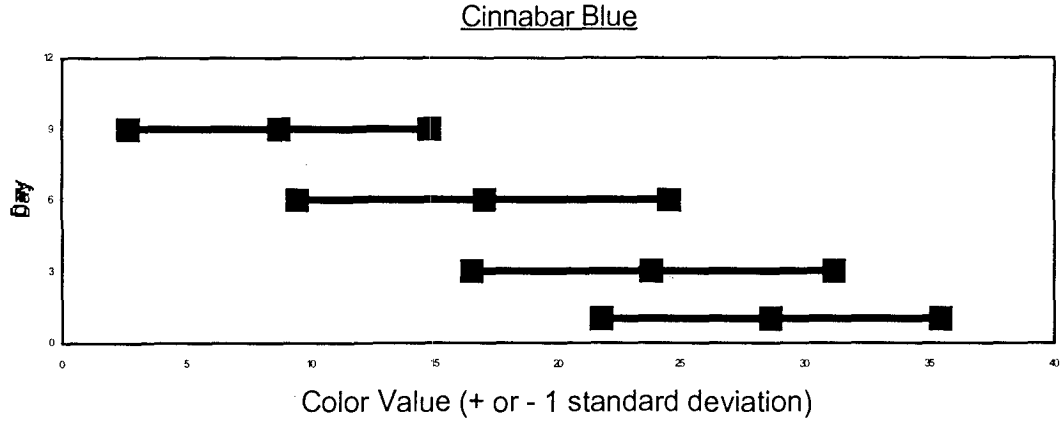


Figure 14: Change in color including standard deviations for cinnabar flies.

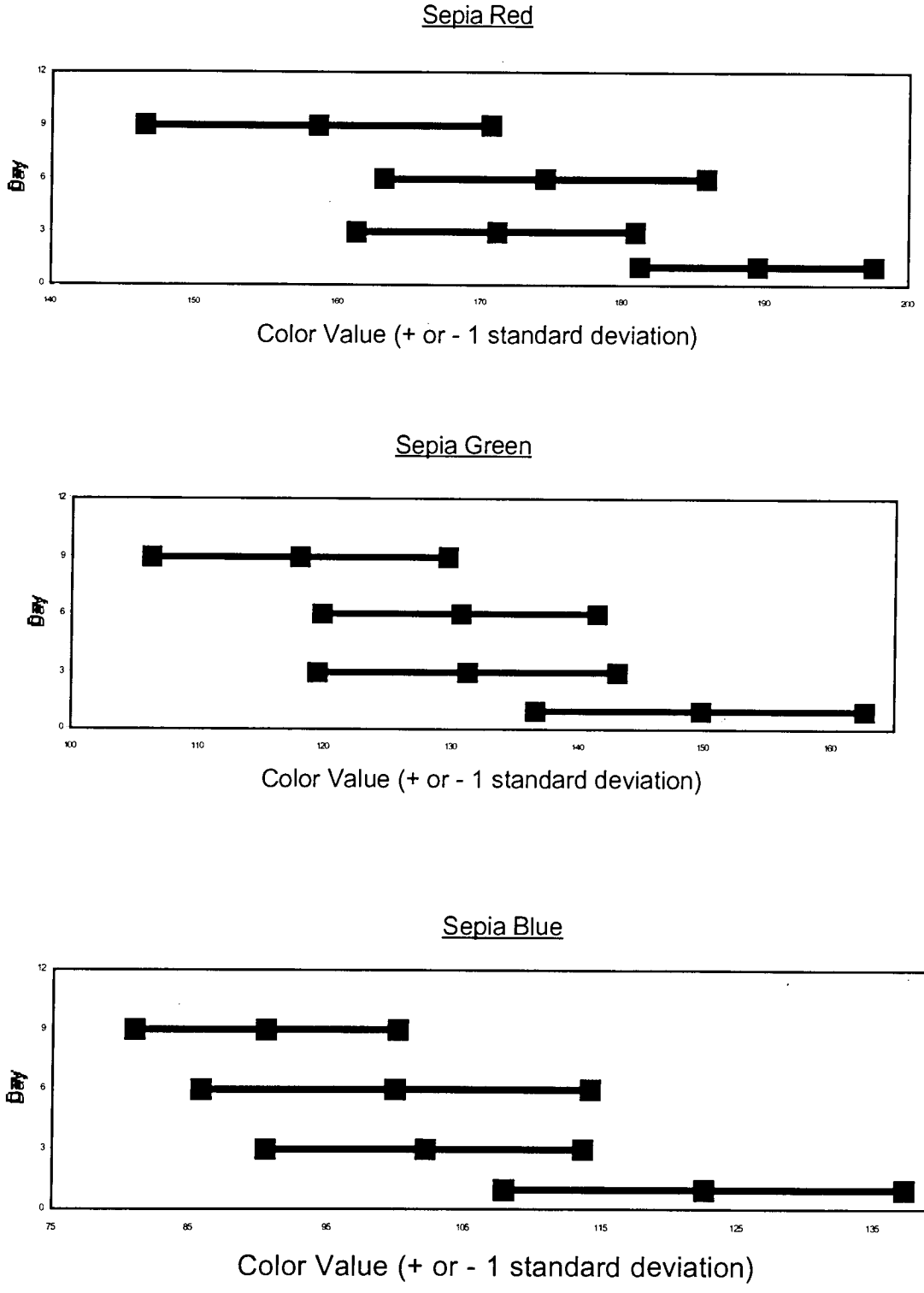
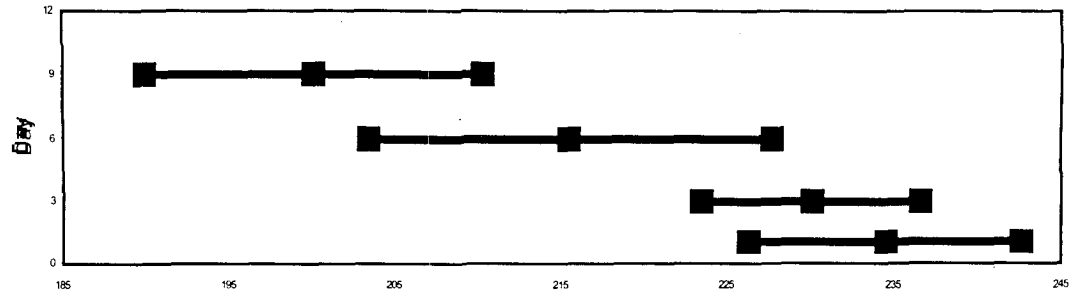
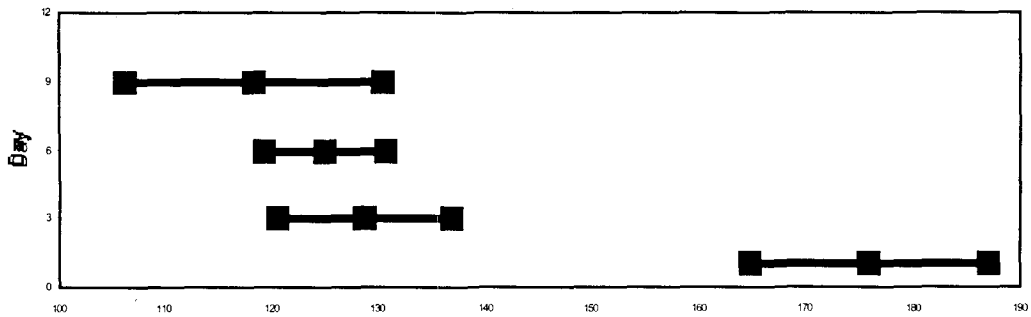


Figure 15: Change in color including standard deviation for sepia flies.



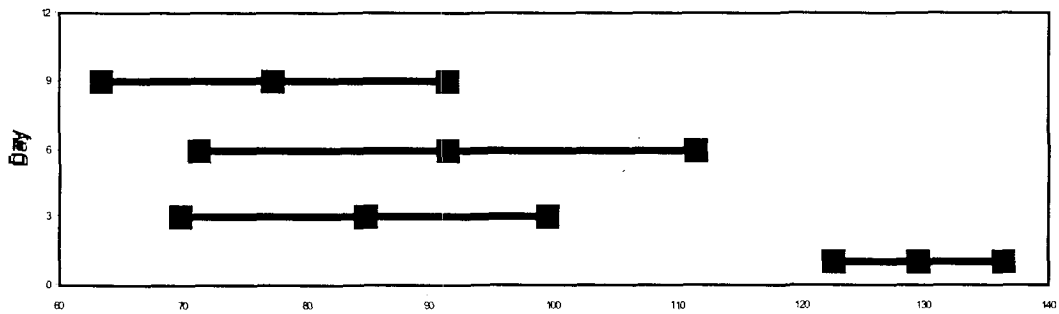
Color Value (+ or - 1 standard deviation)

Apricot Green



Color Value (+ or - 1 standard deviation)

Apricot Blue



Color Value (+ or - 1 standard deviation)

Figure 16: Change in color including standard deviations for apricot flies.

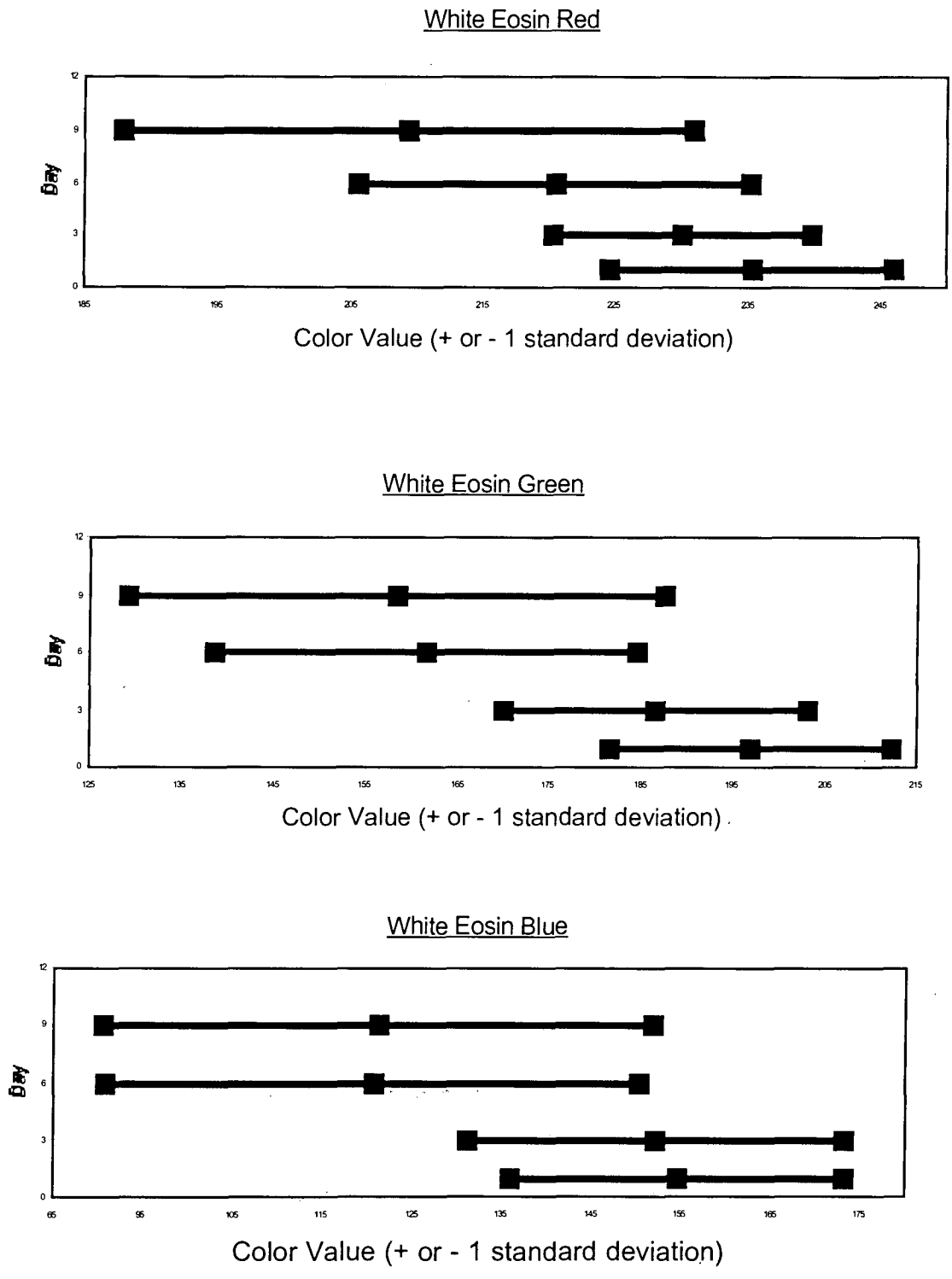
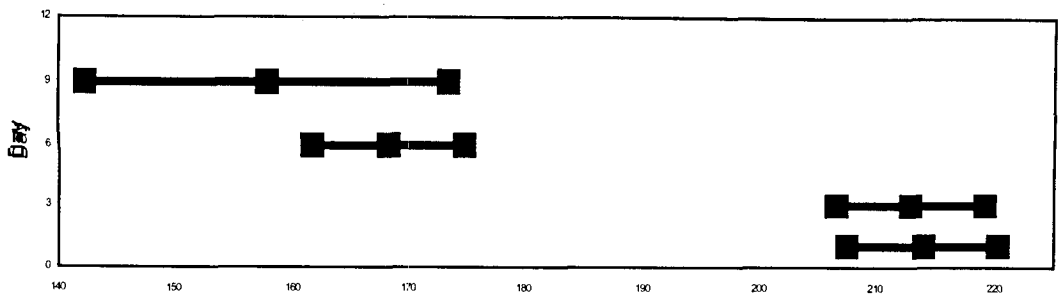
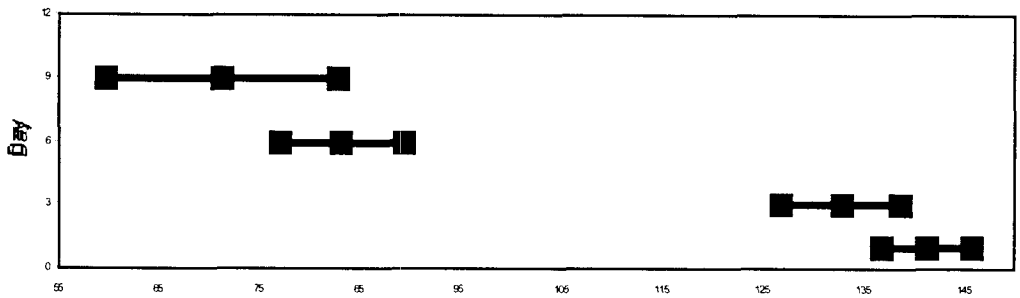


Figure 17: Change in color including standard deviations for white eosin flies.



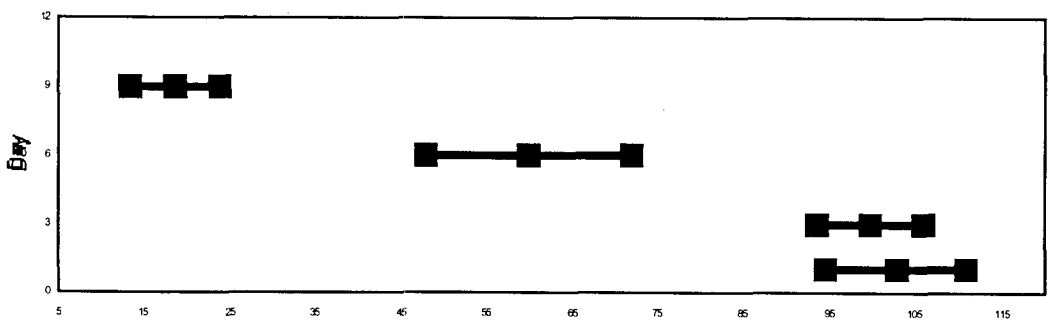
Color Value (+ or - 1 standard deviation)

Brown Green



Color Value (+ or - 1 standard deviation)

Brown Blue



Color Value (+ or - 1 standard deviation)

Figure 18: Change in color including standard deviations for brown flies.

