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<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collector's Relationship to Access-Based Consumption: A Sneakerhead's Perspective</td>
<td>2</td>
</tr>
<tr>
<td>by Naseem Adkinson-Jobe and Stephanie Lawson, Ph.D. (Mentor)</td>
<td></td>
</tr>
<tr>
<td>Business Sustainability: An Analysis of Three Enterprises in Charlotte, North Carolina</td>
<td>8</td>
</tr>
<tr>
<td>by Cera Crowe and Padmini Patwardhan, Ph.D. (Mentor)</td>
<td></td>
</tr>
<tr>
<td>Evaluation of the Potential HMGA1-EF24 Nexus in Human Colon Cancer</td>
<td>20</td>
</tr>
<tr>
<td>by Madeline Diaz and Takita Sumter, Ph.D. (Mentor)</td>
<td></td>
</tr>
<tr>
<td>“I Don’t Fit in a Box; No One Does:” Intersectionality and Gay Male Identity</td>
<td>26</td>
</tr>
<tr>
<td>by Jesse Grainger and Brent Cagle, Ph.D. (Mentor)</td>
<td></td>
</tr>
<tr>
<td>Controlling Oct4 Expression Levels Using Invitrogen's GeneSwitch™ System</td>
<td>39</td>
</tr>
<tr>
<td>by Autumn Leggins; Nicholas Grossoehme, Ph.D. (Mentor);</td>
<td></td>
</tr>
<tr>
<td>and Matthew Stern, Ph.D. (Mentor)</td>
<td></td>
</tr>
<tr>
<td>Targeted Mutagenesis of FOX0 Transcripts Using the Model Organism Ciona intestinalis Utilizing the CRISPR/Cas9 System</td>
<td>45</td>
</tr>
<tr>
<td>by Nicole McMullen and Heather J. Evans-Anderson, Ph.D. (Mentor)</td>
<td></td>
</tr>
<tr>
<td>Mindfulness, Coping, and Psychological Well-being Among Diverse Populations</td>
<td>60</td>
</tr>
<tr>
<td>by Lesley Peña and Darren Ritzer, Ph.D. (Mentor)</td>
<td></td>
</tr>
<tr>
<td>Examining Longitudinal Data of Juvenile Delinquents in Rock Hill, SC</td>
<td>69</td>
</tr>
<tr>
<td>by Jalen Smith and Brad Tripp, Ph.D. (Mentor)</td>
<td></td>
</tr>
<tr>
<td>Contemporary Black Women Artists’ Narratives</td>
<td>78</td>
</tr>
<tr>
<td>by Shannon Snelgrove and Laura Gardner, Ph.D. (Mentor)</td>
<td></td>
</tr>
<tr>
<td>Investigating X chromosome non-disjunction in Drosophila melanogaster su(var)3-9 mutants</td>
<td>81</td>
</tr>
<tr>
<td>by Camerun Washington and Kathryn Kohl, Ph.D. (Mentor)</td>
<td></td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>87</td>
</tr>
</tbody>
</table>
Collector’s Relationship to Access-Based Consumption: A Sneakerhead’s Perspective

Naseem Adkinson-Jobe
Stephanie Lawson, Ph.D. (Mentor)

ABSTRACT
This research focuses on what persuades sneakerheads to partake in access-based consumption. This research is important because the relationship between collectors, particularly sneakerheads, and access-based consumption has not yet been explored. This topic was studied by conducting semi-structured interviews with sneakerheads, to find themes and correlations that depict motives for consumers to participate in access-based consumption. As well as motives, this topic was studied to discover deterrents that may drive sneakerheads away from renting their sneakers. These findings will contribute something new to the literature based upon access-based consumption, because currently there is no literature that depicts what motivates or discourages collectors/sneakerheads to become access-based consumers. To extract those findings, semi-structured interviews were conducted. The sample was a convenient sample from a Charlotte, N.C. mall. After conducting the interviews, it was found that saving money was a major motive for sneakerheads to rent their sneakers. Other than economic benefits, it was also found that for one-time use and status seeking purposes sneakerheads are motivated to rent their sneakers. Deterrents such as anxiety, hygiene, possessiveness, and sneaker condition were all discouragements that drove sneakerheads away from renting their sneakers. Within this study, there is also a proposed plan of how a sneaker-renting platform could benefit the consumer and the market simultaneously.

INTRODUCTION
There has been recent change in the state of mind of consumers, where they would rather gain access to a product without ownership than purchasing the product with ownership. This change has persuaded consumers to seek experiential purchases rather than possessive purchases. Most of these experiential purchases are sought through collaborative consumption, which according to Belk (2014) is “people coordinating the acquisition and distribution of a resource for a fee or other compensation” (p.1597). The particular type of collaborative consumption used in the aforementioned sentence is access-based consumption, which is “defined as transactions that can be market mediated but where no transfer of ownership takes place” (Bardhi & Eckhardt, 2012, p.3). Since access-based consumption is a form of collaborative consumption, it can be challenging to distinguish one from the other. For example, Flight Car, a peer-rental car company that coordinates traveling-consumers to rent their vehicles to one other, partakes in collaborative consumption, while on the hand Zip Car, a car rental company that charges consumers on the basis of car usage but not for maintenance, gasoline money, or insurance, partakes in access-based consumption. Although these car rental companies operate differently from each other, they have both helped the economy by saving consumers money and have helped the environment by reducing the amount of carbon dioxide emissions emitting vehicles on the highways. Research has found many motives for consumers participating in access-based consumption. Lawson (2011) has found that freedom from ownership, variety seeking, value seeking, status seeking, environmentalism, and risk avoidance motivates consumers to participate in non-ownership consumption. Variety seeking becomes a motive when a consumer desires to have multiple products at once or has many options to choose from, while on the other hand, value seeking becomes a motive when a consumer evaluates their options based upon price and quality. Risk avoidance
was not only found by Lawson (2011) to be a motive for access-based consumption, but was also considered in research conducted by other business researchers (Lawson, Gleim, Perren, & Hwang, 2016). Lawson et al. (2016) considered risk avoidance as motivation, when they explained how access-based consumption allows consumers to try a product before committing to owning the product. Depicting choice confusion as the origin of risk avoidance, Lawson et al. (2016) postulated that “consumers are likely to prefer to try a product when they have choice confusion as it reduces their risk, thus leading to positive perceptions of access-based consumption” (p.7). Furthermore, Lawson et al. (2016) included economic benefits, such as saving money from renting rather than buying, as a motive for access-based consumption. Economic benefits are classified as extrinsic motives for consumers to involve themselves in access-based consumption (Hamari, Sjöklint, & Ukkonen, 2015). Receiving more value for less cost is the most common economic benefit sought, when consumers decide to indulge in access-based consumption (Tussyadiah, 2015). For example, if a consumer plans to use a tool once, he/she can rent the tool from a hardware store for $30 a day rather than buying the tool for $400, therefore saving the consumer $370. Lawson’s (2011) suggestion that simplified maintenance is a motive for access-based consumption can be correlated to why some people rent their car from the access-based car rental company Zipcar, which provides insurance, maintenance, and gasoline money to their car renters. Flexibility is another driver that pushes consumers towards access-based consumption, because an access-based business model allows consumers to change-out their merchandise whenever they become bored with their current-rented good (Edbring, Lehner, & Mont, 2015). Most consumers, who are driven by flexibility to participate in access-based consumption, favor products that have fast innovative cycles, for the reason that when these products are upgraded, the consumer can upgrade without facing divestiture or spending excess money (Edbring et al., 2015, p.10). Similar to flexibility, trend affinity is a motive for chic-consumers to take part in access-based consumption, because access-based consumption allows them to keep up with the new trend without going bankrupt (Möhlmann, 2015). As our population is becoming more cognizant of the limited resources that fuel our market, some consumers are persuaded to indulge in access-based consumption because of an environmentalist mind state (Baumeister, 2014). Moreover, environmentalism has persuaded many consumers to become political consumerists and only shop in a way that will enable them to cause desired political and environmental outcomes; therefore, political consumerism has been a recent motive for consumers to involve themselves in access-based consumption (Catulli et al., 2013, p.9). Except for major drivers for access-based consumption, such as environmentalism, economic benefits, flexibility, variety seeking, or political consumerism, there are some consumers who switch over to access-based consumption just based upon enjoyment (Teubner, 2014). This enjoyment stems from the fact that these consumers are participating in a business model that is fairly new, which allows them to become a trend setter amongst fellow consumers.

Other than access-based consumption and the motives for access-based consumption, literature about collections and how they relate to identity was collected, because the goal of this research is to figure out a method to persuade collectors to partake in access-based consumption. Whether people purposely or mistakenly do it, people look at their possessions as part of themselves (Belk, 1988, p.139). In addition, Belk (1988) implies that a person usually starts to regard their possessions as part of themselves, when they become able to exercise power over those possessions. For instance, when a consumer first buys a fast car, such as a Lamborghini, he/she may consider himself/herself as someone who has a nice car; however, once the consumer is experienced at maneuvering their Lamborghini at high speeds, he/she may start to consider himself/herself as an expert Lamborghini driver. Belk’s (1988) statement, “the more we believe we possess or are possessed by an object, the more a part of self it becomes” (p.141), further elucidates the
aforementioned example. Belk (1988) suggested that even though one may not have much control of the world, they can gain a sense of power via having control over their collections. After an unintentional loss of a possession, one may feel as if they have lost a part of themselves; not only because they lost a belonging, but because that possession was perceived by them as part of their extended self (Belk, 1988, p.142). Other than unintentional loss of a possession, when one recognizes a possession of theirs to no longer be compatible with their identity, they are likely to discard the possession (Belk, 1988, p.143). For example, when a child is becoming an adolescent, they are likely to abandon their toys that they now deem to be “childish.” Ahuvia (2005) further expanded Belk’s (1988) research, when he concluded in his research that “loved items were connected to the self both by expressing the self and by transforming the self into some new desired form” (p.180). Although Ahuvia (2005) expanded the research conducted by Belk (1988), he rejects Belk’s (1988) notion that our possessions reflect our extended self and proposes that our possessions are a reflection of our inner self. Throughout his research Ahuvia (2005) depicts how possessions are used to resolve identity conflicts. For example, Ahuvia (2005) interviewed a woman named Cindy, who was having an identity conflict between her rural background and her current metropolitan life. Cindy resolved this identity conflict by collecting antiques, which not only fulfilled her yearning for her past rural life, but also helped her assimilate to her present metropolitan life because antiques are seen as status markers in metropolitan areas.

So far the research found on access-based consumption, motives for access-based consumption, possessions, and the relation possessions have with identity have been discussed, because the intent of this research is to figure out a method to persuade collectors, sneakerheads in particular, to become access-based consumers.

METHODOLOGY

Data was extracted via a qualitative study, where semi-structured interviews were conducted. The semi-structured interviews took place on a Saturday at a local mall in the Greater Charlotte area, for the fact that this location and time was the most appropriate to find sneakerhead-interviewees. The purpose of these semi-structured interviews were to assess how sneakerheads feel about access-based consumption, what motivates or discourages them to participate in access-based consumption, and what they like or dislike about the current sneaker market. During the interviews, interviewees were asked about their sneaker collection like how do they store their sneakers, how do they get rid of unwanted sneakers, and what brands are they most loyal to. Interviewees were also questioned on how they became sneakerheads, so I could better understand the origins of “sneakerhead culture.” After the interviews were conducted, they were analyzed for finding the measures that drive sneakerheads to rent their sneakers and the measures that deter sneakerheads away from renting their sneakers.

RESULTS

After conducting five interviews with sneakerheads, I started to gain insight on the motives that would drive a sneakerhead to rent a pair of sneakers and the discouragements that deter sneakerheads from renting a pair of sneakers. Just as Lawson et al. (2016) inferred, the economic benefit of renting their sneakers was a reoccurring motive interviewees said would influence them to rent a pair of sneakers. As seen in Graph 1 (see Appendix), the economic benefit of renting sneakers made up forty percent of the motives. One interviewee even gave an example where a consumer, who may be saving money for a certain pair of sneakers and does not have sufficient funds to purchase the sneakers, may be interested in renting those pair of sneakers as a substitute. Another interviewee implied that since most young sneakerheads do not purchase their sneakers, rather their parents, parents of young sneakerheads may be interested in renting sneakers to save money. Ranking next to the economic benefits of renting sneakers, the one-time use benefit of renting sneakers also comprised forty percent of motives in Graph 1.
This motive is derived from sneakerheads who are trendy and purchase the latest sneaker release every other weekend. These types of sneakerheads differ from other sneakerheads, where they are experiential with sneakers rather than possessive. Therefore, they are not seeking to collect sneakers but to keep up with the trend of sneakers that are released nearly every weekend. Keeping up with the trend can be costly and consume a lot of closet space, thus renting a new sneaker weekly can clear storage and prevent spending large amounts of money. Most of the time trendy sneakerheads try to sell their sneakers to possessive or laggard-trendy sneakerheads via second-market to regain funds lost from purchasing those sneakers and reinvest those funds into a new pair of sneakers. As you can see, trendy sneakerheads’ consumer behavior can be appropriately accommodated if they can rent sneakers and return them at their leisure. Other than the economic and one-time use benefit of renting sneakers, status seeking was also a motive for renting sneakers. As can be noticed in Graph 1 (see Appendix), status seeking shared twenty percent of the motives. Status seeking is a motive for sneakerheads who like to differentiate themselves from the average sneakerhead with sneakers that expensively cost more than the average sneaker. For instance, a sneakerhead who is trying to seek status, may purchase a pair of $800-$900 Giuseppe sneakers compared to a pair of $150-$200 Nike sneakers. By renting their sneakers, instead of purchasing them, status seeking sneakerheads can still receive their sought status without actually being a member of “high society.” As stated before, Lawson (2011) supports the fact that status seeking can be a motive for consumers to participate in access-based consumption.

Just as I needed to know the motives that would drive sneakerheads to rent their sneakers in order to better understand the relationship between sneakerheads and access-based consumption, I also needed to know the deterrents that discourage sneakerheads to rent their sneakers. Making up forty percent of the discouragements in Graph 2 (see Appendix), anxiety was a key deterrent driving sneakerheads away from renting their sneaker. Majority of the interviewees said anxiety would arise from the thought of having to return the sneaker. The thought of returning the pair of sneakers evoked anxiety in interviewees because they would most likely be more mindful of where and how they are walking to avoid any scuffs that may cause surcharges once the rental period is over. This type of anxiety would distract sneakerheads throughout the day and become very mind boggling. Hygiene and sneaker condition were similar deterrents that each comprised twenty percent of discouragements (see Appendix). Hygiene was a deterrent because some interviewees were worried that the renter before them would have bad hygiene and propose the probability to infect them with a bacterial infection such as MRSA. Other than the interior of the sneaker, the condition of the sneaker’s exterior worried the interviewees. Some interviewees were worried that the renter before them would have poor shoe maintenance and were going to cause them to rent a shoe that is not in appropriate condition. Although most of the deterrents have negative connotations, possessiveness was a discouragement of interviewees who were proponents of the idea of renting sneakers, but would rather purchase their sneakers to keep. Just as hygiene and sneaker condition, possessiveness comprised twenty percent of the deterrents in Graph 2 (see Appendix).

IMPLICATIONS & DISCUSSION

So far, I have been discussing the research I have found on access-based consumption, motives for access-based consumption, possessions, and the relation possessions have with identity; however, the main purpose of my literature review is to explain how I intend to use my research to figure out a method to persuade collectors, sneakerheads in particular, to become access-based consumers. After learning from Lawson (2011) that environmentalism is a major motive for access-based consumption, I plan to create an access-based business platform that advertises the environmental benefits of renting your sneakers rather than buying them. Such environmental benefits would be less cardboard disposal, which decreases deforestation, because
renting sneakers would not require buying a box. Other than lessening deforestation, by consumers renting their sneakers, they could help lessen the 30 pounds of carbon dioxide emissions a typical pair of sneakers generates during the manufacturing process (Chu, 2013). The aforementioned type of advertisements would enable consumers to help the earth become more sustainable, without completely going “green.” Other than environmentalism, value seeking is another access-based consumption motive I learned from Lawson (2011) that I would fulfill with my theorized access-based business platform. Value seeking would be fulfilled by offering consumers access to $250 sneakers for a low weekly fee around the price mark of $50. Moreover, my theorized business platform would alleviate the burden on divestiture for consumers, because they would be allowed to return the rented sneakers whenever they are no longer interested in wearing that particular pair of sneakers. Except learning motives for access-based consumption from Lawson (2011), I have also learned from Ahuvia’s (2005) research that most people desire an object because of competition with another person who also desires the same object (p.180). This lesson learned from Ahuvia (2005) has brought me to the realization why sneakerheads campout for hours each Saturday: sneakerheads are competing for desires. My theorized access-based platform allows sneakerheads who rent their sneakers, to access their sneakers quicker than sneakerheads who buy their sneakers, because they will not have to campout hoping they will be able to receive a pair of sneakers. All in all, my research contributes to the current research on access-based consumption, for the fact that it is dealing with materialism and Lawson et al. (2016) supposed that “more research is needed to understand the relationship between access-based consumption and materialism” (p.20).

CONCLUSION

From this research, it can be observed that although motives such as status-seeking, economic benefits, and one-time use may persuade sneakerheads to rent their sneakers, discouragements such as anxiety, possessiveness, hygiene, and sneaker condition may deter sneakerheads from renting their sneakers. These findings concur the research that predates this research, such as Lawson (2011) stating economic benefits can motivate consumers to participate in access-based consumption. Hamari et al. (2015) also supports the fact that economic benefits persuade consumers to participate in access-based consumption. Möhlmann (2015) supports my finding of one-time use being a motive to rent sneakers, when he implied that trend affinity is a motive for chic-consumers to partake in access-based consumption. From this research, we can also learn or gain an idea of how to market a sneaker-renting platform to sneakerheads. Although possessiveness is a permanent discouragement for sneakerheads to rent their sneakers, other deterrents such as hygiene and sneaker condition can easily be eliminated via rules set by the business owner of the sneaker-renting platform. For example, the owner could set a three-time max for an individual pair of sneakers to be rented, to avoid condition degradation and hygienic issues.

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Business Sustainability: An Analysis of Three Enterprises in Charlotte, North Carolina

Cera Crowe
Padmini Patwardhan, Ph.D. (Mentor)

ABSTRACT
Sustainability is defined in varying ways by businesses for reasons such as industry, stakeholder expectations, compliance, and other factors. In this case study, three companies, Domtar, Duke Energy, and Nucor, are examined on their approach to reporting and practicing environmental, social, and economic sustainability. Content analysis was used to identify themes in corporate paid, owned, and earned media. Interviews with facility managers were used to understand how management implements sustainable practices on a facility level. This case study examines in detail how these companies use different language, tools, and systems to progress sustainable development in a globalized economy. Results showed that in earned media, companies were socially and economically analyzed and not environmentally, unless responding to a crisis. In owned media, companies organized their sustainability priorities in a way they believed most represented their company. In sustainability reports, the more complicated the business portfolio, the more complicated it becomes to differentiate data sets. On all three reports, the most under-reported pillar of sustainability was environmental. All three companies have a huge ecological footprint and very general data regarding it is reported.

INTRODUCTION
Sustainability is a concept that recognizes and aims to balance the complex relationships between ecology, economics, and social equity. In today's society most consumers are not informed where product materials are sourced and what conditions products are manufactured in. With technological innovation and the human race experiencing an exponential growth in population, the world is more connected than ever before in history. Yet world trade operates with very little transparency towards environmental impact. Often sustainability is used as a marketing tool calling consumers into action via purchase of an environmentally branded product. Consider how many times you have seen a brand incorporate shades of green and imagery pertaining to nature in an advertisement. This type of marketing is often used on a product that has sustainable elements but also has externalities and unaccounted for consequences on communities and ecosystems around the world.

As of 2015, Charlotte, North Carolina has an estimated population of 809,958 as the city grew by 2 percent between 2013 and 2014 (Bell, 2015). The Charlotte Observer reported that among the 50 biggest U.S. cities, Charlotte’s 10.7 percent growth rate between the 2010 census and 2014 topped all but Austin, Texas and New Orleans, Louisiana. Charlotte is well-known for a pro-business environment. Seven Fortune 500 companies are headquartered in the Charlotte Metro area, along with 9 other companies on the Fortune 1000 list (Charlotte Overview, n.d.). Charlotte Douglas International Airport is the world’s sixth most-active airport in terms of takeoffs and landings and the nation's eighth busiest in total passengers (Charlotte Overview, n.d.). Charlotte clearly has a large influence economically and socially, but how invested are they in environmental sustainability?

The purpose of this research is to understand how three enterprises with headquarters in metropolitan Charlotte, NC interpret sustainable development using a case study approach. The companies incorporated into the sample are Domtar, Duke Energy, and Nucor. By using a case study approach, it will examine in detail how companies use different
tools and systems, internally and externally, to progress towards sustainable development in a globalized economy.

**Sustainability Defined**

Sustainability first became an international concept in 1983 when the United Nations established the World Commission on Environment and Development (WCED), also known as the Brundtland Commission. Their document entitled Our Common Future published in 1987 firmly placed sustainable development on the political agenda. The report weaves together social, economic, cultural and environmental issues and global solutions.

