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On the Cover: Alias Smith, Senior in Biochemistry, with the model created in his research project on the cyclosporiasis recovery process.
My congratulations go out to all of the MU undergraduates who have participated in the McNair Scholars Program. The papers presented here represent the culmination of more than a year's worth of hard work. They reflect the creativity and energy of the scholars, themselves, but also the careful attention and diligence of their mentors. Although the full papers of only seven scholars are presented here, the work of the other McNair scholars listed in the journal is also to be commended. This is an interdisciplinary journal; therefore, each article reflects the style of the discipline represented.

A University-wide effort, the McNair program attracts students and faculty mentors from a wide range of departments and fields of inquiry. Since 1989, students who might not otherwise have had the opportunity to learn about the importance and excitement of graduate education have gained the insights and the skills that will enable them to excel in advanced studies. The McNair Scholars Program honors the memory of astronaut and scientist, Ronald E. McNair. His hard work and the high goals he set are an excellent example for emerging scholars.

While the papers in this journal truly do reflect the end product of the research and scholarly process, the papers also represent a beginning. For the students who have written them, they mark a transition from membership in a community of learners charged with acquiring knowledge to a community of scholars engaged in producing it. We are so very proud to highlight the work of these talented young researchers in this, the tenth edition of the MU McNair Journal. Our best wishes to all of them as they pursue graduate studies and become the role models of tomorrow.

Suzanne T. Ortega
Vice Provost for Advanced Studies
and Dean of the Graduate School

The McNair Scholars Program

BACKGROUND

College students who are considering study beyond the baccalaureate level realize their dreams through the McNair Scholars Program at the University of Missouri-Columbia (MU). The U.S. Department of Education selected MU as one of the original fourteen universities selected to develop a program named for astronaut and Challenger crew member Ronald E. McNair. The purpose of the program is to provide enriching experiences that prepare eligible students for doctoral study.

PROGRAM ELEMENTS

One of the most exciting aspects of the McNair Scholars Program is the opportunity for junior or senior undergraduate students to participate in research experiences. McNair Scholars receive stipends to conduct research and engage in other scholarly activities with faculty mentors from the areas in which they hope to pursue graduate study. These research internships are either for the academic year or for the summer session and are under the supervision of faculty mentors. For academic year internships, students work a minimum of ten hours per week during the fall and winter semesters. Summer interns work full-time for eight weeks.

McNair Scholars also attend academic conferences with their mentors, go to graduate school fairs, prepare for graduate school entrance exams, receive guidance through the graduate school application process and obtain information on securing fellowships, graduate assistantships, and loans. Participants learn about graduate school life, advanced library skills, and effective ways to present their work. At the completion of the research internships at MU, McNair Scholars make formal presentations of their research to faculty and peers at the McNair Scholars Conference and submit papers summarizing their work. Students who participated as juniors the previous year continue in the program during their senior year for graduate school placement and to further develop their skills.

ELIGIBILITY

Participants must meet grade point average standards; be U.S. citizens or permanent residents; and qualify as either a first generation college student with an income level established by the U.S. Department of Education, or a member of a group that is underrepresented in graduate education.

All students who wish to be involved submit an application to the program. A committee composed of faculty members and representatives from both the graduate dean's office and the McNair Scholars Program selects participants and approves faculty mentors. Research internships are offered to those students who are juniors or seniors and are identified as having the greatest potential for pursuing doctoral studies.
Fresh produce imported from the developing world is forming an increasing proportion of the produce available to consumers in modern western societies. However, the types of quality regulation and the degree to which those regulations are enforced vary dramatically from country to country. Enforcement failures or simple lack of regulation can lead to the increase in food-borne illnesses, such as cyclosporiasis, the result of infection with Cyclospora cayetanensis, a protozoan intestinal parasite.

Past epidemiological studies of Cyclospora cayetanensis have concentrated on specific outbreaks. Consequently, most of the conclusions drawn from these studies are context dependent and limited in scope. Computer modeling, however, can draw on the available literature and create a more broadly applicable profile of Cyclospora. With computer models researchers are capable of drawing general conclusions about the characteristics of infectious diseases.

This paper will present a review of Cyclospora cayetanensis followed by a presentation of the model used in the characterization of the cyclosporiasis recovery process. A discussion of the information gained from the model will then ensue. The knowledge gained from this study and the development of other mathematical models may provide a means to lessen the impact of future outbreaks of such diseases.

LITERATURE REVIEW

The recorded history of Cyclospora cayetanensis spans only about two decades. The first three documented cases of cyclosporiasis, a food or water borne illness, occurred in 1977 and 1978 and were reported in 1979 by Ashford, a British parasitologist who recognized a coccidian parasite in fecal samples of a man in Papua, New Guinea [1-4]. Mature, sporulated Cyclospora oocysts resemble coccidian parasites, but Ashford observed that when first excreted the oocysts were immature (lacking sporozoites) and thus noninfectious [2,3]. Sporulation (development of sporozoites, the infectious units within oocysts) did not occur until 8 to 10 days after excretion [2,3,5,6]. Clinical microbiologists often see just the unsporulated oocysts when examining the stool of an infected patient, which can be misidentified as fungal spores resulting in misdiagnosis of the patient [3]. In 1990, Long et al. recognized Cyclospora's similarity to known coccidian oocysts and cyanobacteria, and referred to it as a coccidian-like-body (CLB) [1,7-9]. This was the accepted nomenclature for the organism, until 1993, when Ortega and colleagues gave it the formal name Cyclospora cayetanensis [12].

The most common route of infection for Cyclospora is through ingestion of water (developing countries) or imported fresh produce (developed countries) contaminated with sporulated Cyclospora oocysts [5,10]. Once the oocysts are inside the gut the sporozoites exit the sporocysts and oocyst to enter the epithelial cells of the small intestine [5,12]. Here they undergo a series of asexual and sexual processes. Cyclospora requires an average of seven days to incubate, after which the patient may begin to experience diarrhea, cramping and abdominal pain, nausea, vomiting, fatigue, and occasional fever [9,10,13-15]. In time unsporulated oocysts are shed from the intestinal wall and passed into the environment with the feces.
Dalton et al. have shown that, once in the environment, sporulation occurs after 7 to 12 days if incubated at 25 - 30°C, or within six months if incubated at 4°C [6-16]. Water directly contaminated with the unsporulated oocysts needs about ten days to become potentially dangerous. Because Cyclospora oocysts become infectious a week to two weeks after they pass from the body to the environment, direct fecal-oral transmission is unlikely [10-17].

Trimethoprim-sulfamethoxazole (TMP-SMX) is the only antibiotic proven to be effective against Cyclospora infection. One 60-800mg tablet of TMP-SMX twice a day has been shown to be an effective treatment for immune-compromised patients [4,5,7,9,10,18]. For immune-compromised patients (HIV-infected patients) one TMP-SMX tablet four times a day for ten days is an effective therapy [7]. Currently, no antibiotic alternative exists for sulfa-allergic patients, and the illness must be allowed to run its natural course. Cyclospora infection is self-limiting and a patient's symptoms may resolve in six weeks, but for immune-compromised patients the infection can last up to six months, with an average duration of four months [10,15].

Cyclospora infection has been reported in many countries but appears to be most common in the tropical and subtropical areas [11]. A one-year surveillance of outpatient health care facilities in Guatemala found cyclosporiasis to be more prevalent during the rainy season in the spring and summer months [17,20]. One explanation for the seasonality and geographical distribution of infection is that certain temperature ranges, humidity levels and other environmental factors facilitate the sporulation and survival of oocysts [3,7,17]. In Kathmandu, Nepal Cyclospora infection is most common between May and August (during the warm monsoon months). The infection has its peak level of occurrence in June and July. In contrast, in Lima, Peru, which receives less than 2 cm of rainfall a year, the peak of infection occurs between December and May, the warmer months for the area [3,4]. These trends are consistent with the postulated explanation of heightened rate of infection due to warmth and moisture.

The prevalence of Cyclospora infection appears to be greater among children. The aforementioned Guatemalan study found the rate of infection to be highest among children between the ages of 1.5 and 9 years [3,17,20]. In a two-year cross-sectional, community-based study in a shantytown near Lima, Peru, the rate of Cyclospora infection was the highest among children aged 2 to 4 years [16,21]. This study also noted that children over the age of eleven were rarely infected, which suggests that immunity develops with repeated exposure [3]. Infected children in developing countries are often found to be asymptomatic or to have relatively mild symptoms, while among non-immune adults (e.g. upper and middle class adults in Peru and case patients in outbreaks in the United States) the illness is characteristically more severe and prolonged [17,18].

Studies conducted among native populations and travelers in developing countries have shown that a number of factors influence the prevalence of Cyclospora infection. These include the design of the study, population investigated (e.g. clinic vs. community-based), season, area of the world, sanitary conditions, and personal attributes, such as age, socioeconomic status, likelihood of prior Cyclospora infection, and immune competence [3,21]. The lack of regulation of sanitary conditions in developing countries may be of particular importance to the spread of Cyclospora. For example, poor maintenance of water supplies could lead to consumption of contaminated water and food [22].

During non-outbreak periods in North America and the United Kingdom, where sanitation conditions are highly regulated, less than 0.5 percent of stool specimens test positive for Cyclospora, suggesting the level of infection in the general population of developed countries is very low [3,5]. However, there have been outbreaks of cyclosporiasis in developed countries, typically associated with international travel or consumption of imported produce [22]. The common factor in both cases is the consumption of contaminated water or food originating from developing countries.

During the spring and summer of 1996 the U.S. Centers for Disease Control and Prevention reported 1,465 cases (740 sporadic and 725 cluster-associated cases) in the United States and Canada [3,5,10,23]. Guatemalan raspberries were implicated as the vehicle of transmission. In the spring and summer export season of 1997 a second multi-state outbreak (over 1600 cases) of cyclosporiasis occurred. This outbreak was also found to be associated with Guatemalan raspberries. In response, the Guatemalan Berry Commissions called a voluntary suspension of exports of fresh raspberries to the United States for the 1997 fall and winter export season [5,10,14,24]. The raspberries may have become infected with Cyclospora through insecticides and fungicides diluted with contaminated water and applied to the fruit throughout the growth period and up to the day of harvest [5,16]. Some water supplies were maintained in wells near deep pit latrines or seepage pits. These wells could have become contaminated from surface-water runoff during the rainy season, the same time of year the 1996 outbreak occurred [3,4,5,16].

The United States Food and Drug Administration (FDA) banned all imports of fresh Guatemalan raspberries in the 1998 spring and summer export season, but Guatemalan exporters shipped fresh raspberries to Canada that season. A multi-cluster outbreak of Cyclospora infection in Ontario in May of 1998 was associated with the fresh Guatemalan raspberries [3,5,14]. In 1999 the FDA allowed the importation of fresh raspberries from Guatemala, but only from four farms that met new hygiene standards. Filters designed to exclude Cyclospora oocysts from water used on berries are now part of the Guatemalan Berry Commission’s new standards for raspberry farms [13].

As Ashford first noted in his 1979 report, it is easy to overlook the presence of Cyclospora [2]. In most laboratories, it is not routine to test stool specimens for Cyclospora, and most laboratories lack the expertise to identify the organism (3,12). In 1996 a few experienced laboratories were vital in detecting the presence of Cyclospora infection at the start of the outbreak, and the resulting news coverage helped in the diagnosis of other cases [4].

It is important to note that berries are not the only source of Cyclospora infection [10,18,25]. The first documented outbreak of cyclosporiasis in the United States occurred in 1990 at a physicians’ dormitory in Chicago [3]. Contaminated drinking water was the suspected mode of contamination. In Tallahassee,
Florida, mesclun (a mixture of young salad greens of various types) from Peru was implicated in a multi-cluster outbreak in mid-March of 1997. Two years later, fresh basil, either from a Mexican or United States farm, caused a cluster of cases in Missouri [3,18].

The increase in documented cases of cyclosporiasis in the United States and Canada in the mid-1990’s is partly due to the advancement of detection methods, but the increase in imports into the United States may be a factor in the spread of this food-borne illness [5]. As the imports increase, important questions are raised, such as whether the fresh produce imported from developing countries is grown in a manner that makes it safe for consumption [22].

In some cases of Cyclospora infection caused by raspberries, patients have reported washing their fruit before eating it, which suggests washing produce may reduce the risk of acquiring Cyclospora infection, but will not eliminate it [3,26]. The high attack rates of Cyclospora reported and the ineffectiveness of washing suggest the infectious dose of oocysts is very low, believed to be between 10 and 100 oocysts [3,5,6,14,18].

The possibility of cross-contamination exists, as shown in the 1999 Missouri outbreak of cyclosporiasis associated with fresh basil [18,25]. A birthday party catered at a country club had seven chefs preparing the food. Three of the seven chefs, none of whom traveled overseas or had been ill in the previous month, were diagnosed with cyclosporiasis after the event, but only one of the three was directly involved in the preparation of any food item containing fresh basil [3,18]. Contamination of utensils and/or work surfaces presumably was responsible for the other two infections, thus adding to the possible risks involved in the transmission of Cyclospora infection.

Several risks, known and unknown, are associated with contracting food-borne illnesses, such as cyclosporiasis. Consuming food or water while visiting developing countries is a well-documented way of developing traveler’s diarrhea. Travelers are often warned against such actions, but over 70 percent of selected produce consumed in the United States is imported from developing countries, making “traveler’s diarrhea” possible without international travel [10,22,27]. Because fruits and vegetables are being imported into the United States and Canada from developing countries at an increasing rate, it is essential to improve our understanding of Cyclospora cayetanensis.

**METHODS**

Information obtained from the epidemiological literature was used to develop a computer model that aided in characterizing the cyclosporiasis recovery process. The STELLA 7.0.3 program was used to create a macro-scale model that simulates the recovery of groups of people categorized by immune-competence and presence of sulfa-allergies. A base model was created and validated with data from past outbreaks. Parameters, such as percent of population to receive treatment and time to proper diagnosis of illness, were systematically varied in order to profile the cyclosporiasis recovery process.

