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About this Issue

Statement of Purpose

The Rhodes Journal of Biological Science is a student-edited publication, which recognizes the scientific achievements of Rhodes students. Volume XXII marks the second year since journal was brought back into regular publication by Mark Stratton and Dr. David Kesler in 2006. Founded over twenty years ago as a scholarly forum for student research and scientific ideas, the journal aims to maintain and stimulate the tradition of independent study among Rhodes College students. We hope that in reading the journal, other students will be encouraged to pursue Scientific investigations and research.

Editorial Staff

Adam Bohnert '07 is a Biology major from Louisville, KY. He is currently a member of the βββ Biological Honor Society and the Men's Tennis Team. While at Rhodes, He has conducted research with Dr. Rosanna Cappellato on the ecosystem services provided by the urban forest of Overton Park. In addition to this research at Rhodes, he has worked at Rutgers University studying deep-sea microbiology. Adam's hobbies include playing sports, visiting the beach, and cheering on the University of Louisville Cardinals. After graduation, he will attend Vanderbilt University for graduate studies in the biomedical sciences.

Matthew Cain '07 is a Biochemistry and Molecular Biology major from Pine Bluff, AR. He is an active member in the Phi Beta Kappa, βββ, Mortar Board, and ODK honor societies and has served as the scholarship chairman in the Theta Chapter of Pi Kappa Alpha fraternity. He has conducted independent research in the lab of Dr. John Schuetz at St. Jude Children's Hospital since May of 2003, both idependently and under the Rhodes-Sponsored St. Jude Summer Plus Program. He also works as a technician in the student computer service center. Matthew enjoys the company of his friends and family and singing with the Rhodes Singers. After graduation, he plans to pursue graduate studies in the field of the molecular and cellular biological sciences at Washington University in St. Louis.

Anastasia Hartzes, '08 is a Biology major from Mobile, Alabama. She is a member of BBB Biology Honor Society, ΗΣΦ Classics Honor Society, Residence Life Staff and the Rhodes Women's Center. She is currently working with Dr. Carolyn Jaslow in researching the effects of Chlamydia on male infertility. During her free time, she enjoys reading, travel, and running. After graduation she hopes to pursue a career in medicine.

Ross Hilliard '07 (Senior Editor, Layout Design) is a Biochemistry and Molecular Biology major from Oak Ridge, TN. He is an active member of βββ, Mortar Board, Order of Omega and ODK honor societies and served as treasurer for the Theta Chapter of Pi Kappa Alpha fraternity for three years. He has conducted research in Dr. Richard Kriwacki's laboratory at St. Jude Children's Research Hospital since May of 2003 both independently and under the Rhodes-Sponsored St. Jude Summer Plus Program. Ross also manages the Rhodes Residential Network program and the student computer service center. In rare free time he enjoys trips to the mountains of North Carolina and the Gulf Coast and spending time with his friends. After graduation, Ross plans to pursue a medical degree and hopes to find a career that successfully combines his interests in clinical medicine and research.

Student Contributors

Brittany Bostick '07 is a biology major from Destin, Florida. Besides persuing her BS degree, Brittany is an active member of BBB Biological Honor Society and has also done several semesters of independent research with the Memphis Zoo and various Rhodes Professors. Brittany enjoys many extracurricular activities, including reading (specifically fiction works), surfing in the Gulf of Mexico, researching maritime history and acting. In her spare time, Brittany also tutors African Refugees in ESL at the United Methodist Neighborhood Center. After graduation, Brittany plans to take a year off from her academic pursuits for traveling, including a trip to Namibia Africa to volunteer with the Cheetah Conservation Fund and help with their ongoing research. She also plans to spend the year visiting various graduate schools and their ethology/animal behavior programs.

Gena Dolson '07 is a Neuroscience major and English minor from Tallahassee, FL. She is an active member of βββ Biological Honor Society and ΨX Psychology Honor Society. She is also involved in Students for Organ Donation. In addition to academics and clubs, Gena is the Student Associate for Career Services. She was selected for the St Jude Summer Plus program, and conducted research in the Radiation Oncology department May 2005 - August 2006. Gena studied psychological side effects of brain tumors and radiation therapy. She is preparing to submit a manuscript to Child's Nervous System. After graduation, Gena plans to attend graduate school in Neuroscience to earn her Ph.D.

Stephanie Juchs ’08 is a biology major and environmental science minor from Bel Air, MD. She is a member of the βββ Biological Honor Society and is currently doing research with Professor Cappellato on urban ecosystems. Stephanie also enjoys her volunteer work at St. Jude and with the Rhodes organization STAND where she mentors the children of Sudanese refugees. After graduation, she plans to attend graduate school in either conservation biology or ecology.

Sandy Obreza '06 is a biology major from Fort Myers, FL. In her four years at Rhodes, she was an active member in both the βββ Biological Honor Society and Fellowship of Christian Athletes, and played on the varsity volleyball team. She was placed on the SCAC Honor Roll multiple times and became a member of the Chi Alpha Sigma College Athlete Honor Society in 2006. In addition to conducting her own independent research at Rhodes, she also interned in the Rehabilitation Services department of St. Jude Children's Research Hospital in the Spring of 2005. In her free time, Sandy enjoys playing and coaching volleyball, running, and reading all the books that she didn't have time to read in college. After graduation, she began work as a Quality Control Technician at Children's GMP, LLC, the therapeutics production facility of St. Jude Children's Research Hospital. Eventually, Sandy plans to pursue a graduate degree in the fields of medicine or biology.

Adam Robinson ’07 is a biology major from Lewisburg, PA. He is a member of the βββ Biological Honor Society. Adam conducted his research under the direction of Dr. Jim Armacost of the Rhodes biology department. During his undergraduate studies he spent a semester at the University of Otago in Dunedin, New Zealand, where he enjoyed backpacking and rock climbing while taking both biology and elective classes. Currently, Adam is interning at the Church Health Center where he is gaining valuable experience in the health field, which he plans to pursue. After graduation, he will spend a year continuing to gain medical experience in Memphis as he prepares to take the MCAT and apply to medical schools.

Rhodes Biology Faculty

Jim Armacost, Jr. has served as an instructor during the 2005/2006 and 2006/2007 academic years. Mr. Armacost is a native Memphian. He has a B.S. in zoology from Louisiana State University, an M.S. in biology from Mississippi State University, and will receive a Ph.D. in biological sciences from Illinois State University. He has studied the ecology and conservation of birds in the United States, Japan, and Peru. His primary interest is in habitat use by birds. Habitat use is of fundamental importance because it is both central to understanding wildlife ecology and central to the management and conservation of wildlife. Mr. Armacost is also interested in the effect of human habitat modification on bird communities, especially in agricultural habitats. He is currently working with Rhodes students on a study of the role of birds as dispersal agents for invasive plants, such as privet, in Memphis city parks.

Dr. Tony Becker received his Ph.D. from West Virginia University with a dissertation on the physiological and behavioral responses of the green crab, *Carcinus maenas*, to severe hypoxia, research conducted at the Marine Biological Laboratory, Woods Hole, MA. Prior to coming to Rhodes in 2000, he had taught Biology at The Pennsylvania State University and Mansfield University of Pennsylvania and worked for The National Faculty, a non-profit professional development organization, as both a science program officer and executive director. At Rhodes, he has taught both lecture and lab sections of both semesters of Core, Evolution, Animal Behavior, Animal Physiology and Students Research. He has directed student research projects in Animal Behavior, mainly at the Memphis Zoo; these research projects have included such topics as courtship in giraffes, grooming behavior in bonobos and handedness in the giant panda.

Dr. Rosanna Cappellato earned her Ph.D. in Ecosystem Ecology/Biology at Emory University in Atlanta, Georgia. Her research focused on the deciduous and coniferous canopies interaction with acidic deposition. Since coming to Rhodes College a year ago, she has initiated two research projects. She

is working on the economic valuation of the ecosystem services , in particular carbon storage and sequestration, provided by Overton Park, and she is promoting the creation of urban green areas in the economically disadvantaged Hollywood-Springdale community. Her teaching ranges from an introductory course in Environmental Sciences to Conservation Biology ; in the summer she also offers an environmental field trip to Namibia. Most of her courses fulfill requirements for the new minor in Environmental Sciences.

Dr. Terry Hill received his Ph.D. from the University of Florida in 1978, and in the same year joined the Biology Department at Rhodes. His research has dealt with various aspects of fungal development – early work on mechanisms of spore formation in human and animal parasites progressed by degrees into studies of the physiology and enzymology of cell wall construction and developmental modification. Most recently this work has transitioned into studies of the molecular and Mendelian genetics of cell wall development in the model organism *Aspergillus nidulans*, which is carried out in collaboration with Dr. Darlene Loprete of the Chemistry Department. Dr. Hill has published numerous research articles on fungal biology while at Rhodes, upon which twelve former Rhodes students are listed as co-authors. Information on the *Aspergillus nidulans* cell wall project can be found at http://www.rhodes.edu/biology/hill/hill/studentresearch.html.

Dr. Carolyn Jaslow received her B.A. in Biology from Mount Holyoke College. At Ohio University she earned an M.S. for studying the functional morphology and digestive efficiency of red and grey foxes, and later she received her Ph.D. from The University of Chicago for research on the functional morphology and biomechanics of skull design and cranial sutures in sheep and goats. Most recently, her research has shifted to the study of reproductive biology, specifically the expression of cell surface proteins linked to fertilization in women undergoing IVF, and the factors related to recurrent pregnancy loss. Dr. Jaslow came to Rhodes in 1988. Courses she usually offers include Histology (360) and Embryology (209), Senior Seminar in Reproductive Biology (485/486), and Introductory Biology II lecture and lab (140 and 141).

Dr. David Kesler came to Rhodes in 1980, having received his PhD from the University of Michigan and teaching stints at the University of Rhode Island and Brown University. Courses he currently teaches and contributes to are Biology of Organisms and its lab (140 & 141), Coral Reef Ecology (253-254), Ecology (315), and Student Research (451-452). His research interests deal with aquatic ecology, and students working with him have spent time in area ponds, the Wolf River, Shelby Forest, and in the laboratory. His current research students are investigating ring formation in mussel shells and trematode parasite loads of Shelby Forest fish . You can learn more about Dr. Kesler's interests, courses, research, and more at http://www.rhodes.edu/biology/kesler.

Dr. Gary Lindquester, Chair received his B.S. in 1981 from Furman University. While at Furman, he spent a summer conducting research on DNA repair mechanisms at the Oak Ridge National Laboratories. He received his Ph.D. in 1985 from Emory University with a dissertation on the molecular genetics of tropomyosin gene expression and alternative splicing. He then spent two years as a National Research Council Fellow at the Centers for Disease Control and Prevention cloning, mapping and sequencing the genome of a newly discovered human herpesvirus. Dr. Lindquester came to Rhodes in 1988 and was initially funded by the Howard Hughes Medical Institute. Since then, he has held one year positions as visiting scientist at the University of Melbourne, Australia Veterinary School (1995-1996) and St. Jude Children's Research Hospital (2002-2003). He is currently studying the role of the Epstein-Barr virus interleukin-10 homolog on viral infection, latency and pathogenesis using a mouse herpesvirus model. Dr. Lindquester's research is supported by the National Institutes of Health and his collaborations with St. Jude Children's research hospital are sponsored by Dr. Peter Doherty, Nobel Laureate.

Dr. Mary E. Miller joined the faculty of the Biology Department at Rhodes College in the fall of 2001. Since this time she has taught in the core biology sequence, genetics, microbiology, and the cancer biology senior seminar. Dr. Miller received a B.A. at the University of Tennessee, and a PhD at the University of Virginia in microbiology. Dr. Miller then studied at the Rockefeller University in New York City as a post-doctoral fellow where she developed her research program. During this time Dr. Miller also served as an adjunct faculty member at Hunter College, City University of New York. Dr. Miller's work continues at Rhodes College where she actively encourages undergraduate research and supports undergraduates in her laboratory where they work to understand the spatial regulation of cyclins and cell division budding yeast. Dr. Miller's research interests focus on understanding the cell division cycle; primarily the regulatory proteins called cyclins, which are required for cells to faithfully duplicate themselves. Cyclin proteins regulate an essential enzyme in the cell called the cyclin dependent kinase or Cdk. Dr. Miller studies the role of spatial regulation in the function of cyclin/Cdk complexes. Dr. Miller makes use of high through put robotic microscopy to analyze genome wide effects on cyclin localization within the cell. These genomic and cytological tools are combined with biochemical and classical genetics readily available in the budding yeast *Saccharomyces cerevisiae*. This work impacts our understanding of the molecular basis of cell cycle progression; and therefore, the molecular basis of cancer. Dr. Miller's research is supported by the National Science Foundation and the AAAS/MERCK undergraduate research program.

Dr. Keith Pecor is in the second year of his two-year position as Faculty Fellow in the Department of Biology. He came to Rhodes in 2005 from the University of Michigan, where he earned his M.S. and Ph.D. in Biology. Dr. Pecor's graduate research included studies of the chemical ecology of native and exotic crayfish in Michigan. Since coming to Rhodes, he

has collaborated with students on both field and laboratory projects involving crayfish and amphibians. Dr. Pecor will be leaving Rhodes at the end of the spring semester to begin a tenure-track position at The College of New Jersey.

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Family Group Interactions of *Potamochoerus porcus* with Newborn Piglets: A Case Study at the Memphis Zoo

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Little research has been done on Red River Hogs in captivity, and virtually nothing is known of their behavior. To improve captive breeding rates, husbandry and self-sustainability, many such in depth studies are needed. A passel of captive Red River Hogs (consisting of two mature individuals and two juveniles from the Memphis Zoo) were studied primarily with an objective of determining the behaviors and interactions of the family group towards a newborn litter of piglets (farrowed by the sounder boar and sow). Specifically, interactions between the newborn litter and their juvenile siblings were speculated to occur more than interactions between the piglets and the sounder pair. The amount of time piglets spent with their Juvenile siblings was significantly greater than the amount of time spent between the piglets and the Boar and Sow. Overall, the two males had relatively low levels of interaction with the piglets throughout the study period as the two females had the majority of the interactions with the young. The interaction rates of the two boars were either stagnant or decreased as the study continued, whereas interactions between the Sow and piglets continued to increase (mostly due to the Sow's role as milk provider and protection). These results suggest that piglets interact significantly more with the Sounder pair than with their juvenile siblings. However, this idea does not disregard the important role that juvenile's may play in piglet development (e.g. acting as a surrogate parent, teaching the young important survival skills, etcetera.)