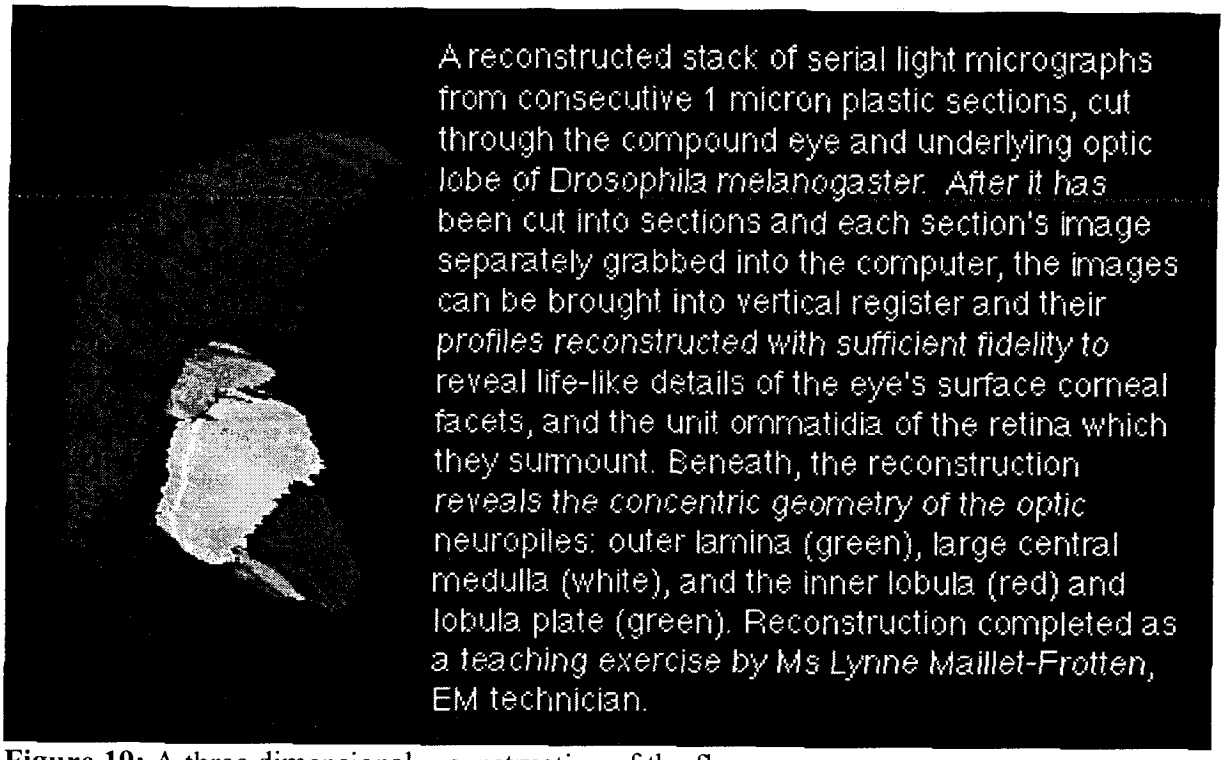


Figure 19: A three dimensional reconstruction of the fly eye.

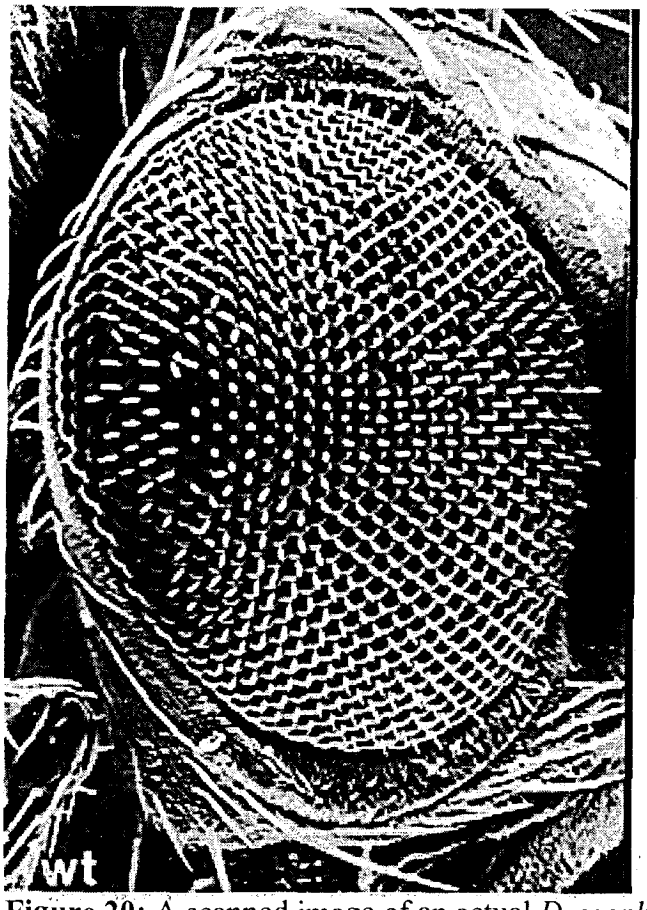


Figure 20: A scanned image of an actual *Drosophila* eye.

Discussion:

Our investigation of eye pigments in *Drosophila* through the use of Photoshop, indicates that as a fly develops their eye pigment darkens. Photoshop also allows us to classify our mutations into the two type of eye pigments, the *pterins* and the *ommochromes.* All of our mutations darken gradually over the period of nine days. Each mutation darkens at a different rate; however, we are able to distinguish a distinct pattern. One group of mutants change more between days one and three where as the other mutants change more between days three and six. These distinct changing patterns illustrate the two main types of eye pigments found in *Drosophila.* The *pteridines* (producing red pigment) contain the pattern of changing the most between day 1and day 3 along with between day 6 and 9. The *pteridine* mutants include wild type, rosy, vermilion, scarlet, cinnabar, and sepia. The *ommochromes* (producing brown pigmentation, derived from trypthophan) obtain the pattern of changing the most between day 3 and day 6. The *ommochrome* mutants include apricot, white eosin, and brown.

The *pterins* and the *ommochromes* are each affected by a different set of genes. Both *pterins* and *ommochromes* function by becoming attached to protein granules, in which form they are deposited in the eye cells, or ommatidia. A fly deprived of its brown *ommochromes* will show only the bright *pterin* pigments, and its therefore appear bright red (e.g., vermilion, scarlet, and cinnabar). However, a fly that is deprived of its *pterins* will have a darker eye color (e.g., brown, apricot). Flies deprived of both the *ommochromes* and the *pterins* will have no eye color at all and will appear whit-eyed. Normal fruit flies, or wildtypes contain a bright red eye color. On the other hand, flies that have orange eyes have a defect in their white gene, which normally produces the red pigments in the eye. In these flies, the white gene only works partially, producing fewer red pigments than it should. White-eyed flies, like the orange-eyed flies, also have a defect in their white gene. In these flies, however, the gene is completely defective and the gene produces no red pigment at all.

Investigations by Caspari and Kuhn in Drosophila identify that vermilion and cinnabar mutations are two of the precursors in the synthesis of one of the *ommochrome* pigments. These gene mutants are blocking essential metabolic steps in the synthesis of the *ommochrome* pigment from tryptophan. This synthesis is not able to be overcome without the addition of either vermilion or cinnabar. Thus, vermilion and cinnabar contain an important role in the process of *ommochrome* synthesis (Strickberger, 603).

In Drosophila, Zeigler-Gunder and Hadorn show that while the effects of normal eye-color genes appear dominant on superficial examination, some recessive mutations affect the amount of eye pigments in heterozygotes. Thus, the sepia eye-color mutant, for example, acts as a recessive gene to normal red eye color, but still reduces the quantity of some of the fluorescent pteridine pigments in the heterozygote (Strickberger, 166).

The significance of this research not only concerns the developmental process of Drosophila and the gradual darkening of Drosophila eye pigments, but it provides exposure to the use of Adobe Photoshop for scientific research. Adobe Photoshop programs provide photo retouching, image editing, and color painting software. Photoshop allows us to recognize the exact color image of the eye pigmentation. We are able to magnify the pixels and get an accurate reading of red, green, and blue values for each Drosophila eye. Photoshop can produce accurate results, however, one must remember the range of human error. The decapitating, smearing, and scanning techniques must be completely accurate and consistent in order for Photoshop to provide correct readings. Measuring Drosophila eye pigments over time with Photoshop provides insight on the developmental process of Drosophila, the two main fly eye pigments, the genetic make up of the pigments, and use of Photoshop for further scientific research.

Works Cited:

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Sincere gratitude to our mentor, Dr. Bob Jones, for his kind guidance and instruction.

Andrea Master's Biography

Andrea Master,a native of Shreveport, La, is a senior biology major at Rhodes College. After graduation, she plans on attending veterinary school of medicine, and specializing in equine medicine and research. At Rhodes, she has completed three semesters of independent genetic research on Drosophila flies. She has enjoyed this opportunity to enhance her lab and research skills. Andrea has also interned with an exotic veterinarian and a research veterinarian. During the summers, she works with large and small animal veterinarians. Her other hobbies include horseback riding, working out, and traveling. On campus, she has also been involved with Kappa Delta sorority, Beta Beta Beta, Campus Green, and the Equestrian team.

Kasey Sweeney

Kasey, a native of Peachtree City, GA, is a junior biology major at Rhodes, College. Upon graduation, she plans on attending medical school. Kasey is involved in various campus organizations like the tennis team and the yearbook staff.

Linda Lasselle

Linda Lasselle is a senior biology major from Tullahoma, TN. She will be attending medical school in the fall at the University of Tennessee, Memphis. At Rhodes, she is a Bonner Scholar, co-coordinator of Kinney Music for Kids, a Kinney Coordinator, and a member of Mortar Board and Beta Beta Beta.

Comparative Analysis of Cotton Fibers Using the Scanning Electron Microscope and the Transmission Electron Microscope

Jaime P. Hook
Supervised by John Olsen, Ph.D. and with the Instruction of Mary Jo Alexander, B.S., Rhodes College, Memphis, TN.

Abstract:

Cotton fibers can differ in their diameter thickness, length, degree of twisting, wall composition, texture, etc. The purpose of this study was to examine six different fiber types (raw stock fine, raw stock intermediate, raw stock coarse, card web fine, card web intermediate, card web coarse) using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). This allowed for a comparison of both surface features and cross sectional interior differences. Quantitative analysis of the SEM data sowed a significant difference in diameter thickness between the fine, intermediate, and coarse fibers of both raw stock and card web fibers, but no differences between the raw stock and card web fiber strains. SEM analysis also suggested differences in the degree of twisting of certain fiber types. TEM analysis was less conclusive, indicating possible differences in the degree of wall smoothness.

Introduction:

Scanning electron microscopy (SEM) allows for a detailed examination of the surface of a specimen. SEM works on the basis of electron emission. A beam of electrons goes over the surface of a sample that is coated with metal. As the electrons hit the sample, this metal surface emits electrons that get detected, and an image is formed (Starr and Taggart, 1995). While electrons act like particles, they also posses wave-like properties. Transmission electron microscopy utilizes electrons with extremely short wavelengths that glass or air would normally scatter. Thus, a vacuum is used, and a magnetic field serves to direct electrons along a specific path toward a focal point. Very thin sections of samples are necessary so the electrons scatter to correspond to the density of structures within a sample. Dense parts stain more darkly. TEM allows for a study of the internal characteristics of a specimen rather than just a surface study (Starr and Taggart, 1995).

Several types of cotton fibers exist, six of which this study looks at (raw stock fine, raw stock intermediate, raw stock coarse, card web fine, card web intermediate, card web coarse). Cotton fibers may differ from each other in terms of diameter thickness, length, degree of twisting, wall composition, texture, etc. This study uses scanning electron microscopy to compare the surface characteristics of these different fiber types both quantitatively and qualitatively. Transmission electron microscopy was also used to compare the internal characteristics of the six fiber types qualitatively.

Materials and Methods:

SEM Study Six types of cotton were obtained from The Cotton Institute in Memphis, TN. For each type, five stubs were coated with the cotton via double stick labels and one stub with carbon tape. All stubs were then coated with a metallic mixture using a sputter coater. Critical point drying was not done prior to sputter coating because cotton has no internal water. Following twenty-four hours in a dessicator, the stubs were ready to be placed into the

scanning electron microscope. Using the SEM with an accelerating voltage of 30 kV and a magnification of 750X, ten measurements of fiber diameter were taken per stub, for a total of 60 measurements per fiber type. Pictures of each stub were taken. Qualitative observations were made comparing the six fiber types. Quantitative measurements of fiber diameter were analyzed. Additionally, one stub of each fiber type was prepared but not sputter coated to determine if images produced were of a different quality than that of coated specimens.

TEM Study

(Much of the procedures for this portion of the study were from personal communication with Eileen K. Boylston, Southern Regional Research Center, New Orleans, LA. Other portions were taken from the EM Laboratory Manual, Dr. Hill, Spring, 1999).)

Using a methacrylate embedding medium (3 gm Methyl Methacrylate: 2 gm Butyl Methacrylate, along with a benzol peroxide catalyst), bundles of cotton fibers of the six fiber types were embedded on glass slides at 65 degrees Celsius over a three hour period. Rapid freeing of the embedded sample from the slide and cover slip was performed by scoring around the cover slip with a razor blade and then spraying both sides of the slide with Freon from an upside down can of Effa Duster. The embedded sample was put into a specimen holder, and, with a razor blade, trimmed into a trapezoid with the aid of a light microscope. These trapezoid samples were then sectioned with a Sorvall MT2-Ultramicrotome. Sections were 600-1000 angstroms thick. Sections were picked up with carbon coated grids (containing no formvar) and soaked overnight in methyl ethyl ketone to dissolve the embedding medium off of the fiber sections. A 2% PTA stain was used to stain the grids. The grids were looked at with the Zeiss EM-109 Transmission Electron Microscope. Pictures of each fiber type were taken and compared to each other. Most pictures were taken at a magnification of X12000, but when it was not possible to focus clearly at this magnification, it was reduced.

Results:

SEM Results

Quantitative Comparison

Sixty measurements for fiber diameter were taken for each fiber type. These were averaged and the fibers compared to each other. It was found that the raw stock coarse was significantly thicker than the raw stock intermediate, which was significantly thicker than the raw stock fine fibers. It was similarly found that the card web coarse fibers were significantly thicker than the card web intermediate fibers, which were significantly thicker than the card web fine fibers. All of this is significant at the $\alpha = .05$ level of significance (Table 1).

Table 1: Average Cotton Fiber Diameters

Fiber Type	Average Fiber Diameter (μm) (n = 60)	Significant Difference
Raw Stock Fine	9.4	
Raw Stock Intermediate	14.6	*
Raw Stock Coarse	21.2	*
Card Web Fine	10.7	
Card Web Intermediate	14.6	*
Card Web Coarse	20.9	*

*Signifies that the fiber type is significantly thicker than the fiber on the line above it to an $\alpha = .05$ level of significance. No other significant differences were present between any of the other fiber types.

Qualitative Comparison

It was observed that the raw stock fine and card web fine fibers tended to show an increased degree of twisting when compared to the degree of twisting of intermediate and coarse fibers of both raw and card web fiber types. No quantitative analysis could be done to support this observation (Figure 1).

It was also found that failure to coat with a metal before putting the fibers into the SEM results in an inability to obtain a clear image of the fibers. The contrast was inadequate (Figure 2). This was found for all six fiber types. Even after lowering the accelerating voltage from 30 kV to 10 kV, clear images could not be obtained.

TEM Results

No major differences were noted between the images of the six fiber types using TEM. Some pictures suggest that the card web fibers (fine, intermediate, and coarse) have a rougher wall than the raw stock fibers (fine, intermediate, and coarse), which seem to have a smoother wall (Figure 3). No definitive differences could be determined between the fiber types in regards to their interior structures.

Discussion:

SEM analysis of the six fiber types indicated significant differences in the thickness of the fiber types. In both the raw and card web fibers, coarse fibers were the thickest, followed by the intermediate fibers, and lastly, the fine fibers (significant at the $\alpha = .05$ level of significance). This was to be expected since the names of the fibers are most likely due to their varying thickness. However, there was the possibility that these fibers also differ in other areas. Yet, no definitive conclusions could be drawn to support this. It seems that the finer fibers have an increased tendency to twist and curl up, but all fiber types displayed twisting to some degree, and there was no precise way to measure the degree of twisting to compare the fibers. Also, no differences were observable between the card web and raw stock fiber types. They both showed smooth outer surfaces with similar twisting patterns and diameter thickness. No significant differences were found between card web fine and raw

stock fine, between card web intermediate and raw stock intermediate, or between card web coarse and raw stock coarse.

TEM analysis proved to be the more difficult aspect of this study, since no thorough protocols for doing TEM with cotton fibers could be found. Thus, much time was spent in simply designing a protocol that would allow us to even get cross sectional pictures of the cotton fibers. The pictures obtained did not reveal any differences between any of the fibers. There did seem to be a rougher wall for the card web fibers than the raw stock fibers, but since so few images were obtained, this cannot be supported. More research would need to be done in this aspect of analysis. Only with many more pictures could the differences be supported or refuted.