A diagram of the STELLA model is shown in Figure 2. An initial population of 3000 individuals infected with Cyclospora is divided into four subgroups based on U.S. national percentages: 96.8 percent, immune-competent with no sulfa-allergies (Control); 0.063 percent, HIV/AIDS patients with no sulfa-allergies (HA); 2.9 percent, sulfa-allergic and HIV-negative (SA); 0.095 percent, HIV/AIDS patients with sulfa-allergies (HA/SA) [28]. The Control and HA subgroups have the opportunity to receive treatment. Due to their sulfa-allergies, treatment is not possible for individuals in the other two subgroups. The percentage to receive treatment, or probability of receiving treatment, is controlled by the converters labeled “Control Prob of Treatment” and “HA Prob of Treatment”, respectively.

The “Recognition” converter is a graphical function, illustrated in Figure 1, representing the time it takes for a treated subgroup to be properly diagnosed with cyclosporiasis and receive treatment. The recognition curve resembles an S-shaped curve, with days on the x-axis and percent recognition on the y-axis. The steepness of this curve and time to 100 percent recognition can be varied to reflect given outbreak conditions.

![Recognition Curve](Image)

**Figure 1.** An example of a recognition curve used in the characterization of cyclosporiasis. As time increases the percentage of individuals who recognize they are infected with Cyclospora increases. The time it takes for the curve to reach 100 % was varied in the recovery simulations.

The “Control Treated Recovery Rate” and “HA Treated Recovery Rate” are set at 7 and 10 days, respectively, as prescribed by TMP-SMX treatment trials [5,7].

A proportion of cases in each treated subgroup are allowed to recover spontaneously without the aid of treatment. However, there is individual variation in the rate of spontaneous recovery from cyclosporiasis. To deal with this, the population is divided into five subgroups, with each subgroup having a characteristic recovery rate. The “Control P at certain rate” and “HA P at certain rate” converters designate the proportions of the Control or HA groups that fall into each of these five subgroups. The days to recovery that correspond to the subgroups of the Control group are 7, 17, 28, 42, and 80 days, respectively. The days to recovery that correspond to the subgroups of the HA group are 10, 24, 40, 59, and 112 days, respectively [5,7]. The longer recovery times for the HA group reflect the difficulty immune-deficient individuals have dealing with infectious diseases. The SA and HA/SA subgroups follow the same recovery pattern as the Control group.

The simulations run included consecutive trials in which...
either the proportion treated or number of days to recognition was held constant while the other was systematically varied.

**RESULTS AND DISCUSSION**

The two parameters varied in the model were percentage of Control and HA patients to receive treatment and the time to proper diagnosis and subsequent treatment. Figures 3 and 4 illustrate the effects of varying the percentage of Control patients receiving treatment. The curves for the HA subgroups follow a similar pattern, but on a smaller scale. The sulfa-allergic subgroups are not affected by the percentage of patients treated, because they are unable to receive the TMP-SMX treatment.

The second parameter varied in the model was the “Recognition”, the time to proper diagnosis and subsequent treatment. Figures 5 and 6 illustrate the effects of setting the “Recognition” parameter at 100 percent recognition in either 14 or 30 days, while holding the percent treated constant.

Varying the “Recognition” did not have an effect on the total population recovery. When the “Recognition” is held constant, there will only be a noticeable decrease in the time to total recovery if the percentage of patients treated is set at 100 percent. In certain outbreak conditions, 100 percent treatment may not be possible. However, when the percentage of patients receiving treatment is increased, the proportion of the infected population that recovers at the faster treated recovery rate also increases, thus lessening the impact of a cyclosporiasis outbreak. When the percentage of patients treated is below 100 percent it is possible that the duration of illness in some individuals can last up to six months. With 100 percent treated all individuals in the Control Spontaneous Recovered subgroup would be shifted into the Control Treated subgroup, allowing a faster total recovery time.
sulfa-allergic or spontaneous recovery subgroups, because neither group receives treatment. Possible reasons a person without sulfa-allergies would not receive treatment include improper diagnosis or not seeking the help of a physician.

Varying the “Recognition” did have an effect on the Control and HA Treated subgroups. The curves for the two settings are the same shape, but there is a rightward shift in the 30-day recognition curve. Since symptoms can persist for more than one month, treatment beginning after 30 days of infection still has a significant effect on patients’ time to recovery. This supports the conclusions of Mohle-Boetani et al., who performed a study on the effects of heightened laboratory surveillance and media coverage on the cyclosporiasis outbreaks [29].

Figure 5. Recovery rates for Control Treated, Control Spontaneous and Sulfa-allergic (SA) subgroups. The proportion treated was 66%. The recognition curve was set at 100% recognition at either 14 days or 30 days. The recognition curve did not affect the Control Sp or SA recovery rates. Due to the nature of cyclosporiasis, every person in a subgroup recovers.

Figure 6. Recovery rates for HIV/AIDS (HA) Treated, HA Spontaneous (HA Sp) and HA/SA. The proportion treated was 66%. The recognition curve was set at 100% recognition at either 14 days or 30 days. The recognition curve did not affect the HA Sp or HA/SA recovery rates. Due to the nature of cyclosporiasis, every person in a subgroup recovers.

■ CONCLUSIONS

Increasing the percentage receiving treatment can lessen the impact of a cyclosporiasis outbreak. The total time to complete population recovery may only be reduced with a 100 percent treatment rate. As the percentage treated increases, more individuals are shifted from the Spontaneous recovery subgroups to Treated recovery subgroups. This shift allows for more individuals to recover at the treated recovery rate and avoid symptoms that can last for more than one month. Since cyclosporiasis can last for more than one month, treatment beginning after an individual 30 days after onset of symptoms can decrease their time to recovery.

Two major limitations of the TMP-SMX treatment of cyclosporiasis are the low “recognition” of the illness and the exclusion of sulfa-allergic patients from possible treatment. The former may be rectified through physician and public education and heightened laboratory surveillance during outbreak seasons, as described by Mohle-Boetani et al. [29]. The latter limitation can be alleviated by the development of a non-sulfa-based treatment. If such a treatment were developed the sulfa-allergic subgroups in the model could be included in the Control and HA subgroups. The sulfa-allergic subgroups are small compared to the non-sulfa-allergic subgroups, though, which raises questions about the significance of being able to move these individuals into the Control and HA subgroups. The issue of increasing antibiotic resistant organisms merits the search for alternative cyclosporiasis treatments. Finding a non-sulfa-based alternative would address both allergy and antibiotic resistance issues. Both finding an alternative treatment and increasing education and laboratory surveillance have the potential to significantly lessen the impact of future outbreaks of cyclosporiasis and other similar food borne illnesses.

It is clear that much can be learned from computer modeling of infectious diseases. The models allow researchers to draw conclusions and characterize future outbreaks without depending solely on retrospective studies. For example, the recovery model presented in this study could be extended and modified to illustrate the effects of possible food safety control measures. It would also be valuable to move from population level modeling to models that incorporate the characteristics of individual people, creating an increasingly more realistic profile of the cyclosporiasis recovery process.

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■ REFERENCES


Henry Roe Cloud, a Winnebago from Nebraska, began his elite education and career at the turn of the century. During this time, the government enacted its policy of assimilation, which intended to make Indian people part of American society. These policies would completely and permanently change American Indian people, including Henry Roe Cloud, who believed that Native people should assimilate and become more like the dominant society in order to survive. He thought embracing Christianity, like he had done, would allow Indian people to continue to exist. Thus, religion became the central focus of his early career and development. In many ways, Cloud, through his contact with other Christian people gained important access to others who were interested in Indian reform and had the resources to assist in this cause. More importantly, Christianity gave him a framework for thinking about the changes he thought needed to be made among Indian people.

**BACKGROUND: National and Tribal**

Henry Roe Cloud grew up and began his career during the government's most intense effort to assimilate Indian people, lasting from the later 1800's until around 1920. Government and sympathetic reformers saw total assimilation into white society as a solution to ever present problems of starvation and poverty on reservations. In order for assimilation to occur, it became important to bring Indian people into the fold of the American values system not only for the sake of the Indians, but also for the greater good of the country. In *A Final Promise*, Fredrick Hoxie stated that, this plan was based upon the idea that “assimilated natives would be proof positive that America was an open society, where obedience and accommodation to the wishes of the majority would be rewarded with social equality.” Hoxie argued that Indian reformers were acting out the perceived national crisis about American ideals and values. In order to administer this societal panacea and solve the problems that plagued Indian country, the government “became involved in the details of Indian land tenure, education, and citizenship” as mechanisms for fostering proper civilization.

Henry's own Winnebago people served as an example of the kind of relationship that the government and tribes established. Winnebago history places Cloud in the proper context. Born in 1884 in Winnebago, Nebraska, Cloud grew up in a time and place marked by numerous, frequent removals and intense conflict.

The Nebraska Winnebago originally called the area around Green Bay, Wisconsin their home. Later, as a result of a treaty negotiation in 1832, the tribe was given a piece of land in Iowa, known as neutral ground, in payment for lands ceded by treaty in Wisconsin. However, this land was anything but neutral. Here, the Sioux and Sauk fought with each other, and the Sauk then fought the newly arrived Winnebago. Despite these difficulties with their new neighbors, it would be about ten years before the tribe split in 1846. This fissure between the groups of Winnebago, or Ho-Chunk, people was precipitated by a treaty negotiation for the lands held in Wisconsin. The Winnebago did not want to sell any more of their land, and in 1837 agreed to send a delegation to Washington. They believed merely sending a group of men to Washington would appease
he gave the prize to his sister "according to Indian custom."15

an event from his childhood, when upon winning a "roan pacer"

and Winnebago people. For instance, Henry Cloud described

childhood. Members along with his uncles played a central role in Cloud's

necessity of warfare. Thus, Henry was named "Wo-Na-Hi-Lay-

meaning War Chief".16

Early Life and Primary Education

Cloud lived a life typical of a Winnebago child during this

time period. Though the date has never been fixed, in an

autobiographical article Cloud stated he was born in 1884.12

Cloud spent the first ten years of his life in a "circular room of

the wigwam."13 Here Henry, his parents, brother, sister and

grandmother "ate, slept, and made merry."14 These family

members along with his uncles played a central role in Cloud's

childhood.

As a child, Cloud also understood his own traditional and

familial roles, which connected him to his immediate family and

Winnebago people. For instance, Henry Cloud described

an event from his childhood, when upon winning a "roan pacer"

gave the prize to his sister "according to Indian custom."15

Early in life, Cloud also received a name reflective of his clan

affiliation, the Bird Clan, whose members determined the

necessity of warfare. Thus, Henry was named "Wo-Na-Hi-Lay-

H unka meaning War Chief".16

Cloud's academic career started in an auspicious way,

which was indicative of the experiences of many Indian children at this
time. Cloud himself states, "an Indian policeman came to take my brother" and "I wanted to go too...because I wanted to

be with my brother...when I was about seven years of age we

were taken to a non-reservation school at Genoa, Nebraska."17

Cloud sheds little light as what he actually learned in Genoa,

yet recalled rather sentimentally that he among other things, "flew

kites, fought John Hunter, and slid in winter."18

Genoa focused upon farming and other manual training. It

produced wheat, corn, and other crops on 300 acres.19 While

attending Genoa, Cloud began to learn English and spoke it so

fluently that he could not remember his own language. On the

wagon trip home he and his father could not communicate with

each other because Henry did not remember how to speak

Winnebago and his father did not speak English.20 Cloud

relearned his own language in about three weeks and never

completely lost this ability again.21

Learning English was significant because Henry like

hundreds of other Indian children were also being taught to

interpret and analyze the world around them in a new way

dictated by the precepts and patterns of Anglo thought.22 The

system of education impressed perfectly upon Henry shaping his

thinking patterns. One of the first points of transition, learning

English in conjunction with relearning his own Winnebago

tongue was important for Henry. He had in essence spent two

years having his patterns of speech and thought conforms to a

Euro-American framework. However, he needed the Winnebago

to communicate with and reconnect to his own

family and tribe. The sheer necessity of being able to think and

work in each of these two mindsets would become a pattern in

Cloud's life.

After a brief time at his home, thirteen-year-old Henry

returned to school, where he would make one of the most

critical choices of his life.23 One "Cross Day" (Sunday), during

meeting, Henry expressed that "he would like to be His (Jesus')

friend."24 Later that evening, the local Presbyterian minister,

Mr. William T. Findley began to tell Cloud about Jesus. From

day Cloud "entered upon a new life" as a "preaching

listener" or Christian.25 The day of his baptism was a

tremendous day for Cloud who announced that he would be

guided by Christ alone and not his parents or family.26 It was

also a significant day for Mr. Findley as well. In converting

Henry Cloud, Mr. Findley made his only Winnebago convert

before he died.27 Years later, Henry remarked that Mr. Findley

had died for him "as Jesus did,"28 Henry said, "He died a

discouraged man. Not discouraged in God and the advance of

his kingdom, but in himself."29

Henry recognized his baptism as a turning point. His life

began to change profoundly. After Henry's spiritual rebirth, his

corporeal existence became difficult at best. For example,

Henry's family did not approve of his conversion. Before her

death, Henry's grandmother explained to him the fate of a Crow

man who also became a Christian. In this story, the Crow

roamed forever lost in the afterlife. Being an Indian, he could

not enter heaven. The man could not come into the Crow

afterlife because he took on the guise of a white man.30 Cloud's

grandmother explained the scenario to scare Henry about the

ture fate of his soul since he had taken the Christian road.