As defined by the United Nations, it is the “development which meets the needs of current generations without compromising the ability of future generations to meet their own needs” ("Our Common Future," n.d.). Sustainability looks to protect human and ecological health, while driving innovation and not compromising our way of life. In the Brundtland Report, the description of sustainability is further elaborated through The Three Pillars of Sustainability Model. The three pillars illustrate the environmental, economic, and social elements that create the need and rationale for sustainability. Environmental sustainability is the rates of renewable resource harvest, pollution creation, and non-renewable resource depletion that can be continued indefinitely. Economic sustainability is the ability of an economy to support a defined level of economic production indefinitely. Social sustainability is the ability of a social system, such as a country, government, or city, to function at a defined level of social well-being indefinitely ("Sustainability,” n.d.). Social sustainability becomes an increasingly important issue as population increases. The current world population is 7 billion and is expected to rise to 9 billion by 2050, with many still living on less than $2.00 a day ("Sustainable Development,” 2011).

These three pillars work together to create a sustainable solution to modern problems. Most problem solving efforts toward sustainable practices focus on only one pillar at a time. Agencies and sectors were designed to “divide and conquer” issues, but often issues of sustainability cross disciplines, regional laws, international laws, etc. For example, the United Nations Environmental Programme (UNEP) and the Environmental Protection Agencies (EPA), focus on the environmental pillar. The World Trade Organization (WTO) and The World Business Council for Sustainable Development (WBCSD) focus mostly on economic growth. The United Nations works to incorporate all three pillars into resolutions, but due to its complex decision making process, shortfall of authority, and scarcity of funding, it has minor impact.

Sustainability uses system thinking as a methodology of solving problems. Economics, biology, and social structures all operate under varying yet interconnected systems. Often issues of sustainability go beyond that of the three pillars and relate to other disciplines and fields. Systems thinking allows problem solvers to be interdisciplinary in finding sustainable solutions to modern issues. Although increasing acceptance of the interdisciplinary nature of the issue of sustainability is a source of encouragement, there is a danger that the prevailing conflicts of views about the environmental crisis, which arise from being locked within the reductionist way of thinking, may harden into inflexible and polarized oppositions (Mebratu, 1998).

Sustainability operates around the Gaia Theory, which generally states that everything in nature, including living and nonliving elements, is interconnected. The Gaia hypothesis has contributed to replacing the image of the “Earth as a machine” with the image of the “Earth as an organism” (Mebratu, 1998). Norwegian philosopher, Arne Naess identified the deeper roots of the environmental crisis in Western culture and, in particular, in the cultural values legitimizing the domination of nature (Braidotti et al. 1994). The contribution of the Gaia theory is to highlight interdependencies within and among the organic and inorganic world and to focus on Gaia-centrism instead of on anthropocentrism, competition, and individualistic aggression, typical of some other biological and social theories (Mebratu, 1998).
Evolution of Sustainability

*Our Common Future* (1987) was produced through an international collaborative effort and created a political change in sustainable development, but was not the starting point of the conceptual development process of sustainability. Thomas Robert Malthus (1766-1834) is considered the first economist to foresee the limits to growth caused by resource scarcity. According to Malthus’s population theory published in his book *Essay on Principle of Population* (1798), the vices and misery that plague society are not due to evil human institutions, but are due to the fertility of the human race (Mebratu, 1998).

In the 1970’s, the world began to realize the implications and consequences of the global population, resource exhaustion, corporate concentration, and the corresponding diminution of individual liberties. The 1972 United Nations Conference on Human Environment in Stockholm, which recognized the “importance of environmental management and the use of environmental assessment as a management tool” (DuBose et al. 1995), represents a major step forward in development of the concept of sustainable development. Major issues discussed were the responsibilities of governments and citizens to the protection of natural resources and biodiversity, the reduction of pollution, and the dedication to economic and social development. At the same time the Club of Rome, formed by stakeholders, citizens, and scientists, created a comprehensive report on the state of the natural environment. Terminology like “eco-development” emerged.

The first major breakthrough in conceptual insight came from the International Union for the Conservation of Nature (IUCN). IUCN is the world’s oldest and largest global environmental organization, with almost 1,300 government and NGO Members and more than 15,000 volunteer experts in 185 countries (“About International,” n.d.). Working closely with the World Wildlife Fund for Nature and The United Nations Environment Programme, IUCN formulated the World Conservation Strategy, which was launched internationally in 1980. This was a major attempt to integrate the environment and development concerns into an umbrella concept of “conservation” (Mebratu, 1998). In the *World Conservation Strategy*, conservation is described as “For if the object of development is to provide for social and economic welfare, the object of conservation is to ensure Earth’s capacity to sustain development and to support all life” (“World Conservation,” 1980).

This led to the formation of UN World Commission on Environment and Development (WCED), also known as the Brundtland Commission. Their document entitled *Our Common Future* defined sustainable development and the conceptual definition contains two key concepts:

- The concept of “needs,” in particular the essential needs of the world’s poor, to which overriding priority should be given; and
- The idea of limitations imposed by the state of technology and social organization on the environment’s ability to meet present and future needs (“Our Common Future,” n.d.).

The WCED established the three pillar model of sustainability by highlighting the strong relationship between poverty alleviation, environmental improvement, and social equitability through sustainable economic growth. Although the WCED created a consensus on the definition of sustainable development, it remained too broad and every stakeholder interprets it differently in application.

The United Nations Conference on Environmental Development (UNCED), also known as the Earth Summit and Rio Conference, was held in Rio de Janeiro in June 1992. Delegates of the Earth Summit established the Commission on Sustainable Development (CSD) and Agenda 21. Agenda 21 is the voluntarily implemented action plan of the United Nations with regard to sustainable development (“AGENDA 21,” n.d.). In 2013, the CSD was replaced by the High-level Political Forum on Sustainable Development (HLPF) that meets every year as a part of the ECOSOC meetings, and every fourth year as a part of the General Assembly meetings.
The HLPF provides political leadership and recommendations for sustainable development. **Business and Sustainability**

Business is the study of the production and distribution of goods and services with the individual functioning as an autonomous, rational, and self-interested actor (Repko, Szostak, Buchberger, 2014). Sustainability of business is when a business has minimal negative impact on the global or local environment, society, government, and economy. The World Business Council for Sustainable Development (WBCSD) is a CEO-led, global advocacy association of some 200 international companies dealing exclusively with business and sustainable development. As stated in the charter of WBCSD, “Business leaders are committed to sustainable development, to meeting the needs of the present without compromising the welfare of future generations. This concept recognizes that economic growth and environmental protection are inextricably linked, and that the quality of present and future life rests on meeting basic human needs without destroying the environment upon which all life depends” (Schmidheiny, 1992).

The law of unintended consequences states that decisions often have unanticipated – or unintended consequences (Acarglu, 2013). In business this is called an externality, which is the cost or benefit that affects a party who did not choose to incur that cost or benefit. An example of this is air pollution effects on citizens from burning fossil fuels at a nearby plant. Business sustainability aims to eliminate unanticipated consequences, unintended consequences, and externalities.

There are numerous business models that aim to encompass environmental concerns, and more broadly incorporate social responsibility. These business models vary across industry, market, and regions. It is important to note what kind of business model is present in regards to sustainability to assess its functionality. At the most basic level there is voluntary reporting on environmental and sustainability performance. Reporting standards continue to be an issue, but a consensus is slowly emerging to use the Sustainability Reporting Guidelines issued by the Global Reporting Initiative (GRI) standards (Strasser, 2011). A second option is companies joining voluntary performance standards programs. Participants in these programs make a commitment to improve their environmental efforts by one or more specific metrics. As a third option, companies create internal company-initiated management systems called Environmental Management Systems (EMS) that are designed to organize and implement a company’s environmental protection efforts and move its environmental performance to government compliance and beyond. Lastly, there is negotiated compliance with legislative or regulatory environmental protection programs, where compliance is commanded by public authorities.

**Sustainability in the United States**

In the United States, the most notable agencies that control environmental regulations are the Environmental Protection Agency, the Food and Drug Administration, U.S. Fish and Wildlife Service, National Park Service, United States Forestry Service, Federal Trade Commission, and the Bureau of Land Management. Important environmental laws include the Clean Air Act (CAA), the Clean Water Act (CWA), and the Safe Drinking Water Act (SDWA).

The Clean Air Act established technology-based emission standards for specific industry categories (SICs). These standards specify the technology and the emission limits that are allowed for pollutants discharged to the air. Companies must adhere to this methodology to calculate air pollution emissions. The Clean Water Act is enforced by the U.S. Environmental Protection Agency (EPA) and established standards that specify the technology used and limits of toxicity that should be in wastewater prior to disposal in a water body. The Safe Drinking Water Act is enforced by the EPA and the Food and Drug Administration and it ensures clean water that is safe for human consumption. The SDWA has four categories of standards that water suppliers must meet. The categories are physical, chemical, microbiological, and radiological (“Environmental Law,” n.d.).
In a document entitled *Guides for the Use of Environmental Marketing Claims: Final Rule*, the Federal Trade Commission explains the requirements of marketers when sharing environmental claims to consumers (Title 16: Commercial Practices, 2012). The document expresses its weaknesses by stating it is not within its authority to set environmental policy. It also supports its general language with ‘more detailed guidance could quickly become obsolete given the rapidly changing nature of this market and consumers.’ If enforcement action is needed, the Commission must prove that the challenged act or practice is unfair or deceptive in violation of Section 5 of the FTC Act.

Some of the key findings on environmental media laws set by the Federal Trade Commission include (Federal Trade Commission):

- Advises marketers to have competent and reliable scientific evidence to support their carbon claims, including using appropriate accounting methods to ensure they are properly quantifying emission reductions and not selling those reductions more than once.
- Marketers should accompany seals or certifications with clear and prominent language that effectively conveys that the certification or seal refers only to specific and limited benefits. This may be particularly challenging with certifications based on comprehensive, multi-attribute standards.
- Advises marketers to avoid making unqualified renewable energy claims based on energy derived from fossil fuels. It clarifies that marketers may make such claims if they purchase renewable energy certificates (‘‘RECs’’) to match their energy use.
- Advises marketers to distinguish between benefits of product, package, and service. Unless it is clear from the context, an environmental marketing claim should specify whether it refers to the product, the product’s packaging, a service, or just to a portion of the product, package, or service.

This document was created to ensure consumers have access to complete information when purchasing products.

*State of North Carolina Sustainability Initiatives*

North Carolina State laws on environmentalism are organized into Air Quality, Coastal Management, Energy, Mineral, and Land Resources, Marine Fisheries, Waste Management, and Water Resources. These laws are found in the North Carolina Environmental Policy Act of 1971.

North Carolina has 14 coal-fired power plants regulated under the National Pollutant Discharge Elimination System, which governs wastewater discharges to surface waters. A coal-fired generating facility turns water into steam, which in turn drives turbine generators to produce electricity. More than a third of North Carolina’s net electricity generation—38.7% in 2014—comes from coal shipped by rail and truck, primarily from West Virginia, Kentucky, and Pennsylvania (“U.S. Energy Information,” 2015). The Dixie Pipeline transports propane, butane, and ethane from Texas, Louisiana, and Mississippi to customers throughout the Southeast and ends in Apex, North Carolina. Of the 737 public and private-access biodiesel fueling stations nationwide, more than 18% are in North Carolina (“U.S. Energy Information,” 2015). In 2014, 6.6% of North Carolina’s utility-scale net electricity generation came from renewable energy resources; all of the generation came from conventional hydroelectric power, biomass, and solar energy (“U.S. Energy Information,” 2015).

North Carolina’s leading industries are aerospace, automotive and heavy machinery, biotechnology and pharmaceuticals, information technology, and finance (“Key Industries,” n.d.). North Carolina is home to 27 Fortune 1000 companies, including Duke Energy, Lowe’s, and Bank of America (Charlotte Overview, n.d.).

North Carolina’s agricultural industry, including food, fiber and forestry, contributes $78 billion to the state's economy, accounts for more than 17 percent of the state’s income, and employs 16 percent of the work force. The state ranks seventh nationally in farm profits with a
net farm income of over $3.3 billion ("North Carolina Ag," n.d.).

Popular conservation topics are banning or having local authority over fracking on North Carolina shores, protecting pollinators such as bees, and finding ways to use sustainable energy. In recent news, North Carolina-based Duke Energy was ordered to close coal-ash pits at eight locations by 2019 and at 25 locations by 2024 by the North Carolina Department of Environmental Quality. Duke has been under pressure to address the way it stores coal waste from power generators after its 2014 ash spill into the Dan River ("Introduction to," 2014).

City of Charlotte and Sustainability

Charlotte is home to Duke Energy, the nation’s largest electric power holding company. It provides energy to more than 7 million residential, commercial and industrial customers in the U.S. using a combination of nuclear, coal-fired and hydroelectric facilities. Natural gas is available from Charlotte-based Piedmont Natural Gas. Charlotte Water receives water from the Catawba River, whose headwaters are in the Appalachian Mountains. Here, dams created a series of lakes, including Mountain Island Lake, Charlotte’s main supply. Charlotte draws less than 3 percent of the lake’s content daily (Charlotte Overview, n.d.).

The Government of Mecklenburg County tracks its performance in reducing its environmental impact using an aggregated measure called the “Environmental Leadership Index.” Mecklenburg County’s Environmental Leadership Index Score reflects four different areas. “Thirty-five percent of the score comes from emission reduction results, which includes energy use and fleet emissions. Thirty-five percent comes from resource conservation results, which includes recycling, land protection, and purchasing. Fifteen percent comes from commitment, which includes employee engagement and department integration. Fifteen percent comes from stewardship enhancement, which includes new and innovative practices and continuous improvement” ("Mecklenburg County: Goals," 2014). In 2014, Mecklenburg County successfully reduced both electrical and fuel use. The County also began resetting the recycling baseline based on new policy regarding waste reduction & recycling, and continued to follow environmentally preferable purchasing habits. The County did report on a decline in employee engagement with fewer public transit riders, environmental volunteering, and environmental education participation ("Mecklenburg County: Goals," 2014).

The Division of Nature Preserves and Natural Resources is responsible for the protection and conservation of Mecklenburg County’s parks designated as Nature Preserves. Land Use & Environmental Services Agency (LUESA) is an organization providing many key services that contribute to a high standard of quality of life for Mecklenburg County residents. LUESA’s role ranges from enforcing building and zoning codes to managing water and air resources for future generations (“About Mecklenburg,” n.d.). The City of Charlotte’s Community Investment Plan (CIP) is a long-range investment program designed to meet the needs of the growing community. The CIP encompasses investments in roads, housing diversity, neighborhoods, storm water projects, transit, water and sewer projects, the airport, and government facilities (“Community Investment,” n.d.).

There are many nonprofit organizations dedicated to keeping Charlotte sustainable. The Charlotte Chamber GreenWorks Council fosters sustainable practices within the local business community by connecting Charlotte Chamber members ("GreenWorks," n.d.). Sustain Charlotte is a nonprofit organization founded in 2010 that has quickly become the city’s voice on sustainable development ("Mission," n.d.). There is also Envision Charlotte, which is a public private plus collaborative that leads Charlotte’s progress as a global Smart City through innovations that strengthen economic competitiveness, environmental sustainability and positive community impacts (“What We’re All About,” n.d.). Envision Charlotte’s board of directors includes representatives from Duke Energy, Wells Fargo, Geosyntec, and Bank of America.

Business Environment in Charlotte

Charlotte is a major financial, distribution, and transportation region.
Businesses range from multinational to microbusinesses. Mecklenburg County is the headquarters to seven Fortune 500 companies. Nine Fortune 1000 companies are headquartered in the Charlotte metro area. Charlotte is home to 48 companies with more than $1 billion sales annually (Charlotte Overview, n.d.). Bank of America grew Charlotte into the second-largest financial center in the United States. Charlotte is also home to a branch of the Federal Reserve Bank of Richmond. The 16-county Charlotte region is by far the largest manufacturing center in North Carolina, accounting for a full third of the state’s 10,300 manufacturing firms (Charlotte Overview, n.d.).

Charlotte is also home to the Charlotte Douglas International Airport (CLT), which is the sixth most-active airport in terms of takeoffs and landings. CLT also offers both domestic freight and international airfreight services from 70 freight forwarders, customhouse brokers and professional international service providers. Interstate 77 passes through Charlotte, connecting Miami to Cleveland, and Interstate 85 connects Atlanta, GA to Washington, DC. More than 500 trucks and transportation arrangement firms operate in the city. Most of the nation’s largest trucking companies have facilities here (Charlotte Overview, n.d.).

**RESEARCH QUESTIONS**

This study focuses on the following research questions investigating varying definitions of sustainability in three different enterprises with headquarters in the Charlotte, North Carolina region using a case study approach. The probes are as follows:

1. What environmental, social, and economic issues do businesses in the region consider important to report on in their annual company sustainability report (Global Reporting Initiative: Sustainability Reporting Guidelines)?
2. How do businesses in Charlotte discuss sustainability through paid, owned, and earned media?
3. How do businesses in Charlotte perform sustainable practices through management or leadership systems?
4. How far reaching are the effects of these businesses sustainability practices socially and environmentally?

**METHODOLOGY**

In order to organize and collect a variety of information on each enterprise’s approach to sustainability, a case study methodology was used. According to Meyer (2001), a case study is “a detailed investigation of one or more organizations, or groups within organizations, with a view to providing an analysis of the context and processes involved in the phenomenon under study.” While there are no specific standards or requirements in this method, it needs to be both rigorous and structured to allow “tailoring the design and data collection procedures to the research questions” (Meyer, 2001, p. 329/31). One major feature of case study methodology is that different methods are combined with the purpose of illuminating a case from different angles: to triangulate by combining methodologies (Johansson, 2003, p. 3). A case study explores many different facets of a phenomenon using multiple sources, including documents, archives, records, and observations (Baxter, 2008) and can offer unique insights beyond other research techniques.

Case study methodology also has limitations. The concept of case is not well defined and remains a subject of debate. The case may be a relatively bounded object or a process; it may be theoretical, empirical, or both (Ragin & Becker 1992). At a minimum, a case is a phenomenon specific to time and space. The constraint of looking at a phenomenon at a certain time and space makes the results less generalizable, but provides an objective and concise view of a certain case within the phenomena. Because methodology requirements are not specific in a case study, it offers opportunity for improper or incomplete research or “loose design” that may not capture all essential aspects of the phenomenon (Meyer, 2001). Alternatively, nonspecific methodology requirements also allow researchers to tailor their design to the context of their case with qualitative and quantitative methods that cover important aspects of the case.
The case study approach was chosen for this research because of the nature of the research questions. Sustainability, as mentioned previously, uses systems thinking to find common ground between the three pillars: economics, society, and the environment. These pillars, applied to a specific case, then break down further into smaller and more manageable sub-systems such as local governments, communities, biomes, etc. Case study methodology allows the researcher to look at each system as a perspective of the research question. In this study, each enterprise has a different approach to how it discusses and practices sustainability.

Sample Selection

As mentioned earlier, the three companies selected for the study are all Fortune 500 companies, with defined sustainability initiatives, and have headquarters in the greater Charlotte region.

Domtar is a leading provider of a wide variety of fiber-based products including communication, specialty and packaging papers, market pulp and absorbent hygiene products. Domtar has approximately 9,850 employees serving more than 50 countries around the world. Domtar's annual sales are approximately $5.3 billion and its common stock is traded on the New York and Toronto Stock Exchanges (Domtar, 2015). Domtar’s principal executive office is in Fort Mill, South Carolina.

Nucor Corporation is the largest steel producer in the United States and is the largest "mini-mill" steelmaker. Nucor is North America's largest recycler of any material and recycled 16.9 million tons of scrap in 2015 (Form 10-K, 2015). In 2015, the company produced and sold over 25 million tons of steel (Nucor, 2015).

Duke Energy is the largest electric power holding company in the United States, supplying and delivering energy to approximately 7.4 million U.S. customers. Duke Energy has approximately 52,700 megawatts of electric generating capacity in the Carolinas, the Midwest and Florida – and natural gas distribution services in Ohio and Kentucky. Duke Energy has $121 billion in assets (Duke Energy, 2015).

Data Sources

Multiple data sources (both secondary and primary) can be utilized in case study analysis. For this study, company materials as well as depth interviews provide the data for analysis. Company materials were content analyzed and included copies of corporate sustainability reports for the selected companies, as well as content delivered through their paid (e.g. placed advertising/promotion), owned (e.g. company website, social media and blogs) and earned media (e.g. media and social media coverage by others). Paid, owned, and earned media content was collected by daily keyword searches operationalized through Google Alerts and Social Mention. Depth interviews were conducted with facility managers and designated spokespersons from each enterprise. These interviews were conducted to understand how management on a facility level practice and encourage sustainability.

Data Analysis Process

Text in each company’s most recently published corporate sustainability report were first coded into the three pillars of sustainability: environmental, social, and economic. Next the report was coded again for discussion of the following topics: 1.) Air quality, 2.) Biodiversity, 3.) Climate change, 4.) Energy affordability and reliability, 5.) Employee well-being, 6.) Customer Satisfaction, 7.) Water quality and availability, 8.) Trusted certification programs or partnerships, 8.) Charity, 9.) Research/Education 10.) Materials for production. Media content collected over the duration of one month was copied chronologically into a single Word file for ease of electronic analysis with similar keywords entered in Word’s “find” function.

Data collection and analysis for the primary interviews was as follows. Before each interview, respondents gave their informed consent. A script (see appendix A) was used for consistency across interviews. The first set of questions inquired about general job responsibilities and the respondent’s personal journey within the company. The second set of questions asked about the facility he or she operates and how it practices sustainability. The third set of questions asked company specific
sustainability questions pulled directly from each company’s most recent sustainability report. These questions were used to understand how management operationally retains and implements corporate beliefs of sustainability in their facility.