Thus, it appears the fibers differ only in their diameter thickness, possibly in the degree of twisting, and possibly in the roughness or smoothness of the walls. Further research should be done to elaborate upon the TEM findings.

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Starr, Cecile and Ralph Taggart. *Biology: The Unity and Diversity of Life, 7th Ed.* Wadsworth Publishing Company, Boston: 1995, 933 pp. total.

Jaime Hook Biography

Jaime Hook is a senior Biology major, Women's Studies minor, from St. Louis, MO. Jaime is involved in several organizations on campus, and is currently the Vice-President of International House, as well as a Resident Assistant. She is a member of Omicron Delta Kappa and Phi Beta Kappa. She plans to attend medical school in the future.

Figures 1-3

Figure 1

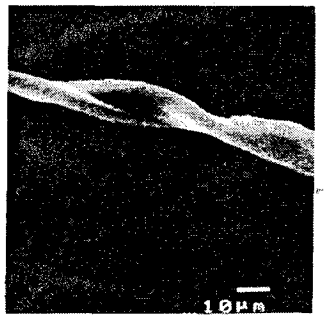

Figure 1: Card Web Fine. X750. Note the extensive twisting of this fiber type. With Scanning Electron Microscope analysis, it was observed that the fine fibers tended to show a greater degree and prevalence of twisting than the intermediate and coarse fibers, suggesting the possibility that thinner fibers twist more than thicker fibers.

Figure 2

Figure 2: Raw Stock fine. X750. Uncoated Specimen. Note the undesirable contrast obtained when coating of the stubs with metal is not don. This suggests that coating is necessary in order to obtain clear images that can be analyzed.

Figure 3

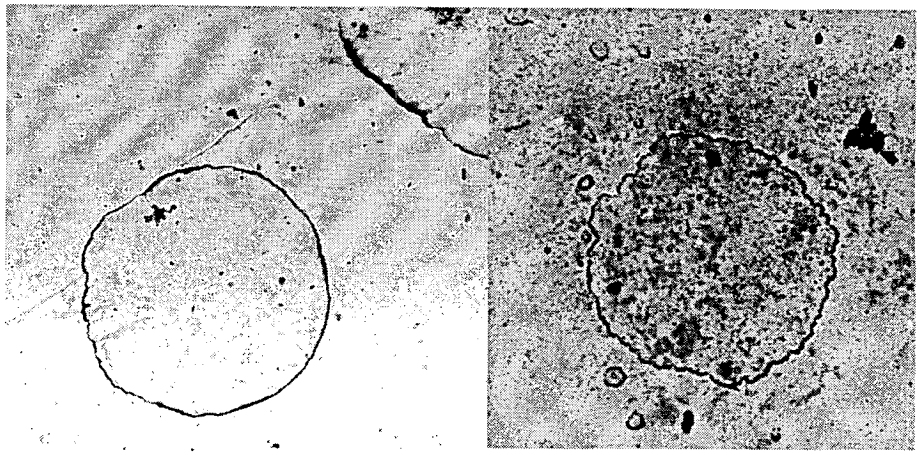

Figure 3: Left: Raw Stock Fine. Right: Card Web Fine. Note the cross section on the right has a much rougher wall than the fiber on the left, which is very smooth around the wall. This suggests that the Card Web fibers may have more uneven, rougher walls than the Raw Stock fibers.

Effect of Dietarily Administered Endocrine Disruptors on Cytochrome P-450 (CYP450) Expression in Male and Female Rat Liver Microsomes

Vanessa Hardin and Dr. Elizabeth Laurenzana

National Center for Toxicological Research, Department of Biochemical Toxicology, Jefferson, Arkansas, USA

Abstract. Certain environmental chemicals mimic or inhibit the action of the gonadal steroid hormones, causing a disruption in the endocrine system – hence the name, endocrine disruptors. By acting through the microsomal Cytochrome P-450-dependent monooxygenase system of the liver, endocrine-disrupting agents can alter the expression of hormonally regulated Cytochrome P-450 (CYP450). The objective of this study was to examine the effect of endocrine-disrupting chemicals (Vinclozolin and Methoxychlor) on hepatic CYP450 expression among male and female rat treatment groups. Both chemicals altered the pattern of expression of CYP450 isoforms; furthermore, these results suggest that endocrine-active agents have differential effects on hepatic CYP450 in male and female rats. This complex pattern of hepatic enzyme induction and inhibition may have consequences for the clearance and toxicity of various compounds metabolized via the liver by altering the overall metabolism.

Introduction

Hormones are ubiquitous in multi-cellular organisms and control many crucial events in practically every metabolic pathway. Steroid hormones (e.g., estrogen and androgens) bind with their specific hormone receptors in the cell, and this combination then activates or represses the transcription process of certain genes. In humans and rodents, exposure to hormonally active chemicals during sex differentiation can produce a wide range of abnormal sexual phenotypes, the degree of which is related to the dose and potency of the chemical administered during this critical developmental period.[1] It is just now becoming clear that certain environmental chemicals in widespread industrial and agricultural use have the potential to alter sexual development and other metabolic processes in wildlife and humans by directly interacting with hormone receptors.[2] Recently, the fungicide Vinclozolin was found to inhibit sexual differentiation in male rats in an antiandrogenic manner, and the pesticide Methoxychlor has demonstrated proestrogenic activity.[3,4]

In addition to interacting with steroid receptors, endocrine disruptors may also act through other mechanisms, such as through the microsomal Cytochrome P450-dependent monooxygenase system of the liver -- the most important enzyme system involved in the biotransformation of drugs, xenobiotics, and endogenous chemicals.[5] Endocrine disruptors can alter the expression of hormonally regulated Cytochrome P450 (CYP450) enzymes, inducing or inhibiting CYP450 activity. *Figure 1* indicates the postulated sites of indirect action for gonadal hormones (and thus, for endocrine disruptors also) on the hepatic/metabolic system.

The objective of this research was to examine the effects of two endocrine-active agents (Vinclozolin and Methoxychlor) on hepatic CYP450 expression in male and female rat liver microsomes. Since endogenous gonadal hormones can indirectly regulate and affect the hepatic metabolism of an organism, we hypothesized that these endocrine-disrupting chemicals will alter the overall metabolism of the organism, as evident by the modification of CYP450 isoform expression.

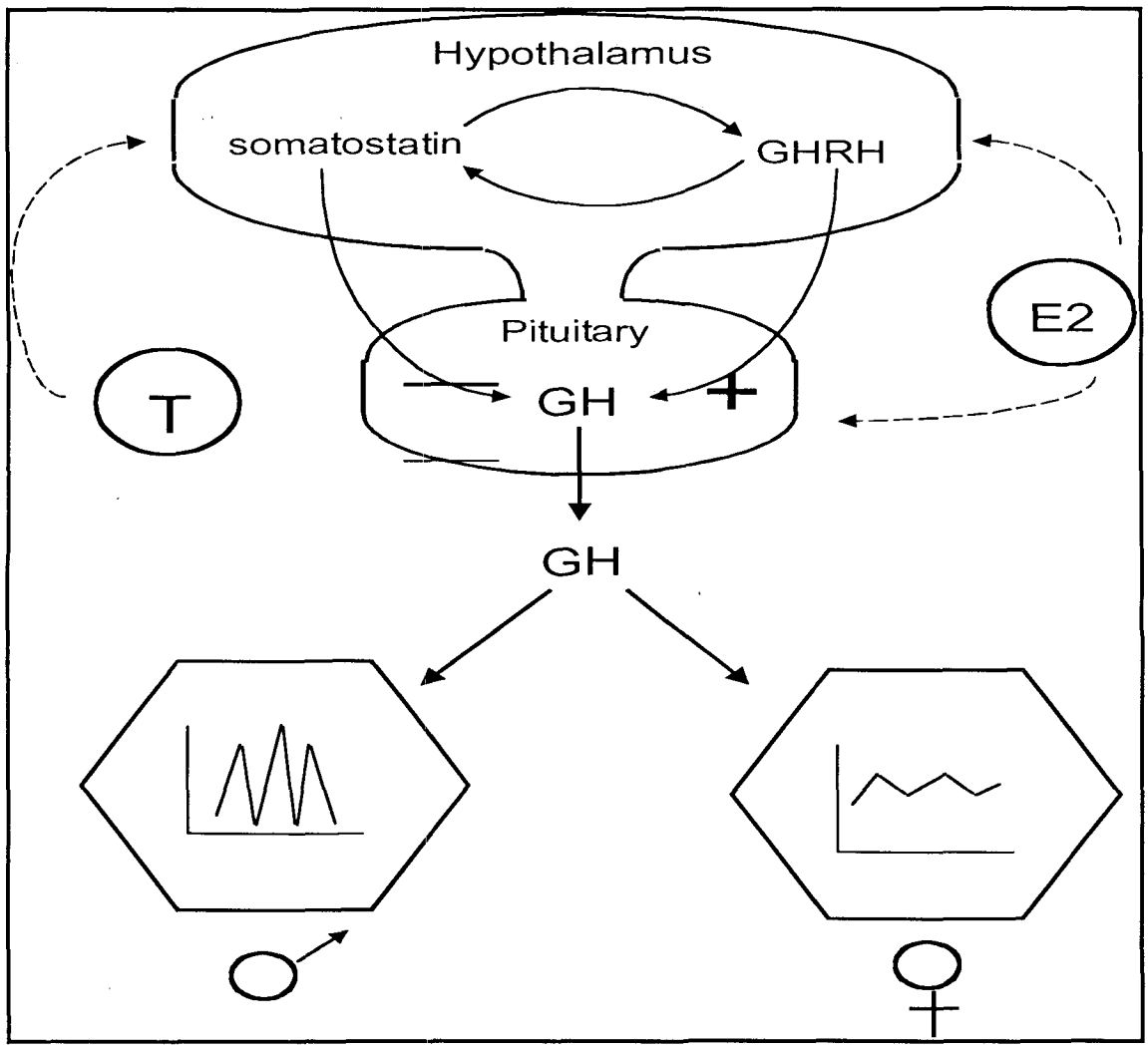

Figure 1. The gonadal hormones (or endocrine-disrupting chemicals) may interact with hypothalamic factors, thereby regulating growth hormone (GH) release. The fashion by which GH is released then determines the type of metabolism that results (male or female).[6]

Methods

As part of a multigenerational study, Vinclozolin and Methoxychlor were dietarily administered to male and female rats (from gestational day 7 through postnatal day 50). To assess the level of CYP450 present, microsomal fractions must be obtained, since these are vesicles of endoplasmic reticulum which contain CYP450. To obtain these fractions, the various liver samples were homogenized via a Dounce homogenization technique using a suitable homogenization buffer. Then a series of ultra-centrifugation steps follows: 1) centrifugation at 10,000Xg, discard pellet which contains cellular debris and nuclei, retain supernatant 2) centrifuge at 100,000Xg, discard supernatant which contains the cytosol, retain pellet, 3) resuspend pellet and centrifuge at 100,000Xg, discard supernatant, obtain microsomes, resuspend in appropriate resuspension buffer. Separate microsomal samples via SDS-PAGE. Immunochemical techniques (ELISA and Western Blotting) were used in order to compare the levels of CYP450 isoforms present among the rat liver microsomal fractions. ELISA (Enzyme-linked Immunoabsorbent Assay) measures the amount of target protein (CYP450 isoform of interest) using specific antibodies. Western Blotting also uses specific antibodies against the target protein, but it mainly indicates which particular isoform is present, serving as a qualitative confirmational test for the ELISA.

Results

We found that both Vinclozolin and Methoxychlor altered the pattern of expression of hepatic CYP450 isoforms in male and female rats. *Figures 2-4* show graphical representations of the CYP450 isoform results ("*" denotes that an isoform is statistically significant from the control group). *Figure 2* illustrates that in females Vinclozolin causes a significant increase in CYP3A isoform expression at a dosage of 150 and 750 ppm, whereas only the highest dose (750 ppm) elicits an increase of CYP2C isoform. In males, Vinclozolin treatment results in an increase of CYP2C at a dosage of 10 and 750 ppm as compared to the control, yet there was no effect on CYP3A expression (*Figure 3)*. As seen in *Figure 4*, Methoxychlor caused a decrease in both CYP3A and CYP2C expression in females at a dosage of 10 and 100 ppm. The effect of Methoxychlor on CYP450 expression in males was not reported for lack of adequate testing.