According to his grandmother, Cloud was doomed to wander

alone in the afterlife. This story also suggests that Cloud's

grandmother thought white society would never completely

accept him even if Cloud were Christian and standing just

outside of heaven. In her thinking, Henry would never really be

one of them, and it was wrong for any Indian to try to turn

himself into a white man. In conclusion to her story, she

explained the Crow had "taken the wrong road."31

While her criticism upset young Henry, this hardship was

minimal compared with the ones he next endured. Soon after

Henry's conversion he lost three family members. Within the

span of about a year both of his parents passed away as well as
his grandmother. Cloud was appointed a guardian by the name of Honest John Nunn who soon passed away as well. After the death of his family members and guardian, he became more religious because God seemed to be the only reliable force in Cloud's life.

Cloud also attended Santee Mission School in northeastern Nebraska, which was composed mostly of Sioux children. The education he received at Santee Mission School was in many ways superior to his previous education. Cloud described the mission school as the place his "soul awoke for the first time to some appreciation of the fact that there is much to learn and much to do." Education here went beyond manual training, though children here were expected to do some vocational work, they were also given a more substantial, high school and theological education than usually afforded to Indian children.

As Henry's academic knowledge expanded, his spirituality seemed to deepen. After the deaths in his family, Cloud read the Bible frequently and sought "to gather new strength in his situation." Though no explanation was given about how God came to Henry, he stated that God had revealed His purpose for Henry who believe it was for him to do God's will and, he thought going to Mount Hermon would prepare him for this purpose. The teachers at Santee and Alfred Riggs, the head of the school, and Mr. Findley encouraged his application to Mount Hermon, the eastern preparatory school.

Although Cloud did not base his religious decisions on white acceptance, his faith also opened doors for him that would not have opened for most full-blooded Indian boys at the turn of the century who were not Christian. The white people who were in charge of Santee Mission School saw Henry's Christianity as sign that he would make something of himself. In a way, Henry's faith elevated him far above the status that most Indian people were classified into by whites. After his years at the mission school, the headmaster, Reverend Alfred Riggs stated in Cloud's application to Mount Hermon that Henry's Christianity would not be at the educational level as a white student, yet Cloud was "better than the average Indian." Mount Hermon and Yale University

From Santee and the plains of Nebraska, Henry made the journey into the next phase of his education in Massachusetts at Mount Hermon, which prepared "less advantaged youth" for the rigors of higher education. The school was known and respected because like many others, it emphasized manual labor, specifically farm work as a means of teaching its students self-sufficiency and self-support. Each student paid part of their tuition by working on the school's farm at least two hours a day. Henry also worked on a farm in New Jersey for a year to pay for his last year of tuition at Hermon.

Mount Hermon became a beloved place for Henry Roe Cloud, and it was home to him in ways that his own home and Santee just were not. For instance, Cloud visited there many times after his graduation in 1906 and went there to celebrate Thanksgiving while he was at Yale. His experience among similar young men guided by the teachers at Hermon gave him a sense of life stability, "buoyancy and power." This sense came from knowing that he could make a life for himself—either by the power of his brain or the power of his physical work.

The duality though was important because it gave him the opportunity to explore and excel intellectually in ways that he previously had not. Cloud's studies in Greek and Latin were just two examples of this exploration. After graduating from Mount Hermon Henry began his academic career at Yale in 1906. Henry's college years at Yale provided him with connections and experiences that influenced his occupation and perspectives. As a freshman, Cloud met the Roe family, Reformed Church missionaries, who nurtured Cloud's religious and personal development. Cloud also explored and cultivated religious beliefs and talents that would become vital to his later life and career.

Though not much is known about his freshman year at Yale, meeting Mary Roe was the "most significant event" of Cloud's year. Mary Roe, while at Yale, gave a speech about her work among Indian people in Oklahoma. Through this speaking engagement, Cloud met one of the most influential people in his life. Mary and her husband, Walter Roe worked as missionaries among the Cheyenne and Arapaho in Colony, Oklahoma. Walter held the post of superintendent of Oklahoma missions for the Reformed Church. Mary and Walter Roe developed a close, familial relationship to Henry and eventually adopted him as a member of their family around 1908. After this adoption, Henry took the name Roe as his own and was forever considered part of their family. The Roes continued to keep home and Yale University

The Roes encouraged his thinking along Pan-Indian lines, which would later become important in his career as Henry became involved in the policy and politics of Indian Country. Cloud gained exposure to different tribal groups in the summer of 1907, while staying with the Roes in Colony, Oklahoma. Cloud, for the first time, in Oklahoma lived around a group of Christian Indian people. Going to various camp meetings in the early 1900's and seeing the possibilities of tribes in a sort of cooperation under Christianity affected him by showing him a small glimpse of the possibilities of survival of Indian communities under Christianity. The Roes continued to keep him informed of the developments. For instance, in Oklahoma the Roes themselves did mission work among the Cheyenne and Arapaho who were at that time organized as one unit and would have one camp meeting. Other Christian Indian people attended these meetings as well. One summer in particular some Comanches and Kiowas also came to the annual meeting. In addition, while in Nebraska, Cloud also had an opportunity to travel among the Sioux, again to attend a religious meeting, which seemed to be a routine part of his mission work there. Each of these experiences gave him a broader perspective about what the Christianity was like among groups of Indian people and what it could become.

During Cloud's career at Yale, Cloud discussed his thinking about religion. Cloud, a Presbyterian, possessed religious beliefs that did not differ from his fellow Christians. Cloud believed in God's will and power in his daily and future life. Yet, he knew that he had to constantly improve his person by waking up in the morning to "pray to God to do better in the future" an expression that exhibited Cloud's discipline in his devotion.
Similarly, Cloud believed in God in a personal way, and thought that God had taken an interest in his life.63 Not only was Henry Roe Cloud’s inner thought focused upon religion, while at Yale his public life also reflected his religiousity. He participated in secret prayer meetings and bible study every day. Cloud also taught bible study to younger boys.64 These activities were important in his life because these practical experiences gave him the experience to understand that he wanted to pursue a career in the clergy.

Henry also cultivated skills at Yale that helped him fund the Ivy League education he received. He did manage to eke out an existence a couple of ways. His brother sent him money, and Henry received the lease money from his allotted land in Nebraska.59 In 1908, Henry began traveling to do speaking engagements at different parishes and for organizations. He gave his first speech in Asbury Park, Connecticut about the missionary work done among the Winnebagos in Nebraska.60 Doing speeches became vital for Henry because they not only helped him fund his undergraduate career, but also allowed him to more fully develop the oratorical skill that he would use in the future.

Cloud participated in many other activities that did not have a direct tie to the church which were also helpful in the development of Cloud’s future. For example, Cloud became quite involved in debate and oratory. He gave speeches such as “Religion and the Indian” and would incorporate these two subjects frequently, for they were not only important to Cloud, but the relationships between America and her original peoples were of current importance.61 Entering speech and debate allowed Cloud to develop the skills necessary to become an excellent public speaker and to prepare for his future vocation. Cloud utilized these skills often, for in one week he would schedule as many as five speaking engagements.62 Despite this demanding schedule, Cloud did participate in more recreational activities. He was a member of Beta Theta Pi and was bestowed the honor of membership into the Elihu Club, one of Yale’s most prestigious senior societies.63

Most of Henry Cloud’s energy was spent outside of Yale’s great halls of learning. He, like many students did face the challenge of balancing their extra curricular activities with their academic careers, in facing this challenge Cloud faltered. He struggled with his early academic career. For example, he had problems with Spanish, and outright flunked a psychology test because he had not prepared for it properly.64 In fact, during his senior year his grade point average was 2.15.65

Although he many not have done much writing for class, Cloud did begin writing about the problems Indian people faced during this time. Cloud believed that Indian people needed to assimilate and he had faith in the ability and capability of Indian people to do so. In “The Winnebago Medicine Lodge” printed in the Christian Intelligencer, Cloud describes the advances made among his people by the Presbyterian Church. The main obstacle in this process was the Medicine Lodge, a religious ceremony held among the Winnebago, though its origins and teachings were “beautiful for primitive man,” Cloud wrote, “They could never stand the test of modern times.”66 He believed because of its teaching about the present and spiritual world scared the rest of the tribe into submission to the Medicine Lodge. However, the brave few were beginning to see the light of “salvation and redemption” and were destroying the power the lodge once held, as evidence of the salvation Cloud discussed the 20 people who had been saved so far.67

This article represented Cloud’s basic attitude toward not only the Winnebago people, but also his attitude about himself in relationship to them. Cloud commented that he lived as a solitary outpost in Christendom; it gave him great joy that “these people” his fellow “tribesmen”, begin to take up the cross.68 Underlying this was the assumption that one only becomes modern if their soul becomes modern and relinquishes ties to the Medicine Lodge. Thus, Cloud only identified himself with this small, but growing population of new Christians who to Cloud were brave souls for going against the grain of Winnebago culture. This article clearly was intended for the eyes of curious, Christian onlookers; thus it was no surprise that Cloud did not readily identify himself with the “primitive men” until the point that they began joining the church. In this description, Cloud seemed to be trying to give himself more credibility because he had lived the life similar to many Indian people at this time, but he also considered himself a modern Indian because he was a highly educated Christian man.

“Missions to the Indians” printed in the Yale Courant expressed similar sentiments. Cloud argued that Christianizing Indian people was the best way for them to experience progress.69 Christianity would teach them self-sufficiency, a hallmark of modern society. Cloud believed that the agency system fostered the development of “the shiftless drunkard of the reservation, or the blanket Indian in his teepee, a savage at heart.”70 He wrote that Christianity would give Indian people the idea “of civic honor, respect for the law and order, and purity in the home.”71 This quote painted a dim scene without the light of Christianity. Cloud believed and promoted these thoughts about his people.

Nebraska Summer Missions

Henry Roe Cloud renewed his connection to Winnebago, Nebraska by becoming a missionary to his own people. Cloud felt that doing this work among his own people was a duty.72 Cloud went back every summer after his sophomore year at Yale for at least part of the summer.73 Cloud and the cohort of missionaries who went to Winnebago, Nebraska made considerable success in their work. Cloud became especially important on these ventures because he could speak Winnebago, though not without practice.74 As summers passed, he conducted Sunday services and Wednesday prayer meetings.75 Because Cloud believed that Christianity was the way of Indian survival, the conversion of the Winnebago was important to Cloud. The greatest wave came into the fold in the summer of 1909, Cloud wrote to Henry Wright, a Yale professor of the joy he felt at the conversion of 120 Winnebago who, “confessed their faith in Jesus before all of the people and joined the church.”76

Cloud had made great strides within the little congregation, which was his intention in coming back to his reservation. Other aspects of that experience did not go as well as the mission work. Cloud’s years away and beliefs distanced Cloud
from his family and other Winnebago people. He lived and felt quite separate from the other Winnebago living around him. Cloud commented, "I am in my room away from the Indians and from everybody else." The education and opportunities he received early in his life changed his perceptions of himself as an Indian. He had enjoyed the most elite education in the country, and in essence became the picture of what early Progressive America wanted and needed from Indian people. Cloud was the modern Indian, a Christian who lived as an individual successfully.

The experiences Henry Roe Cloud had during the early portion of his life showed that his religion dominated his education and the beginning of his career. It afforded him educational opportunities, which allowed him to prepare for the career that lay ahead of him. More importantly, Christianity gave him a way to think about the way in which Indian people needed to change in order to survive in Twentieth Century America.

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The Internalization of Duties As It Relates To Age

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Do people continue to mature in positive ways as they age? In this study we examined this question by asking whether or not older people more willingly perform important societal duties such as voting, paying taxes, buying Christmas gifts, and tipping service people. More specifically, we attempted to measure people’s degree of internalization of these duties, and to predict levels of internalization from age. Internalization is defined as “incorporation of norms and/or roles into one’s own personality, with a corresponding obligation to act accordingly” (Campbell, 1964).

In our society, there are several sources of motivation that encourage us to strive to do our best. The older we become, the more we tend to identify with certain kinds of motivation. The age factor has significant effects on the choices we make and on the things that we do as it relates to the concept of maturity. Researchers explore a variety of concepts and theories that attempt to describe and perhaps explain motivation. As the attempt is made to explain motivation, the internalization process may be seen at the forefront. Internalization does not require an individual to be motivated externally, but rather the motivation occurs within. The question, does this inner motivation as a result of internalization increase overall well being, becomes intriguing as theorists and researchers provide support that makes internalization both beneficial and vital in the process of development.

Researchers Kennon Sheldon and Tim Kasser (2001) propose the question, “Does personality continue to develop in positive ways as people age?” They hypothesize that as we mature in age, we become better people as we grow to becoming more self-actualized. The research conducted by Sheldon and Kasser was a correlational cross sectional study using subjects ranging from ages 15 to 85 years old. It revealed, “older people possess both types of personality integration values and so forth which involves both having true beliefs and the need to grow and connect with others.” In yet another study of students and their parents, the researchers hypothesized that “psychological improvement occurred with aging.” Although it was not tested, there was a reference to the common assumption that physical declination also occurs with age. The results of the study yield that parents internalize significantly more then their children.

In other studies, there is evidence that the internalization process can begin at young ages. Research indicates “the internalization of social practices...is itself as intrinsically motivated process” (Ryan & Stiller, 1991) In addition, “schools are contexts of cultural socialization in which students’ behavior is regulated, and orientations toward learning, achievement, and self-expression are developed...schools transmit strong and lasting affective lessons that can affect one’s confidence, aspirations, interests, and motivational style long after the classroom years” (Ryan & Stiller, 1991) This supports a strong correlation between childhood, influences, and motivation. “Intrinsic” motivation developed by the process of internalization can be affected by early experiences in agents of socialization such as schools.