Introduction

The Red River Hog, *Potamochoerus porcus*, is a member of family Suidae and ranges throughout central, western and southern Africa in a variety of habitats including the savannah and rainforest (Burnie and Wilson 2001; MacDonald 2001; Vercammen et al. 1993; Benirschke 2006). In the wild, most Red River Hogs live in family groups consisting of 4-15 individuals, normally a group structure consisting of a boar with a harem of females and offspring (Burnie and Wilson 2001; Vercammen et al. 1993). These bushpigs are considered to be "locally abundant" in wild populations, but due to poor longevity and low breeding rates these pigs are not self-sustaining in captive populations (Burnie and Wilson 2001; MacDonald, 2001; Vercammen et al. 1993; Sowls and Phelps 1968). Due to anthropocentric issues such as the bush meat trade, agriculture, hunting and habitat loss that could become threatening in the future, it is necessary to have a self-sustaining captive population (Vercammen et al. 1993). As a charismatic species, the colorful Red River Hogs are in high demand in captive environments, with many individuals being imported from Africa. In fact, some wild caught "problem" Red River Hogs are sent to U.S. Zoos to aid in creating a larger captive gene pool (Vercammen et al. 1993; Jacobs 2006). However, without more research on captive husbandry, reproduction and behavior, self-sustainability within captive populations may be difficult to achieve.

Little research has been done on family group interactions of Red River Hogs in captivity. The resident sow and her newborn litter at the Memphis Zoo provided an ample opportunity to record interactions of the juveniles, Sow and Boar*, which reside and interact with the newborns from birth. The sow farrowed four piglets (three males, one female) in July 2006.

The principal object for this project is to monitor behaviors and interactions of the captive family group towards the newborn litter that can aid in updating husbandry and captive breeding techniques. The null hypothesis is as follows: The piglets will interact significantly more (confidence level of $p>.05$) with their juvenile siblings than with their parents. The possibility of the piglets interacting significantly more ($p>.05$) with their parents than with their juvenile siblings is the alternate hypothesis for this project. In addition to the principle objective, behavioral trends (such as time spent with adults and juveniles divided into two segments of 15 observations) for the piglets are included in this study.

Methods

Data were collected though observations of family group interactions outside the Red River Hog exhibit or inside the Round Barn within the Memphis Zoo. In order to reduce observer influence on the natural behavior of the hogs, data were collected on a ledge above the pigs on the opposite side of the enclosure, farthest away from their farrowing nest. Observations from the Round Barn (the indoor feeding facility

* As only the two adult hogs have names, the hogs will referred to as individuals by their sex and age (eg. Juvenile Sow or Sow) as their actual name. If both the Juvenile Sow and the Juvenile Boar are referred at once, it will be stated as "juveniles". "Juveniles" was defined as the progeny of the resident Sow and Boar from the previous breeding season, whereas "piglets" was defined as the progeny of the resident sow and boar farrowed during the current breeding season.

for hoof stock) were made from behind a closed door with a view over the top. Observations took place during morning or early afternoon hours (due to nocturnal/diurnal activity) for a duration of one hour per day for thirty (30) days (Vercammen et al. 1993; Mauget, 1980).

The data collection began three days after the birth of the piglets and continued until they were three months old (at which point the Sow began to wean the young). Observations of interactions and social behaviors were entered into a custom ethogram for analysis. These data were collected via a continuous sampling method, specifically monitoring the interactions of the four piglets with their siblings and parents. The behaviors are quantified as follows: head-butt, group resting, nursing, intercept nursing, aggression towards juveniles, aggression towards young, foraging with young, protective grouping, group resting, challenge and sniff snouts. The "headbutt" takes place when a member of the family group nudges a piglet forward or to one side with their head or snout simply to move the piglet from their path or to move them out of the way when foraging. "Nursing" is characterized by one or more piglets simply drinking milk from the teat of the adult sow, whereas "Intercept Nursing" is characterized by the juveniles or Boar attempting to move a piglet from a teat and sniffing the mammae or attempting to nurse from the sow alongside the piglets. "Aggression towards juveniles" takes place through challenging behaviors, vocalizations or charges of one or more adults towards the juvenile litter. "Aggression towards young" can be exhibited from any member of the family group and includes charges, biting or kicking. "Foraging with piglets" is a behavior that includes a piglet and an additional family member to snuffle in the enclosure's mulch or in the stream for nourishment and treats. "Protective Grouping" occurs when three or more members of the group form a protective circle around the young, often turning their side or back to visitor viewing areas. This behavior occurs in response to a stimulus, and can occur when a visitor scares the family group or in a potentially threatening situation for the newborns. The "challenge" behavior could be exhibited by any juvenile or adult hog and would be recognized by the characteristic raised shoulder bristles, pawing the ground, short charges and tail lashing (Skinner et al. 1976). "Sniff Snouts" is a friendly interaction between two individuals where they briefly touch noses in greeting (Skinner et al. 1976). "Group resting" takes place when the piglets and two more individuals all lie down or sleep together. This behavior can take place anywhere inside the enclosure, including the round barn and in the enclosure's stream. In addition to the previously listed behaviors, several other 'novel' behaviors were recorded for analysis.

A Student's T-test was used to examine the differences in frequency of behaviors exhibited by the juveniles towards the piglets versus the adults towards the piglets thus testing the null and alternative hypothesis.

Results

The data collected from the behavior and group interactions of the Red River Hogs is presented in Table 1. A total of 1,068 interactions between piglet and family group member were observed.

The t-value for the statistical data was t=0.4791, with a standard deviation of 35.46 and a confidence interval of 95%. With a probability of this result (assuming the null hypothesis) equaling 0.996, we fail to reject the null hypothesis. The amount of time piglets spent with their Juvenile Siblings (Figure 1) was significantly greater than the amount of time spent between the piglets and the Boar and Sow (Figure 1). For a graphical representation of the total percentages of time piglets spent with individual family members over the observation period, please see Figure 2.

Table 1

Behavior	Boar	Sow	Juvenile Sow	Juvenile Boar
Headbutt	20	72	121	31
Group Resting	24	51	33	50
Nursing	0	147	0	0
Intercept Nursing	0	0	65	13
Aggression towards Juveniles	11	16	0	0
Aggressing towards Piglets	2	34	16	5
Foraging with Piglets	57	65	87	77
Protective Grouping	7	5	8	9
Challenge	0	0	0	0
Sniff Snouts	8	9	21	4

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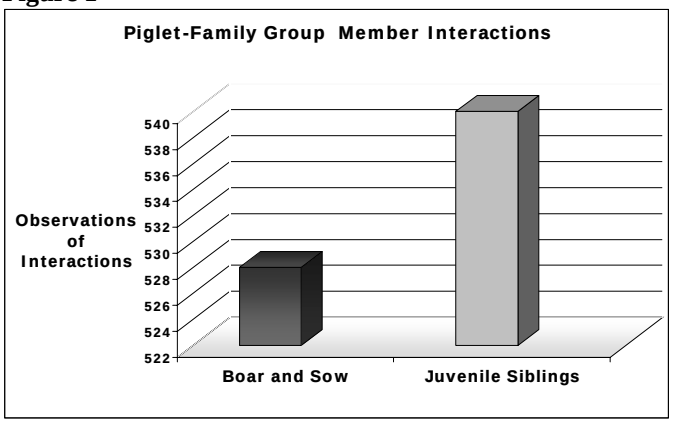
Figure 1



The two boars had very low levels of interaction with the piglets at most times, specifically the adult Boar. The Juvenile Boar's interactions with the piglets were usually limited to foraging or resting (many of the Juvenile Boar's Headbutt behaviors occurred in conjunction with foraging, so the higher frequency could be attributed to simply moving the piglets from his foraging spot). While the Sow had a very active role with the piglets, her interactions (or actual time spent with the piglets) increased as the piglets were over a month old (see figure 3 and figure 4 for comparison). However, this may be due to the decrease of interaction exhibited by all family members as the piglets grew more independent. Many more attempts to initiate nursing (or some other behavior) were exhibited by the piglets than attempts that led to actual nursing (or other interactions), suggesting that the lack of interaction between the Sow and piglets was through the Sow's behavior. Thus, it is possible that to compensate for the Sow's original lack of interaction with the piglets, both the Juvenile Sow and the Boar's interactions with the piglets increased (Figures 3 and 4).

Figure 2

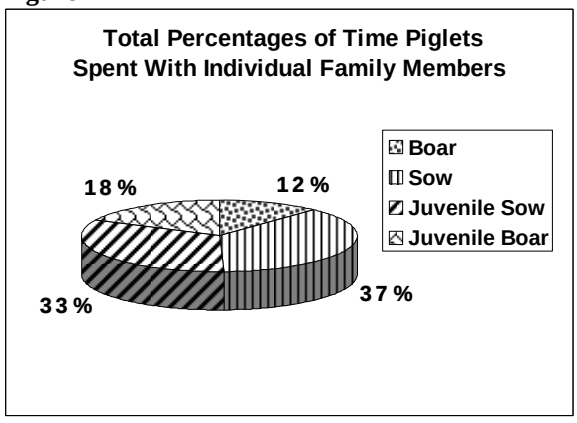


Figure 3

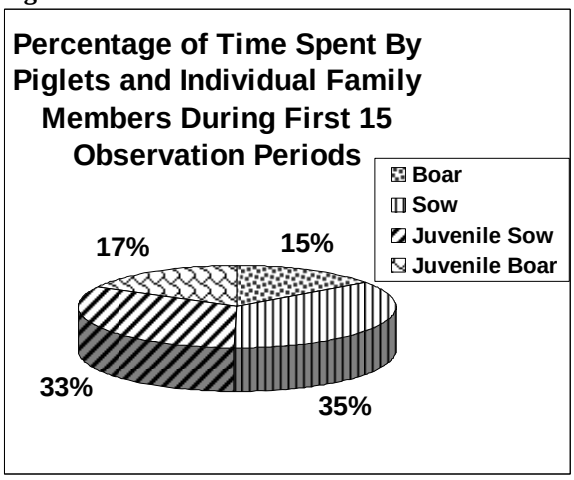
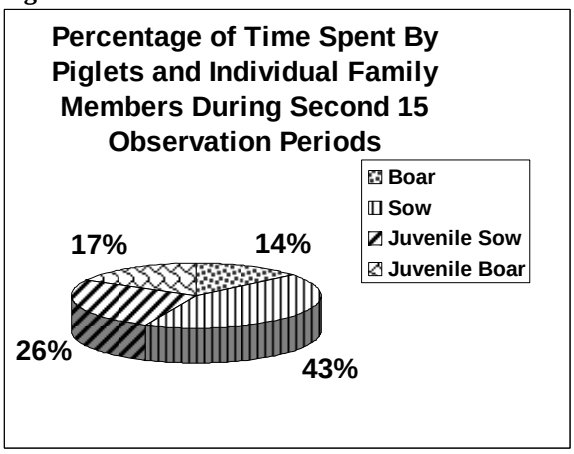


Figure 4



The Sow did have one consistent interaction with the piglets: through nursing. While the rate of interaction may have decreased between Sow and piglets, the Nursing frequency fluctuated but did not have a definite decrease over the observation period (with an average of five occurrences of nursing per observation period) (Figure 5). Interestingly, there seems to be a correlation between the frequency of Nursing and the frequency of Intercept Nursing (Figure 6).

Figure 5

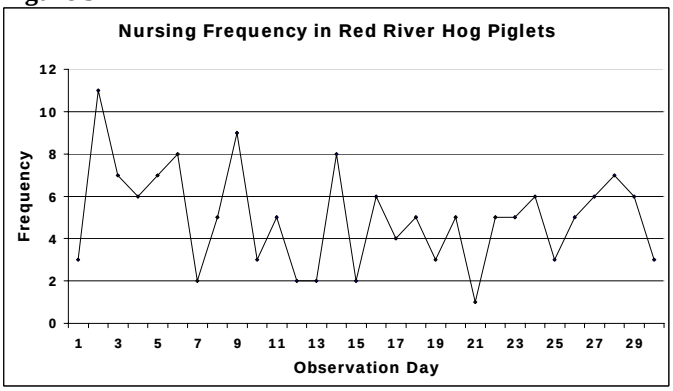
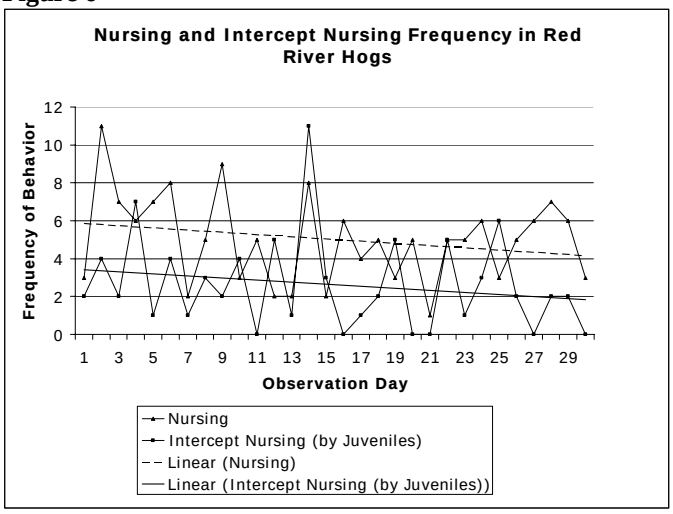


Figure 6



Additionally, many novel behaviors were observed and recorded (although these interactions were not included in statistical analyses or totals). One example of such a behavior is Urine Testing by the Juvenile Boar on the Juvenile Sow (four observations). The Juvenile Boar would approach the Juvenile Sow from behind while the female was urinating and tasted the urine from her stream (looking similar to a flehmen response in other Ungulates **(**Burnie and Wilson, 2001)). Another behavior observed multiple times was Mimicked Play: the piglets would often approach the juveniles, whereupon the two juveniles would begin sparring or chasing each other around the enclosure. At this point, it seemed the piglets closely watched the actions of their older siblings and would then give a close imitation of the play movements they had just witnessed. Lastly, a behavior was exhibited once by all four piglets in conjunction with the Boar: moving in a straight line formation, the five hogs would move forward several paces (together) and stop (once again all together), move forward again and stop once more (sometimes to snuffle in the undergrowth) and continued the cycle several times before the piglets broke formation and went indoors.

Discussion

From these data and statistical results, it is the conclusion that while each member of the family group plays an important role in interaction with young, the Sow's role seems to consist mainly of early protector and food provider, the Boar as simply a overseer, whereas the juveniles play the vital roles of teachers and socialize the piglets. Also, due to the high frequency of aggressive behaviors from the Juvenile Sow, such as Intercept Nursing, Headbutt, and Aggression towards Young, when compared to the male family members, it seems highly plausible that the Juvenile Sow's position within the family passel is as a disciplinarian. Specifically, the Juvenile Sow seems to aid piglets in socialization and in learning survival skills such as foraging techniques and sparring (possibly from mimicked play). Skinner et al. (1976) note that the master boar "is entrusted with the guardianship" of the younger pigs. They also shed light on the behavioral interactions of sow and piglets, stating how piglets and sow are almost oblivious to each other through the trust in the guardianship of the sounder boar. Frädrich (1971) describes three main types of play exhibited by Suidae young: "social play, solitary play and play with objects". "Social play", mainly consisting of fighting amongst similar-sized littermates, was the only type of play from Frädrich's list that was exhibited by the piglets. Additionally, no record was found of mimicked play in Red River Hogs.