1. What environmental, social, and economic issues do businesses in the region consider important to report on in their annual company sustainability report (Global Reporting Initiative: Sustainability Reporting Guidelines)?
2. How do businesses in Charlotte discuss sustainability through paid, owned, and earned media?
3. How do businesses in Charlotte perform sustainable practices through management or leadership systems?
4. How far reaching are the effects of these businesses’ sustainability practices socially and environmentally?

RESULTS

Duke Energy

Duke Energy has facilities in North America, South America, and Saudi Arabia. The energy industry is constantly evolving to meet customer expectations, public policy, and changing technology. In Duke Energy’s earned media, social welfare was notably significant but this could occur because of the nature of the service the company provides and also because of the time frame. The coal ash spill at Duke Energy’s Dan River Steam Station remained a primary topic of earned media throughout the study. On February 2, 2014, officials estimate up to 39,000 tons of coal ash spilled from Duke Energy’s Dan River Steam Station into the Dan River in Eden, N.C., about 80 miles upstream from the Kerr Reservoir. This ended in a huge clean-up and a $6-million-dollar settlement with the EPA for violating the clean water act. This also led to a long chain of closing of coal ash ponds. Political issues were also a focus of earned media because of Mayor Pat McCroy of Charlotte-Mecklenburg County being a former employee of 29 years for Duke Energy. Duke Energy also faced a backlash by the public on development in suburban areas in earned media. Duke Energy had significantly more discussion on both Google Alerts and Social Mention. This could be because Duke Energy supplies services to both the residential and business sectors. Duke Energy’s 2015 sustainability report focused on meeting customer demands and employee safety. Duke Energy provides 24 million people with an important service every day. The company also has education and research initiatives that support business-self interest. Duke Energy consumed 79 billion gallons of non-replaceable water, 32.6 million tons of coal, 44.1 million gallons of oil and spilled 3,425 gallons of oil in 2015. Duke Energy’s sustainability report emphasized their desire to evolve with consumer expectations. Consumer demand could be the driving factor in evolving renewable energy sources. The environmental consequences range depend on the type of facility that is located in an area. Duke Energy’s sustainability report only referred to biodiversity twice. The two claims are that they actively protect eagles in wind turbines and remove vegetation with herbicides approved by the EPA. The amount of environmental reporting in their owned media is alarmingly obsolete.

Domtar

The paper and pulp industry is also in transition with society’s increase in reliance on technology. Paper is still in high demand for hygienic purposes, but paper is in less demand for educational purposes. Domtar reported having 9,850 employees in 2016. Domtar has many human resource initiatives aimed at keeping employees safe. Logging, mulching, and cleaning fibers to create paper can be very hazardous, but with new technology Domtar has worked to make the process as hands-off as possible. Domtar also faces the challenges of having a large percentage of their employees reaching retirement age.

Domtar generally creates paper and pulp in a closed system and returns 90% of water taken to its original source. Also Domtar creates a substantial amount of energy from using biomass energy from unusable bark and other excess. Despite this, Domtar reported 5.64 million tons of greenhouse gas emission in 2015. Domtar primarily uses softwood or coniferous trees. A facility manager stated,
"How I think about it is trees are a crop and are being replanted just like corn. I think of it as a crop where we harvest it in the most sustainable way we can and try to create the most value we can out of that fiber. It's a sustainable crop." The facility manager also stated that he/she could not speak intelligently about the corporate partnership with the World Wildlife Fund or the Rainforest Alliance.

**Nucor**

In regards to employee safety and pollution, the steel industry has significantly changed in the past 50 years. Nucor has unique opportunities for employees like the "NuYou" program that tracks at risk employees to help them make good healthcare decisions. Nucor also hosts preventative Health Fairs. In it's earned media, Nucor was recognized for social contributions in volunteering and community impact. Safety was the most socially reported topic in Nucor's sustainability report. Upon interviewing, a facility manager's dedication to safety was repeatedly mentioned, showing corporate values in practice at a facility level.

Nucor is the largest recycler of scrap metal in the western hemisphere. Nucor also repurposes externalities to be used for other purposes in other industries. One example is using Plant Tuff Silicon Fertilizer or lime "slag" in agriculture. There was no earned media regarding Nucor's environmental impact. A Nucor manager stated "There was a philosophy, that's not shared in our company anymore, that the EPA is an overbearing government facility and it's better rather than telling them what we are doing to let them find out. We cleaned all that up and put pollution monitoring technology on our plant. That was a different mindset, and a different time. We want to get better, make more, cost less. In order to build and buy new things you need to get permits." Although the motive behind environmental compliance is economic, it signifies the importance of government programs that use incentives for business compliance to environmental goals. The Nucor environmental facility manager also stated "Federal (Environmental Protection Agency) has never come since I've worked here (hired in 2011). State comes at least annually."

**DISCUSSION**

A very apparent result in regards to earned and owned media was a hierarchy of what was to be reported on the most in terms of sustainability. In earned media, companies were socially and economically analyzed and not environmentally unless responding to a crisis. In owned media, companies organized their sustainability priorities in a way they believed most represented their company. All three reports were organized in different ways. This often can be attributed to the product or service the company provides. On all three reports the most under-reported pillar of sustainability was environmental. All three companies have a huge ecological footprint and very general data regarding it is reported. In sustainability reports, the more complicated the business portfolio, the more complicated it becomes to differentiate data sets. For example, Duke Energy operates on three continents and creates energy from many different resources, making it complicated to know where the 32.6 million tons of coal were allocated and used. Sustainability issues are not only a global concern but often must be solved at their source on a facility level. Environmental transparency in corporate owned reports could be improved by taking emission testing more frequently at a facility level and compile it in a communicative way. This is an example of how companies can move beyond compliance and reach customer expectations of stewardship. The Global Reporting initiative aims to make sustainability communicative, but standardizing reporting is not full communicative across different industries in different geographical locations. Companies often reported in whole numbers and different measures implying incomplete reporting or simply bad reporting. Interviewed managers stated that they had been visited very few times by state regulators and even fewer had encountered a federal Environmental Protection Agency employee. This could imply a check missing in the reporting and regulating process and could have consequences economically, socially, and environmentally. Another issue raised was management having a specialized perspective on running a business. Long term sustainability
goals set by corporate weren't prioritized in operations by management. Facilities often run on short term and profit-oriented goals and management reflected this. A commonality between all three industries was the attention placed on employee safety beyond compliance. All three companies showed self-interest in investing in employee health and safety initiatives. All three companies also report investing in educational opportunities for perspective employment. They are seeking out young people as current labor employees reach retirement age. Content under earned media did not discuss Domtar and Nucor because their business and facilities are very isolated and only well known by the employees and surrounding geographical area. This is because they are both primarily a business-to-business operation. The impact that these facilities have on the economic and social welfare of the surrounding towns cannot be under stated.

REFERENCES


Evaluation of the Potential HMGA1-EF24 Nexus in Human Colon Cancer

Madeline Diaz
Takita Sumter, Ph.D. (Mentor)

ABSTRACT
The architectural chromatin binding proteins High Mobility Group A1 (HMGA1) are proteins expressed at high levels in malignant cancers and induce neoplastic transformation. The protein is increased as the last step of the Wnt/β-catenin/TCF-4 pathway and mediates drug resistance, therefore correlating with a poor patient prognosis. HMGA1-mediated chemoresistance results from a self-protective process called cellular senescence. Analogs of the antioxidant, curcumin, when used in combination with traditional chemotherapeutic agents, are useful treatment options for drug resistant tumors. This study had two specific aims. The first being to investigate how colon cancer cells HCT116 respond to treatment with EF24. The second aim was to evaluate how hmga1 expression changed as a result of treatment with EF24. Our preliminary findings showed that cell viability decreased after 24-hour treatment with low-dose EF24, as indicated by an MTS assay, with notable discrepancies between cells that underwent a pulsed treatment regimen versus those that underwent continuous treatment. Furthermore, we demonstrated that cells exhibited fragmented DNA when treated with low-dose EF24, which is characteristic of apoptotic cells. At higher, pulsed doses, senescence activity increased, indicating the induction of a senescence pathway. Lastly, gene expression studies indicated that hmga1 was significantly down regulated in cells treated with continuous, low-dose EF24. Further investigation of this pathway could lead to decreased toxicity and increased viability of combination cancer therapies.

INTRODUCTION
The High Mobility Group A1 (HMGA1) family of proteins are non-histone binding architectural proteins. They are aptly named based on their high mobility in polyacrylamide gel (Reeves, 2001). The subfamily consists of two isoforms, HMGA1a and HMGA1b, which are the result of alternately spliced mRNA transcripts derived from the common hmga1 gene. The HMGA1 proteins are characterized by a prominent binding domain that preferentially binds to AT-rich regions on the minor groove of DNA (Reeves, 2001). This allows the protein to alter shape of B-form DNA and control the transcription of a variety of genes and the activity of a variety of proteins (Thanos et al., 1992). Depending on the context, HMGA1 can promote or repress a variety of molecular pathways. As a result, the HMGA family participates in a number of cellular processes including cellular proliferation, neoplastic transformation, aging and metastasis (Reeves, 2001). Hmga1 was identified as a gene that is induced by growth factors, and is required by cells to pass through the G1/S cell cycle checkpoint (Fedele et al., 2001). More importantly, HMGA1 functions as an oncogene when overexpressed in those same cells (Resar, 2010). Therefore, it follows that hmga1 is up-regulated in a number of malignant cancers including, but not limited to: ovarian, breast, lung, thyroid, and colon cancers (Scala et al., 2000). When tissue samples from normal, unaffected mice were compared to their metastatic counterparts, the HMGA1 levels were almost undetectable; suggesting that HMGA1 plays an important role in the transformation of cells from normal to malignant (Xu et al., 2004). Furthermore, when compared to localized tumors, hmga1 was vastly up-regulated in the metastatic counterparts. Similarly, malignant tissues in which hmga1 is overexpressed exhibit increased resistance to common chemotherapeutic drugs. When hmga1 expression is inhibited, sensitivity to the same
drugs increased significantly (D’Angelo et al., 2014). Taken together, these factors suggest that there is a correlation between high levels of hmga1 expression and poor patient prognosis.

When a malignant cell undergoes therapeutic treatment, it triggers a number of responses; the two most prominent responses are apoptosis and a protective cellular process known as senescence (Childs et al., 2014). Previous research shows that HMGA1 disrupts normal cell cycle regulation by preventing malignant cells from undergoing apoptosis while simultaneously propelling it through mitosis (Fedele et al., 2001). Contrastingly, HMGA1 also promotes cellular senescence (Narita et al, 2006). Senescence is characterized by arrest of the cell cycle, expression of SA-β-galactosidase and induction of tumor suppressors p16 and p21. Senescent cells can then induce a change in phenotype in surrounding cells through emission of an intercellular signal (Narita et al, 2006).

Curcumin, the active ingredient of the turmeric plant, possesses novel antioxidant, anti-inflammatory and anti-proliferative properties. Studies show that malignant tissues treated with curcumin exhibited elevated levels of HMGA1 and subsequently induced apoptosis and senescence (Kuo et al., 1996; Jin et al., 2016 and Mosieniak et al., 2016). However, a clinical study showed that curcumin presents with low bio-availability (Ramasamy et al., 2015 and Cheng et al., 2001). EF24 is a chemically synthesized analog of curcumin, however its exact anti-proliferative mechanism remains relatively unknown.

Taken together, we sought to investigate the relationship between hmga1 expression and treatment with EF24, with two specific questions in mind. How does hmga1 expression change upon treatment with EF24? How do HCT116 cells respond to treatment with EF24? It was shown that upon treatment with low-dose, continuous EF24, hmga1 expression was down-regulated. Furthermore, cell viability decreased significantly upon treatment with both continuous and pulsed EF24. Lastly, at low concentrations of EF24, there was evidence of apoptosis, whereas at higher concentrations there was increased evidence of senescence. Further investigation into this nexus could lead to the discovery of a viable, targeted combination cancer therapy.

METHODS

Cell Culture

The HCT116 human colon cancer cell line was obtained from the American Type Culture Collection (ATCC). Cells were maintained in McCoy’s 5A media supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin-Amphotericin B. Cells were maintained in a humidified incubator at 37°C and 5% CO₂.

RNA Extraction and RT-PCR

HCT116 cells were treated with varying concentrations of EF24 (0.0, 0.25, 0.5 μM) for 24h. After 24h, total RNA was isolated using the QuickRNA Mini Prep kit following the manufacturer’s instructions. GAPDH was used as a positive control. The forward and reverse primers for both HMGA1 and GAPDH are shown below:

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMGA- Forward</td>
<td>5’- GAT GGG ACT GAG AAG CGA-3’</td>
</tr>
<tr>
<td>HMGA1-Reverse</td>
<td>5’-CTT CTC CAG TTT CTT GGG TC-3’</td>
</tr>
<tr>
<td>GAPDH- Forward</td>
<td>5’-TGC ACC ACC AAC TGC TTA GC-3’</td>
</tr>
<tr>
<td>GAPDH-Reverse</td>
<td>5’-GGC ATG GAC TGT GGT CAT GAG-3’</td>
</tr>
</tbody>
</table>

The assembled reactions were run using the following thermal cycler program:

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time</th>
<th># of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>15 min</td>
<td>1</td>
</tr>
<tr>
<td>95</td>
<td>15 min</td>
<td>1</td>
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<td>95</td>
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<td>55</td>
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<td>72</td>
<td>1 min</td>
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</tr>
<tr>
<td>72</td>
<td>5 min</td>
<td>1</td>
</tr>
</tbody>
</table>

Cell Viability MTS Assay

Approximately 6000 cells were plated per well in a 96-well plate, and were incubated
for 24h. Cells were then treated with increasing concentrations of EF24 for 24h. For continuous treatment, the treated cells were assayed for cell viability following 24h EF24 treatment using CellTiter Aqueous One Solution Cell Proliferation Assay. For pulsed treatment, the treated media was removed, replaced with drug-free media and cells were incubated for an additional 48h. Following the 48h recovery, cells were assayed for cell viability. Cell viability was quantified by measuring absorbance at 490nm.

**Apoptosis Assay**

HCT116 cells were plated in a 6-well plate and treated with increasing EF24 concentrations (0.0, 0.05, 0.125 μM) for 24h. Treated media was then removed, and cells were cultured in drug-free media for an additional 48h. Genomic DNA was then isolated and the fragments were resolved on a stained agarose gel.

**SA-β-galactosidase Activity Senescence Assay**

Approximately 10,000 cells were plated per well in a 6-well plate and incubated for 24h. Cells were then treated with increasing concentrations of EF24 (0.0, 0.05, 0.125 μM) and incubated for 24h. Treated media was removed and replaced with drug free media and incubated for an additional 48h. Cells were lysed and β-galactosidase activity was assayed using the 96-well Cellular Senescence Assay Kit (Cell Biolabs Inc.) Enzyme activity was quantified by measuring absorbance at 360nm.

**RESULTS**

**EF24 Treatment Down-Regulates hmga1 Expression**

HMGA1 has a well-established link to the phenotype of colon cancer cells. Therefore, we initially analyzed the effect EF24 treatment had on hmga1 expression. Treatment with EF24 had a significant effect on the expression of hmga1 in HCT116 cells. As the concentration of EF24 increased, the expression of the gene was three to four-fold down regulated (Figure 1).

**Continuous EF24 Treatment Decreases Cell Survival**

The HMGA1 is a key player in oncogenic transformation because it allows cells to ignore normal growth pathways and cell cycle regulations, resulting in abnormal proliferation. Upon observing that treatment with EF24 resulted in a down-regulation of hmga1, we decided to test for cell viability after a continuous 24h treatment. Figure 2 indicates that there was a significant decrease in cell viability from 100% to approximately 65% at low doses of EF24. With a gradual decrease in cell viability as the concentration of EF24 increases.
Pulsed EF24 Treatment Decreases Cell Survival and Induces Apoptosis

Chemotherapeutic treatment follows a treatment-recovery model. Patients undergo treatment for a period, which is then directly followed by a period of recovery. We sought to mimic that model by allowing the cells to recover post-treatment. Figure 3A indicates that there was a significant decrease in cell viability from 100% to 15% at low doses of EF24. Cell viability was noticeably low across the board, indicating that cells were unable to recover after the treated media was removed regardless of EF24 concentration.

The prominent decrease in cell viability prompted the need to observe the mechanism by which the cells were dying, particularly at low doses. DNA isolated from untreated cells and from cells treated with 0.05 μM EF24 presented with fragmented DNA. Contrastingly, cells treated with 0.125μM presented with solely whole DNA (Figure 3B).

Increased Concentrations of EF24 Induce Senescence

It has been shown that curcumin, the naturally occurring counterpart of EF24, has the capacity to force cells into a protective process known as senescence. The absence of fragmented DNA upon treatment with higher concentrations of EF24 leads us to believe that the cells may have been forced out of the cell cycle into cellular senescence. Figure 4 is indicative of an increase in senescence associated β-galactosidase activity from untreated to treated. However, the difference in activity was not found to be statistically significant.

DISCUSSION

HMGA1 is overexpressed in a variety of different malignant tumors (Scala et al., 2000). Not only does it facilitate the neoplastic transformation of normal cells, it also facilitates drug resistance and abandonment of the cell cycle while simultaneously inhibiting programmed cell death (Xu et al., 2004; D’Angelo et al., 2014; and Fedele et al., 2001). Recent studies have shown that HMGA1 also plays a role in the promotion of cellular senescence (Narita et al, 2006). The present study investigated the relationship between HMGA1 expression and EF24 in colon cancer cells. The data indicates that treatment with EF24 significantly decreases cell viability at low-doses and down-regulates hmga1 expression. Furthermore, upon pulsed treatment with low
doses, both apoptotic and senescent activity are exhibited.

As previously mentioned, expression of hmga1 results in the inhibition of p53 mediated apoptosis (Fedele et al., 2001). Therefore, it follows that upon treatment with therapeutic agent, EF24, hmga1 expression should decrease. This hypothesis was confirmed by PCR analysis. Upon down-regulation of hmga1, the normal cell cycle should resume resulting in a decrease in overall cell viability. This trend is observed in the cells treated with continuous EF24. Therefore, EF24 is effective in reducing the viability of colon cancer cells as a first wave attack. However, this model of treatment does not accurately represent most modern chemotherapy treatments.

Chemotherapy follows a treatment-recovery model. Patients are treated for a short period of time and allowed to recover. This method is employed as a means of protecting non-malignant cells from repetitive attack by harmful chemotherapeutic drugs. Therefore, an optimal chemotherapeutic compound should not continue to kill cells during the recovery period. Doing so would put vital stem cells at risk of being compromised. Keeping this in mind, the colon cancer cells were treated with EF24 using a pulsed treatment model. The results indicated that cell viability decreased significantly despite the 48-hour recovery period. This prompted us to investigate exactly what was happening to the cells to compromise their viability. The apoptosis assay showed evidence of fragmented DNA in both the untreated cells and cells that were treated with the lowest concentration of EF24. However, at higher concentrations, the band that was indicative of fragmented DNA disappears. Taken together with the data from the senescence assay leads us to postulate that at higher, pulsed concentrations, cells forego the apoptotic pathway in favor of the protective cellular senescence pathway. When cells are continuously treated with drugs, some will die and some will enter senescence (Childs et al., 2014). However, the 24-hour treatment time might not be long enough to allow the senescent cells to signal neighboring cells to enter senescence. Contrastingly, when cells are treated using a pulsed regimen, the cells that have entered senescence can continue to signal neighboring cells even after the treated media is removed resulting in the drastic decrease in cell viability observed in Figure 3A.

Continuing to study the role of HMGA1 in the cellular response of colon cancer following treatment with EF24 may contribute to the discovery and development of less toxic combination cancer therapies.

**FUTURE WORK**

To confirm the hypothesis that treatment using a pulsed regimen promotes cellular senescence, both apoptosis and senescence assays need to be conducted using cells that have been treated with a continuous treatment model. Furthermore, hmga1 expression studies need to be done using cells treated with a pulsed treatment model.

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“I Don’t Fit in a Box; No One Does:” Intersectionality and Gay Male Identity

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ABSTRACT
Using an intersectionality framework, this qualitative study explores how stigma affects identity development and how intersecting identities can compound to either foster resiliency or create health concerns for 11 men who are emerging adults (18-29), same sex identified, African American, HIV +, and homeless. Semi-structured one-on-one interviews were conducted through RAIN (Regional Aids Interfaith Network) in Charlotte, NC. Questions were formulated to understand how participants view themselves and perceived stigmas, current/past health conditions, and their five to ten year prospects. This study uses grounded theory as a guide to analyze and interpret data. Themes explored include: risks (acquiring HIV through homeless status), biographical disruptions, and self-acceptance as a foundation to resiliency through self-empowerment. All participants in this study displayed resilient behaviors post-diagnosis to present, even those who experienced the worse “biographical disruptions.” Participants all spoke about being a survivor of sorts. I found that, for my participants, owning one’s identity created a buffer against the adverse effects of stigma.

Keywords: intersectionality, gay identity, HIV, resiliency, homelessness

INTRODUCTION
White privilege remains a controversial topic. Privilege, in general, is when one has special support due to skin color, gender, or other various aspects of identity. White privilege comes with many benefits. A white person can, for instance, go to a realtor and expect to be shown a home in a neighborhood that reflects their values, and expect to see people with their skin color in the neighborhood. They can also expect, if the house they are shown is trashy, it is not because of their race, it is probably due to their price range. White people can also expect to find shades of makeup in their color, and bandages to match their skin tone. They can expect to find someone who can do their hair in any commercial barbershop. White privilege is when your race is usually represented in history, positively. White privilege is being able to be passed off as cute instead of a criminal. White people do not usually have to fear being pulled over by the police. Many white people often get offended when their privilege is questioned. There is no way that white privilege exists, in the minds of many white people. Though, you can see it when their position in the hierarchy is threatened.