Furthermore, theories such as the self-determination theory and Erik Erikson’s theory of psychosocial development also capture the impact of the internalization process. In examining the self-determination theory (SDT), we find that SDT “is a
macro-theory of human motivation concerned with the development and functioning of personality within social contexts. The theory focuses on the degree to which human behaviors are volitional or self-determined— that is, the degree to which people endorse their actions at the highest level of reflection and engage in the actions with a full sense of choice (www.psych.rochester.edu/SDT). This theory “posits that (a) people are inherently motivated to internalize the regulation of uninteresting though important activities; (b) there are two different styles of self-regulation; and (c) the social context influences which the internalization process and regulatory style occur” (Dici et al., 1994). Furthermore, the theory of SDT “suggests that internalization and integration are natural, intrinsically motivated tendencies, contextual support for self-determination should facilitate these processes... Recent research on social contexts and internalization had demonstrated that an interpersonal factor, referred to as autonomy support, is important for promoting internalization and self determination” (Dici et al., 1994).

Another theoretical perspective is Erik Erikson’s theory of psychosocial development, which “proposed that people continue to gain in maturity and personality integration across the life-span as they take on new roles and broader commitments. O riginic and evolving systems theories also suggest that people tend towards enhanced autonomy and complexity throughout the life-span” (Sheldon et al., 2001).

The process of internalization seems to be quite influential in the developmental process we all take. As previously mentioned, our study was performed to examine whether or not a link could be established between the internalization of societal duties and overall well-being and satisfaction. If so, we propose the question can older individuals achieve overall greater life satisfaction and greater psychological well being as a result of internalizing societal duties?

**METHOD**

**Participants & Design**

160 adults were given a questionnaire that measured motivation and their desire or lack of desire to perform 4 social duties. These duties were selected on the basis that they provide a range of societal and individual duties. Additionally, these duties were common duties performed within our western society and include voting, tax paying, buying Christmas gifts, and tipping service people. The sample included & controlled for the following: sex: 82 men/ 78 women; ethnicity: 54 African Americans, 93 Caucasians, 6 Asians, 2 Latinos, 5 others; marital status: 61 married, 21 divorced/widow(er), 78 never married; education: 92 participants (the majority) had some college; and the mean income was $25,000. The mean age of the participants was 39.4 years old.

**Procedure**

Participants were 160 adults solicited in hospital waiting rooms and retirement centers, ranging in age 20 -82 years old who were given a questionnaire that measured the internalization of 4 social duties as well as participants’ psychological well being. Each participant was greeted and received an explanation for the desire of their participation. If they chose to be included in the study, they signed a list, some choosing to provide their contact information as all participants would be entered into a drawing for dinner for two at a restaurant of their choice. Following signing the list, each participant was asked their age for tracking purposes, and then given a clipboard that included two consent forms and a two-sided questionnaire that thanked them for their participation. Each subject was to first, sign both copies of the consent form, one for the researcher to keep and the other for them in case they had any questions arise. After signing both forms, each participant completed the questionnaire.

Upon completion, each subject was thanked and debriefed. Each subject was reminded that they would be contacted if they were selected for the “dinner for two lottery” drawing. They were further told that if they had any questions that they should refer to their copy of the consent form for the appropriate contact information. Each questionnaire and consent form was collected. Eventually, the data was tallied and entered into a statistical database for further analyzing and interpretation.

**RESULTS**

In the first hypothesis, with age comes greater well being, the independent variable measured was the overall well being while age was the dependent measure. In the second hypothesis, with age comes greater internalization, the independent variable was again age while the dependent variable measured was internalization. In the third hypothesis, we predicted internalization would lead to greater overall well being. In this hypothesis, the independent variable was internalization while the dependent variable was overall well being. We found support for two of three hypotheses. There was no significant correlation (p>.05) between age and overall well being as indicated in table 1. However, we found the correlation between age and overall internalization (p<.01) and between overall internalization and well being (p<.01) to be statistically significant which is indicated in table 2. Both tables 1 and 2 illustrate these significant correlations at the .01 level, .05 level, and .10 level. The .01 level indicates the greatest significance as the probability of obtaining significant statistics is less than 1% due to chance, while at .05 the probability is less than 5% and with a level at .10 less than 10% of the time the resulting statistics may be due to chance.

**Table 1**

<table>
<thead>
<tr>
<th>Age</th>
<th>.23**</th>
<th>.28**</th>
<th>.18**</th>
<th>.05</th>
<th>.14+</th>
<th>-.12</th>
<th>-.24**</th>
<th>.03</th>
<th>-.02</th>
</tr>
</thead>
</table>

Significant values at =>p<.10, *=p<.05, and **=p<.01
Table 2
Correlations of internalization & well-being

<table>
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<th>Voting</th>
<th>Gifts</th>
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<tbody>
<tr>
<td>OVERALL WELL BEING</td>
<td>.28**</td>
<td>.17*</td>
<td>.20*</td>
<td>.19*</td>
<td>.23**</td>
</tr>
</tbody>
</table>

Significant values at *=p<.10, *=p<.05, and **=p<.01

**DISCUSSION**
This study examined the relationships between age, internalization, and overall well-being. The first hypothesis of this study failed, as age was not significantly correlated with well-being. However, good support was found for the hypothesis that age would predict internalization, and internalization would predict well-being. The implications of this research are that greater happiness can be achieved simply by embracing the doing of necessary duties. This greater overall well-being may result in longer life expectancies and better physical well-being as the psychological well-being significantly affects one's motivation to take action. This action may simply be exercising more or volunteering for a good cause. In fact, people tend to be "more satisfied with their lives [who] invest greater effort...in more of their daily activities than those who report less satisfaction with their lives." (Kelly, 1993). No matter what the case may be, the motivation to act and continue being psychologically well adjusted is beneficial. Furthermore, people just may become or continue to be motivated and performing undesired duties such as paying taxes.

This study contains limitations related to the sample selection as most subjects came from the same general area. Additionally, the effects may reflect cohort rather than developmental differences. This leads to the need of additional research. Future research may involve using longitudinal methods to remedy possible cohort effect. Furthermore, other ways of selecting subjects for samples may be used. Finally, observing actual behaviors rather than relying on self-report methods, as used in our study from the questionnaire, may further solidify and validate these findings.

**CONCLUSION**
Our data yields results consistent with previous research as the case with the study in which "[there was link between] internalization of personal goals with overall well being and satisfaction" (Sheldon & Kasser, 2001). Furthermore, "In a cross-sectional study with a large sample, they found that their generativity subscale was positively associated with well being" (Ocshe & Plug, 1986 as cited in Ackerman et al., 2000).

The evidence that with greater internalization comes greater overall well-being implies that people should not complain about the inevitability of some things such as "death and taxes." Our results suggest they would be happier if they fully accepted duties such as voting and tax paying. In other words, mature people are those who willingly do what they "ought" to do; in doing so, they gain significant benefits such as greater psychological well-being and overall life satisfaction. Fortunately, this research implies that aging can be quite beneficial and worthwhile as maturity occurs and greater internalization.

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Analysis of Human Memory CD8+ T Lymphocytes Responding to Influenza Virus

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INTRODUCTION

Humans are equipped with a complex immune system used to combat foreign substances in the body. These foreign substances are called antigens, and the immune system has two main components to fight them: B and T lymphocytes. B lymphocytes recognize antigens outside of human cells. They produce antibodies that fight off further infection. T lymphocytes fight antigens that have invaded cells and are divided into two distinct groups: CD4+ and CD8+. Naïve T lymphocytes are activated by antigen presenting cells that present antigenic peptides bound to major histocompatibility complex (MHC) molecules. Once activated, these T lymphocytes become effector T lymphocytes until the infection is destroyed. After this has occurred, a percentage of the cells remain as memory T lymphocytes that are used first if a second infection occurs (1).

As shown in Figure 1, CD8+ T lymphocytes are particularly important in the clearance of virus infections. These cells recognize virus-infected cells via a pathway in which virus-infected cells display fragments of viral proteins in association with different alleles of HLA class 1 proteins. Once a virus has been cleared, memory CD8+ T lymphocytes develop, and these protect the individual from subsequent infections with the same virus (1). Memory CD8+ T lymphocytes specific to past viral infections clear subsequent infections with the same virus more quickly, because they are usually present at 10- to 100-fold higher frequencies in the immune system than naïve CD8+ T cells, and because they develop effector function in hours as compared to days for naïve CD8+ T cells. One of those effector functions is the secretion of an anti-viral protein called interferon-γ (IFN-γ). One method of detecting the presence of memory CD8+ T cells specific for a virus is the detection of memory CD8+ T cells secreting IFN-γ in response to synthetic viral protein fragments that associate with different HLA class 1 alleles using a technique known as ELISPOT (2).

Influenza is a common virus throughout the world. It is a particularly devastating disease in children and the elderly, and outbreaks occur every winter. Scientists work the year prior to predict the strains that will be most frequent and make vaccines for them. These vaccines contain killed influenza virus and induce an antibody response which is strain specific, but not a memory T cell response that recognizes a broader spectrum of influenza strains (3). In this project, we used ELISPOT to enumerate the number of memory CD8+ T cells specific for influenza virus in different human donors. For future studies, this technique could be utilized to determine whether individuals lack protective memory CD8+ T cells against influenza virus infection. Furthermore, it could be used to assess the efficacy of new vaccine strategies for influenza virus in individuals lacking such memory cells.

LITERATURE REVIEW

I. Immune system

The human body has been equipped with a complex immune system of which scientists have only begun to understand in the last few decades. We now know that the immune system combats foreign substances, antigens, with two main components: B and T lymphocytes. B lymphocytes

Senior, Robert Mahon, grew up in St. Louis and is majoring in Microbiology. In addition to his studies, Robert holds the Paul and Elina Cahn Scholarship as well as membership in Phi Eta Sigma, Tri Beta and Alpha Omega. He has participated in the Honors College Learning Community and spent last year studying in Ireland. Robert’s career plans are to be a research scientist in Virology.
recognize antigens outside of the cells and create antibodies to fight off further infection. Once the infection enters the cell, it becomes the problem of the T lymphocyte. T lymphocytes can be divided into two subsets: CD4+ and CD8+. Each subset has three forms: naïve, effector, and memory cells (1). Naïve T cells become effector cells once they are stimulated by antigens presented by major histocompatibility complex (MHC) molecules on antigen-presenting cells (APC) (4). Once activation has occurred, CD8+ T lymphocytes differentiate into effector T lymphocytes (CTL) which secrete cytokines as a way to alter the behavior of the infection. The main cytokine that CD8+ T lymphocytes produce is IFN-γ which has the ability to interrupt viral replication and in some cases doing it without killing the cell (Fig. 1). The effector CTL also can kill infected cells, eliminating them as viral factories (Fig. 1). After the infection is cleared, a small percentage of the effector cells become memory T cells which are used first upon re-infection with the same virus (2). These memory T cells are long lived and can survive for 15-30 years (5). Many questions regarding memory T cells still remain including the maintenance of memory T cells, and the reasons for the decrease in the immune system of the elderly.

II. Influenza

Influenza A and B viruses, members of the orthomyxovirus family, are some of the most common viral infections. An influenza outbreak can be expected every year with the severity and the strains of the influenza virus changing annually. Scientists have created vaccines to combat these outbreaks; however, these vaccines contain killed virus which only provide protection against the "strain of the year" and thus are only good for that year (6). The strain-specific protection that is provided by current influenza vaccines is due to the fact that killed viruses only induce an antibody response. Since, antibodies recognize only the viral coat proteins, which differ between strains, the immune response induced by current vaccinations only protects against the strain incorporated into the vaccine (7). The vaccine is usually recommended for the very young and elderly due to their heightened risk of mortality and morbidity from the infection. Recent studies though have brought into question of what age constitutes "elderly." Some have asked for routine influenza vaccination to begin at the age of 50 stating that the fatality rate for influenza begins to increase in middle age (8).

III. CD8+ Memory Response

A recent study has used vaccinia virus to test for the presence of memory cytotoxic T cells in adults immunized for smallpox. It has shown that there was a significant level of CD4+ and CD8+ T cell response to the vaccine some 30 years post-vaccination, an indication that memory cells are maintained for many years (5). These results agree with the working model of the immune system that has memory T cells being able to combat multiple infections over a lifetime (9). A memory CD8+ T cell response is generated only when a live pathogen enters the cell. This means that vaccines that contain only dead virus, such as the current influenza vaccine, do not elicit CD8+ T cell memory responses and do not have the advantages brought on by those memory T cells. For one, there is a greater frequency of memory CD8+ T cells found in the body than naïve T cells. Also, memory cells become effector T cells much faster than do naïve T cells, allowing them to inhibit virus infection more effectively (2). Finally, memory CD8+ T cells bring with them a cross-resistance not found in antibodies. Memory CD8+ cells recognize certain proteins and enzymes that are conserved among different strains of influenza enabling them to combat a wide variety of influenza strains in contrast to the strain-specific protection afforded by antibodies (10).

IV. Dendritic cells

In many cases, the immune system requires a co-stimulatory signal for naïve T cell activation, division, and differentiation to begin. This is provided by professional antigen-presenting cells (APC). These include dendritic cells, macrophages, and B cells. Each group of APCs is specialized to deal with a certain type of antigen. For the dendritic cells, they are viral antigens (1). Dendritic cells (DCs) are able to stimulate initial immune responses against numerous epitopes of a virus. There are two forms of dendritic cells: immature and mature. The maturation process takes place when an immature dendritic cell takes up an antigen at the site of infection and takes it to the lymph node. It is important that the dendritic cells become mature because recent research has shown that mature DCs elicit higher IFN-γ production from effector cells than immature DCs. In addition, mature DCs and not immature DCs, are able to stimulate and induce CD8+ T lymphocytes to differentiate into cytotoxic T lymphocyte (CTL) effector cells in 7 days (11). While dendritic cells have not been shown to be necessary for stimulation of memory CTLs, they may be able to increase memory CTL responses.