Interestingly, it has been shown in domesticated pigs that as the piglets reach their third month, they pay more attention to their juvenile siblings than to their mother (who still suckles them) (Stolba and Wood-Gush 1989). While I was unable to find significant documentation of this behavior in feral or bushpigs, this behavior trend was observed with the Red River Hog group at the Memphis Zoo. It may be that the behaviors of the bushpig are more correlated with the domesticated pig than their wild relatives (eg. the Babirusa).

Red River Hogs in general, especially their family group structure and interactions, are poorly documented in captivity, and further research is needed if a self-sustainable population in the future is desired. Broader based studies are needed to determine group interaction trends and to ascertain how natural behaviors could be stimulated though mimicking a wild herd structure. In the future, an intensive study of the role of juvenile siblings with piglets and the piglet's successfulness as breeders could provide important data in achieving a healthy and larger population in captivity. Specifically, as more research is done on captive behavior, the data can be used to help improve self-sustainability particularly by modifying current husbandry techniques and mimicking natural breeding circumstances (i.e. family groups). However, without more research on captive husbandry, breeding, and behavior the long-term survival of Red River Hogs in captivity is in jeopardy.

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Discovery and Implications of Radial Glia Cells as the Stem Cells of Ependymoma

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Pediatric ependymoma tumors account for approximately 10% of all pediatric tumors; current research conducted by Taylor et al. has demonstrated that radial glia cells are possible stem cells of ependymoma (2005). Three types of ependymomas are explored: spinal, posterior fossa, and supratentorial tumors. All of these types are molecularly distinct diseases. Mendrzyk et al. have linked the gain of chromosome 1q to specific clinical prognostic factors in posterior fossa tumors (2006). In addition to chromosomal gains, the Notch pathway and γ-secretase are also prognostic factors. Fortunately, Fan et al. have begun to develop γ-secretase inhibitors that can slow the progress of a tumor. The advent of therapies that directly target stem cells, along with the combination of a better understanding of clinical prognostic factors, will make ependymoma a more comprehensible and treatable disease.

Introduction

Ependymoma tumors originate from the ependymal layer of cells lining either the cerebral ventricles or the central canal of the spinal cord. Ependymoma tumors in the brain typically affect children, and these tumors account for approximately 10% of pediatric brain tumor cases (Mulhern et al. 1989). Spinal tumors are more often seen in adults and are often treated with surgery only. Intracranial (supratentorial and infratentorial) tumors are typically treated first with surgery then followed by radiation therapy (RT). Supratentorial tumors arise from the lateral ventricles and are more likely to be anaplastic (more malignant in appearance, unlike the structure from which it developed) and, due to their location, are more likely to undergo a gross total resection (GTR), ultimately increasing progression-free survival (PFS) rates (Merchant 2002). Infratentorial tumors arise from various places within the fourth ventricle, making them closer to the brainstem and cranial nerves, thereby increasing the difficulty of performing a gross total resection on these patients (Merchant 2002).

Recent research has focused on possible origins of ependymoma: the growing "cancer stem cell" theory has called for genome-wide profiling, xenografts, and microarrays to be performed on cancer cells. Taylor et al. (2005) have found that radial glia cells (RGC) are most likely the stem cells for ependymoma. They have also characterized some molecular distinctions among subtypes of ependymoma that are later explored by Mendrzyk et al. (2006). These include some chromosomal abnormalities, especially the gain of 1q (chromosome 1 on the q arm) that is characteristic of posterior fossa ependymomas (Mendrzyk et al. 2006). Possible treatment options to specifically target cancer stem cells are discussed by Fan et al (2006); the Notch pathway is present in both medulloblastoma and ependymoma and this research could greatly advance the development of a treatment for these tumors.

The cancer stem cell theory stipulates that the majority of the cells within a tumor are actually generated by specific stem-like cells with all the properties of normal stem cells; each cancer or subtype of cancer has its own stem cells. These stem cells, the self-renewing portion of the tumor, actually comprise a small percentage; treatment procedures will change radically once specific stem cells and pathways are identified for a particular type of cancer, like ependymoma (Gilbertson 2006). In order for any new treatment to be effective, it is crucial to identify molecular differences between neural stem cells and cancer stem cells of the brain tumor. This also entails determining a new method of classification for brain tumors: are the subgroups of ependymoma from the same type of progenitor cells? Do these histologically identical but clinically distinct cancers have the same tissue or origin?

Ependymoma Subgroups Are Molecularly Distinct

Gilbertson's initial research on the stem cells of ependymoma revealed an interesting finding: many of these signature genes have been shown to regulate neural precursor cell proliferation and differentiation in the corresponding region of the CNS. These data identified incredible similarities between the gene expression patterns seen in embryonic radial glia and those observed in ependymoma (Gilbertson 2006). These initial findings have generated further research interests that involve isolating specific molecular characteristics of the subgroups of ependymoma.

Taylor et al. (2005) confirmed that the subgroups of ependymoma (supratentorial, posterior fossa, and spinal tumors) are molecularly distinct diseases. An array comparative genomic hybridization (aCGH) was performed to analyze gene expressions. This technique utilizes a large bacterial plasmid that can be transfected with entire portions of a chromosome instead of singular genes, facilitating study of a group of similar genes. The data from this particular experiment supported the idea that there are distinct patterns of both gene expression and chromosomal abnormalities that are not only specific to ependymoma, but also specific to the area in which the tumor arises.

Specific genetic or chromosomal abnormalities revealed in this microarray include the deletion of p16 in over 90% of supratentorial tumors, as well as a gain of chromosome 1q in posterior fossa tumors (Taylor et al. 2005). These results were specific to only one tumor subgroup: knowing that these tumors develop from different progenitor cells can aid in future developments of treatments to target precise developmental pathways. Also, information about genetic profiles can help in determining prognostic factors for subgroups of ependymoma. Mendrzyk et al. (2006) have analyzed genetic profiles of tumors with a 1q deletion and correlated these results with prognostic factors in order to possibly elucidate clinical profiles as well as treatment targets.

Chromosomal Aberrations as Prognostic Factors

According to Mendrzyk et al. (2006), the most frequently occurring chromosomal aberrations found in spinal ependymoma tumors involve gains in 7q and 9q, as well as losses of 22q and 14q. Intracranial ependymomas exhibit gains of 1q and 12q and losses of 22q and 6q. Of the 68 tumors analyzed, 49 were cranial tumors; all displayed DNA copy number imbalances in at least one chromosomal region (Mendrzyk et al. 2006). Once all the genomic profiles were superimposed, it became obvious that there were some overlapping regions of DNA copy number imbalances. These included the loss of the CDKN2A gene (a tumor suppressor gene) located on 9p, an amplification of ependymal growth factor receptors (EGFR) on 7q, extreme losses on 10q and 9p as well as high gains at 7q and 12q (Mendrzyk et al. 2006). The most significant of these imbalances were compared with clinicopathologic variables such as age, gender, chemo, RT, localization, tumor grade, resection, and tumor recurrence. Mendrzyk et al. (2006) affirmed that gains of 1q were significantly correlated with pediatric patients, an intracranial tumor location, and grade III tumors. Even though Taylor et al. (2005) have previously shown that 1q gains occur in posterior fossa, Mendrzyk et al. (2006) have begun to identify specific clinical factors that can help to create prognosis factors for patients.

Although current prognostic factors are helpful in determining treatment, it is more beneficial to isolate certain areas of chromosome 1q in order to obtain a more specific idea of what actually contributes to the prognostic factors for these tumors. There were three large regions of candidate genes for ependymoma in 11 of 49 tumors, and were all found in the 1q21.1 to 1q32.1 range (Mendrzyk et al. 2006). This particular stretch of chromosome 1q was analyzed using mRNA expression by QRT-PCR. Two of the genes within this region include EGFR and DUSP12, a potential oncogene candidate that may be involved in generating lesions in ependymal cells (Mendrzyk et al. 2006). There was an increased variability in the results using the PCR technique so these results were inconclusive about mRNA expression of these genes.

Although EGFR is strongly overexpressed and CDKN2A is underexpressed in the sample of ependymomas, the PCR results were not clear, thus both immunohistochemical analyses and FISH were used to determine specific regions of 1q that are responsible for the prognostic factors of ependymoma. Probes from 1q36 and 1q25 were used for the FISH analysis; the gain of 1q25 was shown to have a significant correlation with a poor prognosis for overall survival ($P < .001$) (Mendrzyk et al. 2006).

Immunohistochemical analyses were performed using p16 (a gene that is deleted in a significant number of supratentorial ependymomas) as well as EGFR and hTERT. There was a severe deficiency of protein expression for p16, but detectable expression was unexpectedly identified with poor prognosis, which merits further experimentation (Mendrzyk et al. 2006). hTERT is overexpressed and shows a significant correlation with an adverse outcome ($P=.01$), as does EGFR overexpression ($P=.002$) (Mendrzyk et al. 2006). These findings did not apply to spinal tumors, although there were similar levels of protein overexpression.

Data from FISH and immunohistochemical analyses were used to create a final model for both overall survival and progression-free survival (PFS) of ependymoma. The model for PFS states that prognosis will improve when a GTR is performed so that all of the tumor is removed; the prognosis becomes poor if there is a gain of 1q25 or if the tumor is classified as a grade III ($P < .001$ for both) (Mendrzyk et al. 2006). Overall survival is dictated by 3 variables: a significant correlation with a good prognosis was found for the delivery of RT, whereas a gain of 1q25 and a classification as grade III once again resulted in a poor prognosis (Figure 1). The conclusion based on these models is that 1q25 is the significant molecular marker in regards to PFS or overall survival. This provides an additional genetic marker that can be specifically analyzed in ependymoma tumors. It is important to know the specific location on 1q that is responsible for these prognosis factors so that this information can be integrated into microarray analyses performed in studies similar to Taylor et al. Once a specific prognosis factor is identified, it will be possible to develop a specifically targeted treatment plan in the future as well as give the parents an accurate idea of the struggle their child will face and a realistic idea of his or her chance of survival.

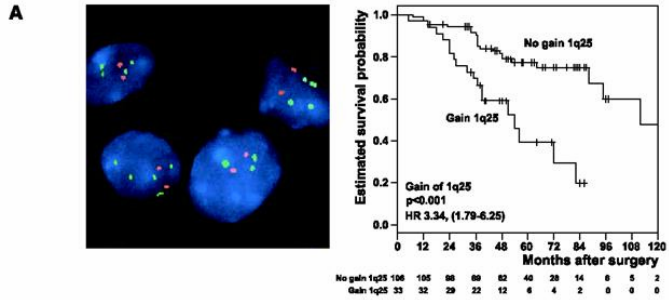

Figure 1: (A) FISH image showing a gain of 1q25 (green) versus 1p36 (red). To the right (B) is a comparison of the survival for several months post surgery for a gain of 1q25 versus those who do not gain 1q25 (Mendrzyk et al. 2006).

Although few of the genes that were isolated and used in analyses in Mendrzyk et al. (2006) were actually significantly involved in ependymoma, these genes have been shown to be involved in other cancers, specifically cancers

that originate from stem cells like breast cancer, prostate cancer, and thyroid carcinomas. Also, the role of p16 in ependymoma is still unknown, although it is associated with a poor prognosis. hTERT overexpression was detected and is also linked with a poor prognosis, but the specific role is also still unclear (Mendrzyk et al. 2006). For tumors classified as grade II, the overexpression of EGFR leads to a poor prognosis because there are more receptors to bind growth factor, thus allowing the tumor to grow rapidly. Although work must be done to elucidate the specific roles of these genes, it is possible to use these genes to identify tumors that may have more malignant lesions and can also help to determine risk stratification for ependymomas. Because the PCR results contained a large amount of variance, it was difficult to determine how significant the role these other genes played in ependymoma: over or underexpression was evident for the genes involved in mitosis and neuronal development and further studies will be conducted to elucidate the role that each of these genes plays in various cancers.

Radial Glia: The Stem Cells of Ependymoma

The additional research by Mendrzyk et al. (2006) that supports findings of Taylor et al. is helpful and provides more specific insight into genetic and chromosomal prognostic factors. However, the idea of stem cells as the origin of ependymoma needs further exploration. In the genetic profiles of each tumor subtype, Taylor et al. (2005) discovered that these genes also normally function to regulate neural precursor cell proliferation and differentiation in each respective area: supratentorial tumors exhibit an overexpression of cells involved in the EPHB-EPHRIN and Notch pathways. Spinal ependymomas show an overexpression of HOX family genes, which play a role in anteroposterior tissue pattering during development (Taylor et al. 2005). Not only did FISH and immunohistochemical analyses confirm that there were overexpressions of particular pathways in ependymomas, but they also confirmed that these patterns are produced by ependymoma cancer cells, not normal cells that are trapped in the tumor. This data provided a breakthrough in determining the stem cells of origin for ependymoma. Knowing that these are actually cancer cells, Taylor et al. theorized that ependymoma stem cells could still maintain some markers of the progenitor cells from which they developed.

In order to support this theory, Taylor et al. (2005) mapped the genetic expression of 77 genes associated with either supratentorial, posterior fossa, or spinal tumors to determine the normal cell populations from which these cancer cells arose. The results from this genetic expression experiment clearly define the normal cell populations that give rise to the genes associated with spinal and supratentorial ependymomas: the genes associated with supratentorial tumors were expressed almost entirely within the walls of the lateral ventricles and the subventricular zone. Also, the vast majority of spinal tumor genes were expressed in the wall of the developing spinal canal and ventrolateral spinal cord (Figure 2). Posterior fossa genes were not as concretely expressed in their area of origin; they were also expressed to some extent in the lateral ventricles and spinal cord, making it much more difficult to draw conclusions about the cell population of origin for this tumor subtype.

The genetic expression mapping revealed that several of these genes are expressed between embryonic days 11 and 15 in mice, which, interestingly, directly coincides with the period of development when radial glia cells give rise to ependymal cells in each respective region (Taylor et al. 2005). Radial glia cells constitute a heterogeneous population of multipotent neural precursor cells that display significant differences in gene expression and function along CNS regions. This strong developmental overlap led to the idea that radial glia cells are the cells of origin for supratentorial and spinal ependymomas (Taylor et al. 2005). This was further supported by the presence of both RGC antigens and the signature genes for ependymomas in both the spine and lateral ventricles. Since posterior fossa tumors did not show a definite genetic expression within its area of origin, it is much more difficult to say that these tumors also arise from radial glia cells; this suggests there is another very similar progenitor cell population from which they develop.

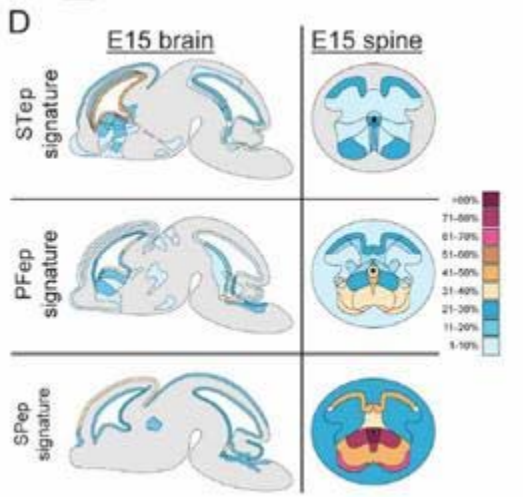

Figure 2: Map of the frequency and location of expression of the 77 genes shown to identify the different subtypes of ependymoma (supratentorial, posterior fossa, and spinal tumors) (Taylor et al. 2005).