It’s hard to confront one’s privilege because it is usually something one doesn’t realize they have. It is something that has always been there, invisible, but ever-present. Whites grew up around other white people who had the same privileges. Many didn’t grow up in or around black neighborhoods where they could expressively see the lack of privilege and inequitable living conditions. Whites usually do not have to fear for their life when they are stopped by the police. The most I worry about is whether I am going to get a warning or an actual ticket. Therefore, it is hard for white people to realize they have this inherited unearned privilege that others don’t. Peggy McIntosh (1988), the author of White Privilege: Unpacking the Invisible Knapsack, states this when addressing her privilege:

In proportion as my racial group was being made overconfident, comfortable, and oblivious, other groups were likely being made inconfident, uncomfortable, and alienated. Whiteness protected me from exhausting daily anxiety, worry, fear, and anger owing to others' treatment of people in my racial group. At the same time, I was subtly trained
People of color face more challenges throughout their life than their white counterparts, which is sure to have an effect on identity development.

In a research study I conducted last year, I explored the experiences of emerging adults who were homeless and LGB; I found that each person’s experience with homelessness was significantly different and unique to themselves, though there were similar themes. Some were HIV positive, some were African American, and all had a varying degree of education. I lumped these individuals together as just “Gay People” with no regards to how their race or HIV status played a role in their experiences with homelessness. Too often researchers and agencies lump individuals together in groups based on gender, race, ethnicity, class, and sexual orientation because we believe that people who are similar share similar experiences. Intersectionality aims to look at an individual on multiple levels of intersecting identities. This is important because everyone has different experiences based on their various identities. These experiences impact their needs. For example, a straight African American male who is not diagnosed with HIV would have different experiences and needs than a gay African American male who is HIV positive.

Moreover, research that examines the LGBTQ community is often problem focused. While it is important to understand problems within the community, it can be detrimental because it creates an environment where LGBTQ individuals are only known to be problematic, suicidal, depressed people who do not possess the strengths to remain resilient and achieve goals. This is not the case at all. It’s important for researchers to examine the strengths of this population, and ask questions that aim to discover how these individuals remain resilient despite the overwhelming number of obstacles they face. This information could be useful in creating new and innovative ways to empower clients.

**THE LITERATURE**

**Identity & Intersectionality**

**Intersectionality.** There are several theories regarding identity and identity development which were conceived by theorists such as Freud, Erikson, and Cass (Cass, 1979; Zastrow & Kirst-Ashman, 2013). These models are quite useful when one wants a generalized view of how identity develops over time; however, these models are somewhat dated and too generic to assess contemporary development accurately. Thus, for this study, intersectionality is used as a lens to view identity. Intersectionality is the study of how different "identities" such as race, sexual orientation, gender, class, age, and religion intersect on multiple levels to create an individual's entire identity. Intersectionality also examines the stigma associated with minority identities. For example, an individual from a lower-class background who is gay, African American, and HIV positive, experiences stigma and oppression at rates much higher than a middle-class, white, straight, able-bodied man (Marsiglia & Kulis, 2015).

**Homeless identity.** Homelessness presents a greater challenge than one just not having decent accommodations. Studies suggest that these individuals “lose their sense of identity and self-worth” (Boydell, Goering, & Morrell-Bellai, 2000). Terui and Hsieh (2015) compare homelessness to illness to describe how homelessness has an impact on identity:

Like illness, although the meanings of homelessness can be determined through “objective measures” (i.e., individuals without homes), the fluid boundaries of homelessness can provide ample opportunities and resources for people to redefine their identities and construct life meanings. Like stigmatizing illness (e.g., HIV or tuberculosis), homelessness entails social stigma and imposes limitations on individuals’ everyday life. Experiencing homelessness has a profound impact on individuals’ identities as one is confronted with the loss of valued attributes (e.g., social roles and personal outlooks). Recognizing that the label of
a homeless identity, just like an illness identity, can be dis-preferred and even traumatizing for some, we propose that people who are (at risk of) experiencing homelessness would adopt various resources to resist, negotiate, and redefine the labeling of a homeless identity. We argue that individuals can experience identity dilemmas as they face the increasing possibility and reality of their homeless status, resulting in widening gaps between a homeless identity and their desired identities (p.2)

They also found that homeless individuals utilize three strategies to manage their identity: differentiating themselves from other homeless, prioritizing certain aspects of life (such as education, past successes, and employment), and embracing the state of homelessness (Terui & Hsieh, 2015). Terui and Hsieh report than the longer these individuals stay on the street, the more likely they are to embrace their homeless identity and the less likely they are to come off the street. Therefore, it is imperative to find resources for these individuals to ensure they can effectively break this cycle of homelessness. Although individuals who couch surf are not roofless, they still are labeled with the same stigmas as those who are homeless. They do not have a stable place to live and are always moving from house to house, or house to shelter to house. The one fundamental difference between couch surfing and rooflessness is privacy away from the public eye. If these individuals are staying in someone’s home, they are not endlessly scrutinized by the public as having a homeless identity. However, they are aware of the stigma that is attached to being homeless, and they are mindful of the fact that they do not have a stable home. One could argue that individuals who couch surf experience the same identity crisis as those who are roofless.

**Gay identity.** Along with a potential homeless status, same sex identified men who live on the street or couch surf must also come to terms with their sexual orientation and how that plays a role in their identity. Many times, these young people lack support and are socially isolated, which stems from the stigma attached to the LGBTQ community. Many of these young people internalize feelings of guilt and shame due to their homeless situation (Grainger, 2015). These young people also feel isolated because of their sexual identity. Family members and peers often reject LGBT people, which can cause internalized homophobia and shame (Doty, Willoughby, Lindahl, & Malik, 2010). Doty, Willoughby, Lindahl, and Malik (2010), terms this as “sexuality stress,” that is, additional stressors that are related to ones’ sexuality (Doty, Willoughby, Lindahl, & Malik, 2010; Grainger, 2015). In western cultures, there is a concept of hegemonic masculinity that associates manhood to power and dominance. Robert Brannon (1979) describes the cultural ideals of masculinity that men in the United States should possess as: no sissy stuff, be a big wheel, be a sturdy oak, and give ‘em hell (David, Brannon, Brannon, & David, 1976). Those who deviate from these cultural ideals face the risk of being stigmatized by mainstream culture.

According to a study conducted by Rosario, Scrimshaw, and Hunter (2012), youth who display non-gender conforming traits at a younger age may be at a greater risk of encountering homelessness versus their more gender conforming counterparts. A common predictor of LGB (lesbian, gay, bisexual) identity in adulthood is gender nonconforming behavior in childhood. Studies have found that people can many times tell the difference between LGB individuals quite accurately based on voice, appearance, and other gender non-conforming cues (Rosario, Scrimshaw, & Hunter, 2012). Perceived sexual identity, regardless of whether youth self-acknowledges it, creates negative reactions from family, peers, and community members (Rosario, Scrimshaw, & Hunter, 2012). This negative stigma creates an environment for these individuals that puts them at a greater risk of becoming homeless due to being socially rejected at a younger age. Once they become homeless, they must cope with both intersecting identities, both of which are stigmatizing. Also, youth who are from a minority background may also face even more stigmatizing reactions from society based on their ethnic or racial identity.
**Racial identity.** Currently in the United States, racism and prejudice can still be witnessed nationwide. Discrimination is still seen in many aspects of life for racially minority groups. These individuals still face housing and job discrimination. Numerous instances of police brutality have been documented in several forms of media. Consequently, same-sex identified homeless men who are also a racial minority not only encounter instances of discrimination based on their orientation and homeless identity, but they also face further discrimination based on their racial identity. These hostile circumstances create an environment where some African American men may feel they need to hide their identity to gain acceptance within their own community, and the broader community (Marsiglia & Kulis, 2015).

Moreover, African American males face racism within the LGBTQ community (Marsiglia & Kulis, 2015). This lack of support from within their community and the gay community can affect their overall well-being, leading to greater health and safety risks for those who are both gay and African American. In fact, according to 2014 CDC (Centers for Disease Control and Prevention) statistics, 44% (19,540) of estimated new HIV diagnoses in the United States were among African Americans, who comprise 12% of the US population (Centers for Disease Control and Prevention, 2016). The CDC reports that “lower rates of linkage to care, retention in care, stigma, fear, discrimination, homophobia, and negative perceptions about HIV testing may also place many African Americans at higher risk and discourage testing” (Centers for Disease Control and Prevention, 2016).

**Health/safety.** Studies suggest that LGBTQ individuals who experience homelessness face greater health/safety risks than their heterosexual counterparts (Rosario, Schrimshaw, & Hunter, 2012). Risks include: mental health disorders, substance abuse, sexual/physical victimization, and survival sex that leads to greater chances of contracting HIV (Grainger, 2015). Rosario, Schrimshaw, and Hunter (2012) reported that LGB (lesbian, gay, bisexual) individuals were more likely to report running away from home due to sexual abuse than their heterosexual counterparts. In a survey conducted by Saewyc, Skay, Pettingell, Reis, Bearinger, Resnick, Murphy, and Combs (2006), gay and bisexual boys reported a higher prevalence of physical abuse in the home, “with nearly 1 in 5 up to 1 in 3 reporting abuse, compared to 1 in 8 heterosexual boys” (Combs, Bearinger, Murphy, Pettingell Reis, Resnick, Saewyc, & Skay, 2006). It is not uncommon for these individuals to run away because of the abuse they face at home. Studies suggest that a high percentage of persons who are homeless couch surfed before they became homeless (Wright, Caspi, Moffitt, & Silva, 1998). Once these individuals are on the street, they face even greater chances of being sexually victimized. In my recent study, I found that many participants who utilized homeless shelters had experienced sexual assault while there (Grainger, 2015). Many reported that staff did nothing when they made reports of sexual assault, citing that the workers made LGBTQ individuals feel unwelcomed (Grainger, 2015).

**Stigma.** Not only must these young people cope with the stigma attached to being a part of the African American and LGBTQ community, but they must do so while facing the stigma of being homeless. According to Terui and Hsieh (2015), homelessness is a socially constructed concept. Meaning we, as a society, define what it "means" to be homeless. Typically, this definition places the blame on the individual who is homeless, rather than the organizational and institutional hurdles and injustices that these individuals encounter (Terui & Hsieh, 2015). Homeless individuals are generally stereotyped as lazy (unwilling to work), addiction-riddled, criminals, and at blame for their situation. In many cities, legislation has been passed that criminalizes common homeless behavior. For instance, panhandling in many states is illegal and sleeping on the sidewalk can get an individual jailed in many cities across America. Cities have gone as far as placing spikes on the pavement in areas where it is common for the homeless to sleep. This anti-homeless behavior sends a clear message to these individuals: you are not valued, and we do not want you here. Moreover, along with the
social isolation that homelessness brings, persons who are LGB are further isolated because of the stigma attached to the community. When these stigmas are compounded on top of each other, it can create the various stressors and mental health disorders that are seen specifically within this population.

Throughout American history, there has been stigma attached to those in the LGBTQ community. Legislation is slowly changing to provide equal opportunities for these individuals, which was witnessed when the Supreme Court ruled that gay marriage was legal in all 50 states. However, legislation such as the recent HB2 law in North Carolina only acts to further stigmatize members of the trans community and members of the LGBTQ community as a whole. Businesses still reserve the right to refuse service to these individuals. Hate crimes and murder against LGBTQ people are common. Stigma related to the LGBTQ community can in and of itself lead to homelessness for these young people. Parents who disapprove of their child's sexual orientation may, in time, throw their children out of the home or create environments where the child feels the need to run away. Once on the street, these young people face the stigma of being both homeless and LGBTQ.

HIV Status and Identity

To further exacerbate the situation, many of these individuals are also dealing with an identity crisis regarding their HIV status. Literature examining how HIV, specifically, has an impact on identity development is sparse. The literature usually classifies HIV as a chronic illness, comparing the two and showing instances of “biographical disruptions” which could be characterized as an intrapersonal conflict as a result of a perceptual loss of self (Baumgartner, 2007). Kralik, Koch, and Eastwood (2001) detail how self and identity are two separate entities: “The ‘self’ reflects international thinking of what it is ‘being a person,’ whereas ‘identity’ is shaped by social interaction” (Kralik, Brown, & Koch, 2001, p. 33). Much like being displaced from one’s home, an HIV/AIDS diagnosis requires one to reevaluate themselves, and examine how they believe the condition influences their life. Since HIV/AIDS is a chronic disease that is no longer a death sentence, individuals with this diagnosis can plan to live longer lives. A longer life also means that they must face the stigma that is commonly associated with this diagnosis. Because of the stigma of HIV/AIDS, a diagnosis can often be quite traumatizing for the individual. The literature on HIV/AIDS and identity suggests that individuals experience a change in identity post-diagnosis.

A study conducted by Destiny Ramjohn (2012), suggests that an HIV diagnosis in young people resulted in a “biographical disruption.” Respondents experienced a loss in their sense of self; post-diagnosis – Many participants “gave up” or suspended the identity development process, the consequences of which included “continued risky sexual behavior (e.g. unprotected sex, sex with a partner they know is infected with HIV); failure to comply with prescribed medication regimens (e.g. missing multiple doses of antivirals); or not pursuing previously set academic goals e.g. dropping out of high school (pp.4-5).

While some literature has established a link between chronic illnesses and negative developmental outcomes, this body of research is not conclusive. Many of my participants have found empowerment through their identities.

METHODS

Study Design and Research Questions

To ensure that research results are as accurate as possible, grounded theorists suggest that one have at least fifty hours of collected data (Charmaz, 2006). This study uses a grounded theory method as a guide to explore how different identities intersect to either foster resiliency or create health concerns for same-sex identified males who are African American, HIV positive, and homeless. There were four major phases of this study: research advertisement, participant selection, one-on-one interviewing, and data analysis. Once my proposal was accepted by the Winthrop Institutional Review Board, I began the initial stage of promoting the study. Since this is a
difficult population to locate, I enlisted the help of several agencies in the Charlotte/Mecklenburg area to advertise and promote the study. These organizations include: Regional Aids Interfaith Network (RAIN), The Powerhouse, and Urban Ministry. The staff at RAIN was by far the most helpful agency in the area. Thus far they have helped secure 11 participants and 8.25 hours’ worth of data. Each participant was asked to distribute information about the project to other individuals who met the criteria and would be willing to participate in the study.

To take part in the study, each participant had to meet certain requirements: They must identify as HIV-positive, same-sex identified males, be between ages 18-29, identify as African American, and have an experience with homelessness that lasted more than six months. Each interview took place in public settings, mainly at the organization that the participant had an affiliation with. Interviews were audio-recorded and lasted between 15-45 minutes. Each participant was compensated with a $20.00 Visa gift card for the interview regardless of full participation.

Data Gathering Procedures

During the initial interview, I ask participants to draw a stick figure or a circle to represent themselves. I then ask them to think about all the identities that make them who they are or that have an impact on their life. I draw a stick figure with the participant to give an example about a couple of my basic identities, such as being an able-bodied, gay, male. I give them time to think about all the different identities they may have. After they are finished writing, I talk with them about intersectionality, and explain how it is a concept that states ones’ identity is not just based solely on one’s race, gender, sexual orientation, status, etc. Rather, one’s identity is formed by all these categories, which overlap to create the unique individual sitting before me, who has his own unique story to tell. I go on to ask them to pick a couple identities from their list that they believe represent who they are the most. I talk with the participants about why they think these identities have an impact on their lives. I also ask them why they chose the identities they did over the others.

Participants are also asked questions based on how they view themselves and perceived stigmas, current/past health conditions, and future prospects. Participants were asked to describe the situation regarding how they first became homeless. From there, the conversation was guided around different aspects of the participant’s lives, experiences, and identity. Questions were based on how participants view themselves and perceived stigmas, current/past health conditions, and 5-10 year prospects. Questions were designed in a way that would not influence participants’ answers. They were neither positive nor negative in nature.

I asked participants to discuss how they first became homeless. Depending on the answer, I asked the participants to describe how they felt during the initial encounter of being homeless. Questions were based on determining how the experience changed how they viewed themselves, their situation, those around them, and society as a whole. I asked participants to then discuss other instances in which they were homeless, and how their prospects possibly changed, and how they viewed themselves and others during those periods of homelessness.

Participants were also asked how living with HIV has affected their lives. Initial questions were structured to gauge how participants first viewed their selves after being diagnosed, and follow-up questions were asked to assess how they view themselves currently. Participants were asked how family and loved ones reacted to their status, and how those reactions impacted how they felt about themselves. I also asked participants to describe how their condition has had both positive or negative effects on their life. To close out the interview, I always revisit and speak on the growth and resiliency that I’ve witnessed from them through hearing their incredible story. Lastly, I asked participants where they see themselves in five years, and how they plan to make their prospects a reality.

Ethics

To ensure that participation was completely voluntarily, I thoroughly went over
an informed consent at the beginning of each interview, which was then given to participants. Participants were informed that this was a voluntary study in which they had the right to withdraw at any time. Participants were also informed that interviews would be audio-recorded and they had the option to stop recording at any point in the interview in which they felt uncomfortable. Throughout the interview process, I took every precaution possible to ensure the confidentiality of participants. Before starting and recording interviews, participants were given the option to choose a pseudonym, which would be used throughout the interview and transcription process. All audio files were secured in a thumbprint locked file. Participants were provided with an informed consent statement and given a chance to ask any questions before starting the interview. Participants were also informed that they could request that audio-taping be stopped at any point during the interview and advised that they did not have to answer any question that made them feel uncomfortable. I also reinforced the fact that skipping any question or asking to turn off audio recording would not impact their compensation.

Data Analysis
For this study, I used grounded theory as a guide to analyze and interpret data. Grounded theory can be compactly summed up through this quote by Strauss and Corbin (1998):

If someone wanted to know whether one drug is more effective than another, then a double-blind clinical trial would be more appropriate than grounded theory study. However, if someone wanted to know what it was like to be a participant in a drug study, then he or she might sensibly engage in a grounded theory project or some other type of qualitative study.

Elements of grounded theory include: question formulation, interview transcribing, data coding, analytic memoing, theoretical sampling, and constructing theory. After formulating my questions and gathering data, I collected roughly 8.25 hours’ worth of interviews, which I transcribed. Once the transcription process was over, I coded all the collected data and developed concepts based on thematic similarities. According to Charmaz (2006), “grounded theory methods consist of systematic, yet flexible guidelines for collecting and analyzing qualitative data to construct theories ‘grounded’ in the data themselves” (Charmaz, 2006). After looking at similarities within the data, I worked with my mentor, Dr. Brent Cagle, to better ensure objectivity when analyzing data and constructing theories.

Researcher Role
As an identifying gay male who has experienced homelessness as a young adult, there is the possibility that bias could have influenced the data gathering process. My experiences may have also influenced the way I analyzed and interpreted the data. As a social worker and researcher, I must continually work towards self-awareness, and separate my experiences from those of the participants and the research project. Throughout this study, I consulted with my mentor, Dr. Brent Cagle, to ensure that my work was as non-biased as possible.

FINDINGS
Risks
For many participants, HIV infection was ultimately a result of a lack of stable housing. Participants spoke about being raped on the street, in the home of someone who was providing temporary housing, or in public shelters.

Demetri: “um, I was younger an livin’ with this guy a friend had introduced me to. He was let me crash on his couch at the time because I had nowhere to go. I had a job, so I would only be there at night. One night I woke up with him on top of me… um, a few months later I started to feel sick, like I had the flu. Turns out I was infected.”

John: “Well, I was at the shelter, and obviously I’m fem… The guys there would expose their junk to me and even try to force their way on me. This one guy tried to get with me in the bathroom but I turned him down. The
next night he cornered me at this spot I used to lounge at, I knew what he was there for. I had nowhere to go and I didn’t know what he would do if I were to try to fight my out. I asked him to leave, but he said no…. I didn’t sleep with just anyone, I know it was him who gave it to me.”

Biographical Disruption

All participants experienced a brief period of “biographical disruption” post diagnosis. This period lasted from six months up to two years. When talking about the post-diagnosis period (period in which participants are likely to experience biographical disruption), participants spoke about suicidal ideation, depression, being reclusive due to feeling ostracized by family and peers. Biographical disruptions were a result of a loss of self and a fear of stigma associated with the virus.

Jay: “I lost my smile for a long time. Nothing made me happy anymore… I just gave up. I was on the streets, I didn’t care. I was trickin’ with other pos people. It was like I was sick, but I wasn’t… ya know? I had dreams, but I couldn’t see myself achieving them.”

Demetri: “Um, its as if everyone pitied you, or was scared of you. And that scared me sh**less. I would wonder if that is how everyone was gonna look at me. I was worried everyone knew. And going to the doctor was awful, I thought they were always scared to touch me. I eventually stopped taking my meds. It was a lot of complications because my body is so sensitive to the virus. It came to a point where I was living on the street in the winter sick as f*** and I refused to go to the doctor. I was going to die. My friend talked me into going. That was the lowest point I’ve ever been. I didn’t fear dying, I feared living and having to tote all of this on my back.”

John: “I feared no one would understand my situation, or how it made me feel. I felt stuck and I kept asking myself what was next? I was sure this was then end. I couldn’t see past the virus. There was no future for me…. Just death. I was sentenced to death by a man I didn’t want to even touch me. People don’t understand. They automatically assume you asked for it. They place you in a little box, and say you did this to yourself. They look at you like it’s your fault. I don’t fit in their box. No one does.”