METHODOLOGY

The sequences for the HLA class1-restricted influenza virus protein fragment epitopes used in this study were obtained from
Table 1

<table>
<thead>
<tr>
<th>HLA Class Allele Restriction</th>
<th>Virus Protein/Epitope</th>
<th>Amino Acid Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Flu PBI/591-599</td>
<td>Val-Ser-Asp-Gly-Gly-Pro-Asn-Leu-Tyr</td>
</tr>
<tr>
<td>A2</td>
<td>Flu M1/58-66</td>
<td>Gly-Ile-Leu-Gly-Phe-Val-Phe-Thr-Leu</td>
</tr>
<tr>
<td></td>
<td>HIV p17gag/77-85</td>
<td>Ser-Leu-Tyr-Asn-Thr-Val-Ala-Thr-Leu</td>
</tr>
<tr>
<td>A3</td>
<td>Flu NP/265-273</td>
<td>Ile-Leu-Ary-Gly-Ser-Val-Ala-His-Lys</td>
</tr>
<tr>
<td></td>
<td>HIV p24gag/259-267</td>
<td>Gly-Glu-Ile-Tyr-Lys-Ary-Trp-Ile-Ile</td>
</tr>
<tr>
<td>B7</td>
<td>HIV env/303-312</td>
<td>Arg-Pro-Asn-Asn-Asn-Thr-Ary-Lys-Ser-Ile</td>
</tr>
<tr>
<td>B8</td>
<td>Flu NP/380-388</td>
<td>Glu-Leu-Ary-Ser-Ary-Tyr-Asn-Ala-Ile</td>
</tr>
<tr>
<td>B35</td>
<td>Flu M1/128-135</td>
<td>Ala-Ser-Cys-Met-Gly-Leu-Ile-Tyr</td>
</tr>
</tbody>
</table>

LaVali et al. (12). HLA class 1 restricted HIV epitopes were utilized as controls. All of these epitopes were synthesized on an ABI 432A peptide synthesizer and are listed in Table 1. Human donors from whom peripheral blood mononuclear cells (PBMCs) were isolated are listed in Table II along with their HLA class 1 alleles. To determine the number of memory CD8+ T cells, PBMCs were isolated from each donor and these were then analyzed via ELISPOT analysis for the number of cells which produced IFN-γ in response to HLA class I restricted influenza virus (FLU) epitopes in vitro. The ELISPOT methodology is a three day process (13). On the first day, a monoclonal antibody (mAb) that binds human interferon-γ (capture mAb) was coated on each well of a nylon coated 96-well plate via incubation overnight at 4°C. The next day, PBMCs were isolated from the human donor, and approximately 250,000 PBMCs were plated per well in CTL medium. A viral peptide, depending on the HLA class I alleles expressed by the human donor (see Tables I and II), was then added to each well to bring the total volume to 200 µl. The plate was then incubated between 6 and 18h at 37°C allowing the memory CD8+ T cells to secrete IFN-γ. The assay was then arrested by washing the PBMCs from each well with PBS with Tween. 100 µl of a biotinylated mAb that recognizes a different epitope on human interferon-γ (from the original capture mAb) was added to each well and incubated for 3 h. The plate was then washed, and 100 µl of StreptAvidin-alkaline phosphatase conjugate was added to each well and incubated for 2 h. Finally, 100 µl of the substrate for the alkaline phosphatase conjugate was added to each well and incubated for 30 min. After incubation, the plate was placed underneath a dissecting microscope, and the number of spots was counted per well. Each spot theoretically represented one memory CD8+ CTL.

For some analyses, PBMCs were isolated twice from the same human donor, and dendritic cells were cultivated from the first set of PBMCs. Dendritic cells were added to the second set of PBMCs to evaluate whether they would help detect weak memory CD8+ T cells in the ELISPOT assay. The use of dendritic cells increases the length of the experiment by a week. DCs were isolated from the PBMCs by a protocol originally described by Bender et al. (14) and subsequently modified by Romani et al. (15, 16). T cell-depleted PBMCs were plated with 1500 U/ml Leukine (granulocyte-monocyte colony stimulatory factor) which stimulates the growth of DCs and 20 ng/ml interleukin 4 which inhibits macrophage differentiation, and cultured for 7 days at 37°C. The media were changed on Day 3 with the addition of 2400 U/ml Leukine and 1000U/ml interleukin 4. During the final 3 days, the DCs were cultured in the presence of monocyte-conditioned medium which was prepared as described by Romani et al (15, 16). This induces the final maturation step for the dendritic cells.

Results

Over the seven month study, we analyzed memory CD8+ T cell response in nine donors. They were selected on the basis of their expression of HLA class 1 alleles for which we had influenza epitopes (Table 1). The PBMCs from all of these donors responded to the positive control stimulus, phytohemagglutinin, that theoretically stimulates all memory T cells. Since all donors in this study were presumed to be HIV-negative, HLA class 1-restricted HIV peptides along with no peptide addition were used as negative controls. PBMCs from the donors showed various degrees of response towards the FLU peptides. Figure 2 presents characteristically high, intermediate, and low responses of memory CD8+ T lymphocytes from different donors. As shown in Figure 2A, the HIV peptide and no peptide controls gave little to no response, while the B35 FLU peptide gave almost zero response when compared to the negative control. The A2 FLU peptide, along with the A3 peptide gave heightened responses. Comparing the two indicates that the HLA-A2 allele is dominant for combating influenza in donor 077. Similar results were found in donors 001 and 045. These results are reproducible, since similar results were found for donor 077 in a previous experiment.

Previous studies had shown that adding dendritic cells to a culture of memory CD8+ T cells had the ability to increase their response (16). For this reason, we performed a few ELISPOT assays with dendritic cells to see if we were missing any responses. After analyzing the data in Figure 3, however, it was determined that the dendritic cells did not increase the response sufficiently to continue using them.

Collectively, when all of the memory responses to influenza virus measured in the nine donors were compared in Figure 4, it was determined that responses to the HLA-A2-restricted FLU peptide tend to dominate the memory responses in individuals expressing that allele.
Figure 2: Graphs showing various degrees of response of HLA alleles towards FLU and HIV peptides. Values were calculated by taking the average of 3 trials per one condition. (A) A representative of a high memory CD8+ lymphocyte responses by donor 077. (B) A representative of an intermediate memory CD8+ T lymphocyte response by donor 001. (C) A representative of a low memory CD8+ T lymphocyte response by donor 045.

Table 2

<table>
<thead>
<tr>
<th>Human Donor #</th>
<th>HLA-A Alleles</th>
<th>HLA-B Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>001</td>
<td>2</td>
<td>66.77</td>
</tr>
<tr>
<td>011</td>
<td>2,11</td>
<td>51.57</td>
</tr>
<tr>
<td>013</td>
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<tr>
<td>077</td>
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<td>35</td>
</tr>
<tr>
<td>089</td>
<td>1,2</td>
<td>8.57</td>
</tr>
</tbody>
</table>

Figure 3. Use of dendritic cells does not significantly increase the detection of memory CD8+ T cells responsive to FLU peptides. (A + B) Comparison of the frequency of memory T cells in PBMCs with and without autologous dendritic cells in Dn089 (A) and Dn013 (B). The background has removed by taking the higher average between the HIV peptide and no peptide controls and subtracting it from the responses towards the FLU peptide.

Figure 4. A2 FLU had the highest average response towards the FLU peptide. A cluster graph of response to all of the FLU peptides presented by different HLA class I alleles. The values used for the graph are the average of 3 trials per one condition. The background has been removed by taking the higher average between the response to HIV peptide and no peptide controls, and subtracting it from the response to the FLU peptide.
DISCUSSIONS

Completing the ELISPOT procedure takes three days but only a few hours each day to complete. The cost for the ELISPOT procedure is considerably smaller compared to other forms of IFN-γ detection (for example, intracellular cytokine staining). The results also appear to be reproducible and reliable. For all of these reasons, detecting the presence of memory CD8+ T cells with IFN-γ ELISPOT is an efficient technique.

However, the addition of dendritic cells to the ELISPOT assay did not significantly increase the detection of memory CD8+ T lymphocyte responses. The cultivation of dendritic cells along with the reagents necessary to grow them makes its addition to the assay both time and cost inefficient.

When human donors who express HLA-A2 were examined for their influenza-specific memory CD8+ T lymphocytes, the HLA-A2-restricted response was found to dominate other HLA class I allele-restricted responses (Fig. 2 and 4). These results suggest that new vaccination approaches should include this peptide epitope, since HLA-A2 is the most frequently found HLA class I allele in the human population.

In the future, this technique will be useful to assess the frequency of memory CD8+ T cells in individuals prior to and subsequent to receiving new influenza virus vaccines. This will become more and more a public health concern as the percentage of people 65 years or older continues to grow rapidly. This will put a strain on the producers of current influenza vaccines.

REFERENCES


ACKNOWLEDGEMENTS and SUPPORT

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The Effects of Epiphysis Shape on Femoral Diaphyseal Proportions in Hominoids

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Carol Ward, PhD, Mentor
Department of Anthropology

Introduction

It has long been recognized that “bone tissue will adapt itself during life to the kinds of mechanical loadings placed upon it” (Ruff, 1992). Knowing this, researchers have reconstructed behavior in fossil animals by modeling long bones as beams using conventional beam theory from the field of engineering mechanics. They have examined the amount and distribution of bone in the femoral midshaft, assuming that this reflects the patterns of stress and strain to which that bone had been subjected. Anthropologists have recognized that human and ape femora have different diaphyseal, or midshaft, cross-sections. Traditionally, these differences have been attributed to locomotor variation within and among species. Because strain within bone triggers bone expansion, by modeling the femur as a beam researchers can predict the strain within bones during activity. Thus, different behaviors should lead to different bone shapes. Recently however, this view has been challenged. Ohman and Lovejoy (2001) suggested that because bones grow from epiphyses near the ends of bones, it is the shape of these epiphyses, mediated by natural selection for joint form that accounts for the observed shape variation in apes and humans. They noted that the mediolateral axis parallel to the knee is broad in apes and round in humans. Following this logic, the proximal end might have the same effect.

Ohman and Lovejoy (2001) wrote “While connective tissue cells are acutely sensitive to mechanical stimuli, the morphological characteristics of an adult bone shaft are not a simple reflection of those stresses as is often assumed.” Rather, these authors proposed that diaphyseal shape merely reflects the shape and size of the closest epiphyseal plates. Long bone growth originates from secondary ossification centers located in epiphyseal plates. As an individual grows, bone cells accumulate and the epiphyseal plates are moved farther and farther apart (Martin et al., 1998). Ohman and Lovejoy (2001) propose that femoral midshaft diaphyseal proportions would reflect the shape of proximal or distal growth plates, rather than loads imposed upon it by variation in locomotor mode. Thus, diaphyseal form would not reflect an individual’s particular activity pattern.

The purpose of my research is to determine whether epiphysis form influences the shape of the femur midshaft in extant hominoids. To do this, I will test Ohman and Lovejoy’s hypothesis that femoral midshaft diaphyseal proportions reflect the shape of its proximal or distal growth plates and do not reflect loads imposed upon it by variation in locomotor mode.

Background

Bone diaphyses can change size, shape, and internal structure in relation to mechanical demands (Martin et al., 1998). Bone cells are thought to have the ability to react to strain by activating a mechanism known as modeling, which is a response to mechanical stimuli, especially where tension is highest. Most researchers argue that the shape of the shaft is determined by the outcome of bone modeling countering the effects of bending strain, which is highest at the middle of the shaft (Nordin, 1989; Frost, 1990a). Therefore, one should be able to infer habitual activity through analysis of midshaft external and internal structure.

Modeling takes place when the amount of bone increases or...
decreases in reaction to the amount of strain incurred during activity. Resorption occurs in places of disuse and formation occurs in areas suffering from increasing strain. The bone's internal makeup is modified by the varying load conditions and resorption and formation occur simultaneously. When muscles pull or weight-bearing places the bone in axial compression, there is tension on the convex surface and compression on the concave surface, also known as bending. This bending influences the bone shape. To resist tension and compression, bone models to accommodate bending stresses by increasing rigidity and strength. Thus, the shape of the midshaft, where strain is greatest, should reflect physiological adaptation to habitual bone strains (Martin et al., 1998).

Long bone growth occurs at growth plates located at the ends of the bone adjacent to the joints. The shape of joints, which is largely genetically determined, governs the shape of the epiphyseal plate (Frost, 1990b). Therefore it is possible that this imprint of the growth plate remains on the diaphysis as it is forming. This should be true primarily at the metaphyses, or ends of the bones closest to the epiphyseal plates, due to their proximity. The closer the bone is to its growth plate, the more influence over its shape the growth plate may have compared to the influences of mechanical stresses during growth (Ohman, 2001).

It is possible, and even likely, that both ontogeny and activity affect diaphyseal form (Frost, 1990a). There are many studies supporting activity-linked bone modification. For example, Ruff (1984) compared human groups with different subsistence patterns along the Georgia Coast. Femoral midshaft diaphyseal cross-sections were analyzed, and while the shapes were found to be quite similar, the distributions of bone types (i.e., trabecular and cortical) were indeed different in the midshaft. This suggests that different activities can affect the distribution of bone types because the stress patterns on the femur are different for each group. Additional studies following this contention are Jungers and Burr (1994), who discussed the use of long bone geometry to predict allometric scaling in quadrupedal monkeys, and Demes and Jungers (1993) that looked at cross-sectional proportions in long bones to study prosimian locomotor adaptations and body size.

While researchers continue to study behavioral changes of the skeleton, there are few testing the importance of the epiphyseal plates. The significant lack of research exploring the relationship between growth plate dimensions and midshaft shape (Woo 1981; Martin et al. 1998; Frost 1990).