It was necessary to confirm experimentally that the RGCs gave rise to ependymoma stem cells. Stem cells have the properties of both self-renewal and differentiation. If these are in fact stem cells, they should exhibit both of these properties, as well as RGC markers. Both medulloblastoma and gliomas have stem cells which can form tumorspheres (clonally derived colonies) that exhibit a marker for CD133, which is indicative of neural stem cells (Taylor et al. 2005). In order to confirm that these ependymoma cells exhibit these necessary properties, single-cell suspensions were generated from freshly resected supratentorial and spinal tumors. All of the resected tumors were found to contain a minority population of cells that generated new tumorspheres. These were replated at one cell per well, and new tumorspheres formed from these primary tumorspheres over one – two weeks, thus demonstrating the property of self-renewal (Taylor et al. 2005).

Next, these cells were analyzed for RGC markers at various stages of differentiation. First, neuroepithelial cells were analyzed because they are the precursor to RGC cells and should exhibit CD133, Nestin, and RC2. Nestin and CD133 are progenitor cell markers, and RC2 is specific to RGC; all three markers were exhibited in neuroepithelial cells. The difference between the precursor cells and actual RGC is the presence of two particular glial markers, an astrocyte-specific glutamate transporter (GLAST) and brain lipid binding protein (BLBP) (Taylor et al. 2005). These were also shown to be present in RGCs (Figure 3). As differentiation of these cells progresses, the markers of the neuroepithelial cells disappears, and other markers specific to the differentiated cell type appear, which will be analyzed in a later experiment. These ependymoma tumor cells were compared with medulloblastoma, another type of brain tumor known to arise from a different type of progenitor cell. These too exhibited CD133 and Nestin, but did not exhibit the RGC-specific RC2 and BLBP (Taylor et al. 2005). This evidence confirms that ependymoma cells are different from medulloblastoma cells and that they exhibit markers from their RGC origins.

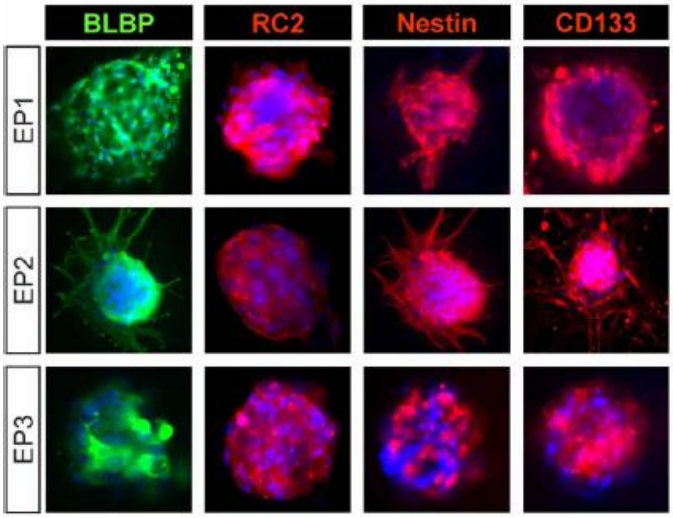

Figure 3: Staining of ependymoma cells showing BLBP and RC2 as markers of RGCs and Nestin and CD133 as markers of neural stem cells (Taylor et al. 2005).

The last step in confirming that these cells are stem cells is to analyze differentiation and specific markers of new cell types. Cells were taken from the secondary tumorspheres and cultures in the absence of growth factors so that differentiation would be induced. After twenty-four hours, approximately 70% of the tumor cells retained expression of BLBP and 30% still expressed RC2; however, very little CD133 was still expressed, indicating that the cells had begun to move away from the neuroepithelial cell profile (Taylor et al. 2005). After 72 hours in culture, there was a dramatic change in the phenotype of the cells: the CD133+/RC2+/BLBP+ phenotype was no longer expressed and specific markers for neuronal differentiation were increased (Figure 4). Neuronal differentiation markers that appeared included beta-III-tubulin, MAP2, astrocytic markers, and oligodendrocytic markers (CNPase and NG2) (Taylor et al. 2005). These experiments have supported the idea that the cells responsible for ependymoma are in fact stem cells because they are self-renewing and multipotent, and they also contain markers for RGC, supporting the theory that RGC are the population of origin for ependymomas.

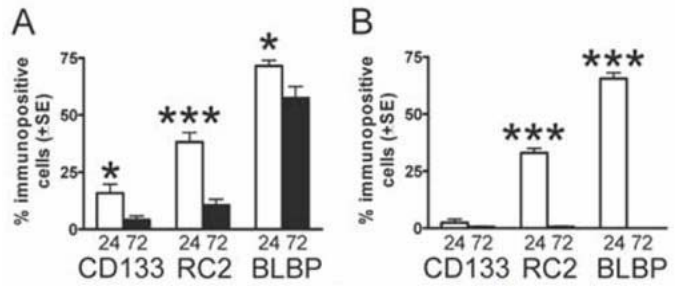

Figure 4: Two populations of ependymoma cells that demonstrate the change in phenotype over 24 and 72 hours as CD133, RC2, and BLBP markers disappear (Taylor et al. 2005).

It is much easier to conduct research on tumors engineered in mice instead of waiting for the rare human tumor. Some experimentation was necessary to ensure that the tumor could grow in mice for further studies as well as proof of recapitulation of the original tumor *in vivo*. In order to determine what markers were necessary for the tumor to develop from a xenograft (human tumor cells implanted into mice), tumor cells were isolated into either CD133+ or CD133- groups using a magnetic bead technique. FISH confirmed that both of these groups contained cancer cells because there was a gain of 1q (Taylor et al. 2005). These two groups of cells were injected into the superficial cerebral cortexes of two groups of seven week-old NOD-SCID (immunodeficient) mice. Mice with as few as 10,000 CD133+ cells developed signs of neurological impairment within four to five months, whereas the mice injected with CD133- cells showed no impairment up to a year after injection (Taylor et al. 2005). The brains of the CD133+ mice were analyzed and the cause of the impairment was indeed an ependymoma tumor that covered the ventricles, causing hydrocephalus. It is important to note that these tumor cells in mice displayed characteristic features of ependymoma, including the rosette shape of the cells. They also exhibited BLBP and glial fibrillary acidic protein, but did not show markers of differentiation (Taylor et al. 2005). Interestingly, these injected cells maintained the genetic profile of the original tumor: if supratentorial cells were injected into a spinal area, the tumor would exhibit characteristic of supratentorial tumors, not the actual location (spine) where it developed in the mouse. This has confirmed that ependymomas arise from a rare population of CD133+/RC2+/BLBP+ stem cells that are necessary to generate the tumor and also transmit some RGC-like properties to these tumors. This confirms that ependymoma stem cells arise from transformed RGCs.

While this supported theory has provided an enormous breakthrough in understanding ependymoma, there are several factors that can influence how RGCs are transformed to produce the mutated stem cells for ependymoma. Previous studies on leukemia have found that

early transformation events are primarily responsible for the altered genetic profile. Therefore, Taylor et al. (2005) reasoned that chromosomal abnormalities may be responsible for driving the transformation of ependymoma cells. Although Mendrzyk et al. (2006) have confirmed various prognostic factors, it is unclear if these also play a role in the development of ependymoma. This is very difficult to study because any human tumors that are resected are fully grown, thus making it impossible to tell which events occurred at each point in development. In order to further study the idea of chromosomal alterations, Taylor et al. (2005) combined all aCGH and gene expression arrays to correlate DNA copy number imbalance with gene expression. Only a very small number of transcripts within identified areas of DNA gain or loss exhibited significant copy number driven gene expression. Since this overlap is very specific, it is easier to identify the genes in the altered genomic regions that contribute to the transformation of RGCs and aid in the progression of ependymoma. It is important to acknowledge that the chromosomal alterations which result in aberrant gene expression contribute to the development of ependymoma; it is not simply the cell population of origin but also specific genetic events. This is a significant start on fully understanding ependymoma, its cellular origins, and the genetic factors or mutations that are also responsible for the progression of the disease. Several other researchers are working on specific pathways to develop different therapeutic agents for ependymoma and for brain tumors in general.

Stem Cells Create New Therapeutic Targets

As discussed in the conclusion of the research conducted by Taylor et al. there has been an increase in understanding of some of the pathways involved in the stem cell phenotype for ependymoma. The RGC population profiles are thought to continue to exist in ependymoma for a reason, not just as a leftover from the cell of origin. The Notch pathway and HOX family of transcription factors are thought to regulate stem cell self-renewal, especially since they are upregulated in supratentorial and spinal tumors, respectively (Taylor et al. 2005). When a Notch pathway malfunctions, it can result in a larger proportion of stem cells than normal and inhibits neurogenesis. This aberrant signaling tends to work on cells whose G0/G1 checkpoint is disrupted. Exposure to JAGGED1, another overexpressed gene in supratentorial tumors, can lead to a greater proportion of stem cells and a decreased number of differentiated cells (Taylor et al. 2005). When JAGGED1 and Notch bind together, γ-secretase cleaves the Notch receptor and activates the signaling of the aberrant pathway. Inhibitors of γ-secretase may be helpful in negating this pathway. The combination of these two upregulated genes could contribute significantly to the self-renewing property of stem cells in ependymoma: this could also create a potentially very effective therapeutic target.

Currently, therapeutic techniques work to target the entire tumor; surgery removes as much tumor as possible and chemotherapy and RT kill the remaining cells and prevent spread of any lingering tumor cells. Unfortunately, these are all incredibly invasive processes that are shown to cause damage. In the years following treatment, a direct correlation develops between IQ and the number of surgeries a patient had during treatment (Mulhern 1989, Merchant and Fouladi 2005). RT is also harmful: it is a clinical variable linked to a decrease in academic performance and increased depression in survivors. Although many ependymoma survivors fall within the normal range of the Child Behavior Checklist, an evaluative method that takes behavior (academically, socially, and problematic behavior) from the previous 6 months into account, many show problems in attention, memory, and learning (Mulhern 1989, Merchant and Fouladi 2005).

Because these therapeutic treatments can cause so many side effects, the development of a more effective, less invasive technique is one of the leading areas of research in terms of stem cells of brain tumors. This allows for a much more targeted area to treat when pathways are identified and understood. Also, prognostic factors are useful in determining the path of therapy that will be most effective. Mendrzyk et al. (2006) have developed prognostic factors that can be helpful in new therapeutic techniques.

This alternative approach of targeting the aberrant pathways (which play roles in development and progression of stem cell and ependymoma) is not currently understood well enough to introduce new therapies. Nonetheless, it is still an existing focus of research. The most important aspect of this approach is to develop a drug that will target the cancerous stem cells and leave the normal stem cells alone, especially in children. For pediatric patients, it is essential that normal stem cells are left unharmed so that they can play a large role in helping survivors recover without many life-altering side effects. One drug that is being considered is a γ-secretase inhibitor. Currently, trials for leukemia are being considered; this could also be applied to ependymoma and other cancers.

Tumors Can Be Dependent on the Notch Pathway

Research involving the notch pathway and γ-secretase inhibitors was conducted by Fan et al (2006). While their research involved pathways in medulloblastoma, several of the same pathways are also active in ependymoma as well; there is a definite molecular link between neural stem cells and these brain tumors. Fan et al. (2006) relied on CD133 markers as well as side populations to isolate stem cells from brain tumors; both of these markers were initially found in nonneoplastic stem cells, thus elucidating the similarities between normal and cancerous stem cells (2006). One particular pathway that could be the target of newly developed therapeutic agents is the Notch pathway; if a tumor is dependent on the Notch pathway it can promote the growth of stem cells and can also inhibit differentiation. The signaling pathway begins with a γ-secretase cleaving the receptor (Fan et al. 2006). It works on the nonneoplastic cells that make up the bulk of the tumor and on which brain tumor stem cells are dependent. Inhibition of this cleavage would slow the growth of a Notch dependent tumor. A small inhibitory molecule (GSI-18) was used in this study in order to further investigate the actual role of the Notch pathway in medulloblastoma (Fan et al. 2006). These results can also be applied to ependymoma

because the same pathway is used with ependymoma stem cells.

The activity of the Notch pathway was measured through Hes1, a target of the Notch pathway. Fan et al. (2006) found that after using 2 μmol/L of GSI-18 both the mRNA and protein levels of Hes1 are significantly decreased. When this amount of GSI-18 was applied to medulloblastoma cultures, the visible cellular mass was greatly reduced as was the individual cell size, but some tumor cells did survive and continued to proliferate (Fan et al. 2006). NICD2, a truncated Notch receptor that does not require ligand binding for activation that makes cells insensitive to the effects of GSI-18, was also used to help estimate effects on these medulloblastoma cultures. When applied to tumor cells, it aided in regenerating the cells and negated the effects of GSI-18. Flow cytometry was used to double check the data; both these measures found the same results: a reduction in the growth, but not a complete cessation. As Taylor et al. (2005) mentioned, the Notch pathway altered portions of the cell cycle: there was in increase in the G0/G1 fraction of cells after GSI-18 was applied to the cultures. Exiting the cell cycle may play a role in the anti-growth effects seen with GSI-18 (Fan et al. 2006).

There was also a noticeable change in the differentiation in both normal cells and in tumors with inhibition of the Notch pathway. It was confirmed that Notch inhibition led to an increase in RNA levels of particular markers of differentiation, Tuj1 and GABRA6 (Fan et al. 2006). GABRA6 is found in mature cerebellar granule cells. Its presence in brain tumor cells represents the similarities between normal and brain tumor stem cells and also suggests that the pathways remain very similar across any type of stem cell.

After GSI-18 was shown to slow tumor growth, the experiment needed to be conducted both *in vivo* and *in vitro*. Colonies were initially formed in soft agar; after treatment with GSI-18 the number of colonies dropped significantly. Constant Notch2 activation caused colony regrowth, supporting the theory that GSI-18 can suppress colony development (Fan et al. 2006). Xenograft experimentation was the next step in the process to determine the role of the Notch pathway in medulloblastoma. Cells were either put into a control group or were treated with NICD2. These cultures were treated with either GSI-18 or DMSO. These cells were counted and injected into mice. Xenografts formed at all 12 control injections; only one small lesion formed in the cells pre-treated with GSI-18 (Fan et al. 2006). While these cells were still alive, they were no longer tumorigenic. After cells were allowed to recover in a medium without γ-secretase inhibitors they were re-injected into the mice. Xenograft tumors still did not form with the GSI-18 treated cells, demonstrating that it GSI-18 is an effective tumor suppressant (Fan et al. 2006). The xenograft tumors that did form were treated with small injections of GSI-18 over five days. New cells failed to form in the group treated with GSI-18; the tumor continued to grow when the tumors were treated with a placebo (Fan et al. 2006). No behavioral or physical changes were noted in the mice, indicating that this treatment directly targeted the cancerous stem cells, not normal stem cells.