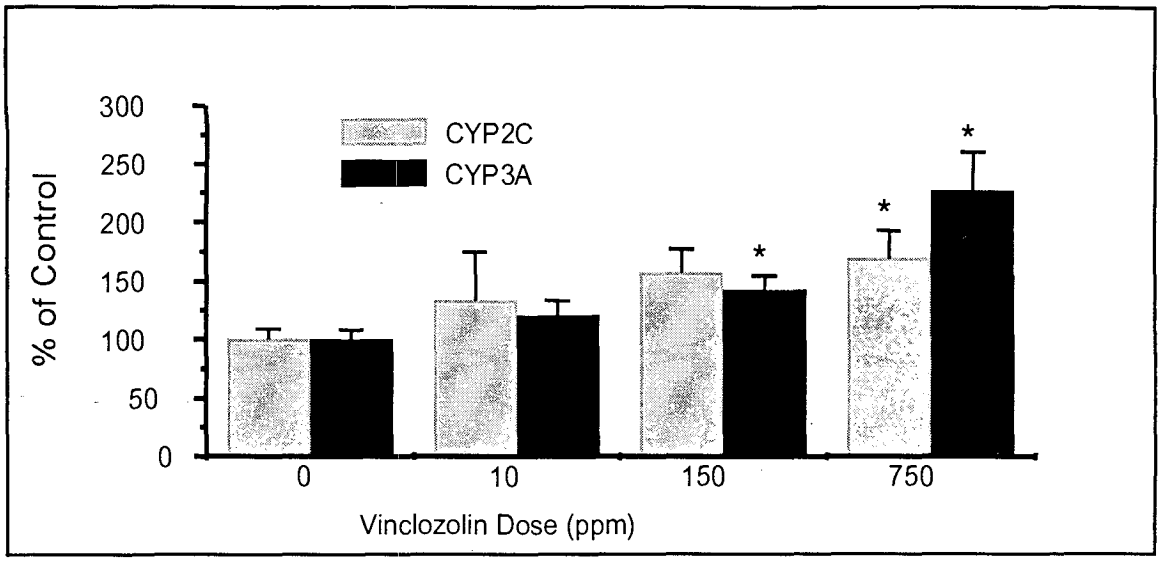

Figure 2. CYP450 Expression in Female Rat Livers Exposed to Vinclozolin

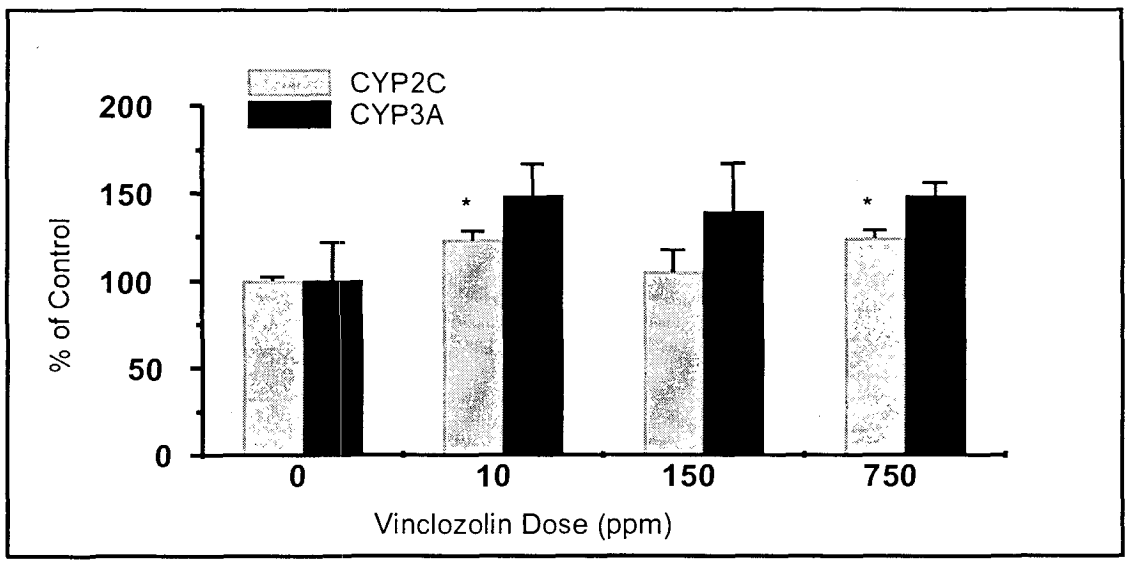

Figure 3. CYP450 Expression in Male Rat Livers Exposed to Vinclozolin

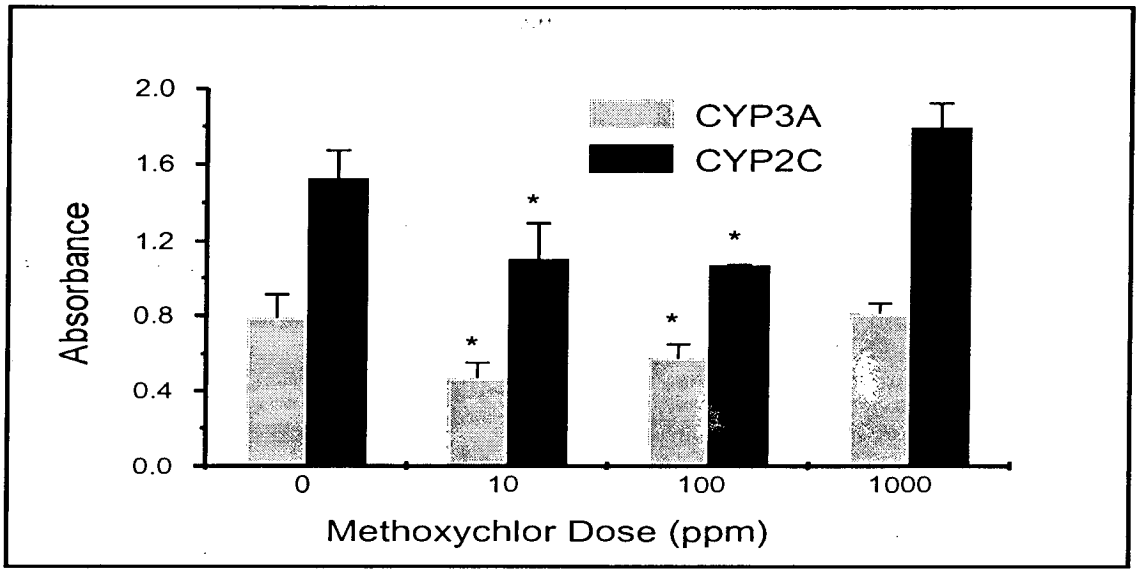

Figure 4. CYP450 Expression in Female Rat Livers Exposed to Methoxychlor

Discussion

The same CYP450 isoforms were expressed differently between male and female rat groups. In females CYP3A expression was induced, yet there was no effect in males. CYP2C was induced in females at 150 and 750 ppm, yet it was expressed in males at 10 and 750 ppm. Such results suggest that endocrine-active agents have differential effects on hepatic CYP450 expression in male and female rats. Although there was not a methoxychlor male treatment group to compare with the methoxychlor female treatment group, it is nevertheless evident that a significant inhibition of both isoforms (CYP2C and CYP3A) occurred at dosages of 10 and 1000 ppm in females. This complex pattern of CYP450 induction and inhibition may have consequences for the clearance and toxicity of various compounds metabolized via the microsomal monooxygenase system by altering the overall metabolism of the organism.

The significance of this toxicity with respect to human health remains to be determined. Human and animal populations are exposed to complex mixtures of many individual chemicals on a daily basis, and thus it is difficult to predict the extent to which these compounds may have their effects. Given the fact that these chemicals are used widely in agriculture, it is plausible that these chemicals can enter into our water systems and food chain, whereby they could have detrimental effects on many living organisms. In fact, industrial farmers would likely be at increased danger from endocrine-active chemicals, since they would be exposed to particularly high levels of these pesticides and fungicides. Furthermore, pregnant women could be putting their babies at risk if exposed to these chemicals, since disrupting normal steroid hormonal processes during development can lead to detrimental, irreversible developmental abnormalities.

The results from this research raise concern about the potential biological effects of excess exposure to endocrine disruptors that could potentially influence normal sexual differentiation, fertility, and metabolism in multicellular organisms. Research into this area is critical since it can ultimately impact the reproduction, and hence survival, of many living things.

Acknowledgments

Sincere gratitude to my mentor, Dr. Elizabeth Laurenzana, for her kind guidance and instruction. This research was supported in part by an appointment to the Student Research Participation Program at the National Center for Toxicological Research administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. Food and Drug Administration.

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[2] Kelce WR, Wilson EM. (1997). Environmental antiandrogens: developmental effects, molecular mechanisms, and clinical implications. *Journal of Molecular Medicine* 75:198-207.

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Effect of Papain on Cellulase Activity in *Achlya ambisexualis*

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Mentor: Terry W. Hill, Ph.D.
May 7, 1999

Department of Biology, Rhodes College, Memphis, Tennessee

Abstract. Cellulases are enzymes involved in cell wall degradation of many organisms. The fungus-like protist *Achlya ambisexualis* demonstrates increased cellulase release during hyphal growth. The mechanism by which it is able to differentially express cellulases when needed is not fully understood, yet it is known that cellulases are associated with cellular membranes and are secreted at times. The objective of this study is to examine the effects of a protease, papain, on activation of cellulases associated with cell membranes. Several treatment groups were exposed to varying levels of papain, and these samples were separated via gel electrophoresis. Intrinsic to the gel is a cellulose substrate, which allows detection of the cellulases at various molecular weights. This treatment released multiple cellulases, including some of the same molecular weights as those normally secreted during hyphal growth. Proteolysis may serve as a mechanism for cellulase activation and release *in vivo* for *Achlya ambisexualis*.

Introduction

The fungus-like protist *Achlya ambisexualis* belongs to the family Saprolegniaceae, a group which usually occur in fresh water and in soil as saprotrophs, growing upon organic debris in freshwater littoral zones and aerated soils. In order for *Achlya* to grow, its hyphae must be able to extend. The mechanism of hyphal growth is not completely understood however. Cytoplasmic vesicles appear to fuse with the apical plasma membrane, thereby donating their contents and their membranes to the growing cell surface, as *Figure 1* illustrates below.

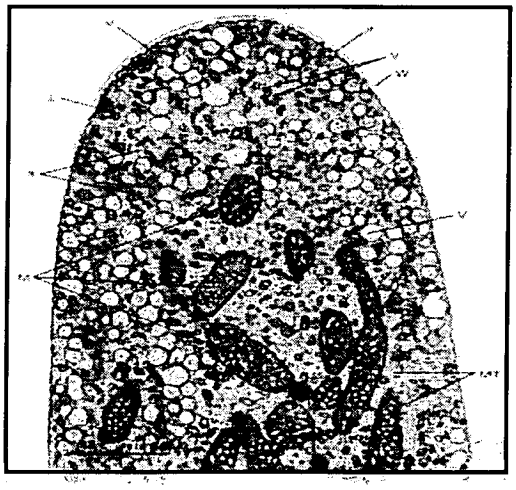


Figure 1. Cytoplasmic vesicles (labeled V in white) appear along the tip of the apical plasma membrane where the cell surface is growing.[1]

As *Figure 2* demonstrates, vesicles are also present where lateral branches are initiated. Lateral walls need to be softened at points where branches form.

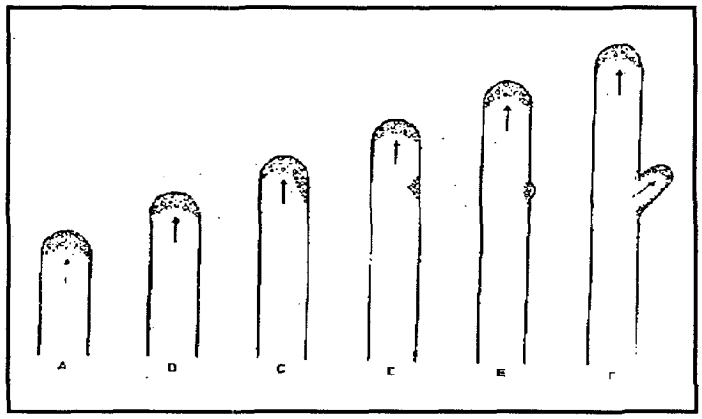


Figure 2. Vesicles appear at the tips of lateral cell walls where branching occurs.[2]

It is possible that these vesicles could contain wall-hydrolyzing enzymes, like cellulases. Cellulases are enzymes involved in cell wall degradation of many organisms. They break down the major component of Oomycete cell walls, called cellulose. Bonds of the lateral cell walls would be broken in order to initiate a hyphal branch. It has been shown that *Achlya* normally releases multiple cellulases during hyphal growth and that osmotically stressing *Achlya* causes an elevated release of cellulase enzymes with a simultaneous increase in wall plasticity.[3] The mechanism by which *Achlya* is able to differentially express cellulases when needed is not fully understood. Former research revealed that certain detergents or proteases released cellulases associated with membranes. The objective of this study is to examine the effects of a protease, papain, on the release and activation of cellulases associated with cell membranes.

Methods

The amount of cellulase secreted by a fungus can be measured by viscometry. The substrate is a 1% CMC (carboxymethyl cellulose) solution in 0.02 M Bis-Tris buffer, pH 5, in merthiolate. The substrate is added to the Ostwald-Fenske viscometric tubes. Then the treated samples are added and the viscometry of the CMC substrate is measured over time. Units are expressed as increase in relative fluidity per hour, times 1000. When cellulases are present in the CMC substrate, they break down the cellulose molecules and cause a lowering of the liquid s viscosity (more fluid liquid). By measuring the viscosity over time, one can assess the levels of cellulase present among the various treatment (and control) groups.

In one of my experiments, the effects of papain, a thiol protease, and a detergent called Triton X-100 on cellulase activity were determined. Triton X-100 is a detergent that completely solubilizes membranes. The following methods were employed:

¥ Homogenize *Achlya* membranes

¥ Several treatment groups exposed to papain (protease) and one with Triton X-100 (detergent):

1) Membrane + Buffer + 2% Triton
2) Membrane + Buffer + 4 units papain/mg protein for 5 , Terminate with Iodoacetimide (protease inhibitor)
3) Membrane + Buffer + 4 units papain/mg protein for 10 , Terminate with Iodoacetimide
4) Membrane + Buffer + 4 units papain/mg protein for 20 , Terminate with Iodoacetimide
5) Membrane + Buffer + No papain (Control)

¥ Run a cellulase assay, using comparative viscometric tubes

Once the levels of cellulase have been assessed, it is necessary to determine which cellulases are present in the various treated samples. In order to do this, homogenized samples are exposed to papain, concentrated, and then separated via gel electrophoresis using the following protocol:

¥ Treatment groups were exposed to varying levels of papain, followed by termination with Iodoacetimide (protease inhibitor)
¥ Membrane and soluble layers (containing the released cellulases) separated via ultra-filtration centrifugation process, using Biomax (5K NMWL, 4mL volume) tubes
¥ Concentrated samples and standard (whole medium) sample containing normally secreted enzymes are separated via gel electrophoresis which contains a cellulose substrate (cellulases will degrade this)
¥ Cellulases are detected by visual cellulose substrate degradation at the corresponding molecular weight of the cellulases

Results

The results of the proteolytic time course experiment are seen in *Figure 3.* It is clear that Triton X-100 results in immediate and maximal cellulase release and activation at 60 units/mL. Papain, however, causes a gradual release of cellulases and appears to plateau after about twenty minutes at approximately 40 units/mL.

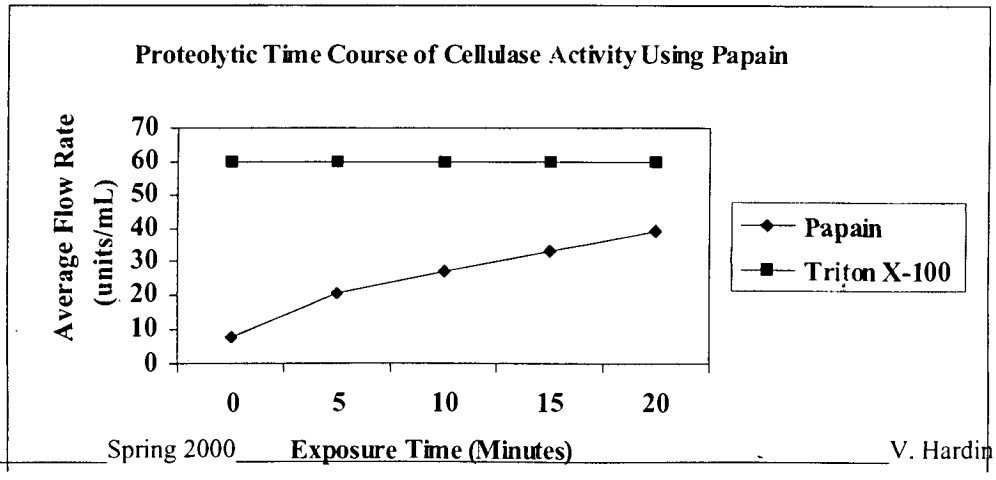


Figure 3. Effects of papain and Triton X-100 on cellulase activity and release.

Proteolytic treatment with papain released multiple cellulases, including some of the same molecular weight as those present in samples (whole medium) containing enzymes that are normally secreted during hyphal growth. *Figure 4* illustrates the secretion of a 25kDa cellulase in the whole and papain-treated groups (8units papain/mg protein for 40), whereas the control (no papain exposure) lacks this particular cellulase. *Figure 5* reveals that different exposures of papain (4 units/mg protein for 60 versus 8 units/mg protein for 40) still yield the 25kDa cellulase, among others of the same molecular weight as those which are normally secreted during normal hyphal growth.

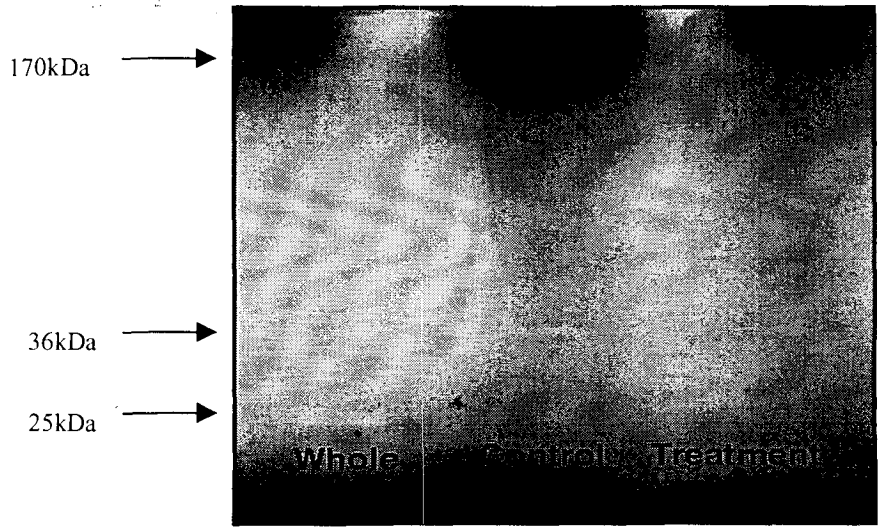
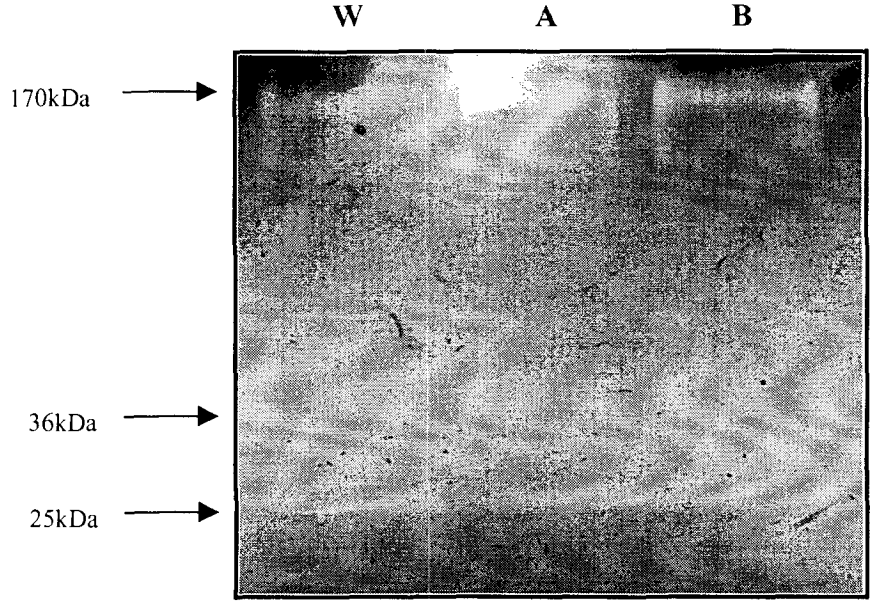


Figure 4. Proteolytic treatment released multiple cellulases, including a 25kDa cellulase that was not present in the control group (no papain).