**METHODOLOGY**

I conducted a comparison among species with different locomotor behaviors using external bone properties. This study does not include information concerning bending integrity, but only the differences between long bone endplates and the midshaft. Humans (Homo sapiens), chimpanzees (Pan troglodytes), and gorillas (Gorilla gorilla) femora from the Cleveland Museum of Natural History were measured. Sample sizes and sex distribution is listed in Table 1. Using sliding calipers, I recorded the maximum anteroposterior and mediolateral breadth measurements of the midshaft and the bicondylar mediolateral and inter-condylar anteroposterior breadths for the distal epiphysis estimation on all specimens. Additionally, neck length was estimated by averaging the superior and inferior breadths of the neck. Anteroposterior breadth of the neck was also recorded.

All specimens were judged to be adults based on epiphyseal closure and have no apparent pathological lesions. Data were tested on bivariate plots and significance was calculated following Sokal and Rohlf (1981). Correlations calculated are tested for significance at p < 0.05. Lines are plotted when the effect of X on Y is statistically significant.

![Table 1. Sex distribution of sample obtained from Cleveland Museum of Natural History](image)

<table>
<thead>
<tr>
<th>Species</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>17</td>
<td>17</td>
<td>34</td>
</tr>
<tr>
<td>Chimpanzee</td>
<td>17</td>
<td>17</td>
<td>34</td>
</tr>
<tr>
<td>Gorilla</td>
<td>16</td>
<td>17</td>
<td>33</td>
</tr>
</tbody>
</table>

**RESULTS**

Figures 1 and 2 compare the ratios of the estimate of the growth plate to the corresponding midshaft ratio. Mediolateral breadths were compared between the midshaft dimensions and neck length (Fig. 3) and distal plate (Fig. 4). The ratio of mediolateral and anteroposterior breadths (AP/ML) was calculated by dividing anteroposterior breadths by the mediolateral breadths.

The quotients have been plotted to conduct a comparison of the measurements recorded. The mediolateral breadth of the midshaft is used because it reflects the curvature developed during growth in response to mechanical stressors. Neck dimensions are used instead of proximal shaft measurements because the femoral neck is a reflection of joint shape, which determines endplate size and shape. These measurements are more indicative of diaphyseal change in response to mechanical stress and strain (Martin et al., 1998).

Figure 1 displays the distal epiphyseal shape against the midshaft shape. Given that no significant correlation is shown, the data do not support Ohman and Lovejoy's (2001) hypothesis that endplate shape determines midshaft shape. Figure 2 compares neck shape to midshaft shape. Again, given that no significance is found, the hypothesis stating that endplate shape reflects midshaft shape is not supported. Both Figures 1 and 2 illustrate that epiphyseal ratios do not affect midshaft proportions. Thus, my data do not support Ohman and Lovejoy's (2001) hypothesis.

Figure 3 plots neck length against mediolateral breadth of the midshaft. Here significance is found only in the gorilla sample. Humans and chimpanzees reveal no significance, which does not support the tested hypothesis. Figure 4 compares distal epiphysis mediolateral breadths to midshaft mediolateral breadths. Here significance is found among humans and gorillas. However, chimpanzees indicate no correlation, stating that distal endplate shape does not predict midshaft shape in this species.
Figures 3 and 4 compare the mediolateral breadth of the midshaft to the neck length and distal epiphysis mediolateral breadth, respectively. Again, according to Ohman’s and Lovejoy’s (2001) hypothesis, one should observe lines within each species indicating correlation. In these plots three lines are observed. However, their significance is considerably low. The gorilla significance line in Figure 3 has an R-squared value of only 0.4698. The gorilla significance line in Figure 4 has an R-squared value of 0.7035 (the highest recorded), while the chimpanzee line has an R-squared value of only 0.3447. True correlations can only be inferred at R-squared values at 0.8 and above (Sokal & Rohlf, 1981). So while, there are correlations observed, but their low values do not completely support the hypothesis.

**DISCUSSION**

The results of the comparisons presented here do not support the hypothesis that growth plates determine diaphyseal axis proportions in hominoids. Femoral midshaft shape does not appear to be determined by the geometry of either the proximal end of the bone or its distal epiphysis. Perhaps not surprisingly, the midshaft external measurements are smaller in overall size than their proximal and distal epiphyses, so clearly some modeling has occurred.

In fact, growth plate shapes are similar across taxa, although the midshaft proportions tend to vary. This can be seen in plots as each species’ cluster is near each other, but vary according to size. In Figure 3, even though chimpanzee femoral necks are shorter than human femoral necks, they share the same midshaft mediolateral shape. Chimpanzees totally overlap with humans in Figures 1 and 2, indicating no locomotor distinction in joint shape. Why is this observed? Perhaps this occurs because joint shape is genetically determined and the difference within each species varies more than any differences between species. Taxonomic and/or behavioral variables appear to influence midshaft shape more than growth plate form. Are the differences due to locomotion or other research areas such as body size or diet? Internal measurements will help understand the epiphyseal and diaphyseal relationship further. Distribution and thickness of bone indicates health and modeling experienced during the individual’s life history (Ruff, 1992). Consequently, future research will include increasing sample size and considering measures of actual femoral midshaft cross-sectional geometry that incorporate internal contours, such as computed-tomography (CT) scans.

**REFERENCE**


INTRODUCTION

As the human population continues to increase, food production must be increased to meet the growing demand. Projections suggest that demand will double over the next 30 years while available land and water continue to decrease (Ignacimuthu et al., 2000). It is estimated that 30% of crop losses are due to pests and diseases (Ignacimuthu et al., 2000). Fall armyworm has become a serious insect pest of corn. Originally, fall armyworm was thought to be a problem only in the southern United States. Recently, northern outbreaks have been occurring more frequently, as far north as Canada.

The fall armyworm hibernates in the southern coastal areas during the winter and migrates north to infest crops in the spring (Sparks, 1979). The average annual crop losses from fall armyworm alone in the United States exceeds $300 million, not to mention the additional cost of insecticides used to prevent damage by these pests (Mitchell, 1979). In addition to being costly and only partially effective, pesticides can be toxic to the consumer and have negative environmental impact such as accumulation in the soil and water. In addition, insects can build up resistance to insecticides over time (Ignacimuthu et al., 2000).

It would be most desirable if endogenous methods of insect control could be found. The diversity already present in maize is a potential source of resistance. Studies in many plant species indicate that some lines are more resistant to insects than others (Overman, 1989). One approach to further understanding of the molecular basis of naturally occurring resistance is comparative protein research among resistant and susceptible lines within a species. Two-dimensional isoelectric focusing (2D-IEF) gels are one method of comparative protein analysis. 2D-IEF gels analyze proteins by separating them by pH in one dimension and then separating them by molecular weight in the other dimension. This research will lead to the discovery of proteins associated with resistance. Development of plant lines with naturally occurring resistance to insects may be a useful alternative for pest control. Already several germplasm lines derived from tropical maize with increasing resistance to fall armyworm have been developed and released (Williams and Davis, 1984). Resistant plants have less damage and fall armyworm recovered from them are smaller than those recovered from susceptible plants (Williams et al., 1989A). Susceptibility leads to crop loss, which increases consumer prices.

All commercially grown non-transgenic maize hybrids are susceptible to fall armyworm. Resistance is found in tropical maize, which is not suitable for growth in the United States. Several quantitative trait loci (QTL) associated with resistance to whorl-stage feeding damage in maize have been identified (Davis et al., 1998). One QTL corresponds to the glossy15 gene (Davis, unpublished). Data from prior experiments indicates that juvenile leaves are more susceptible to insect damage including fall armyworm than adult leaves (Williams et al., 1998B). Juvenile leaves have a thick, bluish wax layer while adult leaves have a shiny, thin wax layer (Moose and Sisco, 1994). Each wax has its own chemical composition (Yang et al., 1993).
The primary purpose of this study is to discover protein differences that are associated with resistance or susceptibility in maize between M p705 (a resistant line) and H 828 (a susceptible line) through 2D - IEF gels. If the proteins that contribute to resistance in tropical maize can be found, then through cross-pollination the resistance can be transferred to commercially grown corn in the United States. Our long-term goal is to identify the molecular mechanism of these differences in resistance.

**LITERATURE REVIEW**

Plant resistance can be classified into three categories: nonpreference, antibiosis, and tolerance (Painter, 1951). Nonpreference occurs when a plant does not possess the desirable qualities for oviposition, food, shelter, etc (O Verman, 1989). Antibiosis denotes adverse effects to the insect when it eats the resistant variety (O Verman, 1989). Tolerance occurs when a plant can function well despite infestations that damage it (O Verman, 1989).

Transgenic plants containing insect-tolerant genes have revolutionized integrated pest management. When integrated with other control methods such as biological, mechanical, chemical, etc. transgenic technology may provide successful pest control (Ignacimuthu et al., 2000). Transgenic plants have a gene for the required trait that is introduced into the host plant through DNA transformation techniques (W Williams et al., 1998A). Insect susceptibility was one of the first problems addressed by transgenic technology. A diverse range of insecticidal proteins has been identified. The first insect resistant transgenic plants were based on δ-endotoxins, which are insecticidal crystal proteins from the soil bacterium, Bacillus thuringiensis (Ignacimuthu et al., 2000). Each Bt protein only affects a specific group of insects. The target insect oviposits normally on the transgenic plant. When the neonates hatch and feed they ingest the toxic Bt protein from the plant tissue. Within three hours of ingestion the midgut epithelium and osmotic balance is disrupted, leading to larval death (Ignacimuthu et al., 2000). Damage to the plant is prevented due to the early death of the insect.

Transgenic crops have become increasingly popular, however, major concern lies in their safety, toxicology, effects on non-target organisms, allergenicity, spread, and the potential for Bt resistant insects to develop (Ignacimuthu et al., 2000). Possible ways to avoid developing resistance to Bt in the insect population is to employ strategies such as gene pyramiding, optimum dosage, or monitoring (Ignacimuthu et al., 2000). In gene pyramiding several insect resistant genes are put into the host plant. This reduces the selection pressure from each individual gene on the insect and decreases the likelihood the insect will develop resistance to any of the genes. Other non-Bt insecticidal proteins are being investigated that could be used in conjunction with current Bt proteins to reduce the possibility of resistance developing. Many of these proteins interfere with the nutritional needs of the insect (Estruch et al., 1997). Examples of non-Bt proteins being evaluated are polyphenol oxidases, proteinase inhibitors, and amylase inhibitors (Estruch et al., 1997).

One natural defense mechanism that seems to have evolved in plants to guard against insects is the synthesis of proteinase inhibitors. These proteins target a wide range of insects with their antimetabolic activity (Lal and Lal, 1993). They are present in the tissues of plants at a relatively high concentration. The downfall to proteinase inhibitors is that they are needed in high levels in order to be effective (Lal and Lal, 1993). Practices such as recurrent selection, test crossing, and backcrossing have been used to increase the levels of natural resistance in maize (Smith, 1989).

Williams et al. (1998A) tested the effect of a Bt corn diet on corn earworm, fall army worm, and southwestern corn borer growth and survival. Of the three insects, fall armyworm was found to be the least susceptible to the Bt protein. Williams et al. (1998A) observed that the survival of fall armyworm on diets containing Bt protein was 35% lower than of the diets lacking Bt; those that did survive had reduced weight.

Fall armyworm, Spodoptera frugiperda, attacks more than 60 species of plants but prefers grass crops such as corn, sorghum, and fescue. Damage to the crop occurs during the insect's larval stage when the armyworm feeds on the foliage. The name armyworm came about because of the manner in which they travel from a depleted food source to another food source. During infestation, fall armyworms can strip a plant of its foliage in a single day. Armyworms continue to consume everything in their paths until they complete development and pupate. On average, fall armyworms complete maturation from an egg to an adult in four weeks. The female deposits eggs on the underside of leaves. Eggs are laid in clusters and covered by protective scales. Females may lay several hundred eggs, which will hatch in 2 to 4 days if the temperature is between 70 and 80 degrees Fahrenheit. When larvae hatch they devour the eggshells and the foliage until they have completed 6 instars and pupated. At this stage they drop to the ground and tunnel 1 to 3 inches into the soil where they spend 1 to 5 weeks pupating depending on temperature. When the armyworms emerge from the soil they cling to plants, inflate their wings and appear as adults (Sparks, 1979).

**MATERIALS & METHODS**

Twenty seeds of each inbred were planted in Columbia, MO during the summer of 2001. Standard cultural practices were followed for the fields in which they were planted. All of the field tests were conducted using a randomized block arrangement with two replications. Juvenile and adult leaf tissue was collected from each plant and stored in a -20 C freezer until ground to a fine powder using a mortar and pestle with liquid nitrogen. A modified version of Hurkman, W.J. and Tanaka, C.K. (1986) was used for protein extraction. Proteins were quantified using the BioRad DC protein assay kit.

IPG ReadyStrips, pH 5-8, 11cm from BioRad were used for the first dimension separations and Criterion PreCast 4-20%, Tris-H Cl, 1.0mm, IPG +1comb 11cm gels from BioRad were used to separate proteins according to manufacturers recommendations. A homemade mixture of known molecular weight proteins was used as a standard. The mixture contains 0.7mg trypsininh, 0.7mg carbonic anhydrase, 1.2mg egg albumin, 2.2mg bovine albumin, 0.5mg phosphorylase b,
0.5mg β-galactose, and 0.5mg myosin. The gels were silver stained according to the following procedure. First the gel was fixed with 40% ethanol and 12% acetic acid for 30 minutes. Then, each gel was washed with 50% ethanol three times for 10 minutes each. The gels were pretreated for a minute with 400ul of 10% sodium thiosulfate in 200mL of water. Then the gel was rinsed three times for 20 seconds each with water. The gels were impregnated with silver using 2mL of 20% silver nitrate, 37% formaldehyde, and 200mL of water for 20 minutes. Again, the gels were rinsed twice with water for 10 seconds each. Finally, they were allowed to develop until the spots appeared. The gel fixative was used to stop the developing. The gels were photographed with a Nikon CoolPix 990 camera and analyzed using Alpha Imager Software.