Resilience

Talking about their experiences was a source of strength for participants’, and owning ones’ identity was self-empowering, and seemed to be the foundation for building resiliency and overcoming instances of biographical disruption. Participants who had stable safety networks such as family, peer and organizational support, displayed more cases of resilient and self-accepting behavior than those who did not. However, I noticed that participants who cut ties with family members who did not embrace their gay and HIV identities were more apt to “own” their identity vs. those who kept tempestuous relationships; and owning identity is essential to self-empowerment.

For many participants, owning one’s identity meant accepting they were African-American, Gay, AND HIV+ and realizing they could still live a fulfilling life regardless of associated stigmas; this resulted in an ability to inhibit self-defeating behaviors like skipping medicine regimes, halting educational endeavors, reclusiveness, etc. Moreover, when I asked participants to write down identities that they thought affected their lives, several participants used words such as fearless, lover, unique, passionate, etc. These self-descriptors of strength and kindness seem to be empowering for these individuals, and important when describing themselves.
Jay: “It took a year, but I made it out of that depression. My family really helped me. My moms never gave up on me, ya know. She was really the push I needed. She pushed me to find help, and I did. I finally have my smile back. And that means everything to me.”

Demetri: “I found myself through finding myself (Laughs). I had to say fu** what everybody else thought about me and take care of myself for me, not for them. I have goals, and this is not a death sentence anymore. I had to figure out what kind of life I was gunna live. I really had to figure out what I was gunna do to get myself off the street…. I cut hair now, and I’m doing alright. I wouldn’t say I’m perfectly happy, I have my moments, we all do. But I accepted myself, every bit. And here I am, I’m alive and kicking.”

King: “My ma was there sometimes, but not usually. She would leave when I was a kid, we never knew when she was gonna check back in. We fended for ourselves, Sh** never could depend on no one. It’s like that now. I sat her down a couple years back and was like, look moms, I’ve got HIV and I’m gay. She didn’t take it well. She’s crazy man. But she is my mom, only one I have. She wasn’t happy about me being gay at first, and she was scared as sh** about the HIV. She didn’t understand what it was, what I had. After a few weeks she came around. It wasn’t no brady bunch shit. We was still struggling. But it was nice to know she was still there for me I guess. If you want to call it that. As far as me I take care of myself now. I gotta. I am who I am, and that’s a bit** who doesn’t want to die, not anymore. I have plans for my life. Write that down.”

Pat: “Um, I am fearless, I found that out through my status. Um, I would say that being fearless is an important part of who I am. I try to love with everything in me. unconditionally. Until it compromises me. I’ve been hurt by plenty of people. My family, friends, people I wouldn’t expect to hurt me. They had to go. I love you, but bye. My health is important to me and if you can’t support that you gotta go honey.”

Some used seemingly negative descriptors as well, but these descriptors also seemed to be a source of empowerment for these individuals.

When I asked King to describe what the identities he chose meant to him, he described being a loner as something that was necessary to maintain his health.

King: “You can’t just let anybody in, people thinks that’s a problem. If you don’t let them in right away, they like what’s wrong with you. Sh** I think its unhealthy to let any ole stranger in my life. I have friends. But ultimately, I’m in charge of me. I do what’s necessary to maintain myself. I know letting people I don’t know in is unhealthy for me. I’m happier this way. Not everyone can understand me, I let people in that can.”
LIMITATIONS

According to Charmaz, one should have 50 hours of collected data to reach saturation. Considering I only have 8.25 hours of data, I have yet to reach any conclusive results. A well-done ground study on this topic would have taken years, if not a lifetime to complete.

The literature is mixed on African American views towards homosexuality. Some research indicates African Americans may be more accepting of LGBTQ individuals than their Caucasian counterparts, while some suggest otherwise. Future research should examine how accepting the African American community is towards the idea of Queer identity.

DISCUSSION

The central theme of emerging adulthood is the discovery of self and one’s place in broader society. Further research using intersectionality to examine how minority identities play a role in this period of self-discovery is needed; the literature is often whitewashed, outdated, or too generic to apply to such a specific population. Searching several databases, I could only find a handful of relevant articles related to HIV and identity formation, development, impediment, etc. (HIV & identity anything). Intersectionality is a relatively new concept, and there is still much to be learned about identity intersection, and how this affects identity development.

Many participants who were homeless spoke of experiences in which people assumed they were homeless because they were HIV positive, when in fact, many times, the acquisition of HIV was a result of unsafe shelter conditions; whether it be public shelter or shelter provided from an acquaintance or stranger. Over the past two years that I have spent working with individuals who lack stable housing in Charlotte, I have heard countless stories about sexual assault in Charlotte’s shelter, and the lack of support from shelter staff.

During a recent presentation at the NASW-SC spring symposium, I passed out an anonymous survey that asked attendees questions about their biases, and comfort level of working with LGBTQ people. Many attendees stated that based on their religious beliefs, they felt being gay, lesbian, bisexual, or transgender is abnormal and sinful. I also remember someone answering that they thought gay, lesbian, bisexual, or transgender people should not tell people their identity unless asked; and a few still opposed same-sex marriage. These oppositional feelings towards queer identity, if left unchecked, can create a rift in the client/professional relationship.

One of my professors frequently used the term “cultural humility” in place of cultural competency during my undergraduate years at Winthrop. Her reason being that we can never say we are truly culturally competent; we can only really strive to be culturally humble. It is important to remain aware that each culture has different customs, and to keep in mind you always have something to learn from them. Educational training that promotes cultural humility is necessary to equip social workers with the knowledge and tools that will enable them to work with this vulnerable population successfully. Furthermore, social work “gatekeepers” need to examine the attitudes and morals of their students carefully, to pinpoint red-flags, and weed out those who cannot put their biases aside to work with clients effectively. As professionals, our sole purpose is to benefit our clients. Any behavior on the part of social workers that could impede or degrade a client’s progress is unacceptable. The NASW (2008) Code of Ethics, Section 4.02 states:

Social workers should not practice, condone, facilitate, or collaborate with any form of discrimination on the basis of race, ethnicity, national origin, color, sex, sexual orientation, gender identity or expression, age, marital status, political belief, religion, immigration status, or mental or physical disability (National Association of Social Workers, 2008).

The Code of Ethics has a clear stance on discrimination; it’s not tolerable—and lacking the capability to work with someone based on their sexual orientation is innately a form of discrimination.
Current literature portrays LGBTQ+ individuals as innately problematic. Focus is placed on what these individuals are doing wrong, rather than on what they are doing right. Despite facing overwhelming obstacles, all participants in this study displayed resilient behaviors post-diagnosis to present, even those who experienced the worse “biographical disruptions.” They all spoke about being a survivor of sorts. I found that, for my participants, owning one’s identity created a buffer against the effects of stigma. Building self-confidence is key for one to truly own their identity. While there are programs specifically for HIV treatment that offer one-on-one counseling and peer support services, they are few and far between, particularly for individuals in rural areas and who lack transportation. Organizations like RAIN, who provide transportation assistance, are making a difference in the lives of those who require their services in the Charlotte-Mecklenburg area.

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Controlling Oct4 Expression Levels Using Invitrogen’s GeneSwitch™ System

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ABSTRACT
Oct4 is a protein that is involved in the retention of pluripotency in adipose derived stem cells (ADSCs). Despite this knowledge, Oct4’s exact role in the complex system used in maintaining pluripotency is not known. One approach to explore Oct4’s role would be through the use of cellular assays to control the expression of Oct4. This can possibly be accomplished by introducing a biological switch and the gene of interest into ADSCs. In this project, the GeneSwitch™ System is used to ultimately induce Oct4 expression. Before the GeneSwitch™ System can be used, the Oct4 gene is extracted from murine embryonic stem cell (ES) RNA. This ES RNA is then used as a template to create complimentary DNA (cDNA) that can then be used to create an insert with the Oct4 gene. In addition to the cDNA, recognition sites for endonucleases must be added on to fully create the Oct4 insert. This insert could then be placed into one of the GeneSwitch™ System plasmids that have the same recognition sites and placed into ADSCs along with the plasmid that will act as a biological switch. With this system put into ADSCs, it is expected that Oct4 levels will be successfully controlled. Once controlled, Oct4 expression can be tested and investigations can be completed to determine how Oct4 expression levels influence pluripotency of ADSCs. This may have significant impact on the creation of regenerative medicine.

INTRODUCTION
Cells are the most basic living component that make up an organism. They divide, communicate, metabolize, transport, and complete many other tasks that the human body requires in order to remain at homeostasis. Remarkably, cells grouped together as tissues, organs and organ systems can accomplish much more complex regulatory processes than individual cells. Since cells have such an important role in the body, losing large amounts of cells would make normal body functions more difficult. The body is often able to replace naturally lost cells, but may not be able to replace large amounts of cells lost due to injury or disease—at least not quickly. Regenerative medicine has made major scientific progress in procedures and tissue repair that can help the body to replace cells on a larger scale. For example, people who have been severely burned can replace the lost tissue through the use of regenerative medicine. Stem cells, immature cells that have not differentiated (i.e. specialized into a specific type of cell), have many possibilities in terms of what kind of cell they can become; this ability or potential to become any type of cell is referred to as pluripotency. This characteristic makes them an important resource in the field of regenerative medicine and holds tremendous potential for the future of all medicine (Pan et al. 2002). There are quite a few sources from which stem cells can be attained, but the most commonly discussed origins of stem cells are bone marrow or embryos.

Despite the potential and major progress in the study of stem cells, there are still some ethical and procedural issues that come with stem cell retrieval. For example, the use of embryonic stem cells presents a serious dilemma for many U.S. citizens. Others raise concern about the procedure to gather bone marrow stem cells (BMSCs), a process that is relatively expensive, intrusive, and has a comparatively
low yield (Zhu et al. 2008). Adipose derived stem cells (ADSCs) have become an attractive alternative source; these cells are cheaper, easier to obtain, and can be harvested in a much less invasive manner. This can be attributed to the abundance of fat available for medicinal use from either the patient or willing donors (Zhu et al. 2008). Not only is the use of ADSCs cheaper and more efficient, it is also still capable of having specified differentiation induced. This can be seen through an experiment conducted by Zhu et. al (2008), where ADSCs were successfully induced to differentiate into fat cells, bone cells, cartilage cells, and heart muscle cells.

While stem cells are able to differentiate, inducing their differentiation into specific cells can be more difficult. Typically, epigenetics—the study of how signals from an environmental or external source affect genes—plays a large role in the differentiation of stem cells. Cells can respond to signals in different ways based on many factors including: what kind of signal is being sent, how strong the signal is, and where the signal originates (Weidgang et al. 2016). Certain signals are able to trigger the stem cells to specifically differentiate into a certain type of cell. Using this same notion, it is possible for scientists to create induced pluripotent stem cells (iPSCs) that allow stem cell differentiation to be controlled through many methods including the use of a signal that can be operated using a biological “switch” (Stadtfeld and Hochedlinger 2010). This “switch,” depending on what genes it controls and what kinds of signals that gene is in control of, would be used to control whether the signal is “on” or “off” and the intensity of the signal. In order to make use of a “switch” to control stem cell differentiation, the gene of interest would have to be a gene that plays a role in the differentiation of stem cells. One such gene could be Oct4, a transcription factor that plays an important role in retaining the pluripotency of stem cells (Kim et al. 2009). Oct4 is usually used alongside many other transcription factors such as Sox2, Nanog, Klf4, and c-Myc in order to regulate the differentiation and retention of pluripotency of a stem cell (Takahashi and Yamanaka 2006). Typically, many transcription factors are needed to control pluripotency of stem cells as noted by the research of Boyer et al. (2005) with embryonic stem cells that are involved in the early stages of embryo development. Out of the transcription factors listed, Oct4 is the only one that is absolutely essential for self-renewal and pluripotency of a stem cell (Jerabek et al. 2014); this means that there is a chance that controlling the pluripotency of stem cells could possibly be done by Oct4 only.

The work described here focused on controlling Oct4 expression through the use of Invitrogen’s GeneSwitch™ System. An insert with Oct4 and two recognition sites had to be made and introduced to the pGene vector of the GeneSwitch System—which should have the same recognition sites—through the use of cloning. From there, further investigation can be done to study whether the expression of Oct4 alone can regulate the pluripotency of murine ADSCs. Once more studies have been completed using murine ADSCs, it will be possible to expect many of the same findings within human ADSCs due to the fact that the Pou5f1 gene sequence that controls expression of Oct4 for humans is 87% identical to that of murine Pou5f1 (Jerabek et al. 2014).

**METHODS**

Embryonic stem cell ribonucleic acid (ES RNA) was used as a template to create complementary deoxyribonucleic acid (cDNA) through the process of reverse transcription. Reverse transcription is the formation of cDNA using RNA as a template rather than the formation of RNA from DNA that is seen with normal transcription. An iScript™ Reverse Transcription Supermix for quantitative reverse transcription polymerase chain reaction (RT-qPCR) from Bio-Rad and thermocycler were used to create the cDNA. The reaction protocol used to create the cDNA was 5 minutes of priming at 25°C, 30 minutes of reverse transcription at 42°C, and 5 minutes of reverse transcriptase inactivation at 85°C. The cDNA acted as a template for the polymerase chain reaction (PCR) process used to amplify the Oct4 gene for molecular cloning. The cDNA, which already contained the Oct4 gene, was
reacted with oligonucleotide primers from Eurofins Genomics containing recognition sites for KpnI and NotI in order to make the Oct4 insert. The 5’ primer (5'-CTTGTTACCATGGGACACCTGGCT TCAGACITCCGC) contained the recognition site for KpnI and the 3’ primer (5'-CGAG CGGCCGCCCTGGTTGAGCATGGGAGAGCCCAGAGCAGTG) contained the recognition site for NotI. A 3’ primer with a stop codon and the recognition site for NotI (5'-CGAGCGGCGCCCTTCAGTTTGAATG CATGGGAGAGCCCAGAGCAGTG) was also used during PCR. The reaction protocol used for PCR was 2 minutes of denaturing at 94°C, 30 seconds of denaturing at 94°C, 30 seconds of annealing at 50°C, 3 minutes of elongation at 72°C, 1 minute of elongation at 72°C, and storage of PCR reaction at 4°C until the PCR reaction is retrieved. The PCR reaction went through 41 cycles of 30 second-denaturing, 30 second-annealing, and 3 minute-elongation before moving on to the step of 1 minute-elongation. Gel electrophoresis was completed and the gel was viewed through a Gel Doc™ XR+ System to ensure that the insert was the size it was expected to be. The insert was removed from the gel and gel DNA extraction was completed to purify the insert. The insert and pGene vectors—from Invitrogen’s GeneSwitch™ System—were then digested in KpnI and NotI endonucleases at 37°C in a thermocycler to create the correct cuts on both the insert and vector. The DNA of the insert and vector was purified using gel electrophoresis. Each sample was loaded on to a 1% agarose gel and separated for 30 minutes at 115V. The agarose gel containing the samples was viewed under a Gel Doc™ XR+ System from Bio-Rad using ethidium bromide to invoke DNA fluorescence. The DNA was carefully extracted from the gel and further purified using the Gel DNA Extraction protocol.

RESULTS & DISCUSSION
ES RNA went through reverse transcription to create cDNA with the desired Oct4 gene. The presence of Oct4 in the cDNA was confirmed by running a gel electrophoresis on a PCR reaction that incorporated cDNA and Oct4 primers that could be used to identify Oct4 using only a small segment of the Oct4 gene. It was expected that a band around 0.1kB would appear in the agarose gel and a band was present in the expected location as seen in Figure 1.

![Figure 1](image1.png)

Figure 1. Gel Electrophoresis results for the PCR reaction including cDNA as the template and primers to identify a short sequence of Oct4. The lowest lane with bands contains the base pair ladder. The middle lane with bands contained the PCR product for the reaction using Oct4 primers and 0.5µL of cDNA. The top lane with bands contained the PCR product for the reaction using Oct4 primers and 2µL of cDNA. The expected band size for this PCR reaction was 100 base pairs or 0.1kB.

Once the cDNA was made, a PCR was completed using a 5’ primer and 3’ primer to add on recognition sites to the cDNA, but the PCR product did not appear in the region it was expected to be when it was viewed after running a gel electrophoresis. It was expected to appear around the 1kB region, but the band was found well below the 0.5kB region as seen in Figure 2. PCR reactions using various concentrations of MgCl₂ and DMSO were completed, but neither reaction resulted in bands within the 1kB region, as seen in Figure 3 and Figure 4; all reactions resulted in bands well below the 0.5kB region. A PCR reaction incorporating one of the Oct4 primers used to confirm the successful creation of cDNA and the 5’ primer was completed along with a PCR reaction with the other Oct4 primer and the 3’ primer. The resulting gel, shown in Figure 5, indicates that the reaction with the 3’ primer worked, but the reaction with the 5’ primer did not.
Figure 2. Gel Electrophoresis results for the PCR reaction including cDNA as the template, 5’ primer with a recognition site for KpnI, and 3’ primer with recognition site for NotI. The lowest lane with bands contains the base pair ladder. The second lowest lane contains the control PCR reaction using WT NUR, T7 promoter primer, and T7 terminator primer. The middle lane contains the PCR product for the reaction with cDNA as the template, a 5’ primer with a KpnI recognition site, and a 3’ primer with a NotI recognition site. The second highest lane with bands contains the PCR product for the reaction with cDNA as the template, a 5’ primer with a KpnI recognition site, and a 3’ primer with a NotI recognition site and a stop codon. The highest lane with bands contains the PCR product for the reaction with cDNA as the template, a 5’ primer with a NdeI recognition site, and a 3’ primer with a BamHI recognition site. The expected band size for the PCR reactions (excluding the control) were 1000 base pairs or 1kB.

Figure 3. Gel electrophoresis results for PCR reactions with varying MgCl₂ concentrations. Each PCR reaction made use of cDNA as the template, a 5’ primer with a KpnI recognition site, a 3’ primer with a NdeI recognition site, and a specific concentration of MgCl₂. The MgCl₂ concentrations ranged from 0mM to 4mM and increased in increments of 0.5mM.

There are two possible reasons why the PCR of the 5’ primer failed. One reason is that the 5’ primer was not made correctly. Since the primer was designed while looking specifically at the expected cDNA sequence, there is very little reason to believe that primer design is the cause for this PCR to fail. The other possible reason is that the reverse transcription of ES RNA to make the cDNA did not completely work. When reverse transcription happens, the primer used during the process will find the template’s poly-A tail in order to anneal to the 3’ end of the mRNA. Once annealed, the primer will be used to copy the mRNA to cDNA, but it may not copy the entire template. This means that some of the 5’ end of the cDNA is ultimately missing. Without this 5’ end, the designed 5’ primer will not have an overlapping template sequence to bind to and will not be able to amplify the cDNA.

A PCR to figure out where the 5’ end of the cDNA started was completed. Primers that started at various intervals of the 5’ end of the cDNA were made before being used in separate PCR reactions. Each reaction also used the 3’ primer. A gel electrophoresis was completed after the PCR and bands were seen just below the desired region of 1kB, as seen in Figure 6. The bands were also sequentially increasing—which is what was expected—because of the fact that each primer was closer to the 5’ end of the cDNA than the last. With this PCR, it was now known that the cDNA was completed at least to the 50th nucleotide of the expected cDNA sequence. It was decided that another PCR would be completed to manually build on the first 49 nucleotides. This PCR was completed using two primers that would build onto one another to form the remaining nucleotides needed for the cDNA. A gel electrophoresis was completed to see if the appropriate bands could be found after each build was added on to the cDNA and 3’ primer. Bands around the 1kB marker were expected and found after viewing the agarose gel as seen in Figure 7.
Figure 4. Gel electrophoresis results for PCR reactions with varying DMSO percentages. Each PCR reaction made use of cDNA as the template, a 5’ primer with a KpnI recognition site, a 3’ primer with a NdeI recognition site, and a specific concentration of DMSO. The DMSO percentages ranged from zero percent (0µL) to 3 percent (1.5µL).

Figure 5. Gel electrophoresis results for PCR reactions using cDNA as the template, one of the Oct4 identifying primers, and either the 5’ primer or 3’ primer. The lowest lane with bands contained the base pair ladder. The second lowest lane with bands contained the PCR product for cDNA and the Oct4 identifying primers. The third lowest lane with bands contained the PCR product for the cDNA, 5’ primer with a KpnI recognition site, and one of the Oct4 identifying primers. The third highest lane with bands contained the PCR product for cDNA, 3’ primer with a NotI recognition site, and one of the Oct4 identifying primers. The highest lane with bands contained the PCR product for cDNA, 3’ primer with BamHI, and one of the Oct4 identifying primers.

Figure 6. Gel electrophoresis results for PCR reactions with cDNA as the template, a 3’ primer with a NotI recognition site, and of the primers starting at various intervals on the 5’ end of the cDNA.

Figure 7. Gel electrophoresis results for PCR reactions with cDNA as the template, a 3’ primer with a NotI recognition site, and either one or both of the Build primers. After the cDNA received the needed recognition site from the 5’ primer, the newly made Oct4 insert just needed to be cloned into the pGene plasmid before the pGene and pSwitch plasmids could then be transfected into the murine ADSCs and Oct4 expression levels could be investigated.