W=whole medium
A=4 units papain/mg protein, 60
B=8 units papain/mg protein, 40

Figure 5. Various proteolytic treatments secrete cellulases of same molecular weight as those normally secreted during hyphal growth.

Discussion

Following proteolysis, we did find cellulases that were of the same molecular weight as those normally secreted by *Achlya* during hyphal growth. Therefore, proteolysis may serve as a mechanism for cellulase activation and release *in vivo* for *Achlya amibsexualis.*

Researchers have been interested in fungal cell walls for many reasons, including their biotechnological applications. Antifungal agents could potentially target proteases and prevent the release of cellulases, and hence, prevent fungal growth. The next step in research for *Achyla ambisexualis* involves identification of proteases, presumably thiol proteases, which can activate cellulases in a manner similar to that which I have found.

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Vanessa Hardin s Biography

Vanessa Hardin was born in Pine Bluff, Arkansas in January 31, 1978. She is will be a graduate of the Class of 2000 at Rhodes this May with a major in Biology. She has done research at Rhodes for 2 years: her junior year under Dr. Terry Hill and her senior year under Dr. Darlene Loprete. She is a member of the Chi Omega Fraternity, Order of Omega and Mortar Board Honor Societies, Vice-President of Beta Beta Beta (Biology Club), and she is the recipient of the 2000 Rhodes Student Research Award. Vanessa claims that Frazier-Jelke has become her second home, since she spends more hours there than in her own apartment! Next year, she will be doing research at St. Jude Children's Research Center

Investigation of the Possible Interaction of Plasma Membrane Calcium ATPase Isoforms with Two Synapse-Associated Proteins

Anna Pinchack, supervised by Jay Blundon, Ph.D.

INTRODUCTION AND BACKGROUND

Calcium ions (Ca^{2+}) act as important signaling molecules in eukaryotic cells. They are involved in many diverse activities including cell division, motility, and secretion (Carafoli, 1987). Ca^{2+}'s ability to serve as a signaling molecule relies on the tremendous concentration gradient across the cell's outer membrane. When ion channels open, Ca^{2+} crosses the membrane, and this signals the cell through various pathways. Once the signal has been received and processed, the former Ca^{2+} level must be re-established. (Strehler, 1996)

Cellular Ca^{2+} homeostasis is aided by proteins which serve as extrusion pumps known as plasma membrane calcium ATPases (PMCAs). There are four mammalian PMCA isoforms, (PMCA 1-4) which are encoded by separate genes. In some cases these isoforms are expressed in a tissue-specific manner. In addition to this specificity, within some cells the PMCAs are localized to specific membranes and regions of the cell, such as the basolateral membrane of kidney cells. This suggests that the specific variants play an active role in the local control of Ca^{2+} within special regions of the cell (Kim *et al.*, 1998). To illustrate this significance of the functional role of the PMCA, Street *et al.* (1998) recently reported that a strain of deaf mice, which also exhibit difficulty balancing, suffer from a mutation in the gene that codes for PMCA 2. Normally, when auditory hair cells are moved by sound waves, Ca^{2+} channels open, and signals are transmitted. In these mice, the mutated PMCA 2 is present but does not work effectively, and the mice's hearing and balance are compromised. Kozel *et al.* (1998) disrupted the PMCA 2 gene and produced a line of PMCA 2-deficient mice, lacking the protein entirely. These mice were also deaf and demonstrated difficulty balancing. There was an absence of otoconia, small calcium carbonate crystals needed for proper functioning of the vestibular system, suggesting that PMCA 2 may provide a major source of Ca^{2+} for their formation (Kozel *et al.*, 1998).

The mechanism by which PMCAs localize to appropriate regions of the plasma membrane is currently unknown. Fanning and Anderson (1996) have recently shown that some membrane proteins are gathered into functional complexes by interaction of their C-terminal tails, with other proteins, located just inside the cell membrane. These interior proteins contain one or more PDZ protein-binding domains (Ponting *et al.*, 1997). Proteins that contain PDZ domains may be involved in organizing complexes of other proteins at specialized membrane locations (Ponting *et al.*, 1997). For instance, the C-termini of the N-methyl D-aspartate (NMDA) receptor and the Shaker-type K^+ channel bind the PDZ domains of two proteins found at the neuromuscular junction. These particular synapse-associated proteins (SAPs), PSD-95 and PSD-93, are mammalian homologues of the *Drosophila* Dlg protein (one of the three proteins in which PDZ domains were first found). Mutations in the Dlg gene of *Drosophila* have been shown to disrupt the localization and clustering of Shaker K^+ channels (Tejedor *et al.*, 1997) and the localization of a cell adhesion protein, Fasciclin II, at the neuromuscular junction (Zitko *et al.*, 1997).

The focus of this research was to examine the possible interaction of the PMCA isoforms 1b-4b, with selected synapse associated proteins (SAPs). (The b designation refers to the RNA alternative splice variant in the C-termini of the PMCAs.) These variants contain a consensus sequence of amino acids similar to a sequence that has been implicated in the interaction between PDZ-containing proteins and Shaker K^+ channels and NMDA receptor proteins. Specifically, Kim *et al.* (1998) have shown that the human PMCA4 (hPMCA4) and post-synaptic density protein of 95 kDa (PSD-95) interact within COS cells using co-immunoprecipitation (co-IP) techniques. They concluded that there is direct binding between the hPMCA4 and several proteins in the SAP family

(of which PSD-95 is a member) via their PDZ domains.

This past summer at the Mayo Clinic in Rochester, Minnesota, I was involved with a project studying the hPMCAs and their possible binding to two synapse-associated proteins: SAP-102 and PSD-93. These two proteins have both been shown to bind the NMDA receptor and the Shaker K^+ channel via their PDZ domains (Fanning and Anderson, 1998). Since Kim *et al.* and others have shown that the PMCA binds proteins via their PDZ domains, we decided to look at the possibility of interaction between the hPMCAs and each of these two PDZ domain-containing SAPs. We had thought that PMCA4b interacts with SAP-102, while PMCA2b does not (unpublished results). Experiments on PMCA2b and 4b with PSD-93 had not been concluded. This current work was a continuation of those efforts to assess the possible interactions between these same two synapse-associated proteins (SAP-102 and PSD-93) and two other PMCA isoforms, hPMCA 1b and 3b. Knowing with which SAPs the various PMCA isoforms interact may help to elucidate the possible roles of the SAPs in both clustering the PMCAs into multi-protein complexes and localizing the pump isoforms in specialized membrane domains, such as the synapse. This investigation was designed to determine if the PMCA isoforms really do bind these two SAPs with variable specificity.

EXPERIMENTAL PROCEDURES

Full-length cDNAs encoding hPMCA1b, hPMCA2b, hPMCA3b, and hPMCA4b (from now on referred to simply as PMCA1b, 2b, 3b, and 4b) were cloned into pMM vectors (for original description of the expression vector, see Adamo et al., 1992). cDNAs encoding SAP-102 and PSD-93 were individually cloned into pGEX2TN vectors. COS-1 cells, grown in Dulbecco s modified Eagle s medium containing 10% fetal bovine serum and antimycotic (50 g/ml), were transfected at 50% confluence by the LipofectAMINE method (Life Technologies) as described by Kim *et al.* (1997). The constructs above were transfected in the following combinations: SAP-102 and PMCA1b, SAP-102 and PMCA2b, SAP-102 and PMCA3b, SAP-102 and PMCA4b, SAP-102 and empty pMM vector. The same transfections were repeated using PSD-93 DNA in place of SAP-102 DNA. The empty vector and SAP not in question were used as negative controls in the experimentation. Cultures were harvested 48 hours after transfection in 1 mL lysis buffer (Ausubel *et al.*,1999). 50 l of lysate was removed for control lanes and expression electrophoresis. Co-IPs were performed on the remaining lysate using monoclonal 5F10 (anti-all-PMCA) antibody and protein A and G sepharose beads according to the methods outlined by Ausubel *et al.* (1999). Following collection, washing and denaturing of the immunoprecipitate as described (Ausubel *et al.*,1999) the proteins were electrophoresed on a 7% SDS polyacrylamide gel. Immunoblotting analysis of the membranes was performed using isoform and protein-specific monoclonal and polyclonal antibodies (1:600). Polyclonals NR1-1 (anti-PMCA1), NR2-2 (anti-PMCA2), NR3-1 (anti-PMCA3), anti-PSD-93 (Synaptic Systems), and anti-SAP-102 (Dr. J. Hell, University of Wisconsin, Madison) were recognized by a secondary, goat anti-rabbit HRP antibody (1:5000). Monoclonal antibody JA-3 (anti-PMCA 4b) was recognized by a secondary anti-mouse HRP antibody (1:5000). The presence of HRP antibodies was detected using chemiluminescence methods.

RESULTS AND DISCUSSION

Data collected previously seemed to indicate that SAP-102 interacts with PMCA4b but not as strongly, if at all, with PMCA2b (unpublished results). Figure 1 shows the lysates and the co-IP lanes probed with anti-SAP-102. The presence of SAP-102 in both lysate lanes indicates that this protein was over-expressed in the COS cells, and a very strong band in the SAP-102/PMCA4b lane supports the conclusion that SAP-102 was pulled down by interaction with PMCA4b. The very light band in the SAP-102/2b co-IP lane may have resulted from the over-expressed SAP being pulled down by some endogenous PMCA in the COS cells and not necessarily by the transfected PMCA2b.

This would explain the lightness of such a band. However, when this experimentation was recently repeated, SAP-102 did not appear to interact with any of the four PMCA isoforms, calling into question the earlier SAP-102/PMCA4b results.

In order to determine whether the co-IP results are reliable, we needed to ensure that the proteins were over-expressed in the COS cells and that the transfected pumps were pulled down by the 5F10 anti-all PMCAs in the co-IPs. Figures 2, 3 and 4 show recent co-transfection lysates and co-IPs probed with antibodies recognizing PMCA 1b, 3b and 4b, respectively. The PMCA2b-probed blot is not shown because the antibody did not recognize anything on the membrane in this trial. (The fact that nothing showed up even in the lysate lanes indicates that something may have gone wrong in the transfer of that gel or with the preparation of the antibody). The presence of the other three PMCAs is accounted for in all of the appropriate lanes indicating both that there was adequate co-expression of the PMCA isoforms and that the pumps themselves were pulled down during the co-IP. (This was expected since the antibody used in the co-IP was 5F10, which binds all isoforms of the PMCA.)

The heavier bands in the IP lanes of Figure 3 are probably due to the fact that more protein was loaded in those lanes. That is, only a small amount of lysate was loaded in the lysate lanes (25 l) while the remaining approximately 500 l was used for the co-IPs. If a significant portion of the PMCA3b in that lysate was pulled down by the 5F10 antibody during the co-IP, more protein would have been loaded in those lanes, possibly explaining the relative heaviness of those bands. The lab had not previously expressed PMCA1b or 3b in COS cells, so figures 2 and 3 represent small successes in the overall course of the project.

The lack of a distinct band in the PSD-93/PMCA4b lysate lane of figure 4 may indicate poor expression levels due to inefficient transfection or the presence of a bubble in the transfer process. The latter would inhibit the transfer of the protein from the gel to the membrane. The fact that both the IP antibody, 5F10, and the immunoblotting JA-3 anti-body are monoclonal explains the dirtiness in the IP lanes of figure 4. When the anti-mouse secondary antibody is used to probe for the presence of the JA-3 primary antibody, it also recognizes the 5F10 antibody from the IP. This could not be avoided in the PMCA4b case because polyclonal antibodies are not available for this isoform.

Having shown that the PMCAs were expressed and pulled down during the co-IP (figures 2-4), the immunoprecipitates were probed for SAP-102 to determine if there is any interaction between this SAP and the PMCA isoforms. Figure 5 shows the lysates from the co-transfections and the co-IPs probed with polyclonal anti-SAP-102. Strong expression of SAP-102 in the lysate lanes indicates that the protein was over-expressed in the COS cells and was present in the lysate before the co-IP was performed.

Figure 5 seems to indicate that no interaction exists between any isoform of the PMCA and SAP-102. This conclusion is supported by the expression of both the pumps (figures 2-4) and SAP-102 (figure 5) in the lysates and by the presence of the PMCAs in the IP lanes of figures 2-4. Since the PMCAs were pulled down in the IP portion of the experiment, if SAP-102 were bound to any of those isoforms, its presence should be noted in the IP lanes. This conclusion, however, calls into question the earlier results regarding SAP-102 and PMCA4b (figure 1). This earlier work was also supported by strong expression of both proteins and the presence of the pump isoforms in the IP lanes on the gel (data not shown).

There are several possible explanations for these conflicting results. The presence of the SAP-102 band in the SAP-102/PMCA4b IP lane could have been due to spill-over of the SAP-102/PMCA2b lysate loaded in the adjacent lane. This does not seem entirely likely though because the band in figure1 in the SAP-102/PMCA4b IP lane is quite strong. Alternatively, the co-IP conditions in the second experiment could have been different, particularly at the washing stage. A wash that was too stringent (because of an incorrect preparation of the 1% triton wash) could have disrupted the interaction of SAP-102 after the co-IP, leaving no SAP-102 in the precipitate to be run

on the gel. This type of mistake, while relatively unlikely, is a possibility because a new wash solution was prepared between these sets of experiments. Further experimentation is needed to resolve this conflicting evidence.

While the PSD-93 co-transfections and co-IPs were performed and the presence of the PMCA isoforms from these experiments is shown in figures 2-4, no data was obtained from the immunoblotting with anti-PSD-93. We experienced great difficulty with this antibody and were not able to draw clear conclusions based on data gathered from PSD-93 experimentation. Although our earlier work seemed to indicate an interaction between PSD-93 and PMCA2b (unpublished results). It is possible that poor transfection efficiency of PSD-93 is responsible for the difficulty in observing PSD-93 with the anti-PSD-93 antibody.

Each of the problems and inconsistencies discussed thus far could most likely be resolved with additional experimentation. From this project s beginning, countless problems with transfection efficiency plagued the work. Many trials were abandoned, prior to the IP stage, after poor expression levels were observed. In addition several co-IPs were performed on lysates that exhibited only minimal expression levels. These IPs did not produce readable results. Toward the end of this work, the DNA was re-precipitated and this seemed to improve the expression levels as shown in figures 2-5. Many blots prior to this cleaning did not show distinct bands of the correct size when the expression levels were checked. This may have been due to dirty DNA and/or other problems with transfection efficiency such as cell confluency etc. Alternatively, difficulty with the antibodies may have masked expression that really did exist. Using fresh antibody for immunoblotting seemed to improve the visualization of the blots. Also, probing the membrane for more than one protein called for stripping the membrane between immunoblotting. This caused difficulty with the antibodies in early experiments, perhaps because the process of stripping the membrane can remove some of the protein in question. In order to resolve this, several gels were run and transferred so that each membrane only had to be probed for one protein as shown in figures 2-5.

Repeated experimentation, using all of that was learned from the many trials described above, may allow for more concrete conclusions than can be drawn from the data presented here. This work, however, does seem to illustrate that there is variable specificity in binding between full-length PMCA isoforms and the two SAPs in question, which was not previously known. Also, the successful co-expression of PMCA1b and 3b with the SAPs in COS cells was a step that had not been completed in the lab prior to this most recent experimentation. When stronger results are reached, a thorough examination of the specific binding partners will be warranted looking to their sequences for clues to explain the specificity.

Additionally, further work in this field may lead to the determination of the exact function of the SAPs with respect to specific PMCA isoforms and other membrane proteins. Elucidating the process by which the PMCAs and other such proteins are guided to appropriate regions of the cell will be an exciting breakthrough. This successful completion of this investigation may help to place one piece in this complicated and interesting puzzle.

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Anna Pinchak is a senior biology student. Next year she will be attending Duke University for Medical School.