**RESULTS & CONCLUSION:**

Protein differences between the resistant and susceptible parents were identified. Protein differences were also identified between the juvenile and adult leaf tissues. Types of protein differences found include: presence and absence of proteins, changes in protein intensity, and protein modifications. Figure 1 shows a representative set of gels. A protein of approximately 75Kda is absent in M p705 adult but present in M p705 juvenile and O h28 adult tissues. The arrow indicates an example of presence vs. absence of a protein. Presence of this protein may confer susceptibility. The blue circle in Figure 1 indicates a protein intensity change among the tissue types. The right hand spot in the circle appears much more intensely in the M p705 juvenile tissues than the M p705 adult tissue and is in between in the O h28 adult tissue. The M p705 adult tissue appears to have some protein modifications compared to the M p705 juvenile and O h28 adult tissue (pink circle, Figure 1).

There appear to be 5 protein spots in the M p705 adult, in the M p705 juvenile there are only 2 spots and only 3 in O h28 adult. The large number of spots indicates that phosphorylation or post-translational modification of a protein has occurred.

We have identified protein differences between resistant and susceptible maize lines. As other morphological differences exist between the lines further studies are needed to confirm their association with resistance. In order to do this, the protein differences found between the parents will be compared to those found in the F3 derived progeny segregating for resistance and susceptibility of the M p705xO h28 cross. For example, a protein present in the resistant parent and also found in the more resistant progeny is absent in the susceptible parent and susceptible progeny then it is likely that this protein is associated with resistance. Once these putative resistance proteins are identified they will be cut out of the gel and sent to the protein core where they will be microsequenced and further characterized. Using the sequences the public databases can be searched in order to determine the gene identity. Inserting these genes, which are known to be associated with insect resistance into commercial maize would allow plant breeders to use less pesticides, saving them money and improving the quality of the environment.

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When *The Awakening* was originally published, it was not well received by the critics at the time. They were offended by Chopin’s open discussion of sensuality and described the novel as “sex fiction,” “disagreeable glimpses of sexuality,” “not a healthy book,” “an essentially vulgar story,” and “unwholesome in its influence” (Culley 161-178). Today *The Awakening* has a much different reputation. The novel is considered part of the canon of feminist literature. Emily Toth’s description of Kate Chopin reflects the way many people look at *The Awakening* and its author: “She was a pioneer in her own time, in her portrayal of women’s desires for independence and control of their own sexuality” (Toth, *Kate Chopin’s* xxii).

Many modern critics discuss *The Awakening* in terms of feminist theory. They describe the novel as one woman’s struggle to balance her desires and her traditional role in society. Jules Chametzky discusses Edna’s conflicting feelings between wanting to be a mother and wife and finding fulfillment as a person. When Edna becomes aware of her physical desires, she changes the way she sees her place in the domestic sphere. Her awareness of this contrast causes her to become a solitary soul (Chametzky 221-222). Lee R. Edwards also discusses *The Awakening* in terms of physical desire: “The plot [of *The Awakening*] demonstrates how the process of Edna’s waking into an awareness of her body’s life alters the psychic and social structures that orient her in the world” (Edwards 282). Edna is unable to reconcile her desires with her limited options in life, and thus chooses suicide. Other critics discuss how *The Awakening* shows the effects of women being treated as personal property. As Margit Stange explains, “Chopin’s dramatization of female self-ownership demonstrates the central importance of the ideology of woman’s value in exchange to contemporary notions of female selfhood” (Stange 204). Still other critics, such as Elaine Showalter and Sandra M. Gilbert, discuss *The Awakening* in terms of how it connects Edna’s sexual awakening with her intellectual awakening. Under such criticism *The Awakening* transforms from a shocking story of adultery to a woman’s quest for personal meaning.

It’s hard to know what Chopin would have made of the modern interpretations of *The Awakening*. As Elaine Showalter points out, Chopin never aligned openly with the suffragist’s movement (Showalter 177). She was never overtly political, though she transgressed the norm of womanhood with her eccentric modes of dress, her smoking habit, and her famous salon held in her own home in St. Louis (Toth, *Unveiling* 121-147). In the same way *The Awakening* is an unusual portrayal of a woman’s inner life, but it never overtly states any political agenda. One of the most fascinating and frustrating aspects of *The Awakening* is that Chopin never tells us what to think about Edna. She simply presents Edna’s changing inner life, without ever condoning or condemning the changes.

Similarly Chopin does not condone or condemn the act of adultery in *The Awakening*. As a result the adultery theme in the novel is not radical because it includes sexually explicit descriptions or because it presents a feminist agenda. Rather it is radical in that Edna Pontellier’s physical adultery is a reflection of her growing changes as a person. She is changing and testing the boundaries of who she is on many levels. The challenging and testing of the boundaries of a woman’s inner life through the...
theme of adultery is explored in several of Kate Chopin's short stories as well as in the more famous _The Awakening_. Despite the variations in the way that adultery is presented in these works, they are united in that they all attempt to show the complex inner lives that women are capable of living rather than concentrating directly on radically changing patriarchal institutions such as marriage.

When Kate Chopin created in Edna Pontellier a complex character who is neither a victim nor an evil seductress, she allowed Edna to be a character who strove to come to terms with the struggles in her inner life rather than being a character used only to illustrate a moral lesson. Edna cannot be easily labeled as either a force that perpetuates or destroys the institution of marriage. Instead she's a flawed (yet highly sensitive and remarkable) woman who can't make up her mind about what she should do with her life. Regardless of what Chopin really thought of Edna, she does a remarkable job of showing the reader the inner life of her character, which is filled with contradictions. Edna becomes more than a stereotype of what a woman can and cannot be.

Arguably the greatest accomplishment of _The Awakening_ is the open-ended portrayal of a woman's inner life. Even the ending of _The Awakening_ is open-ended. Some critics such as George Spangler find the conclusion very unsatisfying: “For in the final pages Edna is different and diminished: she is no longer purposeful, merely willful; no longer liberated, merely perverse, no longer justified, merely spiteful” (Spangler 211). Others have seen Edna's walk out into the sea as a triumphant moment for her because she finally transcends her existence. Her suicide becomes “the crowning glory of her development from the bewilderment which accompanied her early emancipation to the clarity with which she understands her own nature and the possibilities of her life as she decides to end it” (Seyersted, “Kate” 207). The different interpretations of the ending of the novel reflect the possibility for different interpretations of Edna herself. Chopin is more interested in showing Edna's inner turmoil than in expressing an opinion about her actions one way or the other.

Edna's inner turmoil is much more vividly portrayed than the physical manifestations of Edna's temptation. For example the passages in _The Awakening_ describing Edna's physical relationship with Alcée are surprisingly vague. This vagueness is evident in a description of the ending of one of their evenings together: “He did not say good night until she had become supplie to his gentle, seductive entreaties” (Chopin, _The Awakening_ 114). The description takes up only one line and is not explicit in the details of the relationship between Edna and Alcée. Edna's disloyalty to Léonce begins way before she actually physically betrays him with another man. The steps that lead to the physical adultery begin with her feelings about Robert and her vague dissatisfaction with her life. It is telling that the moment when Alcée physically tempts her, she does not feel remorse about hurting her husband. “The thought was passing vaguely through her mind, ‘What would he think? She did not mean her husband; she was thinking of Robert Lebrun’” (Chopin, _The Awakening_ 98). Edna had broken with her husband long before the trysts with Alcée that were so shocking for readers during Chopin's time.

The book also spends very little time actually overtly critiquing the position of women in Edna's society. Chopin makes an occasional comment about the limitations of a woman's life. Edna, for example, says, “I always feel so sorry for women who don't like to walk; they miss so much—so many rare little glimpses of life; and we women learn so little of life on the whole” (Chopin, _The Awakening_ 127). Still most of the episodes of the book deal more directly with Edna's changing inner world. One of the most vivid passages takes place in chapter six. It describes the vague emotions that Edna is experiencing: “A certain light was beginning to dawn dimly within her,—the light which, showing the way forbids” (Chopin, _The Awakening_ 31). During those kinds of moments Chopin presents Edna's thoughts, but does not try to interpret whether or not such thoughts are good or bad for Edna. They simply are her thoughts. Chopin shows that a woman can have a complex inner life, and that she doesn't have to be a caricature of a purely good or purely evil woman.

Some of Kate Chopin's short stories tend to work in similar ways as _The Awakening_ in that Chopin presents various women's personal reaction to the married state and the choices they then make, but without ever passing personal judgment on the woman presented. Kate Chopin wrote “Athénaïse,” “ _A Respectable Woman_,” “ _Her Letters_,” and “ _The Storm_” over a four-year period, dating at about the same time that she wrote _The Awakening_. Though none of these short stories delves as deeply into the inner life of the female characters as _The Awakening_, they do show women as having rich private lives in that for all four of the women the mental temptation to commit adultery is as important as whether or not they physically decide to commit it.

Adulterous temptation is certainly a key factor in both “ _Athénaïse_” and “ _A Respectable Woman_.” Both women in these stories appear to choose to stay faithful in their marriages, but the fact remains that both felt tempted to stray. They faced a moment when they had to decide if they wanted to seek out something outside of their marriages. Just as in _The Awakening_, Kate Chopin simply presents these moments, without sermonizing about or justifying the women's thoughts. For example, she explains the moment when Mrs. Baroda, the respectable woman, is tempted in these terms: “She wanted to draw close to him [Gouvenaire] and whisper against his cheek—she did not care what—as she might have done if she had not been a respectable woman” (Chopin, _Bayou_ 347). Though Chopin does tell us that Mrs. Baroda refrained from acting on her desires because she is a respectable woman, she gives no other indication of what we should think about Mrs. Baroda's desires. Even the line about her being a respectable woman has to make one pause. One would not expect a “respectable woman” during this time period to be thinking about whispering romantically suggestive words in the ear of a man who was not her husband.

This contradiction is what makes “ _A Respectable Woman_” so interesting. Mrs. Baroda is a respectable woman who may behave in respectable ways, but who is still capable of sensual feelings. She is explained in terms of being respectable and sensible. Her relationship with her husband is also respectable and sensible. They are a society couple on easy terms with each other. They entertain often and have a good relationship together. At one point in the story they are depicted as “making
a bit of toilet sociably together in Mrs. Baroda's dressing-room" (Chopin, Bayou 345). They appear on the surface to have a satisfying relationship, and yet for an entire year Mrs. Baroda struggles with her affections for another man, a man who has roused passion in her by reciting lines of poetry from Whitman. This passion is something that Mrs. Baroda has to work out alone, without the help of her husband who is supposed to be her primary source of companionship. She is well aware that this struggle is a private affair: "Besides being a respectable woman she was a very sensible one; and she knew there were some battles in life which a human being must fight alone" (Chopin, Bayou 347). Mrs. Baroda is aware and struggles with her own private emotions.

Gouvernail awakens Mrs. Baroda's private emotions in ways that are similar to the way Robert Lebrun awakens Edna. Both men stir passion in the wives of other men by offering alternatives to their marriages. Mrs. Baroda reacts to Gouvernail's sensitive manner just as Edna reacted to Robert's sensitive attention to her. Gouvernail's sensitivity awakens the more sensual side of Mrs. Baroda's personality: "Her [Mrs. Baroda] physical being was for the moment predominant. She was not thinking of his words, only drinking in the tones of his voice. She wanted to reach out her hand in the darkness and touch him with the sensitive tips of her fingers upon the face or the lips" (Chopin, Bayou 347). The difference between Edna and Mrs. Baroda is what they choose to do after the moment when they are awakened. Edna chooses the road of sensuality, while Mrs. Baroda appears to head back to the path of respectability. She goes away to avoid the influence of Gouvernail and then requests that her husband not invite him back.

A year later she seems to have conquered her affection. She tells her husband in the last line of the story: "I have overcome everything! You will see. This time I shall be very nice to him" (Chopin, Bayou 347). Here, Chopin gives us another ambiguous ending. She does not specify what Mrs. Baroda has overcome. The tender kiss she gives her husband suggests she has overcome her affection for Gouvernail and has come to a greater love for her husband. Or maybe she has overcome her respectable hesitations and is now ready to give herself up to sensuality. It does not really matter which version one chooses to believe because in either version Mrs. Baroda spent a whole year thinking about another man. While her husband thought he was the only man in her life, another man held a place in her consciousness. Despite this fact, the only opinion of Mrs. Baroda that Chopin gives us is that she is a "respectable woman." We are left unsure of what it is that Chopin means about being respectable. Perhaps it is meant ironically. Chopin wants us to understand that Mrs. Baroda is not respectable. Or maybe she wants us to see that even a respectable woman can have unrespectable thoughts. She can have an inner life that does not conform to the traditional social ideals.

In "Athénaïse" Chopin creates a character who is less concerned with retaining her status as a respectable woman and more with adjusting to married life. Athénaïse is a confused young girl who has had "deficient preparation for marriage" (Thomas 207). Her family is not too concerned about preparing her because they feel that marriage will make a woman of her. It does not occur to them that perhaps Athénaïse is not ready. As a result, she is unhappy in her marriage. She is not unhappy because her husband is cruel or unloving. On the contrary, he cares very much about her though he does not understand her. She just hates being married. As she says in her own words, "It's just being married that I detest an' despise. I hate being Mrs. Cazeau, an' would want to be Athénaïse Miché again. I can't stan' to live with a man!" (Chopin, Bayou 197). It's not the actual day-to-day state of marriage that she is unhappy about. She is unhappy with the idea of being married, the idea of belonging to another. She yearns on an intellectual level for something different.

When Athénaïse meets Gouvernail, the very same character who tempted Mrs. Baroda, she has already moved away emotionally and mentally from her marriage with Cazeau. Now she is tempted to stray physically with another man. Though Chopin never directly asserts that Athénaïse has romantic feelings for Gouvernail, the implications are there. She constantly seeks him out while they are staying at the same hotel. We know that Gouvernail is interested in Athénaïse, but restrains from acting on his affections right away. One night when she is homesick, she is the one who puts her arms around Gouvernail and cries on his shoulders. She kisses him on the neck: "She had to love someone in her own way" (Chopin, Bayou 218). She is the one acting aggressively towards Gouvernail, seeking him out and initiating physical contact. Her aggression gives at least a suggestion of romantic feelings. Chopin then tells us that Athénaïse feels that "Henceforward she would not be lonely and unhappy, with Gouvernail there to comfort her" (Chopin, Bayou 218).