Experimentation has shown that only CD133+ cells are capable of multipotency and self renewal. In cells enriched with CD133+ there was a significantly higher expression of Hes1, a marker of the Notch pathway. This suggests that the Notch pathway is especially active in brain tumor stem cells, and also supports the idea that inhibition of this pathway may be a new therapeutic target for brain tumors and other cancers that utilize this pathway. When GSI-18 was applied to these CD133+ enriched cells, the size of the cell fraction significantly decreased, as expected (Fan et al. 2006). Also, it could be recovered by NICD2, which functions without receptors and cleavage. Thus, blockage of the Notch pathway can decrease the size of enriched subpopulations along with a decrease in actual tumor growth. This constitutes a significant step forward for the development of a drug like GSI-18 that can play a role in killing brain tumor stem cells.

Nestin, another marker of progenitor cells, may be more prone to apoptosis when the Notch pathway is blocked. Nestin is identified in neural stem cells and is also continually present in neurospheres formed from primary brain tumor stem cells (Fan et al. 2006). When GSI-18 was applied to a population of cells containing the Nestin marker, the population was decreased four-fold. It was rescued by NICD2, confirming that it is the blockage of the Notch pathway that causes the decrease in the population size. There was a difference in apoptosis rate for cells stained for Nestin (37% increase) versus the cells lacking Nestin (3.9% increase) with the application of GSI-18 (Fan et al. 2006). This indicates that more differentiated cells (where Nestin is not present) are less likely to undergo apoptosis when the Notch pathway is blocked.

In conclusion for this study, GSI-18 was found to have a slowing effect on tumor cells *in vitro* but there was a much larger effect on the xenografted tumors and the cell colonies in soft agar. There were no side effects on the mice when they were treated with GSI-18 and the tumors were also greatly reduced. γ-secretase may become a very important part of future therapeutic treatments for tumors that involve the Notch pathway, especially medulloblastoma and ependymoma. GSI-18 appears to eliminate the stem cells of the tumor. The lack of stem cells will cease tumor progression so that the remainder can be safely removed through surgery. However, it is important to note that blockage of the Notch pathway works only to eliminate stem cells, and will frequently leave most of the more differentiated cells within the tumor.

Fortunately, the Notch pathway is found in many cancers: GSI-18 could be used in medulloblastoma, ependymoma, breast, lung, and pancreatic cancer, and leukemia. This known pathway could help to reformat the use and structure of chemotherapy. It could directly target stem cells with GSI-18 to induce apoptosis as well as manage the better differentiated cells through another technique. The only concern is that normal stem cells would be depleted as well. However, that was not evident in the mice used in this experiment.

Conclusions and Future Directions

A fair amount of recent literature focuses on isolating the possible stem cells from different types of tumors. It is not likely that all brain tumors come from neural stem cells. In fact, plenty of tumors originate away from the ventricular surface and many arise from other progenitor cells or other types of differentiated glia (Nakano and Kornblum 2006). Some of the stem cells found in other types of brain tumors are similar to neural stem cells; the actual neuron-like and glia-like cells are quite similar from tumor to tumor, although the proportions are very different across ependymoma, medulloblastoma, astrocytoma, and glioblastoma multiforme (Nakano and Kornblum 2006). Once specific stem cells are isolated for tumor types, it is easier to analyze the genetic profile so that new therapeutic agents can be developed to specifically target these genetic and chromosomal alterations that may be the source of stem cells for tumors. One particular type of immunotherapy that has shown some promise in gliomas is the targeting of special antigens that are expressed by the cancer stem cells (Nakano and Kornblum 2006). The development of therapies for one type of brain tumor can ultimately be useful in other types of brain tumors and eventually be applied to other types of cancers.

With the discovery of stem cells as the source for several brain tumors and other types of cancers, the world of therapy for cancer has greatly expanded. Most current therapies do not distinguish between the stem cells and the cells that make up the mass of the tumor. Drug sensitivity could be an important factor for determining new treatments. Stem cells may inherently be more resistant to particular types of chemotherapy or drug therapies. Targeting specific pathways that are known to be involved in stem cell self-renewal and multipotency could make brain tumor treatment much more effective with fewer negative side effects.

The discovery that cancerous stem cells are responsible for the origins and maintenance of brain tumors provides a colossal advance in the understanding of these tumors. Classification systems can be based on genetic and chromosomal alterations instead of histology or clinical behavior, neither of which are incredibly accurate predictors of the outcome for the patient. Specific therapies can be developed to target known pathways used by the stem cells for self-renewal and differentiation. Investigation of these specific pathways can be done in one type of tumor and similar ideas or even the same pathways can be present in the development of therapeutic agents for another type of tumor. For example. the Notch pathway study was conducted using medulloblastoma stem cells but this pathway also exist in ependymoma. Inhibition of the Notch pathway has shown that a small inhibitor can be quite effective in slowing tumor growth and inducing apoptosis for the stem cells of medulloblastoma.

Not only is it important to understand the pathways that are used in the maintenance of tumors, but it is also important to determine how chromosomal abnormalities can affect gene and protein regulation, which can also play a role in the mutation of these pathways. The identification of specific prognostic factors, including the presence of an overexpression of EGFR and the gain of chromosomal region 1q25, aids in determining specific therapeutic techniques that can be used, assists in giving both the patient and parents a realistic idea of the risk factors associated with the tumor, and can also lead to a better method of classification that is not supported on histology alone. Taylor et al. (2005) have shown that these subtypes of ependymoma are molecularly distinct tumors and should be treated as such. Although the stem cells arise from transformed RGCs, there are specific genetic alterations that occur in each location to create the genetic profile specific to each tumor type. The combination of research on molecularly distinct subtypes, specific chromosomal alterations, and new pathways that could be targeted by drug therapies all lead to a much greater understanding of the disease itself so it will be easier to treat in the future.

It is also important to understand that the techniques used in Taylor et al. can be applied to other cancers to elucidate the origins and possible new therapies that can be used for several cancers. The advancement of the treatment of cancer is made possible through research concerning tissues or cell populations of origin, molecular distinctiveness, chromosomal abnormalities, pathways used by stem cells, and the collaboration of cancer, molecular, and developmental biologists so that all stages of the tumor can be understood for better treatment and higher survival rates for the patients.

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Spatial Heterogeneity of Zooplankton in the Water Column during Homogeneous Oxygen and Temperature Conditions

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Thermal stratification is a characteristic common to all lentic ecosystems. In lakes, periodic mixing occurs when cooler, denser water sinks and warmer, less dense water comes to the surface which causes not only a homogeneous temperature throughout the water, but uniformity in other abiotic factors as well. This cycle suggests that a lake that has mixed would be uniform in the distribution of nutrients and therefore support a uniform distribution of organisms like zooplankton. The data collected from Yellow Poplar Tree Lake in Tennessee on the temperature and oxygen concentration as well as plankton sampling performed over a 24-hour period allowed for the observation of the daily cycle of lake composition. After observing these trends, this study attempted to test the claim that homogeneous conditions due to lake mixing would support a homogeneous concentration of zooplankton. However, data analysis did not substantiate the claim that when abiotic factors are homogeneous throughout the lake there will also be a uniform distribution of zooplankton. This suggests further research is necessary for an understanding of the mechanism controlling zooplankton distribution in the water column.

Introduction

Lakes and other still-water ecosystems have well-defined physical characteristics which lead to fairly predictable behavior over time. Thermal stratification is common to all lakes in varying degrees (Smith and Smith 2006). For at least some part of the year, all lentic ecosystems have a vertical profile with a defined epilminion, thermocline, and hypolimnion. The epilimnion consists of warm, low-density surface waters, and the hypolimnion encompasses cold, high-density, deeper waters. The thermocline is the zone between these two layers where a rapid temperature change occurs. Lakes go through periodic mixing when surface waters cool, become more dense than the water beneath them, and subsequently sink (Smith and Smith 2006). Mixing of the entire lake basin to a uniform temperature occurs during spring and fall turnover but circulation of the epilminion and part of the thermocline can occur throughout the year (Smith and Smith 2006). The effects of wind and convection currents contribute to this mixing which results in a uniform temperature in this thicker epilimnion (Wetzel 2001).

Mixing not only affects the temperature of a lake but other conditions as well. Homogeneous temperature conditions correlate with uniformity in the oxygen distribution of the upper layers of a lake after mixing occurs (Cole 1994). The stratification of various conditions, such as temperature or oxygen concentration, dictates the types of organisms that different strata of the lake will support. The location of organisms throughout the day or throughout a whole season depends on the abiotic and biotic factors at various depths.

Zooplankton are a vital part of most lake ecosystems and exist at particular locations due to specific environmental requirements. Zooplankton consist of members from classes of Crustacea, Cladocera, Copepoda, and rotifers (Wetzel 2001). The zooplankton studied here are *Chaoborus*, Cladocerans, Copepod nauplii, and Copepods. Most types of zooplankton have a limited ability to propel themselves through the water and instead must rely on mixing or turbulence for dispersal purposes (Wetzel 2001)**.** This mechanical force of the water along with whatever swimming capability a zooplankter might possess are utilized to combat sinking due to gravity (Clarke 1934).

The location of zooplankton reflects a balance between depths that provide the most nutrients for growth and reproduction and depths that offer protection from predation. Many previous studies have focused on zooplankton diurnal vertical migration as a type of predator avoidance (Loose and Dawidowicz 1994). However, few if any studies explore the passive movement of plankton due to water mixing or turbulence throughout homogeneous conditions. In addition, few studies have examined epilimnion and thermocline mixing not related to fall turnover. This study attempts to test the claim that homogeneous conditions, due to mixing, of the temperature and oxygen concentrations of the upper depths of lakes will result in homogeneous zooplankton concentrations at those levels.

Materials and Methods

We studied the various abiotic and biotic factors of Yellow Poplar Tree Lake in Tennessee in order to further our understanding of the interactions between organisms and their environments. To maintain consistency over the 24-hour period under observation, sampling was conducted at one location of the lake, marked by a buoy. We utilized a HydroLab Data Sonde to determine oxygen concentration and temperature at 0.5- meter intervals from the surface of the lake to a depth of seven meters. Data was collected every hour from 14:00 on Friday, September 29, 2006 until 13:00 Saturday, September 30, 2006. Every two hours over the same period of time, we obtained plankton samples using a Schindler trap at 1.0-meter intervals from the surface of the

water to a depth of seven meters. We kept these samples in Lugol's solution to preserve them for later examination when we counted the number of *Chaoborus*, Cladocerans, Copepod nauplii, and Copepods by utilizing microscopes.

Data indicates that the temperature of the surface water from the time period 24:00 to about 10:00 was much lower than temperature of the water at greater depths suggesting the surface water had become more dense and mixing had occurred. This claim is further supported by the uniformity in temperature for the first three meters of the lake over the same time period where mixing was suspected. After observing this trend in the oxygen concentration data as well as the temperature data from 24:00 to 10:00 until a depth of three meters (Figure 1 and Figure 2) I independently sought to determine homogeneity through a T-test. I first calculated the coefficient of variation (later referred to as cv) for the oxygen concentration at each time for the depths up to three meters. This coefficient allows for the comparison of the variation of data that have significantly different mean values. I then separated these coefficients into two categories; cv homogeneous and cv heterogeneous, which are the time periods where I suspected homogeneity (24:00-10:00) and heterogeneity (14:00-24:00, 10:00-13:00) for the data. These time periods were chosen after observing similarities in oxygen concentration and temperature over the period from 24:00-10:00 as compared to the distinct variation seen from 14:00-24:00 and 10:00-13:00. The homogeneous period is also the suspected time period where mixing would occur. As seen in Figure 2 the surface water during this time period was cooler and thus denser than the waters below leading to the sinking of this surface water and subsequently, the formation of a homogeneous epilimnion.

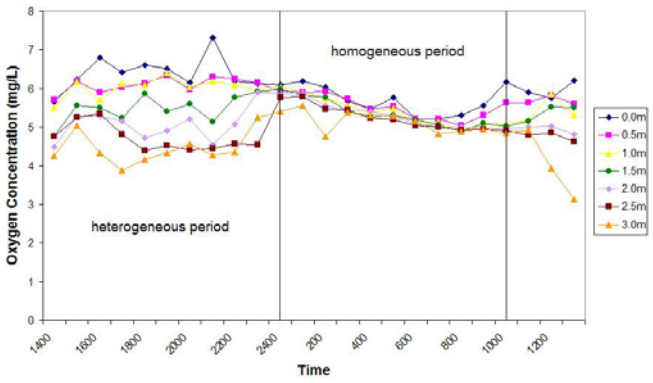

Figure 1: Oxygen Concentration over time of Yellow Poplar Tree Lake

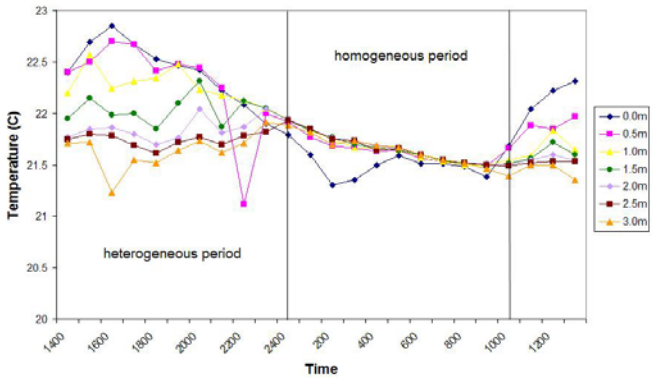

Figure 2: Temperature over time of Yellow Poplar Tree Lake

To substantiate this premise of lake mixing and homogeneous conditions I took the mean of each of these categories (cv homogeneous and cv heterogeneous) and then performed a T-test assuming unequal variances. The T-test allows for the evaluation of the means of these different groups to determine if they are statistically different from each other. I then repeated this procedure for the temperature data. The method of performing statistical analysis for the plankton data required some revising due to the fact that we collected samples every two hours at 1.0-meter intervals in contrast to oxygen concentration and temperature data taken every hour at 0.5-meter intervals. The category of cv homogeneous for plankton is the time period from 24:00-10:00 while cv heterogeneous is from 14:00-24:00 and 10:00-12:00 for the upper three meters of the lake. Once again, I calculated the coefficient of variation at each time and then found the mean of the homogeneous and heterogeneous categories. I then performed a T-test assuming unequal variances for this data to determine whether the means I was examining were significantly different.

Results

Graphs compiled of the coefficient of variation for oxygen concentration and temperature suggested a statistical difference between these factors over the two time categories (Figure 3 and Figure 4) but a T-test was required to confirm the significance of the variation.