Figures 1-5

Figure 1

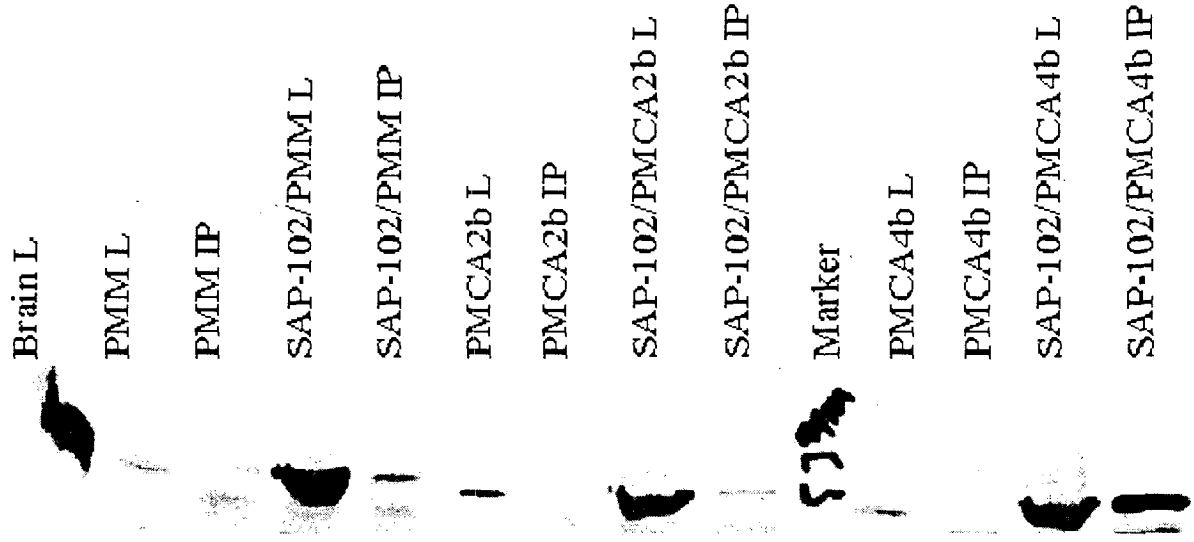

Figure 1: SAP-102 and PMCA2b and 4b co-IPs (IP lanes) and lysates (L) probed with anti-SAP-102 seem to indicate an interaction between SAP-102 and PMCA4b. Interaction between SAP-102 and PMCA2b is questionable.

Figure 2

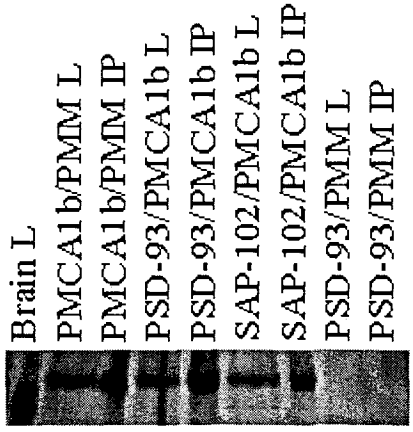

Figure 2: SAP-102 and PSD-93 co-transfected lysates and co-IPs with PMCA1b were probed with NR1-1 anti-PMCA1b. The presence of PMCA1b can be observed in each of the expected lanes. Note that the brain lysate band runs lower on the gel. This is due to the fact that the PMCA1 splice variant in rat brain lysate is slightly smaller in size than the human PMCA1b used in our experimentation.

Figure 3

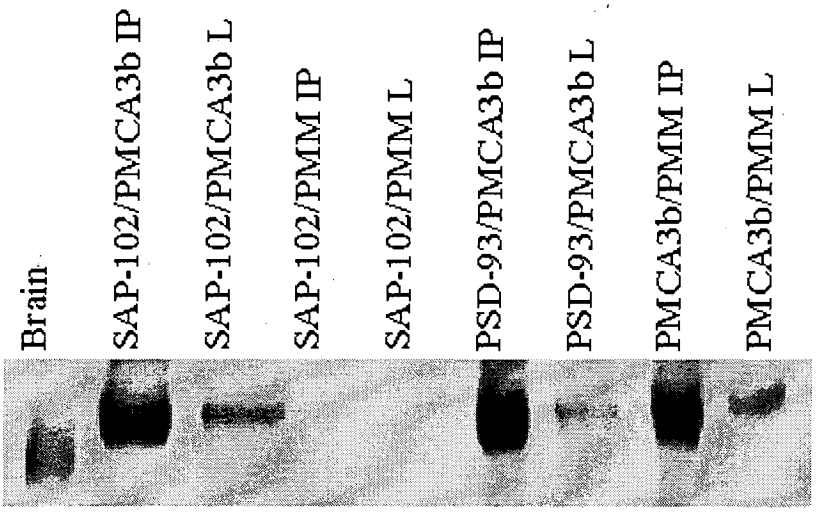

Figure 3: SAP-102 and PSD-93 co-transfected lysates and co-IPs with PMCA3b were probed with NR3-1 anti-PMCA3b. The presence of PMCA3b can be observed in each of the expected lanes. Note that the brain lysate lane band runs lower on the gel for the same reason listed above.

Figure 4

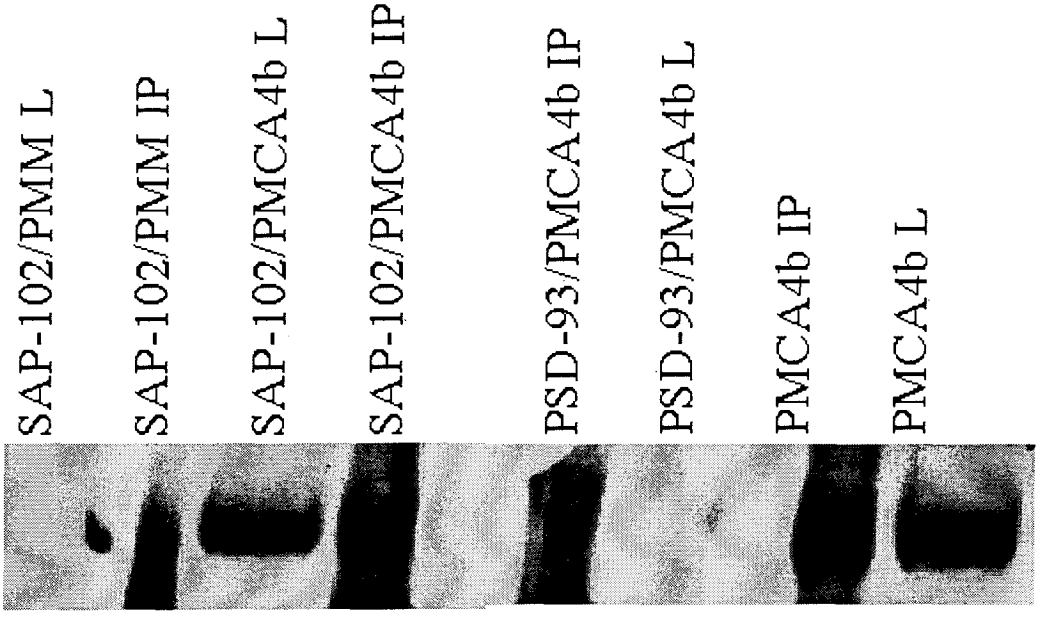

Figure 4: SAP-102 and PSD-93 co-transfected lysates and co-IPs with PMCA4b were probed with JA-3 anti-PMCA4b. The presence of PMCA4b can be observed in each of the expected lanes except the PSD-93/PMCA4b lysate lane.

Figure 5

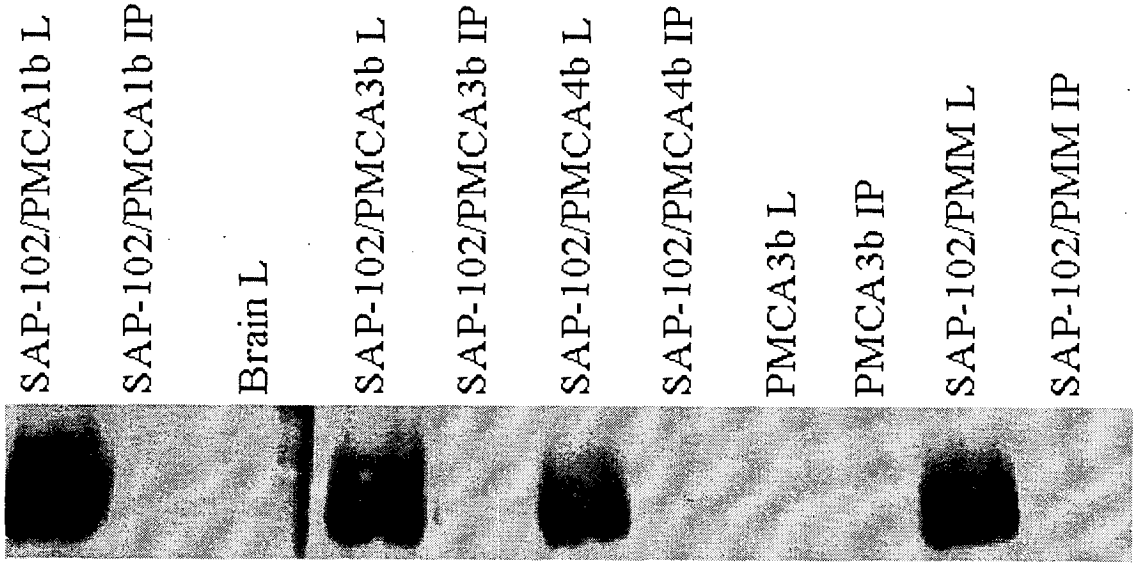

Figure 5: SAP-102 co-transfected lysates and co-IPs with PMCA1b, 2b, 3b and 4b were probed with polyclonal anti-SAP-102. The presence of SAP-102 can be observed in each of the expected lysate lanes; however, there does not appear to be any SAP-102 present in the IP lanes.

A long-term study of vertical migration in *Chaoborus* larvae: Patterns due to changes in time and oxygen concentrations

Heidi Rine, supervised by David Kesler, Ph.D.

INTRODUCTION

Diel vertical migration has been observed as a behavior characteristic of many types of zooplankton, including the aquatic *Chaoborus* larvae, which belong in the order Diptera and family Culicidae, which also includes the mosquitoes. The larvae of this insect are also known as phantom midges because of their transparency. The phantom midges are able to capture other types of zooplankton for food while the adults do not feed. In addition, the adult forms may cause a great nuisance when they leave the water due to their large numbers.

Diel vertical migration is a behavior that consists of a daily migration cycle in which the larvae remain in the deeper waters of a lake during the day and ascend to higher waters during the nighttime (Dodson, 1990). The most commonly accepted explanation for the vertical migration of zooplankton is that this behavior reduces predation by fish (Dawidowicz et al. 1990). However, by remaining in deeper water during the day, zooplankton do suffer reduced growth rates (Dawidowicz and Loose, 1994). In addition, this behavior is very costly to zooplankton in other ways as well, including reduced feeding time and energy losses (Dodson, 1990).

Some types of zooplankton are able to avoid most fish by remaining in the hypoxic waters of a lake. *Chaoborus* larvae are able to survive in anaerobic conditions for periods of two-three weeks (Cole, 1994). However, most fish species can not survive in such conditions for any extended period of time. Although the oxygen concentration below which fish can not survive varies among species and with changes in environment conditions, the general critical threshold has been found to be 2.0-2.25 mg/l (Wilding 1939; Moore, 1942).

Although numerous studies have been performed on the diel vertical migration of zooplankton, I could find no study that examined these patterns over a period of years. This study examines the migration patterns of *Choaborus* larvae in the same body of water over a nineteen-year period. Information was collected on the depths of *Chaoborus* larvae and the oxygen concentrations of Poplar Tree Lake since 1981. I compared average day and nighttime depths of the larvae to see if the larvae exhibited a consistent pattern of change in depth over this nineteen-year period. In addition, I compared these average depths with the depth at which the concentration of oxygen was 2.0 mg/l, the threshold below which fish can not survive. With this comparison, I could examine the hypothesis that zooplankton avoid fish predation by undergoing vertical migration. According to this hypothesis, the average daytime depth of the larvae should be below the depth of the critical oxygen threshold while the average nighttime depth should be above the critical oxygen threshold. This pattern would allow the larvae to avoid visual predation by fish during the daytime. I also wanted to examine if there was a correlation between the depth of the critical threshold of oxygen and the average depth of the larvae.

In addition to these considerations, this study also considers how a population of *Chaoborus* larvae can respond to drastic habitat changes. Poplar Tree Lake was completely drained between the years of 1994-1996. Thus, the *Chaoborus* population was completely destroyed in these years. However, the lake was replaced in 1997. Therefore, average depths of *Chaoborus* larvae before and after this period can be compared to give an idea of how resilient this species is to such changes.

METHODS

Research was carried out at Poplar Tree Lake located in Meeman Shelby Forest State Park in Memphis, Tennessee. Samples were taken between the years of 1981 and 1999 by a different group of undergraduate biology students each year. The lake was drained during the years of 1994-1996; consequently, no data are available for these years. In addition, no data were available for *Chaoborus* populations in the years of 1983, 1985, or 1998. Data for oxygen concentration levels were unavailable for the years of 1981, 1988, and 1991. Therefore, these data sets have not been included in this report.

Plankton were sampled by using the same plankton trap each year. By using the same trap, the volume of *Chaoborus* sampled has remained constant throughout the study. The trap was released and closed in the lake at eight depths, beginning at the surface and at every one meter interval until a depth of seven meters. Samples were taken every two hours over a twenty-four hour period, for a total of 96 samples per year. After a sample of water was captured in the trap, the plankton in the water sample were sieved into sampling jars and treated with formaldehyde. The bottles were labeled with the depth of the sample and the time of day at which they were collected. The formaldehyde served to kill and preserve the plankton for further research. At a later date, each bottle was analyzed to determine the number of *Chaoborus* larvae found at each depth at each time sampled.

After the number of *Chaoborus* larvae were counted for each sampling time, the mean depth at which the larvae were found at that time was determined. To determine the mean day depth of the larvae, the average depths of the plankton at 8:00, 10:00, 12:00, 14:00, 16:00, and 18:00 were averaged together. The mean night depth was calculated in the same manner, using average depths at 20:00, 22:00, 24:00, 2:00, 4:00, and 6:00.

Oxygen concentrations were determined in two manners: using an oxygen probe that provided a direct reading of the oxygen concentration and by the Winkler titration method. To perform the titrations, water samples were taken in a water trap at each of the eight depths every two hours for the same twenty-four hour period.

For this study, the mean depth at which the oxygen concentration was 2.0 mg/l was determined. This depth was found by first finding the two depths closest to a concentration of 2.0 mg/l– one depth with a higher concentration and one depth with a lower concentration. These depths were always 1 meter apart. Then, for these two depths, the oxygen concentration was multiplied by that depth and the two products were added. This sum was then divided by the sum of the oxygen concentrations at both depths. For each time at which the water was sampled, the average depth at which the oxygen concentration was 2.0 mg/l was determined. This gave 12 average depths. These depths were then averaged to find the mean level for that year. The standard deviation and standard error of the mean were also calculated.

RESULTS

Average Day Depth of Chaoborus

The average daytime depth at which *Chaoborus* larvae were found did not exhibit a consistent change over the time period from 1981-1999. The daytime depth of the larvae ranged from 2.97 to 4.89 meters below the surface of the water. However, regression analysis shows that the averages are not related to date (P=0.37). Figure 1 shows average day depth, along with standard errors, of *Chaoborus* over time.

Average Day Depth of Chaoborus vs. Oxygen Concentration

The average daytime depth of *Chaoborus* shows a correlation with the depth at which oxygen concentrations reached 2.0 mg/l in the water. That is, as the level at which this oxygen concentration moved to shallower depths, *Chaoborus* daytime average depths also moved towards the surface. Likewise, as the depth of this oxygen concentration dropped, *Chaoborus* average depths were also lower in the daytime (Figure 2). There was a significant correlation between the average daytime depth of *Chaoborus* and the depth at which oxygen concentrations equaled 2.0 mg/l (P<.05) (Figure 3). In addition, the larvae were found to always remain below the critical oxygen threshold of fish during the daytime (Figure 2).

Average Nighttime Depth of Chaoborus

The average depth to which *Chaoborus* larvae migrated during the night did not exhibit a consistent change between the years 1981-1999 (Figure 4). These average depths ranged from 1.89 to 3.27 meters below the surface of the water. The average nighttime depth for each year, along with standard errors, can be found in Figure 4. By regression analysis, I determined that average nighttime depth of *Chaoborus* was not related to time (P=.22).

Average Nighttime Depth of Chaoborus vs. Oxygen Concentration

As with the average daytime depths of *Chaoborus* larvae, the average nighttime depths also showed a correlation with the critical oxygen threshold depth. Average nighttime *Chaoborus* depths rose toward the surface when the oxygen concentration of 2.0 mg/l was nearer the surface and *Chaoborus* depths dropped when this oxygen concentration reached lower depths (Figure 5). The average *Chaoborus* nighttime depth was significantly correlated with the depth at which the oxygen concentration reached 2.0 mg/l (P<.05) (Figure 6).