Perhaps Gouvernail is just a surrogate for Cazeau until she realizes that it is really her husband that she loves. The fact that she does return to Cazeau at the end of the story seems to suggest that this is the case. The question that arises though is whether or not Athénaïse really has had a change of heart. Possibly she just decides to give in to the pressure of her family and friends. She then makes the decision to be happy as a wife. Martha Cutter describes the story in these terms: "Chopin depicts a woman who indoctrinates herself into the 'cult of domesticity,' foregoing her earlier attempts to use language as an instrument of resistance" (Cutter 22). When Athénaïse becomes pregnant, she gives up the language of resistance and gives in to the language of domesticity. Athénaïse's discovery of her pregnancy takes place almost immediately after the scene where she puts her arms around Gouvernail. Before she is even able to think of pursuing a relationship with Gouvernail, she finds out she is pregnant. Just as Edna's children become a burden in The Awakening, Athénaïse's pregnancy strengthens the hold her marriage has on her. While in such a vulnerable state, she could hardly have terminated one marriage in the hopes that another man would take care of her.

Athénaïse does seem to take the news of her pregnancy happily and goes back to Cazeau filled with love, but perhaps it is a false happiness. If Athénaïse has been fully unprepared for marriage, it hardly seems possible that she has been prepared for motherhood. If she thinks marriage restricts her freedom, how will she feel when her children demand all of her time? Chopin's story leaves that question unanswered. Chopin here presents a character who appears to return joyfully to her marriage, but the fact still remains that Athénaïse did not naturally adapt herself to the mental and emotional state of marriage. Whatever Athénaïse's reasons are for going back, she is the one who made that decision. She has made the decision by herself (perhaps
reluctantly) to go back and start again.

In these two short stories Kate Chopin remains within the social frames that the readers of her local color tales would have expected. The stories end with passionate kisses between husbands and wives, seeming to reinforce the social rules. Yet more is going on in these two stories than affirmations of marriage. They are examples of what Cutter would call the “voice couvert.” She explains it as that “which (at its most effective) undermines the patriarchy from within its own paradigms” (Cutter 18). Chopin remains within the patriarchal institution of marriage, and yet manages to bring it into doubt by showing women whose inner lives reveal that they have desires and ideas that extend beyond just housekeeping. Chopin does not explicitly show adultery in these two stories, but the mere hints of the possibility show these two women as being both passionate and capable of making decisions about what to do with that passion.

While “A Respectable Woman” and “Athénaïse” portray women who chose not to commit adultery, both “Her Letters” and “The Storm” are similar to The Awakening in that they do explicitly deal with adultery. They differ from The Awakening in that both feature women who ultimately go back to their husbands, but do not regret the adulterous affairs. In this way they are as controversial or more so than The Awakening because the two stories feature women who are not destroyed by their adulterous affairs. Instead the affairs seem in some ways to enhance the lives of both women. In part of Martha Cutter’s article, she discusses some of Kate Chopin’s later works, which include both “Her Letters” and “The Storm.” She says, “In some of Chopin’s later works, however—particularly those written during or after 1894—she moves towards depicting women who are more active and more vocal” (Cutter 17). Both of these stories are examples of what Cutter would consider more active and vocal women because they challenge the institution of marriage more directly, though not only in terms of language as Cutter explores in her article, but also in terms of the inner lives that they lead. These women both decide to act outside the bounds of traditional marriage because they have desires and feelings that they feel compelled to explore.

By presenting a woman who loves her husband, but who commits adultery, Chopin shows in “Her Letters” a woman who has an emotional life that is separate from her husband’s world. She is afraid that after her husband dies she will find the letters from her adulterous affair and will be crushed by them. And yet she cannot bear to destroy them. The letters, a symbol of the passion she felt for the other man, become her comfort during her illness. As the narrator tells us, “Whit unbounded thankfulness she [the wife] felt at not having destroyed them [the letters] all! How desolate and empty would have been her remaining days without them” (Chopin, A Vocation 96). She takes every chance she can to look over them and remember the past.

Many critics, including Martha Cutter, have looked at the way the letters affected the woman’s husband. They argue that the idea of the letters is so painful for the husband, not just because they represent a possible adulterous affair, but because they suggest that his wife had a separate life that he had no part of. Martha Cutter explains the letters in this way: “Covert texts such as these letters take away the pedestal for the existence of the subject by suggesting that men do not really know the women they marry, live with, and believe they construct” (Cutter 27). The letters force the husband to look at his wife in terms that do not include him, and he has difficulties coming to grips with this idea. This idea is key to understanding the story, but not just because the letters represented the wife’s separate identity to the husband.

The letters also reveal this separate identity to the reader. The story becomes a more open challenge to the institution of marriage because it directly shows a woman who has an inner life that is separated from the life of her husband. When she needs comfort during her illness, she does not go to her husband, the choice that generally would be expected of a wife. She has shared her life with this man. Surely he would be the best source of comfort. Instead she goes to the letters because they provide memories of an experience she never shared with her husband. In fact the letters even become a sort of substitute for the man she committed adultery with. Long after the physical affair has ended she continues to live out the adultery through the letters. Her rather sensual interactions with the letters themselves prove this point: “She crushed it [the letter] between her palms when she found it. She kissed it again and again. With her sharp white teeth she tore the far corner from the letter, where the name was written; she bit the torn scrap and tasted it between her lips and upon her tongue like some god-given morsel” (Chopin, A Vocation 96). She may have ceased the physical part of the affair, but in her mind the affair is not over.

Kate Chopin presents a woman who ends the physical side of her adulterous affair and worries about the effects on her husband, but she also presents a woman who never regrets ever actually having the affair. She does not express guilt when she thinks of the effect this other man had on her: “This man had changed the water in her veins to wine, whose taste had brought delirium to both of them” (Chopin, A Vocation 96). In the end Chopin shows the harsh effects the mysterious letters have on the wife’s husband, but she never gives any forthright condemnation of the wife’s actions. Unlike in The Awakening, we see a woman who chooses her husband. Also unlike The Awakening, we see a woman who feels little confusion or guilt about her adulterous affair. Perhaps one can see this lack of guilt as a lack of morals, but one can also see the wife in “Her Letters” as a woman with the strength of character to face what she has done and to make sense of her actions so that she can deal with them. She may not have found peace exactly, but she had found a way in her mind to cherish the memory of the adultery and to stay loyal to her husband.

“The Storm” also features a wife who does not feel any guilt about her adulterous affair. In this story the reader is presented Calixta, a woman who is able to go back to her marriage refreshed after her adulterous affair. At the end of the story she greets the return of her husband and child with genuine happiness for the first time in a long time. “Bobinot’s [her husband] explanations and apologies which he had been composing all along the way, died on his lips as Calixta felt him to see if he were dry, and seemed to express nothing but satisfaction at their safe return” (Seyersted, Complete 596). She too cares for her husband, but she has always carried a passion for this other man. They were kept apart though because of class differences. After their passion erupts, she no longer is tortured...
by the memories of this other man. She now knows what it is like to be with this other man and can go back to her husband feeling real affection again.

The story itself has a much more graphic love scene than what was depicted in The Awakening. Chopin did not even try to get "The Storm" published during her lifetime because of the lovemaking scene in the story (Toth, Unveiling 206). She describes to the reader in surprising detail the lovemaking that occurs between Calixta and Alcée (who is a different character from the infamous Alcée in The Awakening) as the storm rages on outside: "They did not heed the crashing torrents, and the roar of the elements made her laugh as she lay in his arms. She was a revelation in that dim, mysterious chamber; as white as the couch she lay upon" (Seyersted, Complete 594-595). This scene is a reflection of Chopin's growing interest in pushing the boundaries of what was proper subject matter for literature.

Still despite the more graphic love scene, "The Storm" is still interested in emphasizing a woman's inner life through the strong portrait of Calixta, the main female character. She is the one who proposes to her husband at the end of "At the Cadian Ball" to which "The Storm" is the sequel: "You been sayin' all along you want to marry me, Bobinôt. Well, if you want, yet, I don' care, me" (Chopin, Bayou 150). Then she closes the deal in an assertive manner, holding "out her hand in the business-like manner of a man who clinches a bargain with a hand-clasp" (Chopin, Bayou 150). Though she is not the one who initiates the love scene in "The Storm," she is a willing and eager participant. At the end of the story she does not struggle with feelings of guilt. Chopin shows that Calixta is a strong woman who is capable of making her own decisions. Alcée does not seduce her, and neither is she stuck in a loveless marriage that was not her choice. Whatever the outcomes of her decisions are, she was the one who made them.

As with The Awakening, critics differ about the ending of "The Storm." They disagree over whether or not they think the adulterous affair really results in greater happiness for all involved. Lawrence Berkove feels that Kate Chopin meant for the happy ending at the end to be taken ironically. He believes that Kate Chopin implied that it was a false happiness because the adultery would eventually bring harsh consequences for everyone (Berkove 194). In contrast Per Seyersted describes the adulterous meeting in "The Storm" as one that was filled with joy as they pursued s*x pur (Seyersted, "The Storm" 145). The very fact that the ending of the story can produce such different interpretations from critics shows that Chopin did not make it clear which way she wanted readers to interpret the story. "The Storm" may be most famous for its open depiction of sensuality, but it also has to be looked at in terms of its open portrayal of a woman who made the decision to marry one man while still being emotionally and physically attracted to another man and the results that may occur because of this decision.

"The Storm" seems more controversial than a story like "Athénaïse," but ultimately both are vital examples of Chopin's style. They both reveal the capabilities of women to think and feel outside of the bounds of marriage. Looking at short stories like these alongside The Awakening can help give greater understanding of both. Though originally her short stories met with much better success than her novel because they were seen strictly as local color stories, they still explore some of the same ideas that Chopin explored in greater depth in The Awakening. The theme of adultery is crucial in both The Awakening and these four short stories. The physical acts of adultery though are not what are important. What is important is that the temptation of adultery suggests that these women all were capable of complex emotions and desires. Chopin was more interested in showing the complexities of women than in offering a direct challenge to the institution of marriage. Jane Gallop explains that "Infidelity is not outside the system of marriage, the symbolic, patriarchal, but hollows it out, ruins it, from within" (Cutter 18). Whatever Chopin's political views were, she knew and was not afraid to show that women had rich inner lives that had to be explored, not in terms of their moral implications, but as reflection of the lives of real and diverse women.

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FEATURED SCHOLARS

STARLA IVEY, PHD

As a first generation college student, my parents provided me with love and a lot of support; however, they were unaware of many of the situations that I would face. I found myself alone in the effort of searching for answers about my love for learning and higher education. With no immediate family members having obtained college degrees, the McNair program provided the direction that I needed. I was able to form relationships with peers and faculty members who had similar goals and interests. McNair also gave me the exposure and experience of presenting my research at conferences. My presentations were praised for using cutting edge technology that other programs had not prepared their students to utilize.

My McNair faculty mentor, Dr. Craig Israelsen, was genuinely concerned about my academic progress, me as an individual and keeping a balanced life. He continued his support by serving as one of my committee members throughout my doctoral program.

In May 2002, I received a PhD in Career and Technical Education from the University of Missouri - Columbia. I am currently employed with Academic Retention Services here at the MU, where I assist in the academic pursuits of other students like myself. I hope that I can be a positive influence on them and encourage them to pursue academic excellence. Thank you, McNair, for showing me how to achieve academic success so that now I can help someone else.

DANA DUREN, PHD

I was awarded an internship from the Ronald E. McNair Post-baccalaureate Achievement Program in 1992 while attending the University of Missouri. The program offered me the opportunity to design my own research project under the supervision of a gifted faculty mentor, Dr. Carol V. Ward, in the Department of Anthropology. The McNair program provided me with several career-shaping opportunities in my chosen field of study. First, I was able to travel to the Cleveland Museum of Natural History to gather data for my McNair research project. While in Cleveland I met and worked with established scholars from around the world. Second, the McNair Program sent me to the annual meeting of the American Association of Physical Anthropologists, where I was able to reinforce academic relationships with several scientists I had met in Cleveland, as well as network with other anthropologists. These opportunities reinforced my commitment to pursue an advanced degree in the human biological sciences.

Having said that, I believe that the most important aspect of my McNair experience was the faculty mentor. Having the opportunity to work with Dr. Ward on a specific research topic prepared me for interactions with other scholars. She always treated me as an equal and challenged me with questions regarding the methodology, results, and implications of my project. I have come to appreciate these interactions because they prepared me so well for graduate school, and have benefited me as I develop my career as a scientist.

During my fellowship year, the staff of the MU McNair Program offered both encouragement and support in every aspect of the program. In many ways my McNair experience continued long after my fellowship ended. The research that I conducted as a McNair scholar formed the basis for my master’s thesis, and later the same principles developed into my dissertation at Kent State University.

I received my Ph.D. in 2001, one of my personal goals and the goal of the McNair Program. I am very proud to have been a McNair scholar, and believe that my graduate career was strengthened because of my experience in the McNair Program. I am currently a Post-doctoral Research Fellow at Wright State University School of Medicine, and study growth, development, and aging of the human skeleton in the world’s largest and longest-running study of human development, the Fels Longitudinal Study.
### 2001-2002 Research Topics

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2001-2002 McNair Scholars

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Fourth row: Havis Wright, Robert Kimes, Troy Johnson, Alias Smith
Third row: Robert Mahon, Kimberly Moore, Thierra Nalley, Justin Phillips, Jarrett Dickerson
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