REFERENCES


Targeted Mutagenesis of FOX0 Transcripts Using the Model Organism *Ciona intestinalis* Utilizing the CRISPR/Cas9 System

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ABSTRACT

The chordate, *Ciona intestinalis*, has become an excellent model organism for the study of cardiac development. *C. intestinalis* is a member of the phylum Tunicata, which is the sister phylum to vertebrates. Since both phyla share a common ancestor, an orthologous gene has been identified in *C. intestinalis* and vertebrates, the FOX0 transcript. Multiple reasons makes *Ciona intestinalis* an interesting model organism. For instance, ease of access to the animal, close relation to vertebrates, and mapping of the genetic code of *Ciona intestinalis* makes this an excellent model organism. The FOX0 family of transcripts plays an important role in cardiac, muscular, and neural development in humans. Humans have four copies of the transcript while *Ciona intestinalis* only has one. However, the function of FOX01 in *Ciona intestinalis* is unknown. This study aims to investigate the function of FOX0 transcripts in *C. intestinalis* by incorporating transgenic DNA into embryos using fertilization, dechorionation, electroporation and the CRISPR/Cas9 system.

INTRODUCTION

The development, modification, and utilization of the CRISPR/Cas9 system has revolutionized biomedical research. Clustered Interspaced Short Palindromic Repeats (CRISPR) refer to short segments of DNA that contain repeated base pair sequences, which are then followed by short segments of spacer DNA. Through the addition of short guide RNA (sgRNA) segments, Cas9 is guided to the target DNA (Stolfi et al., 2014). Through the utilization of this ‘genomic editing tool’, researchers can now ‘cut’ genes at a desired loci and ‘paste’ the desired gene in its place. This can allow for the development of new drugs, new vaccines, and more efficient methods of treatment for genetic diseases. This system also allows for the manipulation of genes to determine their functions. Here, we seek to use the CRISPR/Cas9 system to alter the function of a specific gene in *Ciona intestinalis* in order to determine the role of this gene during cardiac development. *C. intestinalis* is a sessile, marine organism. They filter water, food, and other nutrients through the buccal siphon and expel excess water and metabolic wastes through their atrial siphon. This filter feeding process has earned them the nickname ‘sea squirts’ because through the process of expelling water, it ‘squirts’ water out of their bodies. [Figure 1](http://www.marlin.ac.uk/assets/images/marlin/species/web/o_cioint4.jpg)

![Figure 1: A picture of a *Ciona intestinalis*. A.) The buccal siphon, which is also called the incurrent siphon, draws water in the body. B.) The atrial siphon, which is also called the excurrent siphon, expels water from the body.](http://www.marlin.ac.uk/assets/images/marlin/species/web/o_cioint4.jpg)

*C. intestinalis* have been used in developmental, molecular, and regenerative biological research for more than one hundred and twenty five years (Jeffery, 2014). There are numerous reasons why *C. intestinalis* make excellent model organisms for biological research. The first of which is its unique position on the phylogenetic tree (Girino et al., 2002). Tunicates are the sister phyla of...
Vertebrata. This allows for certain genes to be highly conserved throughout the evolution that both humans and *Ciona intestinalis* have in common. Such as, the FOX0 transcription factor that this study wants to investigate. Humans have four copies of the transcript and *Ciona intestinalis* has only one. This study aims to investigate the role of the FOX0 transcript in cardiac development and may gain insight into human cardiac development. The following figure illustrates the close proximity of vertebrates and tunicates.

![Phylogenetic position of Tunicates and Vertebrates](image)

**Figure 2: Phylogenetic position of Tunicates and Vertebrates.**

The ease of access to *Ciona intestinalis* is another reason that makes these organisms so attractive as a model organism. *Ciona intestinalis* is relatively easy to obtain on both coasts of the United States. Also, they are readily available in most all marine environments and some estuaries. This ease of access makes them attractive to researchers because of their abundance for research projects. Furthermore, *Ciona intestinalis* may also be cultivated and maintained as colonies in a laboratory setting. *Ciona intestinalis* are transparent and only have three tissue layers. This makes dissection easy for researchers as well. Moreover, the entire genome of *C. intestinalis* has been sequenced and annotated.

Another compelling reason researchers are attracted to *C. intestinalis*, is due to the rapid development of fertilized embryos to mature adult organisms. The first cell division becomes visible approximately forty-five minutes post-fertilization. The prospect of controlling all *in vitro* conditions is appealing and almost essential for researchers. This control allows researchers the ability to reduce extraneous variables and ensure optimal growing conditions for the sensitive organism. All of these reasons combine to make *Ciona intestinalis* an attractive model organism for researchers.

The FOX0 transcription factor plays an important role in cardiac development. However, there has been very little investigation into the function of this gene. Through genetic breakthroughs, the entire genome of *Ciona intestinalis* has been mapped and orthologous gene identified in both vertebrates and chordates (See Appendix A). The research that has been completed on this gene shows that it plays an important role in cardiac, muscular, and neural development, but the exact function is not known. As stated earlier, *Ciona intestinalis* has only one copy of the transcript while humans have four copies of the gene. It is logical that humans have four copies of the gene because of FOX0's role in not only cardiac but muscular and neural development as well. *Ciona intestinalis* does not have a muscular system like humans nor do they have a brain or central nervous system. This investigation aims to define the function of the FOX0 transcript in cardiac development in *Ciona intestinalis*.

The processes of fertilization, dechorionation, electroporation and the CRISPR/Cas9 system will allow manipulation of the DNA of *Ciona intestinalis*. Fertilization will allow this study to have ample specimens to manipulate because some may not develop to the juvenile stage. Dechorionation will enable this study to manipulate the DNA of *Ciona intestinalis*. Electroporation will allow this study to put the desired DNA strand into the DNA of *Ciona intestinalis*. The CRISPR/Cas9 system will allow this study to isolate the FOX0 transcript and examine its function through the process of ‘knocking out’ the gene. After these steps are completed, then the development of *Ciona intestinalis* can then be monitored to see the role FOX0 plays in cardiac development of *Ciona intestinalis*.

**MATERIALS & METHODS**

**CRISPR/Cas9 System**

In order to investigate the function of FOX0 in *C. intestinalis* using CRISPR/Cas9, a
specific *C. intestinalis* FOX0 sequence must be identified. The protocol for designing and cloning a single guide RNA (sgRNA) expression vector for use in the CRISPR/Cas9 system was adapted from Stolfi et al, 2014. In order to design the sgRNA guide construct, a series of nineteen nucleotides, 5’ to Protospacer Adjacent Motif (PAM) is identified. Due to the ease of use of the CRISPR systems, the vast amount of research using it, and the complete annotation of the genetic information of *Ciona intestinalis*, E CRISP is a tool that researchers can use to identify potential target sequences for their gene of choice. In order to make sure that the targeted gene has been specifically identified and to reduce the chance of non-specific ‘off targets’, sequences will be sorted using the Basic Local Alignment Search Tool or BLAST using (https://blast.ncbi.nlm.nih.gov). Once the target sequence has been located and isolated, then a proto-spacer nucleotide ‘G’ and an overhang sequence ‘agat’ will be inserted to the 5’ terminus end using PCR; this will then become the top oligo. Another overhang sequence of ‘aaac’ will be inserted on the 3’ terminus end of the reverse complement strand of the target sequence; this will become the bottom oligo that is necessary to anneal to the top oligo for cloning.

![Figure 3: An illustration of the CRISPR/Cas9 process isolating and annealing the desired target sequence in place.](image)

The target sequence will be incorporated into a vector that also includes a promoter sequence 5’ to the inserted target sequence as well as a sgRNA(F+E) scaffold at the 3’ end. The vector will be used for amplification of the target sequence to produce abundant plasmid DNA that can then be electroporated into *C. intestinalis* embryos that will express the exogenous DNA along with the CRISPR/Cas9 components. In order to get embryos that can be electroporated, two processes must occur: in vitro fertilization and dechorionation of fertilized eggs.

![Figure 4: An illustration of the process of electroporation of a desired plasmid into vector DNA.](image)

*Ciona intestinalis Maintenance and Husbndry*

*C. intestinalis* are sensitive organisms and pH, water temperature, salinity and overall water quality must be strictly maintained. Optimal pH is approximately seven. Water temperature must be maintained at 56°F and the optimal salinity is 3.5%. Strict attention to the overall quality of the water must be maintained after the biological load (i.e. a batch of *Ciona intestinalis*) was added to the aquarium. This was achieved using a water quality testing kit that tested for ammonia, nitrite, and nitrate levels. These levels must be maintained because these products of the natural biologic processes of *Ciona intestinalis* are harmful and toxic to the animals. Ammonia, nitrite, and nitrate levels were monitored using an API Salt Water Quality. Salinity was measured using a refractometer. Artificial seawater was made using Instant Ocean. The organisms were obtained from M-Rep in San Marcos, California.

![Figure 3: A.) Protein filter that detoxifies the metabolic wastes from the biological load. B.) Chiller that moderates the temp of the water C.) C. intestinalis](image)
organisms D.) Digital thermometer that constantly monitors water temp.

Ciona were received from M-REP every 2 weeks. Once received, they were allowed to acclimate to the aquarium for at least 24 hours prior to initiating any procedures. In addition, gamete production in *C. intestinalis* is affected by periods of darkness, a process called light induced spawning (Wantanbi et al., 1973). To alleviate this problem, a light is added to the tank to provide constant illumination in order to stimulate gamete production prior to in vitro fertilization.

**In vitro Fertilization**

Cross fertilization was achieved following the Cold Spring Harbor protocols (CSHP, 2009). The eggs and sperm of the organism were collected and placed in separate dishes. The eggs were collected in an egg basket that was then placed in the sperm dish that contained filtered artificial sea water, Tris 9.5 buffer, and sperm. The eggs and sperm were allowed to mix for one minute and thirty seconds and then sent through a series of washes to remove any excess sperm. Some of the fertilized eggs were then pipetted into a plate and their development was monitored. The rest of the fertilized eggs were then pipetted into a formaldehyde coated plate. The plate was prepared according to Cold Spring Harbor Protocols for coating dechorionation plates. This coating keeps bacterial growth to a minimum and also coats the plates for dechorionated eggs. Dechorionated eggs are extremely susceptible to rupturing if they come into contact with anything plastic. They will also attach to and grow if not properly placed. Then the eggs could proceed to the next step, dechorionation.


**Dechorionation**

Dechorionation was achieved following the Cold Spring Harbor Protocol (CSHP, 2009). The fertilized eggs were collected in a conical tube that had been altered to include a filter in the exposed end of the tube. This would allow for the fertilized eggs to come into contact with the dechorionation solution and the ensuing washes that occurred. The eggs were then passed through the series of washes in formaldehyde coated plates. As stated earlier, the intention of the formaldehyde coating was to keep bacterial growth to a minimum. The plates were also coated with filtered artificial seawater and the dechorionation solution in an effort to keep the fertilized eggs submerged in water. The eggs were then sent through five subsequent washes to remove excess solution. Some of the eggs were then plated on a formaldehyde coated plate lined with FASW. It was then placed in an incubator held at 18°C and monitored for development. The other eggs were then sent through the next process, electroporation.
Electroporation was achieved using the Cold Spring Harbor Protocol (CSHP, 2009). DNA constructs are then placed in a cuvette along with embryos, mannitol and topped off with FASW if necessary to ensure that all samples contained the same volume. The machine was turned on, the voltage was set at 0.05 kV, 1.00 µF X 1000, and samples were then placed in the Gene Pulser electroporator. The construct was then electroporated into fertilized embryos.

TRIzol® Reagent
Isolation of RNA was achieved using the TRIzol® Reagent protocol from Thermo-Scientific (THSP). RNA was extracted from specimens at different stages of development; larval, juvenile, and the first ascidian stage to detect when FOX0 is expressed in C. intestinalis. After adding phenol and chloroform to RNAse free tubes, the samples were then incubated on ice for ten minutes and then centrifuged. After, the aqueous supernatant that contains RNA was removed and placed in another RNAse free tube and isopropyl alcohol was added to the tube. The samples were then incubated for ten minutes and centrifuged for ten minutes. The pellet was then air-dried under the dryer hood for ten minutes and then the pellet was dissolved in RNAse free water and then incubated again for ten minutes. The results were then obtained using PCR.

RESULTS

CRISPR/Cas9
At the time this paper was written, the constructs were unavailable. However, the expected results from this investigation are that there will be altered cardiac development through the manipulation of the FOX0 transcript.

In vitro Fertilization
The results of in vitro fertilization were as expected. The embryos reached the 2-cell stage 45 minutes post-fertilization at 18°C (Figure 6) and then progressed to the larval stage by 12-18 hours post-fertilization (Figure 7). Larvae were then moved to uncoated plates to develop into juveniles. Juveniles were evident 3 days post-fertilization (Figure 8).
Dechorionation

During the process of fertilization, a chorion is formed to protect the fertilized egg. However, the chorion protects the fertilized egg so much so that it inhibits any manipulation to the fertilized egg. To circumvent this process, the chorion is removed during dechorionation. The fertilized eggs were moved to a container with a 10 micron filter to keep the fertilized eggs in the dechorionation solution. The solution was comprised of sodium thioglycolate and sodium hydroxide was placed in a series of petri dishes to perform a serial wash of the fertilized eggs to remove the chorion.

FUTURE DIRECTION OF INVESTIGATION

Although this research was not completed in one investigational time period, there are still plans to continue this study. The aims of this study will be to identify the role of the FOX0 transcription factor in cardiac development of Ciona intestinalis. This study can give researchers keen insight into the not only the role that FOX0 plays in cardiac development in Ciona intestinalis, but also to perhaps gain insight into our own cardiac development. There are many directions that this investigation can take. All of them will be beneficial to our current understanding of cardiac development.

LITERATURE CITED
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APPENDIX A

APPENDIX

Appendix 1: pMiCiTnIG (PMIC) Construct Sequence

Gray: SV40ter

Green: GFP

Blue: NLS

Yellow: promoter

Pink: Minos ITRs

GGTACCAAGTGTGGAYATGCTAAATGTTTTCAATTTTCGCCATTAAGACAA
GCCTACACAATGTGTCATCATAAAATATAGTTACACGTTAGCACAGCTTCGACGA
GCCCAACAACTATTAAATTCAAGACCATGTTTTCGAGTGGCAATGCTTATGAC
AACACATATATATTCAATACACTAAAGATAACATACCATACATGCTATTCTGT
CTCAAAGAGAATTATTTATTTTCTTACGAGCAAAATCAAAAAATTTTGGTCTCTATTT
CACAACAAACAAATATAGGTAAATTTTATTCAAACCCGTGTTAGTAAAGAGAT
AAAGAAAAAGTGGACACCATATTAAATTCAAGACGGCGGCGGTACGTTACCTAAATC
TCAGAAGACAGAACAACAAAAGCAAAACTAAATGTAACGAATCTAATTATCTGTTA
TGATCTGCATAATAGCCATGCCAGGCGACAGCAATAACAGCGTCTGCTGCGT
GTCAACCTCAGACGCGGTTAGGTGCTTGTCACGTACATACGTGACGCTGTTATC
GCCTGAGCAGCAGTGTTCTCCCGTGATTTTTGGTCTGGATTTCAAATTTATTGATT
AACAATATAAAATTTTATTTTATTTATTTATTTATTTAACTATATGAAAACTATGATAT
AAGCATATTTCGTTAATTTTAAACCGAAAATTAGCTATAGTTAAGACACTTCCAG
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GGTTATCAGCTCAATTATTAAACCTTGTATTTTCTGGAGGGTTTAAAAATTA
AAAGCCTTTAAGCATTATTATTATTTTATTAAATAAAGTTTTATCTTCTAGGGTTG
TATAGATATACCTTTGTTAACATATAGCAGAAAATCAATAGGAAAAATTTTTCTTTA
GCTGATAAGACTACCATACCTGCGTAAACCTTTAGGAAGTCATATAATGAT
ACTTACTCTTCAGTTACTGAAATGCACTGCTATAAACCGGAAATTTGCGAGTT
TCGGCCTATTGGTTAAAATAAGCTGATTAAACAAAAATTATAACGCGAATTT
TAACAAAAATTATAACGCTTACAAATTAGGTGGCACTTTCGGGGAAAATGTGC
GCGGAAACCCCTATTGGTTAAAATTTCCTAAATACATTCAATATGATATCCGCTCA
TGAGACAATAAACCTGATATAATGTCCTCAAATAATATTGAAAAGGAAGAGATAT
GAGTATTCAACATTTCCGTGTCGCCCTTATTCCTCTTTTTTGCGGCAATTATTGCCT
TCCTGTGTTTGTCTCCACCCAGAAACGTGTTGGAAGTAAGAAGATGCTGAAGAT
CATTTGCTGACGAGTGAAGAGTGTTTTCTATGAGTCGCCTTTAAGAGCTATCATTAA
GTTCTGCTATAGGGGCAGGTATTATCCCGGTTATTGTCACCCGCCAGAAGACCCAGAC
CGTGGCCGCTACATACATTCTACAGATGAATCTGTGATGACTCAAACTGCAACTTAC
CAGAAAAAGCATCTTAGGCAAGATGTCAGTAAATGTTGATGAAGACTCAGTCACTC
GCTAACAATGAGTATAACACTGCGGCAAATTAATTCTCTGGACAACAGATCGGA
GGACCGAAGAGGCTAAGCCTTTTTGGCAAGAACATTTGGGGGATGACTAACCTC
GCTTGTGCTCCTTGGGAAACCCGGAGCTGATGGAAGCATCCAAACACGCCAGAGCG
TGACACCACAGATGCTGCTGCAATGCAAAACACCTGCCAGAAttAACATACCTTT
TGCTAAGAGGCTAGATTATGTCACGAGCTTTTGAAGATCTTATGCTTAC
AGATTGATTTTAAACCTTCATTTTTAAATTTAAAAGGATCTAGGTAAGAGATCCTT
TTTGATAATTCATCAGCCAATAAATCCCTTAAATCCCTGAGTATTGTCGTTGCAG
GTCGAGCCCTGGTGAAGAAAGATCAGAAAAGGTATCTTGTGACGTATCCTTTTTTTCTGC
GCGTAAATCTGTGCTTGGCAGAACAAAAACCCACGGCTACCAGCGGTGTTTTG
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CAGTTAGCTGCTGCTGCCAGTTGGCAGTAAAGTGCCTGCTTCCCAGGTTGAGACTCAAG
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ACACACCCAGATCAGACGAGAAGCTACACCCGGAAGATACACCTAACG
GTGAGCTAGTGAAGAAAGGCAGCCACGCCCTCCCGAAGGGAAGAAGGCGGACAGGT
ATCGGCTAGCCGCGAAGAGAGGAGAGGACCGACGAGGAGCGCTTCTCAG
GCGGAAACCGGCTGGTATTATGCTAGTGGCTGCTGCTGGACACCTCTGACTT
GAGCTGCTAGTATTCTTTGATGCTGCTGCTGCTAGGGGGCGCCGAGCCTATGGAAGGACG
CCACACCCGCGGCTTTTTACGTTCTGGCCTTGGCCTTTTGCTCACAC


Appendix 2: pMiCiTniGCiprmG (PMIC cip) Construct Sequence

Gray: SV40ter

Green: GFP

Blue: NLS

Yellow: promoter

Pink: Minos TRs

TGTTCCTTTCCTGCCCTATCCCCGGATATTCTGTGGATAAACCCTATTTACCGCCTTTG
AGTGAAGCTGATACCCTCGTCGCAGCCGAGCCGAGGAGCGGCGAGTCAGT
GACGAGGAAGCGGAAGAGCGGCCCATAACGACACCGGCTCTTCACGGCCGC
TTGGGCCGATTCTAATAAGCGATGGCCACGACAGTTCCTCAGCTGACTGAGGAG
GGCAGGTAGCGGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACC
AGGCTTATACCTTTATGCTTGCCGCTTCTGTTGTGTTGGATTTGGAGCCG
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TTAACCCCTCACTAAAGGGAAACAAAAAGCT

GGTACCAAGTGCTTGAAATGCTAAATGTTTTCCATTTTTCCGATTTAAGACAA
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FACGA
GCCGCCAACCACATTTAATTCCGAAACAGCATTTTTTGGCAGCTGGCAATTGTTC
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TGTTGCTGCTTTTGCTGCTATCTAAGAGAC
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CGGGCGATGTGAGCGCAACGCAATAATGTGAGTGGATGCTACTATAGGCCACC
CCAGGCTTTACACTTTATAGTCTCATCCGGCTGATGTTGTTGGAATTGTGAGCG
GATAAACAATTTCAACACAGGAAACAGCTATGACCATGATTACGCAAAACGCGC
AATTAACCCCTCAGTAAAGGGAACAAAAGCT
Mindfulness, Coping, and Psychological Well-being Among Diverse Populations

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ABSTRACT
Racial minority populations in the United States have increased in the past decade. The purpose of this study was to examine how mindfulness and other coping methods affect stress levels among a diverse population. One hundred-thirty-two participants took an online or paper survey including a General Health Questionnaire, Psychological well-being and physical health symptoms, and a Five Facet Mindfulness Questionnaire. Results show African Americans report more religious and overall mindfulness coping, and Hispanics report lower levels of wellbeing and lower active coping including mindfulness. There was a positive correlation between mindfulness and well-being and a negative correlation between mindfulness and maladaptive coping. Hispanics have lower psychological well-being reports than African Americans and Caucasians. Since mindfulness was linked to psychologically well-being, it is likely that adults were using it as a strategy to counteract avoidant and maladaptive coping skills. These findings extend our understanding of mindfulness to previously overlooked minorities.