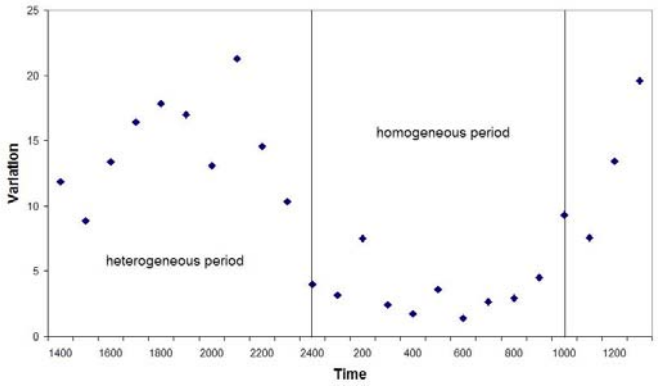

Figure 3: Coefficient of variation values for oxygen concentration over time

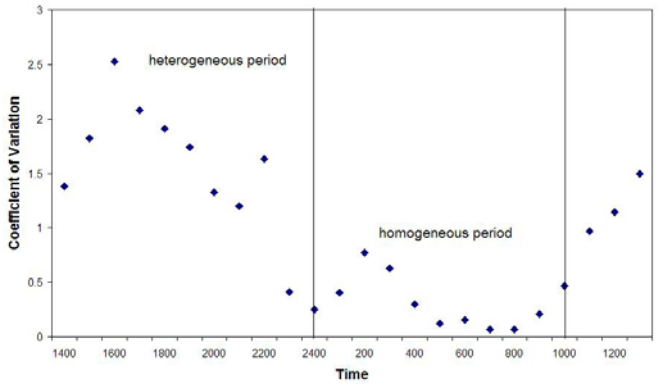

Figure 4: Coefficient of variation values for temperature over time

The T-tests performed for oxygen concentration and temperature demonstrated a significant difference in the mean coefficient of variation from the different time categories I examined. For oxygen concentration the means were found to be significantly different (t= -5.35; df=19; p< 0.01) as well as the means of the temperature data (t= -7.27; df= 17; p< 0.01). These T-tests suggest the homogeneity of the upper levels of the lake that was the premise for the examination of homogeneity in the plankton distribution.

Graphs compiled for the coefficients of varaiation for *Chaoborus*, Cladocerans, Copepod nauplii, and Copepods (Figures 5, Figure 6, Figure 7, and Figure 8) show more variation than those graphs complied for oxygen and temperature. T-tests further demonstrated that the mean coefficients of variation for the two time categories are not significantly different for any type of zooplankton. The results of the T-tests were *Chaoborus* (t=2.07; df=8; p=0.07); Cladocerans (t=2.75; df=10; p=0.02); Copepod nauplii (t=1.89; df=9; p=0.09); and Copepods (t=0.00; df=7; p=1.00).

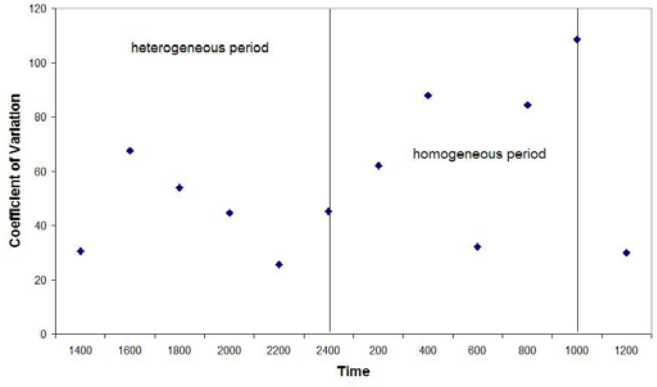

Figure 5: Coefficient of variation values for *Chaoborus* over time

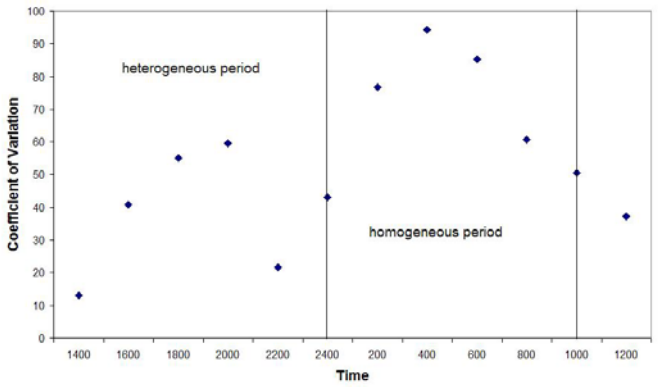

Figure 6: Coefficient of variation for Cladocerans over time

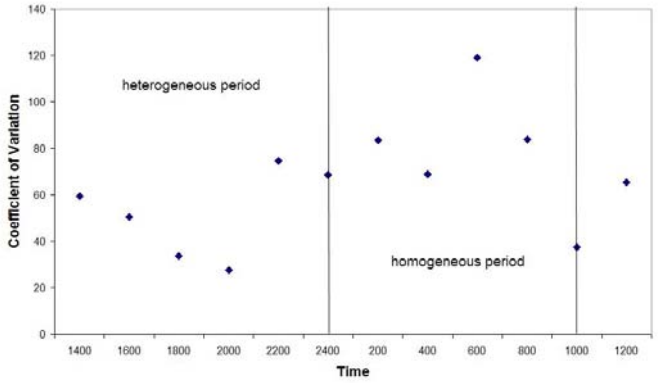

Figure 7: Coefficient of variation for Copepod nauplii over time

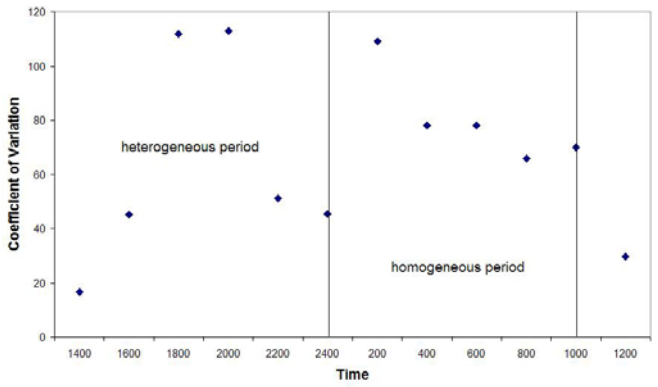

Figure 8: Coefficient of variation for Copepods over time

Discussion

The hypothesis that homogeneous oxygen concentration and temperature in the first few meters of a lake would correspond with a homogeneous distribution of zooplankton in the water column was unsubstantiated by T-tests. The limited research on zooplankton movement during lake mixing makes it difficult to demonstrate whether these results are real or the result of sampling errors. While studies on phytoplankton have found that phytoplankton are homogeneous in the water column during mixing, less is known about zooplankton (Marshall 1966). Zooplankton are often observed to travel passively through the water column most of the time but this study suggests that perhaps zooplankton use their swimming abilities more than is often thought.

This study is also very limited in its scope as it ignores numerous variables to test a specific hypothesis. Further study to investigate the role external influences play in the movement of zooplankton could ultimately lead to a better understanding of these organisms' movement and general behavior. Abiotic factors such as salinity, pH, and various nutrients such as phosphorous or nitrogen could be providing a directional effect for zooplankton movement which was suggested by G. Clarke (1934). Migratory patterns such as diurnal migration for the acquiring of food or protection from predation might also influence zooplankton movement and facilitate the need for swimming abilities. It is thought that "the presence of other organisms may influence the distribution of copepods" and presumably other zooplankton. These other organisms may take the form of potential mates or potential predators (Clarke 1934). While little is known about the movement of zooplankton through the water column, especially during lake mixing, further studies could eventually determine factors that influence zooplankton behavior. Understanding these minuscule yet vital organisms could be the first step in a greater understanding of aquatic ecosystems.

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Generation of a recombinant murine herpesvirus containing the Epstein-Barr virus interleukin-10 gene driven by the mouse phosphoglycerate kinase (pgk) promoter

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The human Esptein-Barr virus (EBV), an incredibly pervasive human pathogen, is the etiological agent of infectious mononucleosis and is associated with several forms of cancer. The murine gammaherpesvirus (MHV) may provide a viable animal model of EBV infection. Viral interleukin-10 (vIL-10) is a gene unique to EBV, and it is very similar to the host IL-10 gene, which works to suppress cytokine synthesis of T_H1 helper T cells as part of the host's immune response. It is hypothesized that the introduction of the EBV vIL-10 gene to MHV-76, a MHV variant that has lost a subset of its immune evasion genes and has minimal pathogenic effects, will restore the more extensive pathogenic effects of the wild-type virus. The specific aim of this experiment is to generate a recombinant MHV-76 virus using plasmid constructs containing the pgk vIL-10L version of the vIL-10 gene. The products of the co-transfections were screened using a series of limiting dilutions and PCR analysis. The purified viral stock will require further genetic analysis to test the vIL-10 recombinant virus' ability to infect in vivo and to characterize its pathogenic properties.

Introduction

Herpesviruses represent an extensive group of viruses that contain large genomes of double-stranded DNA, which allow these viruses to carry both essential and non-essential genes. While the essential genes play a role in infection and replication, non-essential genes allow the herpesviruses to regulate and escape the immune response of the host. Because herpesviruses infect a host and then lie latent, they are often responsible for making their host cell malignant (Keiff 1996). For example, the human Epstein-Barr virus (EBV), which is commonly transmitted through saliva, causes infectious mononucleosis (CDC 2005). However, after the infection clears, the virus will remain latent in the host. This latent EBV has been implicated as the source of several types of cancer including Burkitt lymphoma, nasopharyngeal carcinoma, and Hodgkin's disease (Keiff 1996). Because of this, the mechanisms of EBV need to be better understood.

EBV expresses a unique viral interleukin-10 (vIL-10) gene. The vIL-10 gene is incredibly similar to the human IL-10 gene, which plays an important role in regulating the immune response of the host (Moore et al. 2001) by suppressing cytokine synthesis by T_H1 helper T cells. The fact that EBV contains vIL-10 suggests that EBV may have obtained this mammalian gene as a survival advantage (Howard and O'Garra 1992). Currently, a small animal model of the EBV infection is needed to characterize the function of vIL-10 and understand its role in disease.

The murine gammaherpesvirus (MHV) is genetically and pathogenically similar to EBV, and therefore may be used as a small animal model of EBV infection (Virgin et al. 1997). Specifically, MHV-68 has sequence homology with EBV, although it does not contain the vIL-10 gene (Simas et al. 1998). The genome of MHV-76, a naturally occurring variant of MHV, is essentially identical to the genome of MHV-68, except for the deletion of a 9,538 bp region of the genome that encodes a number of immune evasion genes (Figure 1). Consequently, MHV-76's pathogenic effects are markedly reduced compared to MHV-68 (Macrae et al. 2001). It is hypothesized that the introduction of the EBV vIL-10 gene into the MHV-76 genome using a plasmid construct containing the vIL-10 gene driven by a mouse gene promoter will restore the pathogenic effects of the virus, thereby supporting the importance of the vIL-10 in EBV-associated disease. Ideally, the generation of a recombinant MHV-76 virus containing the vIL-10 gene will provide a method to study the *in vivo* effects of vIL-10 on viral replication, latency, and pathogenesis in mice.

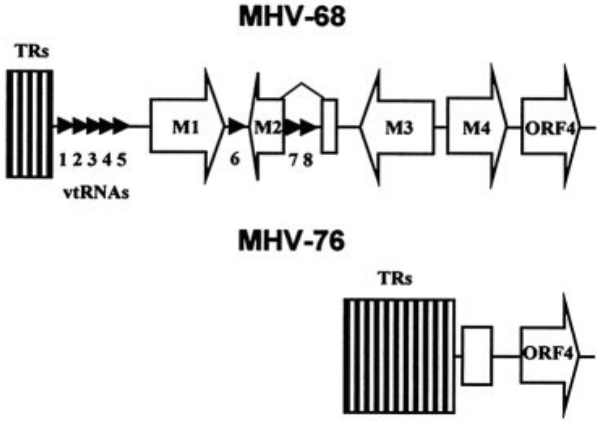

Figure 1: Genetic map of the left hand end of the MHV-68 and MHV-76 genomes. MHV-76 is a naturally occurring variant of MHV-68. Their genomes are essentially identical, with the exception of a 9,538 bp at the left end of the unique region of the MHV-68 genome which is deleted in MHV-76, resulting in its reduced pathogenicity (Macrae 2001).

Materials and Methods

Cells, Viruses, and Plasmids Used

NIH-3T3 cells are a rapidly-growing mouse fibroblast cell line that are permissive for MHV replication. NIH-3T3 cells, grown in DMEM supplemented with 10% fetal bovine serum and penicillin/streptomycin, were used to generate viral stocks and recombinant viruses through co-transfection.

MHV-76 is a type of gammaherpesvirus and a naturally occurring variant of MHV-68. However, it lacks the pathogenicity of MHV-68 and EBV because its genome does not contain the vIL-10 gene.

The plasmid was constructed previously. The plasmid (Figure 2) contains the EBV vIL-10 gene and the phosphoglycerate kinase (pgk) promoter. The MHV sequences on either side of the insert allow for homologous recombination upon co-transfection with viral DNA because they are homologous to either side of the location where the insert should be located within the MHV genome. Following transfection and recombination, the vIL-10 gene is incorporated into the genome in the left orientation relative to the prototype genome, such that transcription of the MHV genome will occur from right to left.

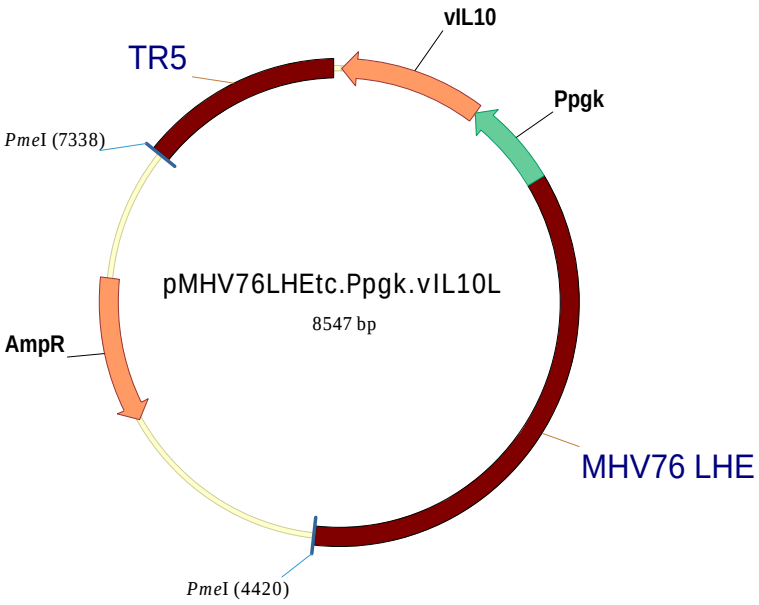

Figure 2: Map of the 'targeting construct' plasmid. The DNA insert is comprised of the EBV vIL-10 gene and its promoter, phosphoglycerate kinase (Ppgk), as well as the left-hand end of the MHV-76 genome (MHV76 LHE) and the terminal repeats (TR5). The enzyme PmeI is responsible for releasing the insert from the plasmid so that co-transfection with viral DNA can occur.