DISCUSSION

Diel vertical migration patterns observed in *Chaoborus* larvae do not exhibit a consistent pattern of variation in relation to time. Between the years 1981-1999, the *Chaoborus* larvae in Poplar Tree Lake revealed no such pattern in either their average day or nighttime depths. The average depths at which the larvae were found over these years did change; however such changes must have been due to fluctuations in environmental conditions which did not change consistently over time.

This study provides evidence that one such environmental condition that influences zooplankton depth is the oxygen concentrations in the lake water. During both the day and night, *Chaoborus* larval migrations followed the changes in oxygen concentration of the water. This finding is especially interesting due to the variation among research team members over the nineteen-year period.

As stated earlier, numerous studies credit vertical migration as a behavior to avoid predation. The data collected in this study show that during the daytime, *Chaoborus* do in fact always remain below the critical threshold for oxygen. Thus, during the day, the larvae do remain in areas of the lake where fish can not survive. This clearly supports the hypothesis that vertical migration is an anti-predator defense mechanism.

However, one interesting finding of this study is that during the nighttime, *Chaoborus* larvae were often also found at depths with oxygen concentrations below the critical oxygen threshold. This pattern was not consistent, but it holds true for the majority of the years between 1981-1999. Thus, even during the night, some of the larvae are not exposing themselves to predation by fish. This finding suggests that other factors could in fact be the driving force for vertical migration. Future studies could investigate this finding to try and find what these driving forces may be.

The destruction of Poplar Tree Lake between the years 1994-1996 seemed to have little effect on the average depths of *Chaoborus* larvae. Upon replacement of the lake in 1997, the larvae exhibited average day and nighttime depths very similar to depths observed in earlier years (Figures 1 and 4). This shows that the larvae are very resilient; they exhibited the ability to return to normal behavior patterns immediately after the lake was refilled.

The data collected do show some variation in the average depths of the zooplankton over the sampled time period. In particular, average depth of the larvae, during both the day and the night, is much higher in the years of 1990, 1993, and 1998. The depth of the critical oxygen threshold is higher than normal in 1990 and 1993, but not in 1998. These exceptions could be indicators of other conditions that were abnormal at these times, such as temperature. Further research could investigate this phenomenon to see if any correlations can be found between these higher than normal depths and environmental conditions at those times.

In conclusion, this study shows that while *Chaoborus* larvae show variation in their spatial patterns over time, these patterns were significantly related to water oxygen concentration. This robust pattern was uncovered by relatively inexperienced research teams that were different each year and suggests that visual fish predation is a strong selective force on the behavior of aquatic insect larvae in lakes.

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Figure 1: Average Day Depth of *Chaoborus*

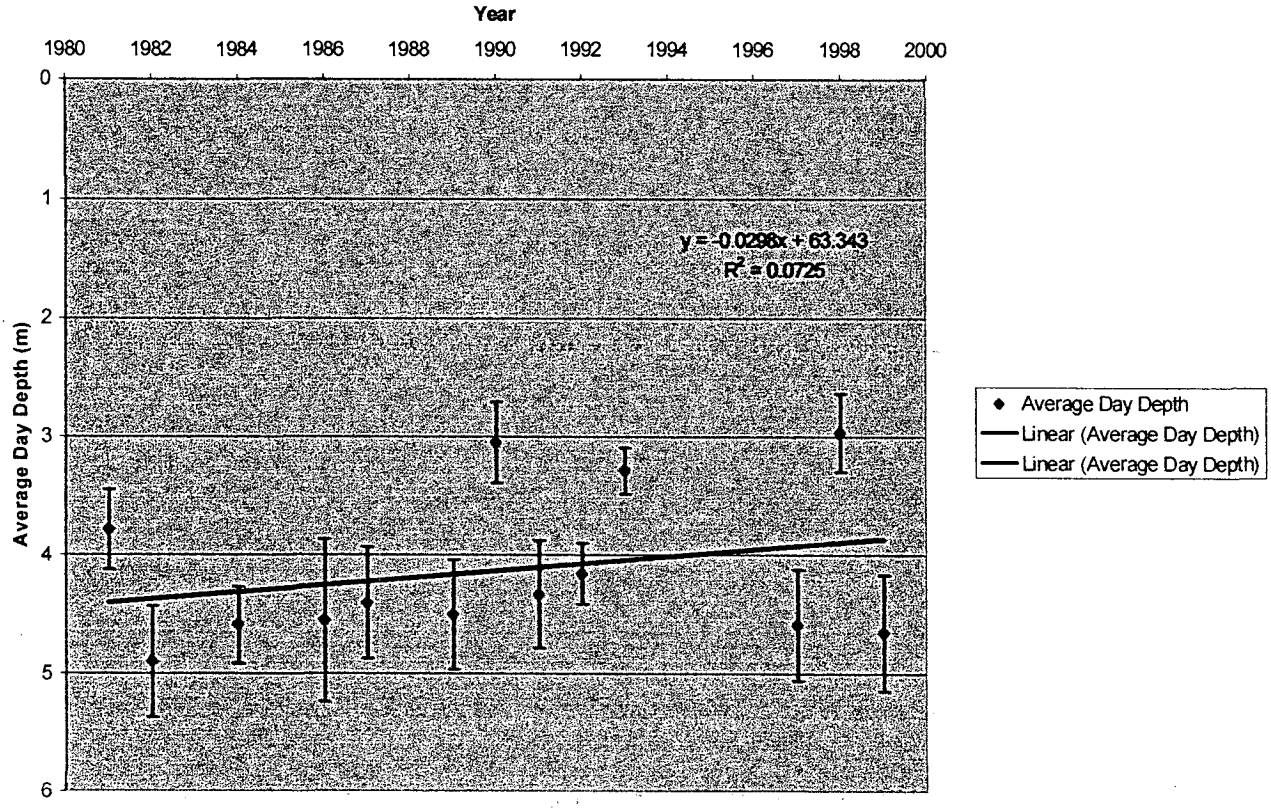


Figure 2: Average Day Depth of *Chaoborus* and Depth of Critical Oxygen Threshold

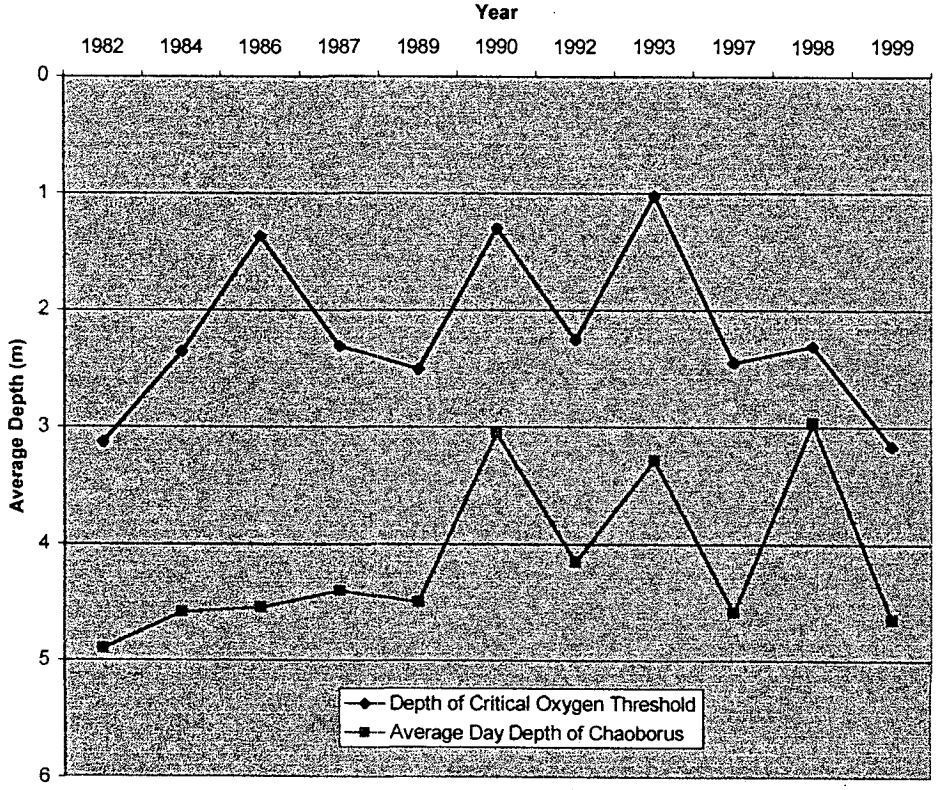


Figure 3: Correlation Between Average Day Depth of *Chaoborus* and Depth of Critical Oxygen Threshold

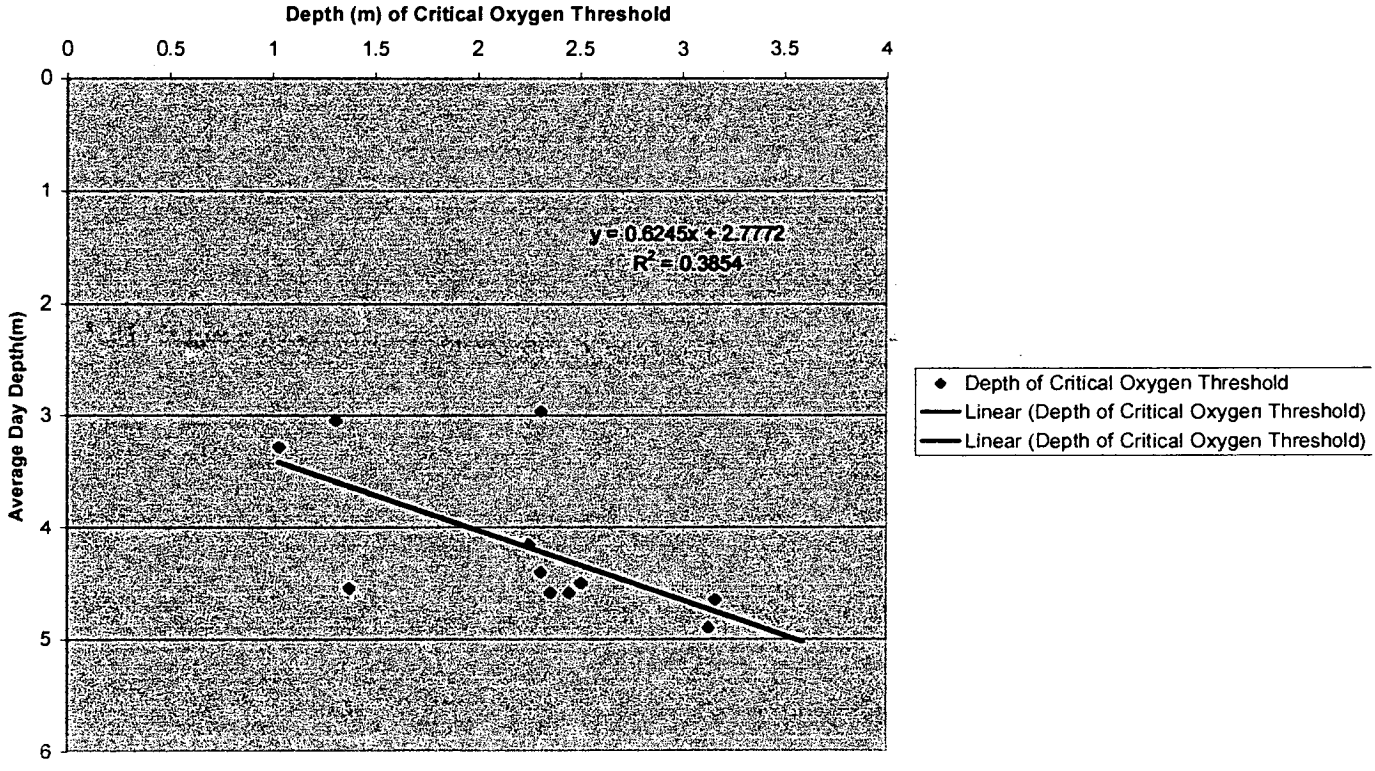


Figure 4: Average Night Depth of *Chaoborus*

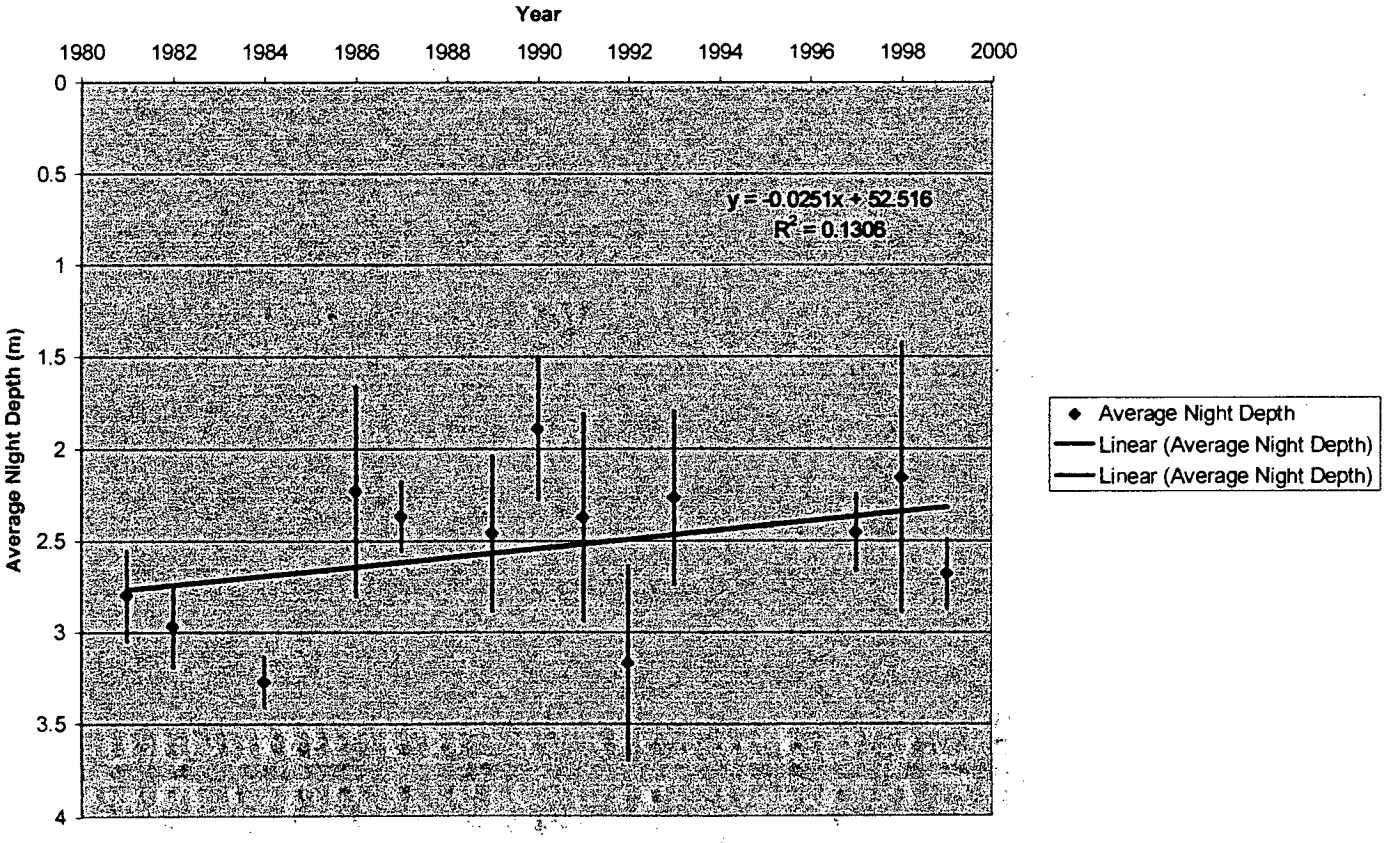


Figure 5: Average Night Depth of *Chaoborus* and Depth of Critical Oxygen Threshold