INTRODUCTION
Racial minority populations have increased over the past decade. Hispanics have become the largest and fastest growing ethnic minority group in the United States. According to the 2012 Census, of the total population they make up 19%, and between 2000 and 2010, they accounted for more than 50% of the total population growth (Cheng & Mallinckrodt, 2015). With the racial and ethnic minorities increasing, specifically the Hispanic population, there is a profound need to better understand the factors and coping strategies that contribute to the mental health of these populations. Studies have shown a correlation between racial discrimination, stress, and negative coping strategies (Polanco-Roman, Danies, & Anglin, 2016; Torres, & Vallejo, 2015; Villegas-Gold, & Yoo, 2014). However, there has been very little research on the use of mindfulness as a coping strategy in non-Caucasian populations.

Stress
Stress is defined as the interaction between stimulus and the responses it creates (Woods-Giscombe, & Lobel, 2008). When an individual is exposed to stimuli they perceive as demanding and/or threatening and they do not believe they have the capacity to manage the event, stress occurs. Being exposed to constant stressful situations that drain an individual’s ability to cope may lead to feelings of anger, anxiety, fear, frustration, and hopelessness. Unsuccessful coping may result in a physiological stress response that affects the immune, nervous, endocrine, and cardiovascular systems (Butcher, Hooley, & Mineka, 2014) and lowers the overall health of the person.

Stress Among Racial Minorities
Carter and Reynolds (2011) found that incidents of racial/ethnic discrimination that accumulate in African American’s lives can be experienced as traumatic since they often happen suddenly, are beyond the control of the person, and have the potential to be psychologically stressful. Discriminatory events that create emotional stress can promote physiological stress. For example, compulsive focus on the possible threat of ethnic discrimination is associated with higher levels of
stress and worry. Stress and worry have been linked to negative health. Utsey, Chae, Brown, and Kelly (2002) showed that there is a significant difference between Asian American, African American, and Hispanic American participants when psychological well-being was compared to race-related stress. Polanco-Roman, Danies, and Anglin cited a study by Carter and Forsyth (2010) that found that 78% of racial/ethnic-minority adults who reported direct experiences with racism stated that the events were stressful, and 44% reported feeling stressed for a long period of time after the event. Polanco-Roman et al. (2016) found no significant difference in racial discrimination between males and females. They did, however, find that females were more likely to engage in active coping rather than passive coping. There are a variety of ways in which individuals respond, cope, and adapt to stress and emotional distress. These include hypervigilance, avoidance, or numbing, and are known as dissociative symptoms.

Engagement, Disengagement, and Alcohol Coping

Villegas-Gold and Yoo (2014) examined different engagement and disengagement coping strategies in Hispanic college students. They defined engagement as any attempts to actively manage the stressful situation or event through social support, cognitive restructuring, expression of emotion, and problem solving. They also defined disengagement coping as attempts to be removed mentally, emotionally, and physically from stressors; examples include problem avoidance, self-criticism, wishful thinking, and social withdrawal. They found that only problem solving (behavioral and cognitive strategies designed to eliminate the source of stress by changing the situation) was strongly linked with higher intrapersonal well-being. They also discovered that self-criticism (blaming oneself for the situation and criticizing oneself), wishful thinking (cognitive strategies that include denial that an event occurred, reframing, or symbolically altering the situation), and social withdrawal (withdrawing from family and friends) were associated with lower personal well-being. This is consistent with other studies that suggest that negative coping strategies can lead to alcohol and substance abuse, thus lowering personal well-being.

Bodenlos, Noonan, and Wells (2013) found that stress levels are positively associated with alcohol use and alcohol-related problems. They also found that males reported more usage of alcohol than females, indicating that males could experience more stress or have lower levels of stress coping skills.

Cheng and Mallinckrodt (2015) theorized that among Hispanic college students, more discrimination led to higher stress levels and high consumption of alcohol. Previous research (Rodriguez-Esquivel, D., Webb-Hooper, M., Baker, E. A., & McNutt, M. D. (2015); Torres & Vallejo (2015)) shows that Hispanics reported greater avoidance/numbing and hyperarousal than non-Hispanic Whites. As a result of ethnic discrimination, a Hispanic can experience recurrent emotional stress. Stress taxation can lead to significant depletion of an individual’s physiological, cognitive, and emotional coping resources, compromising the ability to effectively manage stressful situations. The study conducted by Torres and Vallejo (2015) found two things: alcohol usage was higher in Hispanics born in the United States compared to those born in other countries, and alcohol usage among Hispanic males was linked with reaction to discrimination. This suggests alcohol abuse is used to numb or avoid the feelings of stress. The Centers for Disease Control and Prevention stated that one of the risk factors for suicide is a history of alcohol and substance abuse.

Coping Strategies: Suicide and Religion

Emerging racial and ethnic minority adults (ages 18-25) have a heightened risk for suicide when compared to Caucasian emerging adults. The Centers for Disease Control reported that in 2010, between 24 and 32% of suicides among Asian, Black, and Hispanic individuals were in young adults aged 18 to 29, compared with 15% of suicides among similarly aged Caucasian individuals. Additionally, the Center reported that among Hispanic high school students, 18.9% seriously considered attempting suicide, 15.7% of students made a plan, and 11.3% actually attempted suicide. The Center stated that this was consistently higher
than Caucasian and African American students. There is evidence to suggest that avoidant strategies such as acceptance and resignation, while common, prove to be more harmful when compared with more active approaches like problem-solving or seeking support (Polanco-Roman, Danies, & Anglin, 2016). Avoidant strategies tend to lead to alcohol and substance abuse, which are known to be comorbid with other harmful, often self-destructive disorders like depression.

Utsey, Chae, Brown, and Kelly (2002) found that African Americans reported experiencing higher levels of race-related stress; however, when comparisons test was performed, they found that compared to Asian Americans, African American participants had significantly higher psychological well-being scores than Asian American participants. Their study found that African Americans scored significantly higher on the quality of life and psychological well-being scale when compared with Hispanics and Asian Americans. African Americans may seek social and psychological support from their ethnic and spiritual community in response to racial stressors.

Spiritual beliefs are often important in forming perceptions of self, others, and the world. Reinert, Campbell, Bandeen-Roche, Sharps, and Lee (2015) found that both Caucasian and African American women were more religious than men and more likely to use religious coping when facing stress. However, Sherman, Harris, and Erbes’ (2015) meta-analysis and case study found that survivors of trauma could also face spiritual and/or religious challenges such as blaming their higher power, thinking they are being punished for sinning, or completely distancing themselves from their faith. Sherman, et. al (2015) suggested that intrusive memories and flashbacks can be distressing because of their interruptions in daily life. In addition, intrusive memories may disrupt spiritual practices (e.g., prayer, meditation), leading to further distance from religious practices and the loss of a potentially useful coping skill.

One coping skill Sherman, et. al (2015) suggested for veterans with intrusive memories and flashbacks was mindfulness techniques because mindfulness requires mental discipline and focus; it has been shown to help veterans either reduce intrusive symptoms or more effectively redirect mental activity during or after distressing events. However, veterans who have difficulties with flashbacks or intrusive memories may struggle, since it could prove difficult to focus on breathing or to empty one’s mind of intrusive thoughts without a main point of focus. For these veterans, Sherman, Harris, and Erbes (2015) suggest that a point of focus may help keep intrusive thoughts at bay. Mindfulness and yoga approaches that invite participants to become aware of and slow breathing may lower stress levels that in turn increase the spirituality, psychological, and physiological well-being of an individual.

Mindfulness

Mindfulness is a 2,500-year-old practice recently introduced in the West, and it has been associated with reduced stress in adults. Mindfulness is usually defined as directing one’s attention so acceptance of the present moment experience can occur (Baer, Smith, & Allen, 2006; Bluth, & Blanton 2014; Boatright, & McIntosh, 2008; Bodenlos, Noonan, & Wells, 2013; Bodhi, 2011). Baer, Smith, and Allen (2006) created a survey to measure four mindfulness skills: observing, describing, acting with awareness, and accepting without judgment. Mindfulness focuses on the relationship with thoughts, feelings, and sensations in the present moment. Bluth and Blanton (2014) revealed that higher mindfulness levels, as measured by the Children and Adolescent Mindfulness Measure (CAMM), increased self-compassion and lowered perceived stress. Mindfulness as stated by Call, Miron, and Orcutt (2014), can be thought of as a skill set used to adapt the experience of stress and anxiety through the development of self-regulation by paying attention to the present moment and acceptance of internal and external emotions, thoughts, and experiences.

Mindfulness and Coping
Mindfulness prompts a state of conscious presence where the person has an accepting and nonjudgmental attitude towards events. Mindfulness has been associated with improved emotional well-being and social
functioning of adolescents (Bluth, & Blanton, 2014; Edwards, Adams, Waldo, Hadfield, & Biegel (2014). Higher levels of mindfulness have been associated with less reactivity to threatening emotional stress, positive self-regulation, better awareness and comprehension of experiences and emotions, higher acceptance of emotions, and a stronger ability to find an answer to troubling thoughts.

Bodenlos, Wells, Noonan, and Mayrsohn (2015) examined the mindfulness facets that are similar to the mindfulness skills with the addition of no reactivity to inner experience (the tendency to allow thoughts and feelings to come and go). They found that students who reported alcohol and substance abuse had lower levels of acting with awareness, were more judgmental, and were more reactive than those with no history of alcohol and substance abuse. Bodenlos, Noonan, and Wells (2013) found that higher mindfulness levels were negatively associated with stress and other negative emotions in adults and college students. Both studies found that certain facets of mindfulness such as acting with awareness and describing thoughts and/or feelings were negatively associated with alcohol use in college students. Both studies found that males consumed more alcohol in a week than females. Females reported more stress than males. Mindfulness levels did not differ greatly between gender, and men scored higher in no reactivity to inner experience than women. The results of Bodenlos, et al. (2015) indicate that the awareness and not judging facets of mindfulness are related to positive functioning and are associated with better health. The authors suggested that mindfulness based interventions with college students must continue to ensure specific facets are developed. The findings suggest that acting with awareness and no judgment play the biggest role in emotional and social health.

Carmody, Reed, Kristeller, and Merriam (2008) found that a person had a greater sense of control if they were capable of bringing mental processes under control and directing them in a positive way. They found that when thoughts and feelings do not overwhelm the person, psychological and physiological well-being was being reported. Their study also showed that decreases in medical symptoms and psychological stress were related to an increase in mindfulness. It is possible that through mindfulness, one can reduce mental preoccupation with everyday stressors and thus can increase overall psychological, physiological, and spiritual well-being.

Mindfulness and Suicide

Collins, Best, Stritzke, and Page (2016) conducted a study in which they sought to understand the relationship between mindfulness and suicide. They discovered that students who had been exposed to mindfulness interventions had lower stress levels and that mindfulness increased overall persistence in the face of perceived burdensomeness and thwarted belongingness (theorized to be possible components of suicidal behavior and suicide completion). The study shows that mindfulness acted as an acute form of resilience that protected against interpersonal difficulties. Students who had received 10 minutes of mindfulness interventions before the study showed constantly less desire to quit the study across time compared to the control group. This agrees with the idea that mindfulness enhances persistence in adversity. Mindfulness does not prevent the experience of hardship, but rather can be used as a coping mechanism. These findings with other research show that even short periods of mindfulness training can reduce emotional reactivity. By being mindful, a person learns that he/she does not have to problem-solve immediately; instead, by staying in the present, the person learns to step back and dissociate from maladaptive coping and thinking.

PRESENT STUDY

Most mindfulness studies that relate to stress and coping have focused on Caucasian adult participants (Baer, Carmody, & Hunsinger, 2012; Bodenlos, Noonan, & Wells, 2013; Bodenlos, Wells, Noonan, & Mayrsohn, 2015; Collins, Best, Stritzke, & Page, 2016; Greeson, et al. 2015; Hathaway & Tan 2009). Not many studies focus on the stress levels, coping strategies, and mindfulness skill levels of other racial and ethnic minorities. This study was
designed to help determine benefits from mindfulness skill training and teaching among different racial/ethnic groups. Males and females of multiple racial and ethnic backgrounds (Caucasian, African American, and Hispanics) will take part in the study. It is anticipated that the higher mindfulness levels are, the less stress and maladaptive coping the person will report. Additionally, it is expected that females of all racial backgrounds will report higher levels of stress. African American females will report more religious coping; Caucasians will report more mindfulness coping; and Hispanics will report higher stress levels, lower levels of mindfulness, and lower levels of active coping.

MATERIALS & METHODS
There were 132 participants (97 female and 34 male). Participants were age 18–64 years old. There were 68 Caucasian, 27 African American, 29 Hispanic, and 8 others. After obtaining approval by Winthrop University’s institutional review board, participants were recruited by using several different recruitment strategies: email, social networks, and word of mouth. All participants were given an informed consent, and all participation was voluntary. Participants filled out a series of self-report surveys either online or on paper that took approximately 15–20 minutes to complete.

MEASURES
Demographic Characteristics
Background data and lifestyle information were collected, including age, gender, ethnicity, college class, major, and grade point average, and religion.

The General Health Questionnaire
The 12-Item General Health Questionnaire (GHQ-12) asks the participants questions such as: have you been able to concentrate on whatever you’re doing, lost much sleep, felt constantly under strain, and been losing confidence in yourself, etc. Each one assesses the severity of a mental problem over the past few weeks using a 4-point Likert-type scale (from 0 not at all to 3 a lot more than usual). High scores indicate worse health.

Psychological well-being and Physical Health Symptoms
This is a coping scale on how well people deal with stress. It consists of 24 questions asking how often an individual engages in coping activities such as smoking, daydreaming, exercise, avoiding the problem, and seeking religious guidance when stressed. It is a 5 point Likert scale (1 never to 5 always). Halverson, Bliese, Moore, Castro (1995) used the psychological well-being and physical health symptoms on soldiers deployed for Operation “Uphold Democracy.”

Five Facet Mindfulness Questionnaire
The Five Facet Mindfulness Questionnaire (FFMQ) is a 39-item questionnaire used to examine individual’s mindfulness. Baer, Smith, Hopkins, Krietemeyer, and Toney (2006) created the questionnaire to examine five mindfulness subscales: observing, describing experience, acting with awareness, not judging the experience, and non-reactivity to inner experience. The FFMQ uses a Likert method (1 never or very rarely true to 5 very often or always true) to determine the rate with which each mindfulness facet occurs and to conclude the total mindfulness level. Questions include: I watch my feelings without getting lost in them; When I have distressing thoughts or images, I “step back” and am aware of the thought or image without getting taken over by it; and I disapprove of myself when I have irrational ideas. The higher the overall scores, the higher levels of mindfulness are represented.

RESULTS
Hypothesis One: The higher mindfulness levels are, the less stress and more maladaptive coping the person will report
This hypothesis was supported. Running a Pearson correlation test showed there was a positive correlation between mindfulness and well-being (r (132) = .461, p=.000) and a negative correlation between mindfulness and maladaptive coping (r (132) = -.207, p=.017).

Hypothesis Two: Females will report greater stress and less wellbeing than males
The second hypothesis was not supported. A T-test revealed there was a not a significant
difference in the scores for gender and wellbeing (t (129) =-.211, p>.05.)

Hypothesis Three: African American Females will Report a Higher Level of Religious Coping
This hypothesis was partially supported. A One-Way Anova test revealed there was a significant difference in race, though not gender, when reporting levels of religious coping. F (3, 117) =5.91, p <.05. Tukey Post Hoc test revealed that African Americans showed more religious copings (M=7.1154, SD= 2.355) than Caucasians (M= 4.89, SD= 2.79) and Hispanics (M=4.62, SD= 2.26). African American males reported more religious coping (M=7.29, SD= 1.98) than African American females (M= 7.05, SD= 2.52), though not significantly.

Hypothesis Four: Caucasians will report higher levels of overall mindfulness
This hypothesis was not supported. A One-Way Anova test revealed there was not a significant difference between race and mindfulness. F(3, 121)= .647, p>.05. African Americans showed more overall mindfulness (M=24.68, SD= 4.72) and Hispanics showed the least overall mindfulness (M= 23.43, SD=4.07).

Hypothesis Five: Hispanics will report lower wellbeing, lower mindfulness, and more avoidant and maladaptive coping.
This hypothesis was not supported. A One-Way Anova was performed and it showed that between race and wellbeing there was an approaching significance F(3,121)= 2.499, p=.063. The test showed that African Americans reported higher levels of wellbeing (M= 32.77, SD= 5.88) and lower levels of maladaptive coping (M=10.48, SD= 2.83). Hispanics reported lower levels of wellbeing (M=28.34, SD= 6.28) and higher levels of avoidance coping (M=12.17, SD=3.15). The wellbeing reports for Caucasians was (M= 31.10, SD=6.27) and they had the highest levels of maladaptive coping (M= 11.61, SD= 2.57) reported.

Additional data found
Additional data found that there is a high correlation between age and overall mindfulness (r (130) =.204, p=.020) and the less avoidance coping (r (130) =-.316, p=.000). The nonjudgement facet of mindfulness was shown to be most effective and positively correlated with age (r (130) =.213, p=.015) and overall wellbeing (r (132) =.480, p=.000). Nonjudgement was also negatively correlated with avoidant coping (r (132) =-.268, p=.002) and maladaptive coping (r (132) =-.186, p=.033). There was not a significant correlation between the nonjudgement facet of mindfulness and religious coping (r (132) =.169, p=.053).

DISCUSSION
This present study examined the relationship between mindfulness; different forms of coping like avoidant, active, maladaptive, and religious; and psychological wellbeing. Our results show that higher levels of active coping are associated with psychological well-being. Our results further show that though active coping can be used for psychological wellbeing, mindfulness as a coping mechanism can have a highly significant effect on wellbeing. The higher the mindfulness levels, the more psychological wellbeing was reported. People with higher levels of reported mindfulness also showed lower levels of avoidant and maladaptive coping. Results support research from Collins, Best, Stritzke, and Page (2016) that shows that by being mindful, a person learns that he/she does not have to problem-solve immediately; instead, by staying in the present, the person learns to step back and dissociate from maladaptive coping and thinking.

Research from Bodenlos, Noonan, and Wells, (2013); Bodenlos, Wells, Noonan, and Mayrsohn, (2015); and Woods-Giscombé and Lobel, (2008) report that females showed higher levels of stress and thus lower wellbeing. Our results suggest there is no significant difference between males and females in psychological wellbeing; additionally, our results show that males report slightly lower psychological wellbeing than females. Stress reduction and well-being promotions can be tailored for both genders.

Greeson, Smoski, Suarez, E. C., Brantley, Ekblad, Lynch, and Wolever, (2015);
Hathaway, and Tan, (2009); and Johnson, Williams, and Pickard, (2016) all reported that higher positive religious coping, that is seeking religious guidance and praying, lowers stress and promotes wellbeing. Reinert, Campbell, Bandeen-Roche, Sharp, and Lee (2015) reported that females tend to use more religious coping than males. Our results support the research that states that religious coping promotes wellbeing. Our research findings show that African Americans report significantly higher levels of religious coping followed by Caucasians and Hispanics. Our results show there is no significant difference between gender and religious coping; however, African American males report slightly higher religious coping than African American females. Caucasian males reported the lowest religious coping, right below Hispanic females. This indicates that perhaps religious or spiritual faith has an impact on psychological well-being.

The majority of mindfulness studies that relate to coping and wellbeing have focused on Caucasian adult participants (Baer, Carmody, & Hunsinger, 2012; Bodenlos, Noonan, & Wells, 2013; Collins, Best, Stritzke, & Page, 2016), in particular females. This would indicate that Caucasians would have more mindfulness than African Americans or Hispanics. Our results suggest, though not significantly higher, that African Americans show greater mindfulness levels than the other two ethnic groups. In fact, Caucasians reported slightly higher maladaptive coping than African Americans and Hispanics.

We hypothesized Hispanics would report significantly lower mindfulness and wellbeing levels and higher avoidant coping. Our results found that Hispanics, in fact, did report lower mindfulness and wellbeing levels and higher avoidant levels though not significantly different. One possible theory is that we needed more Hispanics’ input.

Our results also found that the nonjudgment facet of mindfulness (to allow reality or what is there, to be as it is without judging, avoiding, changing, or escaping it) was associated with better overall wellbeing. This makes sense. In today’s society, where judgements are done quickly and mostly without thought, nonjudgement about self, others, and situations can impact a person’s view on life and overall well-being. Psychologists should promote a nonjudgement viewpoint on life. There was no significant difference between the nonjudgement facet and religiousness, which suggests that one need not be religious to be nonjudgmental. This suggests that perhaps we need to focus on developing this facet in all populations. Additionally, older is wiser. The results showed the more years of life a person has, the more overall mindfulness and the less avoidance coping they had.

This study has limitations. First, one of the authors did not put two survey questions on the online version of the survey. Additionally, self-report measures are also a weakness of this study.

In conclusion, our results add to the literature that supports mindfulness as a coping method to improve psychological wellbeing. Additionally, we found that all groups could benefit from mindfulness training to promote wellbeing. Mindfulness training should be promoted within the Hispanic community who reported the lowest levels of wellbeing. Our next step is to translate the survey into Spanish and get a wider Hispanic population. We will also focus on different mindfulness facets and see what makes a person have higher nonjudgmental levels. Through mindfulness training like full body relaxation, mediation, or simply teaching how to be more aware and not so judgmental, psychological well-being can be improved.