Co-transfection of NIH-3T3 Cells

Recombinant viruses were generated through co-transfection of NIH-3T3 cells with viral and plasmid DNA. First, the restriction enzyme PmeI was used to release the insert containing the vIL-10 gene, terminal repeats, and the left-hand end (LHE) of the MHV-76 genome from the plasmid DNA (Figure 3). DNA was quantified by spectroscopy. For each transfection, a mixture of DNA and FuGene (Roche) reagent was combined according to the manufacturer's protocol and added dropwise onto the media surface of a well of a previously seeded 6-well plate, where each well contained 3×10^5 cells in 5 mL of DMEM. The plates were incubated for 5-6 days at 37 ºC and 5% CO_2 until the plaques were well-developed.

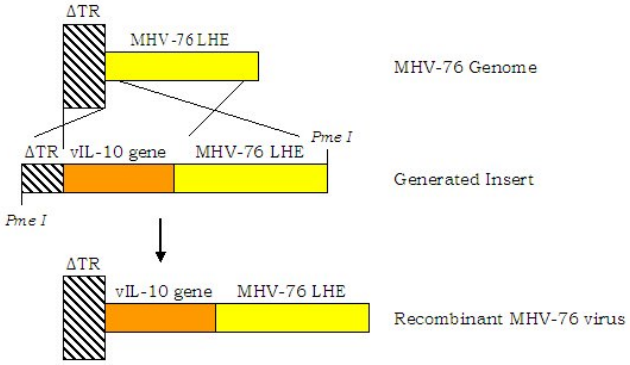

Figure 3: Generation of a recombinant MHV-76 virus. To facilitate homologous recombination, the PmeI enzyme was used to release the insert containing the vIL-10 gene bounded by the terminal repeats (TR), and an adjacent 3 kilobase sequence from the left-hand end (LHE) of the MHV-76 genome. The pgk vIL-10L insert was thus incorporated into the MHV-76 genome in the left orientation.

Next, the plaques, which represent the point of cell death due to viral replication, were harvested and frozen at -80 ºC until they were needed for limiting dilution analysis. Four different co-transfections were performed: (1) a mock transfection, which contained no MHV-76 DNA, (2) a control transfection, which contained only MHV-76 DNA, (3) a transfection which contained 1 µg MHV-76 DNA and 2 µg of insert DNA, and (4) a transfection which contained 1 µg MHV-76 DNA and 3 µg of insert DNA.

Limiting Dilutions in 96-well Plates

To lyse the cells and release the recombinant virus, the harvested cells from either the co-transfections or the previous limiting dilution analysis were frozen in liquid nitrogen and thawed in a 37 ºC water bath three times. 1-5 µL of the co-transfection stock were added to the first column of wells in a previously seeded 96-well plate, where each well contained 1×10^4 cells in 200 µL of DMEM. Next, by transferring 100 µL from one column to the next and repeating this process, a 2-fold serial dilution was performed across the wells of the plate.

The 96-well plates were incubated for 4-6 days at 37 ºC and 5% CO_2 until plaques were well-developed. The wells that contained either 1 or 2 well-defined plaques were harvested and stored at -80 ºC.

PCR Analysis

Samples from the limiting dilutions were frozen in liquid nitrogen and thawed in a water bath three times in a row to lyse the cells and release the viral DNA. 100 µL from each sample was diluted with 100 µL of PBS and purified using the QIAamp Blood Mini DNA kit (Qiagen) according to the manufacturer's protocol.

To assay the presence of a recombinant virus, PCR was used to amplify the vIL-10 gene using primers that were specific to the insert. The PCR samples were assayed on a 1% agarose gel. Samples with a band at the predicted 563 bp were declared positive for the recombinant virus. Stocks from which positive results were obtained were removed from the -80 ºC freezer and used to infect another set of previously seeded 96-well plates for the next round of limiting dilutions. Dilution analysis was continued until two successive rounds gave all positive results from wells contained single viral plaques. A summary of the procedure for this experiment is illustrated in Figure 4.

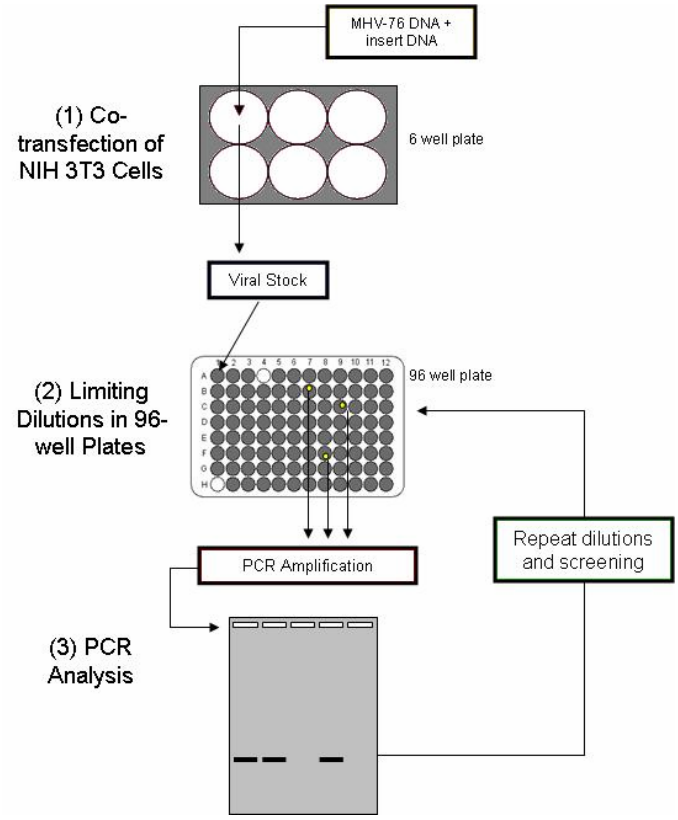

Figure 4: General procedure for the generation of a viral stock containing the MHV-76 recombinant virus: (1) Co-transfections were performed and NIH-3T3 cells were infected in 6-well plates. Viral plaques were harvested after 5-6 days of incubation; (2) Each well of column 1 in the 96-well plates containing NIH-3T3 cells were infected with 1-5 µl of the co-transfection stock, and a 2x dilution was performed by transferring 100 µl from the column 1 to column 2 wells and repeating this process across the entire plate. After 4-5 days of incubation, viral plaques were harvested; (3) PCR was used to amplify the vIL-10 gene using primers that were specific to the insert. The PCR samples were assayed on a 1% agarose gel. (4) 96-well plates were infected again until two successive rounds of limiting dilutions gave all positive results from wells containing single viral plaques.

Results and Discussion

The specific aim of this project was to generate a recombinant MHV-76 virus using plasmid constructs containing the pgk vIL-10L version of the vIL-10 gene. Overall, five rounds of screening were performed, with the final two rounds providing all positive results from wells that contained single viral plaques. In the first round of screening, 3 out of 10 plaques were positive for the recombinant virus. In the second round of screening, 1 out of 7 plaques was positive for the recombinant virus. In the third round of screening, 5 out of 6 plaques were positive for the recombinant virus. In the fourth and final rounds of screening, 7 out of 7 and 6 out of 6 plaques were positive for the recombinant virus, respectively. The PCR samples from the final screening on a 1% agarose gel are shown in Figure 5. In summary, of the 36 PCR reactions that were performed, 22 turned out to be positive (Table 1).

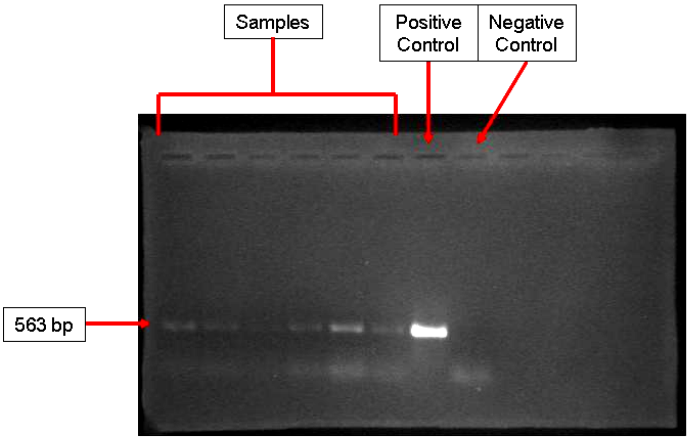

Figure 5: PCR samples from the fifth and final round of limiting dilutions on a 1% agarose gel. The positive control used was the plasmid construct shown in Figure 2, and the negative control was water. The vIL-10 gene is expected to yield a 563 bp fragment.

Screen	PCR Results									
1	-	+	-	-	-	+	+	-	-	-
2	-	-	-	-	-	+	-			
3	+	+	-	-	+	+				
4	+	+	+	+	+	+	+			
5	+	+	+	+	+	+				

Table 1: Summary of the PCR results from five rounds of screening. Green represents samples that were positive for the recombinant virus, while red represents samples that were negative for the recombinant virus.

By screening the products of the co-transfections using five rounds of limiting dilutions and PCR analysis, a purified viral stock was created. The purified viral stock will require further genetic analysis to test the vIL-10 recombinant virus' ability to infect *in vivo* and to characterize its pathogenic properties.

Future Steps

To confirm the insertion of the vIL-10 gene into the desired location, restriction fragment analysis, Southern blotting, and sequence analysis will be performed. Next, tests of the *in vitro* growth properties of each virus will be performed to ensure that the titer of the recombinant virus is comparable to that of the wild-type MHV-76 and MHV-68. An ELISA assay will be performed on the viral cultures to confirm that the virus is expressing vIL-10. Finally, when the screening of the recombinant virus is complete, the virus can be used for mouse studies.

Mice used in the future mouse studies will be infected with three different variations of the MHV virus. The groups will be infected with either (1) the recombinant virus, (2) the unaltered, normal MHV-68 as a positive control for spleen enlargement, or (3) the variant MHV-76 as a negative control for spleen enlargement. Several measures of the infection will be recorded, including the growth rate of the virus, the time course of the disease, the establishment of latency, and the final outcome of the infection (tumors, survival, death, etc.). Finally, by comparing the results between mice infected with recombinant MHV virus to those infected with the non-recombinant MHV, conclusions concerning the role the EBV vIL-10 gene plays in infection and immunity may be made.

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Tree species selectivity of four mid-south birds and implications for ecological restorations and invasive plant control

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The loss and fragmentation of forests across America have contributed to a decline in species richness and abundance in many communities. Understanding the relationship between forest composition and species diversity will provide helpful insight to future conservation and reforestation efforts. Through preferential selectivity in foraging, many avian species demonstrate significant influence on vegetation structure. Particularly, bird species may influence the distribution of common invasive plants. Observed foraging behaviors within an urban park old growth forest reveals patterns of selectivity for four mid-south birds in accordance with known diet categories. The foraging patterns of the Northern Cardinal (Cardinalis cardinalis), Ruby-crowned Kinglet (Regulus calendula), White-throated Sparrow (Zonotrichia albicollis), and Yellow-rumped Warbler (Dendroica coronata) may suggest roles in preferentially selecting particular vegetative substrates. Further investigation, with larger sample sizes, will offer key insights to the relationships between forest and avian structures, which will lead to more effective planning for conservation.

Introduction

In the last century the loss or fragmentation of our nation's forests has increased dramatically. These reductions have been driven by several anthropogenic sources including urbanization, industrial development, expanding grazing land, and agriculture. Fragmentation and forest losses are responsible for the conversion of vegetation structure and the alterations of key floristic communities that cause major impediments to the diversity of wildlife that are supported by these habitats (Knutson et al. 2005; Pimm et al. 1995; and Scott et al. 2003). For North American avian communities, habitat loss has been a chief contributor to declines in species richness and abundance (Knutson, et al. 1997). The ecological consequences of forest loss and these wildlife risks are of concern to conservationists and wildlife managers. It is absolutely paramount to research the best practices for reducing or reversing forest loss.

Scientists have predicted that the fastest reforestation methods that would result in the growth of a mature forest with native tree species diversity and structural diversity are the best for meeting the needs of bird species of management concern (Knutson et al. 2005). However, it is important for studies like this to consider not only the speed of such reforestation but also the quality of habitat for the supported biota. Gabbe et al. (2002) suggest that the attempts being made to restore forests and ecosystems do not incorporate the strong correlations between floristic composition and avian community structure. In their study they found that the structures of both tree and avian communities interrelate. Askins (2002) claims "conservation of terrestrial birds depends on a clear understanding of their habitat requirements and the physical and biotic processes that create and maintain those habitats" (Askins 2000 quoted in Scott et al. 2003).

Birds are key players in ecosystems, as Gabbe et al. explain because of their abilities to preferentially select for successional patterns by their foraging behaviors. Conversely, tree-species composition can lead to changes in avian populations by variables, such as foliage, seed type, and distribution. Thus there is a dynamic relationship between vegetative strata and avian community structure. When current restoration projects neglect these connections between avian and floristic composition the reforestation of habitats is driven by natural succession alone. By these mechanisms many of the early colonizers fill niches of the more highly preferred tree species of the avian community and thus to some extent restore a habitat having low tree-species diversity, which does not support a diversity of birds or other biota. In order to achieve optimal restoration success, the tree species that offer the most highly preferred food resources for birds (based on their foraging behaviors) should be planted (Gabbe et al. 2002).

In order to determine tree species preference it is necessary to study avian foraging behaviors. For our research we observed the foraging behaviors of bird species in an old growth forest remnant within an urban park. Data on four of these species, Northern Cardinal (*Cardinalis cardinalis*), Ruby-crowned Kinglet (*Regulus calendula*), White-throated Sparrow (*Zonotrichia albicollis*), and Yellow-rumped Warbler (*Dendroica coronata*), were collected from August to November 2006 in Overton Park, Memphis, Tennessee, and are presented in this paper. Of primary interest was the role of birds in the distribution and abundance of a particular invasive plant, privet (*Ligustrum* spp.). By determining the tree species preferences of these four avian species we aimed to discover whether there is a preferential selection for privet, which may correlated with the spread of this invasive species.