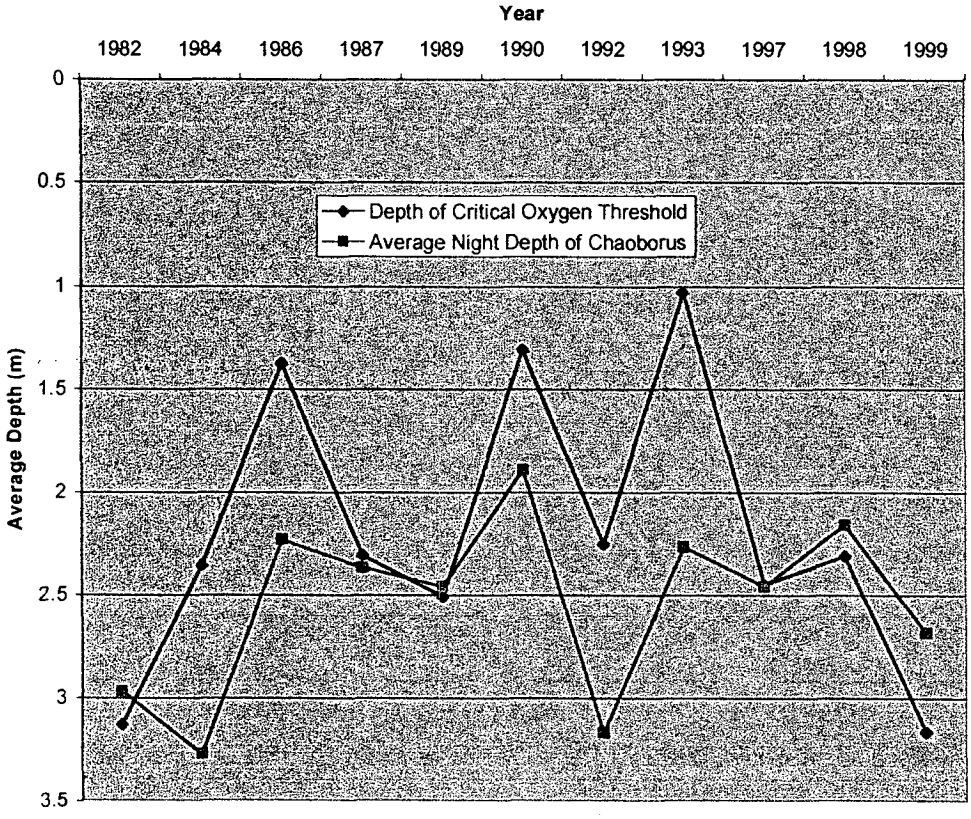
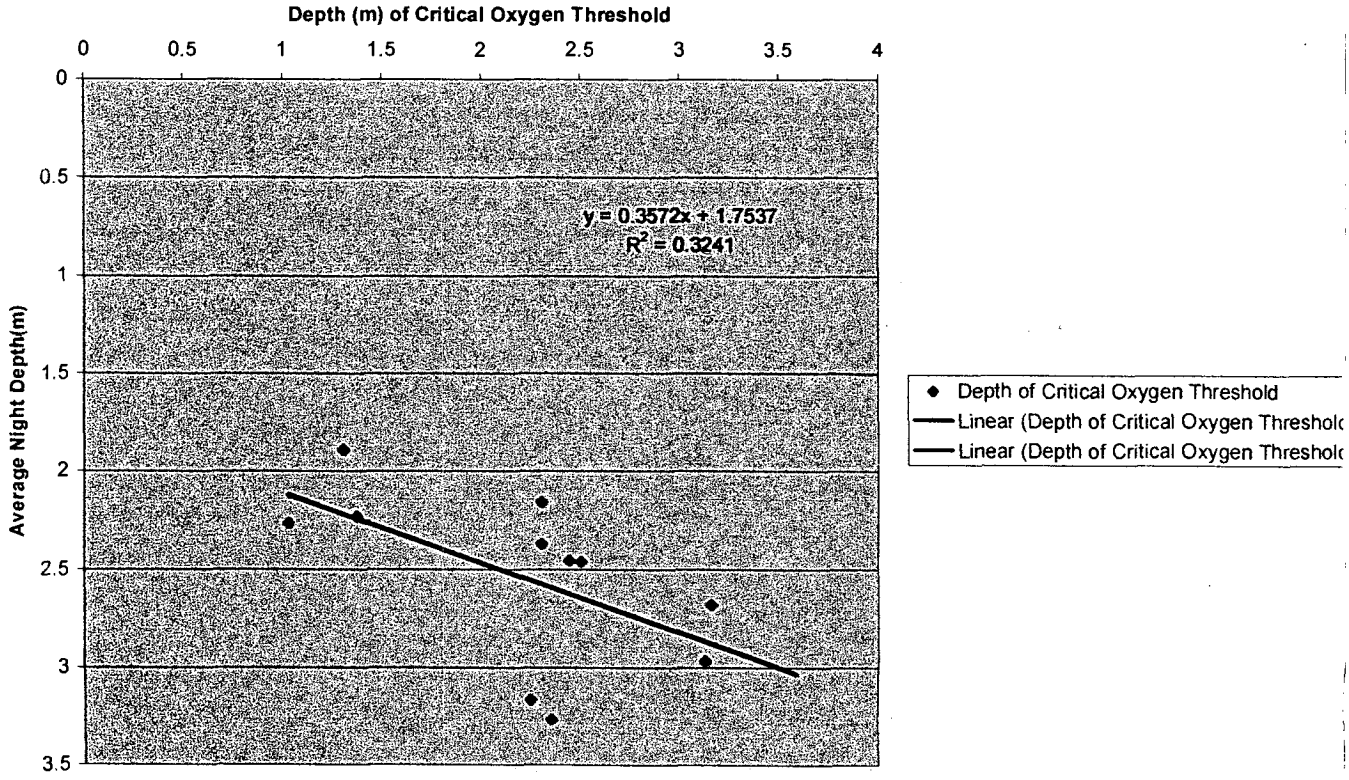


Figure 6: Correlation Between Average Night Depth of *Chaoborus* and Depth of Critical Oxygen Threshold



Migration of the Giant Cloudless Sulphur, *Phoebis sennae*; where and how?

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Abstract. The fall of 1998 witnessed an unexpectedly large appearance of the Giant Cloudless Sulphur, *P. sennae*, across the eastern USA. Not only were they abundant, but they were migrating in a southeasterly direction contrary to their previously documented migration patterns. The direction of the migration was 136 ±29 degrees at a speed of 206 ±107 meters per minute. This study examines the nature of the migration and also attempts to reproduce migratory behavior by *P. sennae* in a laboratory setting.

Introduction

Every year millions of butterflies migrate thousands of miles and nobody knows how they do it. In order to get at how they do it, one must first make sure that the butterflies are actually migrating and determine their origin and destination. Witnessing an unusually large number of butterflies provides a wonderful opportunity to record and analyze just such behavior. This fall *Phoebis sennae* has been sighted all across the eastern USA in large numbers (Barber,1998; Gibo, 1998; Netherton, 1998; Soukup, 1998) and provides ample opportunity to determine if they are migrating and how. Most butterfly migration studies concern the abundant and well known *Danaus plexippus*, the Monarch butterfly, yet other species migratory patterns need to be better documented. Data on migratory behavior in other species could be very important to comparative studies of how butterfly migration evolved. The purpose of this experiment is to provide just such data.

Once it is verified that a particular group is migrating, then one can proceed with testing their method of navigation. Mechanisms for insect migration have been studied for over three decades and many theories have arisen (Baker, 1968). The variety of insect navigation includes the use of the Earth s magnetic field, solar cycles, lunar cycles, polarized light, scents, and even wind currents (Walker, 1981; Baker, 1987). Current research into butterfly navigation has revealed that the Earth s magnetic field is important to butterfly navigation, but is not the only source of orientation (Kiepenheuer,1984). This study will also attempt to reproduce migratory behavior in a laboratory setting. Reproducing migratory behavior in the laboratory would be very useful in elucidating the mechanisms of navigation in further experiments.

Methods

To determine if the *P. sennae* were migrating 40 individuals were observed during one afternoon on a football field located on Rhodes Campus in Memphis, TN. Compass headings were taken from the spot where the butterfly was when it was initially spotted to the spot where the butterfly was when we stopped following it . We followed the butterflies at a distance of 20-30ft until it began altering its flight due to buildings south of the field. Time was recorded from the moment of initial spotting of the butterfly to the moment of the end of the tracking. The distance between the two spots was divided by the observed time to determine the speed of the

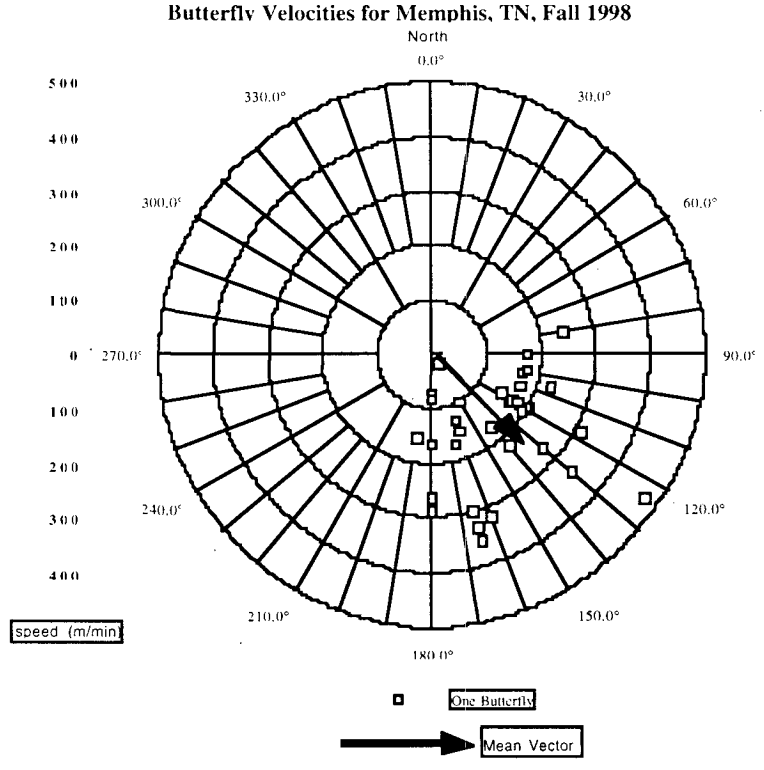
butterfly. The null hypothesis predicts that the vectors will be randomly distributed about the compass.

Hypothetically, the P. sennae in an enclosure should prefer the region of the enclosure closest to their migratory destination. Butterflies were captured for the laboratory experiment using a butterfly net and were kept in a shaded, ventilated jar until they could be used in the experiment. Butterflies were not stored for more than two hours between capture and experimental use. The experiment took place in an empty 50 gallon aquarium with screen covering the top. The aquarium was marked on its sides to divide it into two sections along the long axis. The aquarium was placed in direct sunlight and the butterflies were released into the aquarium. 2-3 butterflies were tested at once since no agonistic behavior was observed while they were being stored. The butterflies were then observed for ten minutes with the position of each butterfly being recorded at each minute. Position was recorded at being in one half of the tank or the other. Initially the aquarium was aligned with its long axis pointing due north. After observing the butterflies this way the aquarium was aligned pointing due east. The controls used in this experiment were one Variegated Frittilary (*Eupotoieta claudia*) and one Orange Sulphur (*Colias eurytheme*). Three *P. sennae* were used as the experimental group.

Results

The combined observations of the directional data of our class can be seen in figure 1. The mean heading is 136 $\pm$29 degrees and the mean speed is 206 $\pm$107 meters per minute.

Figure 1

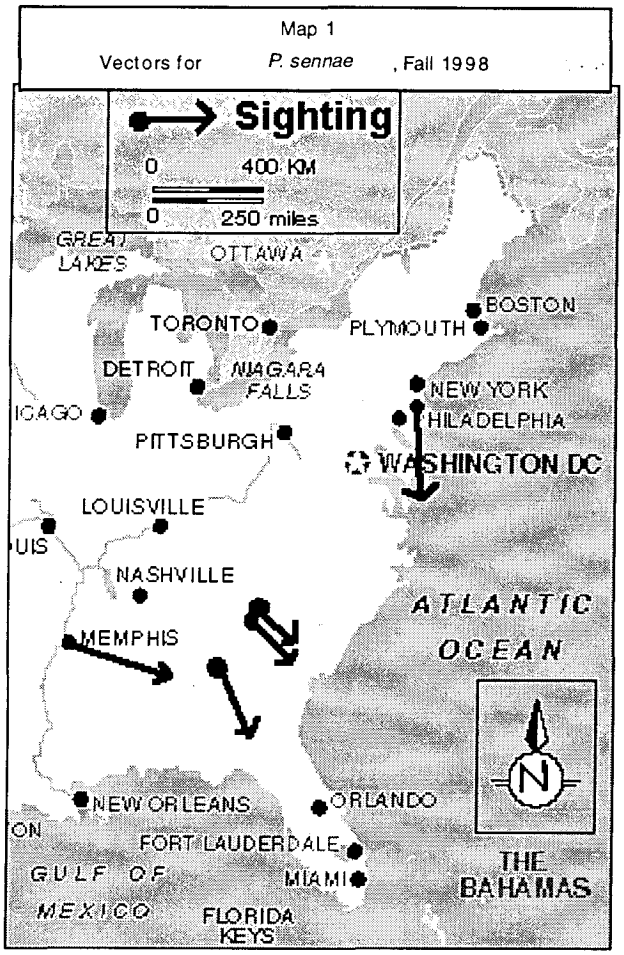


The results of the manipulative experiment are far less interesting. The number of observations for the controls and the experimental group were too few to facilitate any analysis. Once placed in the testing aquarium the *P. sennae* in the experiment did not exhibit any behavior, much less migratory behavior. Once the experiment was concluded and the butterflies were released, the *P. sennae* in the experiment took off and immediately headed in a southeasterly direction, displaying perfect migratory behavior.

Discussion

The behavioral data from the field observations indicates a migration in a southeasterly

direction. These data, along with other migrational data from the fall 1998 season in the eastern USA (Barber, 1998; Gibo, 1998; Netherton, 1998; Soukup, 1998), and one previous observation of southern migration in *P. sennae* (Gibo, 1987) may provide evidence for a southern migration of *P. sennae* that is not yet documented (See map 1). It is known that *P. sennae* steadily migrate northward from Mexico and the southern USA during the summer until they travel as far as southern Canada. It has been observed that many *P. sennae* die without returning south in the Fall (Pyle, 1981; Barber, 1998). The fact that normally few *P. sennae* are seen in the mid-south moving in any direction in the fall is evidence of this fact. The exceptional numbers of *P. sennae* migrating south easterly this year is truly a remarkable event. The destination of the *P. sennae* is unknown but by assuming constant rate and heading, the butterflies would be in Florida in under three weeks. Unfortunately it is known that other butterflies alter their course mid-migration (Kiepenheuer, 1984) and thus *P. sennae* could be migrating anywhere.



The manipulative experiment was less interesting, though still informative. Apart from demonstrating the need for superior handling techniques, this experiment showed that different butterflies may respond differently to enclosures. It would be informative to observe the activity of various butterfly species in a variety of different types of enclosures to determine the best type of enclosure for this experiment. The reproduction of migratory behavior in butterflies in a laboratory would go a long way towards understanding how butterflies navigate.

The documentation of migratory patterns is an important part of understanding the ecosystems traveled from/through/and to by the migrant population. If the southerly migration is an emerging behavior of the species, this would be a very interesting event indeed. By observing the evolution of a migratory pattern as it happens a lot of questions about butterfly migration could be answered. Finally, the addition of another butterfly species migratory behavior to our current knowledge would facilitate further comparative studies of insect migration.

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Cody Nash Biography

Born November 12th, 1976 to Tom and Nancy Nash in Vernal, Utah. Graduated valedictorian from Southern Boone County R-1 High School in Ashland, Missouri in May of 1995. High School activities included 1st chair alto saxophone in the school band, captain of the quiz bowl team, and numerous academic honors. Matriculated to Rhodes College as a Cambridge Scholar in the fall of 1995 with the intention of double majoring in Mathematics and English. Took first biology courses sophomore year and declared Biology Major, Math Minor the spring of 1997. Went on Semester at Sea in the fall of 1997 and spent the summer of 1998 at the Coral Reef Ecology program and at an internship with the Pink Palace Museum identifying coral specimens. Began working for St. Jude Children's Research Hospital conducting genetic mapping in the fall of 1998. Presented research on butterfly migration at the Tennessee Academy of Sciences in the spring of 1999. Spent the summer of 1999 at a Mathematical Biology Workshop at Duke University and attending the Professional Oncology Experience at St. Jude. Presented the genetics research at the 13th annual International Mammalian Genome Conference in Philadelphia in the fall of 1999. Future plans include graduating in May, 2000, spending the summer at the Space Life Sciences Training Program and hopefully Biosphere 2. Next year I hope to work for the Student Conservation Association conducting field research, and attending graduate program in ecosystem modeling in the Fall of 2001. After that, time will only tell.

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