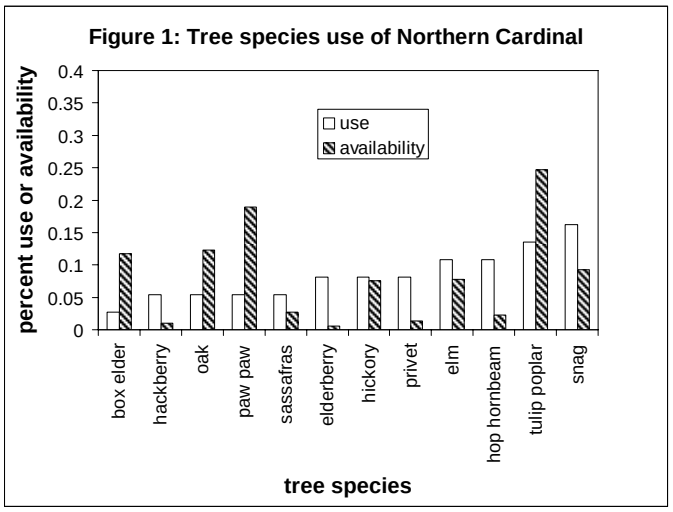
Materials and Methods

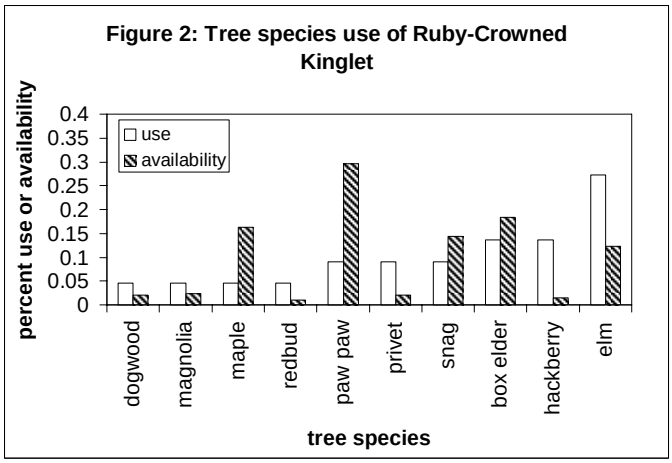
A forest composition index for the 342-acre park was generated through sampling the forest using the Point Centered Quarter Method at 88 sites. This method is a measurement of tree species density. Additionally, tree diameter was measured and later calculated into basal area (BA). Sampling was conducted along parallel transects running north to south through the park at 200 meter intervals. Use of GPS defined the sampling transects and monitored our sampling positions along transects. Data were transcribed into an Excel spreadsheet for analysis, which involved the calculation of important values (IVs) for each tree species. IV calculations were modeled after those described by Gabbe et al. (2002).

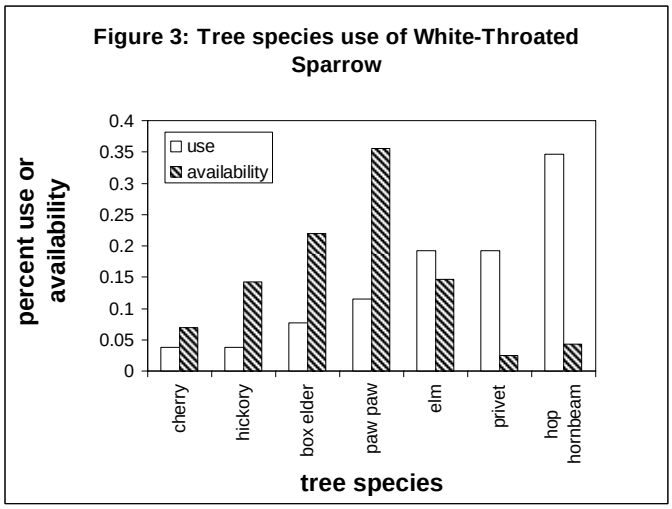
Field observations were made in Overton Park along paved pedestrian roads and single-track trails. In the field, upon sighting a foraging bird the observer made the following recordings: the time and date of observation; bird species and gender; tree species on which foraging occurred; fruit on tree (yes or no); whether bird takes fruit (yes or no); foliage density (percentage of coverage within 1 meter radius of bird); height of bird (in meters); canopy height (in meters); and various other optional notes such as attack method, substrate, and prey type. All data were recorded in the field by hand and later transcribed into an Excel spreadsheet for analysis. Data analyses followed the methods used by Gabbe et al. (2002) and produced quantifiable support for the selectivity of birds foraging on different tree species. Selectivity was determined by whether birds foraged on trees in proportion their availabilities. These calculations compared the observed frequencies of bird foraging to the expected frequencies, which were functions of the IVs of the trees (Gabbe et al. 2002). Bird species that were used in these foraging selectivity analyses were restricted to just eight species having abundances > 15. Foraging selectivity patterns for four of these species (identified previously) are discussed below.

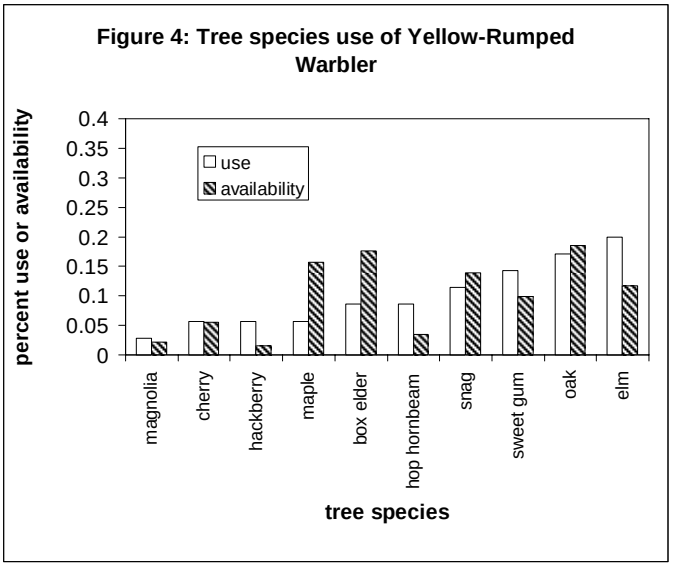
Results

We found several trends in tree species use by four bird species, the Northern Cardinal, the Ruby-crowned Kinglet, the White-throated Sparrow, and the Yellow-rumped Warbler. Figures 1-4 depict the use of tree species by these four foraging birds within Overton Park. Excluding snags, Northern Cardinals were observed to most readily forage upon tulip poplar. This high use pattern is consistent with the high availability of these trees. Tulip poplars actually had the highest importance values of all of the trees identified in our forest composition index. As the figures display, cardinals have the greatest distribution of tree species use and because of this the values for use are lower than what is seen for other birds.



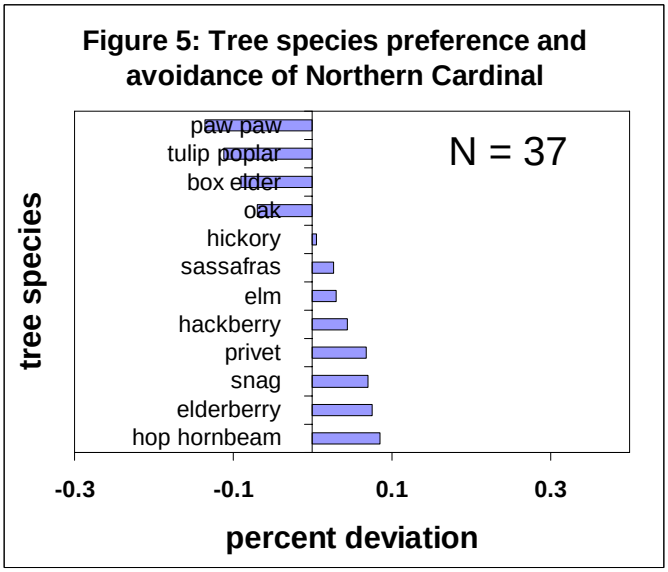


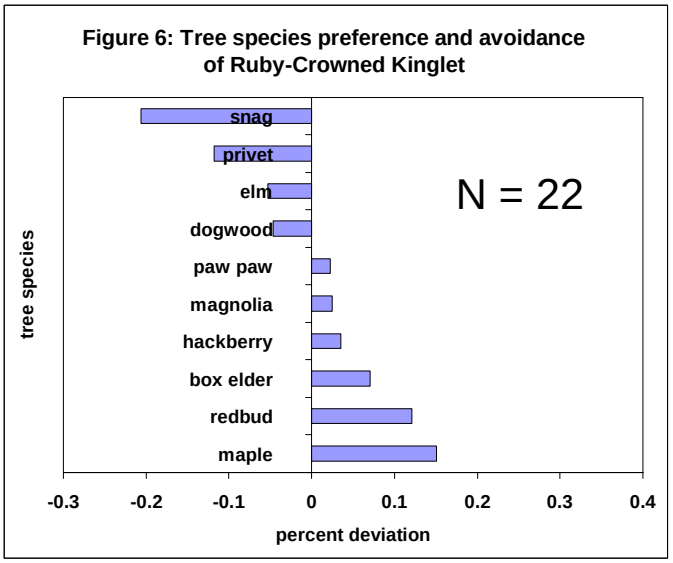




Our results showed that Ruby-crowned kinglets use elms more than any other tree species and dogwoods the least. These data are not necessarily what would be predicted because the use of elms and dogwoods by this bird species are both higher than actual availability of these two tree species. For the White-throated Sparrow, its usage was highest for hop hornbeam. This tree species had very low availability values but surprisingly was highly used by the cardinals as well as moderately used by Yellow-rumped Warblers. Elms and oaks were used most by the warblers.

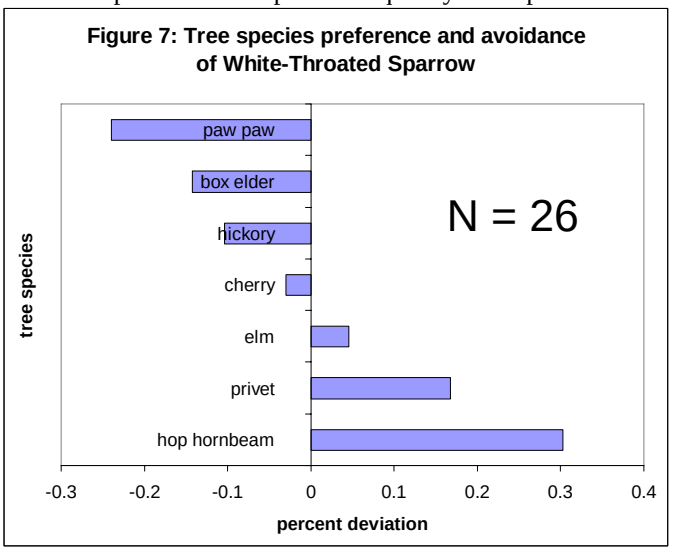
Differences between use and availability of tree species by all four birds was quantified as percent deviation. These variances, shown for all birds in figures 5-8, indicate whether usage was more or less than expected and can determine preference or avoidance. For all four species of birds, elms were in the top four most frequently used trees. Additionally, each bird species, except for the warblers, depicted the greatest avoidance of paw-paw. One last trend that is shown by these data is that for all birds, excluding the warblers, privet was within the top four most frequently used trees.

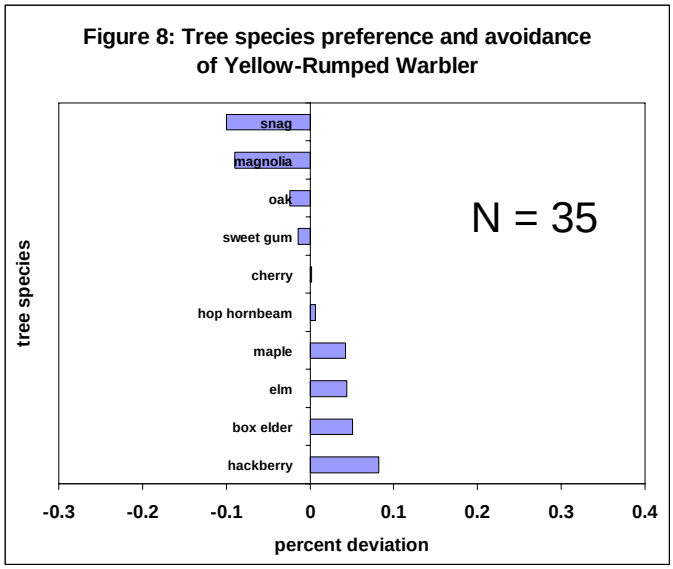




Discussion

The data show the selectivity of particular trees for four species of birds. These patterns do not necessarily correspond to the level of use birds had for each tree species. For instance, tulip poplars were the second most frequently used trees by cardinals, however as figure 5 displays, cardinals demonstrated a clear pattern of avoidance for thesse trees. This discrepancy can be accounted for with the understanding that avoidance and preference are indicators of the deviation (either positive or negative) of the observed frequency from the expected frequency of foraging for each bird species on each tree species. The expected frequency is the predicted





amount of use of a given tree species by a particular bird species based on that trees importance value (IV). In this case, since tulip poplars had such a high IV the expected frequency of their use by birds was also high. Thus, for cardinals, which typically forage on many different trees, there was a large negative deviation from the expected frequency, which resulted in a pattern of avoidance.

The tree species that were preferred by cardinals (those that had positive deviations of observed from expected frequencies) were hop hornbeam and elderberry both of which bear favorable seeds or fruit that are conducive to the cardinal's typical diet. This reasoning also explains the high preference for hop hornbeam trees by the sparrows. Avoidance of paw-paw may be because groves are most densely concentrated in forest interiors and subsist amongst the under story. Foraging observations were primarily made along forest edge and patch habitats. Thus, foraging observations were restricted to tree species within these sections. Greater sampling of forest interiors would reveal a better a understanding of bird preference or avoidance of paw-paw and other under story trees. Furthermore, importance values of trees taken from the forest composition index could relate more closely to foraging observations by applying the methods for sampling tree composition along the paths and paved roads used for making observations. Although parallel transects through the park provide accurate tree-species composition for the whole park it may be advantageous to know the tree-species composition of just the areas where observations are made.

Since three of the four bird species showed high use values for privet (in top 4 of all trees for all species except the Yellow-rumped Warbler) this might suggest preferential selection and indicate the special roles birds play in invasive plant distribution (Reichard et al. 2001). In every case, excluding the warblers, the use of privet was higher than expected, based on its availability. Therefore, birds showed a preference for this plant. Privet does produce small berries that were observed being picked off by these birds on several occasions. So, even as non-native inhabitants of Overton Park, these plants do provide optimal food resources that are necessary within the dietary habits of these birds. The Yellow-rumped Warbler may not have showed preference for privet because of its greater preference for insects. Many invasive plants do not attract as many insects as native plants and thus are generally more uninhibited by their destructive effects (Reichard et al. 2001). Warblers may not use the privet within Overton Park because they fail to produce the food resources that warblers most readily consume. Nonetheless, the primary frugivores displayed preference for privet berries. The fruit on these plants also tend to ripen later than other fleshy-fruited plants and thereby remain an optimal partner for avian species, particularly toward the end of Fall and the beginning of Winter (White et al. 1991). Future studies on the roles birds play in privet seed dispersal should involve the collection of fecal samples and the germination of seeds to test viability after gut passage. Studies like this will provide helpful understanding for the controlling of invasive plants. Additionally, by combining our results with the results of other similar studies on the selectivity of birds for tree species, we can confirm the dynamic connections between vegetative and avian structures. A thorough understanding of these connections will promote strategic reforestation yielding successful restoration and conservation of habitats particularly within urban parks.

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