REFERENCES


Examining Longitudinal Data of Juvenile Delinquents in Rock Hill, SC

Jalen Smith
Brad Tripp, Ph.D. (Mentor)

ABSTRACT

Examining factors that contribute to the initiation, continuation, and desistance of criminal activities is crucial in determining how the criminal justice system can be reformed in an effort to decrease recidivism rates, as well as halt the initiation of juveniles into the criminal realm in the first place. This study examined longitudinal data from the daily reports of the Rock Hill Police Department, as organized by the Crime Mapping Division. The study examines juvenile suspects between the ages of 10-17 during 2003-2007. Wave One looked at subjects ages 10-13 in 2003/2004. Wave Two looked at subjects ages 12-14 in 2005/2006, and Wave Three looked at subjects ages 15-17 in 2007/2008. Using the concepts of Criminal Careers and recidivism, the goal was to examine continuation or desistance of criminal behavior over six years. Indicators of race, gender, residence in gang areas, hotspots, as well as residence in a single dwelling or an apartment were used to predict continued criminal behavior. The majority of the subjects were black or white with all other races representing less than ten percent of the population. Therefore, only suspects coded as black or white were utilized. The data was examined using Linear Regressions Analysis and Chi Squares test. The Linear Regressions Analysis found that there was no significant association between offending and race, gender, gang areas, and residence in a single dwelling or apartment for Wave One only, Wave One and Three only, and Wave One and Two only. When examining offending across all three waves, there was a significant association between residents in hotspots and gang areas, as well as race.

INTRODUCTION

The purpose of this research was to identify variables that may correlate with early delinquency, continued delinquency, and recidivism. Previous research has explored life-course persistent delinquents versus adolescent-limited delinquents in terms of mental state and familial factors, but has not gone far enough to identify how variables like: gender, race, gang areas and/or hotspots, and place of residence in a single dwelling or an apartment affect the initiation, continuation, and desistance of a person’s criminal career (Bacon, Paternoster, & Brame, 2009; Elder, 1998; Farrington, 1986). The present study sought to explain the initiation and continuation of juvenile offending utilizing longitudinal data.

Sherman and Gartin (Sherman, Gartin, & Buerger, 1989) created the term hotspots in reference to the concentration of certain crimes. They found that the routine activities of hotspots may either be generators of crime or receptors of crime (Sherman, Gartin, & Buerger, 1989). The routine activities theory is premised by a criminal event occurring by an offender, suitable targets, and the absence of a capable guardian against crime converging together in time and space predictably (Sherman, Gartin, & Buerger, 1989). In regards to hotspots and crime mapping, this study aimed to use the geographic location of adolescents to gain insight as to what environmental factors influence individuals to offend. We predict strong, positive, correlations for the variables of gender, race, gang areas and/or hotspots, and place of residence in a single dwelling or an apartment.

REVIEW OF LITERATURE

The criminal career approach provides the field of criminology the notion that chronic offenders are a unique group (Sampson & Laub, 2003). Chronic offenders were believed to be a unique group because they offend persistently at
a higher rate than others, even as they grow older (Sampson & Laub, 2003). The term criminal career itself is defined as within-individual trajectories that occur over time (Sampson & Laub, 2003). Within-individual trajectories is movement of a person, individually, in a sequence of activation, aggravation, or desistance of criminal activities throughout the life course (Sampson & Laub, 2003). Sullivan and Piquero (2016) analyzed criminal career research over the past 30 years and expressed the importance of development of the concept criminal career itself. Their article reviewed past reports on criminal careers so that the strengths, as well as limitations, of those reports could help future research. The key dimensions of criminal careers (frequency, participation, duration, and seriousness) have remained an integral component in the criminal career debate. Previous research has explored what life course criminology, as well as criminal careers, mean for the field of criminology; but, it has not gone as far to identify how specific variables like gender, race, social class, place of residence, and birth year affect one another and how all of those variables can ultimately affect the initiation, continuation and desistance of a person’s criminal career (Bacon, Paternoster, & Brame, 2009; Elder, 1998; Farrington, 1986). This research examined the initiation and continuation of juvenile offending utilizing longitudinal data.

Sampson and Laub (2016) believed that there was a need for breadth and depth when it came to trying to understand individuals and institutions in relation to criminal career patterns over that individual’s life course. The Criminal Career report (2016) helped emphasize the need to describe and understand offending patterns, which is why criminologists pay homage to both its advantages and disadvantages. By viewing the Criminal Career report (2016) and other reports related to criminal offending, researchers can develop more distinct frameworks that delve deeper into patterns of desistance and persistence. As the term criminal career began to gain acceptance in the field of criminology, criminologists believed that studying the history of the criminal career concept, the elements of a criminal career, as well as the methodology involved with studying criminal careers was vital to understanding the term and its meaning.

Criminal Careers

The topic of criminal careers, as well as the length of a criminal career, has become increasingly popular within the past few decades due to the changing nature of research. Piquero and Brame (n.d.) attempted to access more information about criminal career length due to the fact that most knowledge about this topic is more than 30 years old and does not factor race into delinquency and recidivism. In order to measure criminal career length, Piquero and Brame took the difference in ages between the last and first criminal justice contact. They found that parolees who scored higher on cognitive abilities tests had shorter criminal careers and that those who came into first police contact at a later age had longer lengths of prison stay. However, parolees who were older at first police contact tended to have shorter criminal careers. Piquero and Brame’s research showed that early age was a significant predictor of a long criminal career and that the duration of time a person spends in jail affected their overall criminal career length. While researching criminal career length through the observation of parolees is important, knowing the basis of why individuals offend throughout the life course is just as equally important, which is why Sampson and Laub’s opinions and findings are examined.

Sampson and Laub (2016) took part in an important debate surrounding life-course criminology in which the National Research Council’s (NRC) report was the center of attention. The major argument of the NRC report was that scientific knowledge about crime and delinquency had been prohibited by the lack of data. Sampson and Laub created a coding scheme that was longitudinal for the basis of criminal offending in order to combat the shortcomings of the Glueck’s coding scheme. Sampson and Laub used the life-course perspective of criminology because this perspective holds the notion that individuals have continuity and change in behavior as they age. Concepts that were important in relation to crime over the life span were turning points and
trajectories. They demonstrated how turning points in a person’s life could lead to desistance of criminal careers/activities. Their findings reflect the changing nature of thought in the field of criminology and outlines specific concepts, like life-course criminology, that may produce variables that demonstrate how the decisions a person makes in their life affect the course of their offending (Sampson & Laub, 2016). To understand why an individual offender, researchers must examine that individual’s history and that includes their childhood, as well as the environment surrounding said childhood.

A study conducted by Farrington and Ttofi (2011) found that the relationship between bullying and later offending juveniles was significant, even after controlling for early risk factors. They also found that bullying was a strong predictor of antisocial outcomes, which can contain elements of offending behavior. This study was longitudinal, indicating the importance of examining life-course criminology. This article demonstrates how bullying is a unique action and that there is a possibility that interventions that aim to reduce school bullying can ultimately shape reductions in a person’s criminal lifestyle. In observing adolescents, monitoring the change in behavior over a period of a few years can yield paramount findings and possibly pinpoint when and where non delinquency meets delinquency.

Loeber and Stouthamer-Loeber (1991) addressed the concurrent and longitudinal patterns of offense seriousness for boys in their research. They found correlations within initiation, escalation, and desistance. They found that of the three age cohorts studied, the largest proportion of the youngest cohort were a part of the non-delinquency category. On the opposite end of the age cohort, the oldest cohort held the smallest proportion of the non-delinquent group category. Non delinquents in the young adults sample decreased over time, while the proportion of moderate to serious delinquents doubled. These findings are important for researchers today because this study allows the public to see how changes in the percentage of adolescents go from non-delinquent to delinquent in relation to changes in age. This study observed the initiation, as well as escalation of a criminal career. The Life-Course Perspective examined three processes within offending and those were activation, aggravation, and desistance (LeBlanc and Loeber, 1998). Activation was referred as the way in which the development of criminal activities once stimulated was continued, frequented, and diversified (LeBlanc and Loeber, 1998). After the process of activation came aggravation, and aggravation to LeBlanc and Loeber (1998) meant that a developmental sequence of delinquent activities increased in seriousness over time. The final process in Life Course and Developmental Criminology was desistance (LeBlanc and Loeber, 1998). Desistance was the slowing down in frequency or seriousness of offending (LeBlanc and Loeber, 1998). An important element in life-course criminology is the presence of re-offending. Re-offending, also known as recidivism, can often arise for a number of reasons (Sampson and Laub, 2003).

Recidivism

Recidivism is the risk of continued offending across unique groups, as defined by Grunwald, et al. (2010). Criminologists study recidivism and variables associated with criminal careers in order to better understand patterns of offending. Before criminologists can study recidivism, they must first have a concrete idea of what this term means. In a study concerning juvenile offenders who were housed in a rehabilitation center, Ganzer and Sarason (1973) found that there was only a slightly higher proportion of recidivists who came from a broken home, than non-recidivists. Ganzer and Sarason (1973) also found that males who offended but later did not become recidivists, were significantly older at the time of their first commitment than either male recidivists or female non recidivists. The most prominent predictors of recidivism found were: family background, age at first offense/commitment, and diagnostic classification. In this research, they used diagnostic classification to mean mental and behavioral disorders like antisocial personality disorder and neuroticism. These predictors help explain how external factors in a person’s life can influence their criminal career.
Ganzer and Sarason’s (1973) article showed that there are sex differences in offending, as well as reasons why individuals offend. While Ganzer and Sarason chose to examine factors that surround recidivism, Livingston and Stewart (2008) chose to turn their attention to trajectories and whether or not juvenile trajectories lead to adult offending.

Livingston, Stewart, Allard, and Ogilvie (2008) examined how many distinct offending trajectories could be identified. They discussed how sex, indigenous status, and other variables were related to trajectory membership. They also examined whether or not juvenile offending trajectories are predictors of adult offending. Sex, indigenous status, and socioeconomic disadvantage were shown to be related to offending trajectory group membership, although remoteness was not. Remoteness, in this study, was viewed as remoteness of residence. Remoteness of residence was based off of where the juvenile resided at the time of their court appearance or where cautions were given. Regarding juvenile offending trajectories and their ability to predict adult offending, Livingston, et. al., (2008) found that chronic offenders were twice as likely as other offenders (early peaking-moderate offenders or late onset-moderate offenders) to have finalized adult court appearances. Findings that chronic offending trajectories were five to 15 percent of the total cohort reinforce prior studies’ results. This shows that group membership, a social factor, is related to juvenile offending (Livingston, et. al., 2008). Family life plays a humungous role in shaping an adolescent’s future; whether that be socially, economically, or otherwise. It is logical to believe that familial demographics would have a hand in juvenile offending as well.

Spatial Variations

Spatial variation in crime is linked to both the physical and social environment of a neighborhood (Sherman, Gartin, & Buerger, 1989). This concept is important to criminology because where a crime takes place matters just as much as how many crimes occur. Sherman and Gartin (1989) attempted to provide a better description of how crime varies across place. They found that police call data is one of the most reliable ways of getting information about time and place variations in crime. Sherman and Gartin created the term hotspots in reference to the concentration of certain crimes. They found that the routine activities of hotspots may either be generators of crime or receptors of crime (Sherman, Gartin, & Buerger, 1989). It is also important to note that according to research and implementation of new practices, routine activities of people do not necessarily have to change in order for places to be less criminogenic. Lastly, in relation to crime and space, it was thought that variation of crime within communities is larger than variation of crime across communities. Hotspots tell police agencies and the public where crime is most concentrated. If agencies can know who is committing crime the most and where, intervention methods and prevention methods for present and future crime can be implemented (Sherman, Gartin, & Buerger, 1989). Sherman and Gartin (1989) focused on crime varying across place, but failed to mention how a community’s structure and culture affect crime, so Sampson and Wilson (1995) attempted to do that.

Sampson and Wilson (1995) attempted to incorporate structural and cultural aspects of society into race, crime, and inequality for the purposes of demonstrating how those variables affect and are affected by community life. They found that structural factors like low economic status, ethnic heterogeneity, and residential mobility gave way to community social organization being disrupted, which thus caused variations in crime and delinquency rates. Sampson and Wilson (1995) also found that family disruption had an effect on juvenile violence and juvenile delinquency. A main point found is the distinction between poor blacks versus poor whites and their ecological dynamic. With the emergence of social isolation, institutions faltered which made social bonds weak. The weakening of social bonds has the potential for other aspects of society to change, which can lead to an individual’s decision of a turning point to be affected. Changes in the urban structure of minority communities in the 70’s and 80’s spurred changes in the activities of juveniles. Sampson and Wilson’s (1995) article
provided the historical context of how affecting the residency of people also affects criminal activities. In keeping with the theme of community in relation to crime, Roncek and Maier (1991) examined specifically the relationship between crime and bars/taverns.

The association between traditional index crimes and property crimes as well as violent crimes was examined by Roncek and Maier in 1991. Roncek and Maier (1991) found that 499 residential city blocks with either taverns or lounges had 21,099 index crimes committed. They also found that crime of every type except murder was significantly higher on blocks with taverns or lounges than on blocks without them. The first two predictors of assaults, which had the strongest effect of any individual crime type, was block population and residualized crime potential (Roncek & Maier, 1991). Bars and taverns were looked at specifically due to their affiliation with a suspect, a victim, and a chance opportunity that may not have happened if the location was different. Their research used routine activities theory to link crime to specific establishments in a city. Routine activities theory could also work for establishments like stores in which delinquents may visit. An establishment’s acquired environment can further increase the probability that crime will occur and that the individuals who have a presence in that certain environment have previously been or will be associated with crime. Crime and the circumstances of where or when it occurs shapes not only a criminal’s career, but the reputation or mark on a specific place.

DATA

With the occurrence of a disproportionate amount of crimes in a certain location comes the term “hot spots.” A hot spot is a group of incidents that are clustered together. Data collected from the Rock Hill Police Department Crime Mapping Division allows conclusions to be drawn as to specifically what types of crimes are being committed and where geographically. The articles discussed previously all present the theme that crime is a product of many dimensions of society being poorly affected in some type of way; whether that be socially, economically, environmentally, or otherwise. Previous research on the topic of crime over the life course lacks a broad overview of an adolescent’s life and the criminal activities they engage in that could potentially signal the initiation of a criminal career. Many factors that make up the broad overview of an adolescent’s life were examined in this study. Another unique trait of this study is that it was longitudinal, so criminal activities over the life course of an individual were studied. With regards to crime mapping, this study aims to use the geographic location of adolescents to gain insight as to what environmental factors may influence them to offend. We predicted strong, positive correlations, main effects, and interactions for the variables of gender, race, social class, place of residence, and birth year.

METHODS

Participants

Subjects were studied from a population of 10-17 year olds who were suspects in Rock Hill, SC according to the Rock Hill Police Department. This was a longitudinal cohort study. Data was broken up into three waves with Wave One beginning in 2003-2004, and subjects being ages 10-13. Wave Two encompassed the years 2005-2006, in which subjects were between the ages of 12-15. Lastly, in Wave Three, subjects were between the ages of 14-17 during the years 2007-2008. The majority of the data focused on black and white subjects since there was minimal data on individuals of other racial classifications. Subjects were coded according to gender and race, with females being coded as “0,” males as “1,” and Whites coded as “0,” Blacks as “1.”

The other key variables were based on residence in particular areas. Not living in a hotspot was coded as “0,” while living in a hotspot was coded as “1.” Not living in a gang area was coded as “0,” while living in a gang area was coded as “1.” Finally, living in a single dwelling was coded as “0,” while living in an apartment was coded as “1.”

The dependent variable was an examination of offending over different waves and patterns across these waves. Patterns of offending were coded, with Wave One only
coded as “1,” Wave One and Two only coded as “2,” Wave One and Three only coded as “3,” and Wave One, Two, and Three only coded as “4.”

<table>
<thead>
<tr>
<th>Waves</th>
<th>Black Males</th>
<th>White Males</th>
<th>Black Females</th>
<th>White Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
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<td>84</td>
<td>57</td>
<td>38</td>
<td>18</td>
<td>197</td>
</tr>
<tr>
<td>One and Two only</td>
<td>17</td>
<td>10</td>
<td>13</td>
<td>3</td>
<td>43</td>
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<td>One and Three only</td>
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<td>17</td>
<td>14</td>
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<td>One, Two, and Three</td>
<td>50</td>
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<td>13</td>
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<tr>
<td>Total</td>
<td>179</td>
<td>95</td>
<td>78</td>
<td>31</td>
<td>383</td>
</tr>
</tbody>
</table>

Table 1-1 indicates the race and gender of the juveniles suspected of criminal offenses, as well as the number of times they offended in a given time period, further indicated by the category waves.

**PROCEDURES**

Two different forms of statistical analysis were utilized, and those were Log linear analysis and chi-squares test. Both Log linear analysis and chi-squares test were used because the study dealt with dichotomous variables, (0, 1) or (yes, no), and because the study wanted to examine variables that could predict offending. As noted above, independent variables examined across the different waves were gender, race, residents in gang areas, residents in hotspot, and residents in single dwelling or apartment.

**RESULTS**

Log Linear Analysis was run first with minimal findings. Gender was the only variable that had some significance using Log Linear Analysis. Wave One, Two, and Three only was the only wave that showed gender predicted offending. There was marginal significance for men offending in all three waves, $\chi^2 (1) = 3.72$, $p=0.054$. These results showed that men were more likely to offend across all waves. A chi squares test was also run and results were examined by wave.

For Wave One and Two only, there was no significant association between housing in a single dwelling or apartment, hotspot, gang area, race, gender and offending. Wave One and Three only saw similar insignificant associations between the above mentioned variables and offending as well. In examining Wave One, Two, and Three, there was no significant association between housing and offending, $\chi^2 (1) = 0.29$, $p\leq0.6$ but $p\geq0.05$. **There was a significant association between hotspot status and offending, $\chi^2 (1) = 6.42$, $p=0.011$. There was also a significant association between gang area and offending, $\chi^2 (1) = 7.53$, $p=0.006$. Race and offending were found to be significantly associated with one another, $\chi^2 (1) = 7.26$, $p= 0.007$.** There was, however, no significant association between gender and offending, $\chi^2 (1) = 0.63$, $p\leq0.5$ but $p\geq0.05$. Results showed that the variables hotspot residence, gang area, and race were more likely to predict offending across all three waves, while housing and gender were not.
Chi-Square Tests

<table>
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<tr>
<th></th>
<th>Value</th>
<th>df</th>
<th>Asymp. Sig. (2-sided)</th>
<th>Exact Sig. (2-sided)</th>
<th>Exact Sig. (1-sided)</th>
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<td>Pearson Chi-Square</td>
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<td>.054</td>
<td></td>
<td></td>
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<tr>
<td>Continuity Correction</td>
<td>2.748</td>
<td>1</td>
<td>.097</td>
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<tr>
<td>Likelihood Ratio</td>
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<td>.060</td>
<td></td>
<td>.063</td>
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<td>Fisher's Exact Test</td>
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<tr>
<td>Linear-by-Linear Association</td>
<td>3.678</td>
<td>1</td>
<td>.055</td>
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<tr>
<td>N of Valid Cases</td>
<td>88</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

a. Gender = Men

Table 1.2 indicates the marginal significance found for men offending in all three waves.

<table>
<thead>
<tr>
<th>Wave 1 &amp; 2</th>
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<th>No</th>
<th>Wave 1 &amp; 3</th>
<th>Yes</th>
<th>No</th>
<th>Wave 1, 2, 3</th>
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<th>No</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>23.1% (12)</td>
<td>16.5% (31)</td>
<td>24.5% (13)</td>
<td>24.2% (50)</td>
<td>25.9% (14)</td>
<td>29.6% (66)</td>
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<tr>
<td></td>
<td>21.4% (15)</td>
<td>16.5% (28)</td>
<td>24.7% (18)</td>
<td>24.1% (45)</td>
<td>39.6%* (36)</td>
<td>23.7% (44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22.1% (15)</td>
<td>16.3% (28)</td>
<td>27.4% (20)</td>
<td>23.0% (43)</td>
<td>39.1%* (34)</td>
<td>24.2% (46)</td>
<td></td>
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</tr>
</tbody>
</table>

Table 1.3 indicates a Chi Squares Relationship between Predictors and Percentage of Subjects who Offended in Each Wave.

**DISCUSSION AND CONCLUSION**

The present study examined variables that could potentially be associated with early delinquency, continued delinquency, and recidivism. The issue of which variables were associated with juvenile delinquency was addressed by the selection and examination of the variables race, gender, gang areas, hot spots and single dwelling or apartment.

Since subjects were chosen solely based on whether or not they had been a suspect in a crime, it was a weakness in this study. This was a weakness in the study because the criteria in which subjects were chosen failed to obtain a control group. In addition to how the subjects were chosen, another potential weakness of this study was the small sample size, 383 subjects. For example, there were zero white women who had offended in the second wave, lived in a single dwelling, lived in a gang area, but did not live in a hotspot. An additional weakness of this study was that there was no control group to compare with offenders. Without a control group, differences between delinquents and nondelinquents could not be examined. The data that was collected exhibited offending patterns of subjects, but the data cannot establish whether or not the subject desisted from crime, died, or moved away. Since this data was public, there were limitations as to what information, like mental state, was available about the subject.
While this study had a number of weaknesses, it also contained a number of strengths. Since this study was longitudinal, there was a greater amount of information that could be examined and thus used to support the findings. The data that was observed was official data obtained from the Rock Hill Police Department, so that was another advantage of this study. Another strength of this study was that the data showed variables that are associated with the continuation of offending.

The results of this study show that subjects who offend only in a maximum of two Waves, Waves One and Two or One and Three, do not have significant associations with the dependent variables studied. This study does, however, show that subjects who offended in Waves One, Two, and Three have significant associations with all of the independent variables with the exception of gender and housing. Previous literature had not shown associations in delinquency longitudinally with the combination of race, gender, gang areas, hot spots and housing as variables.

FUTURE STUDIES
Future research should contain a control group when examining longitudinal data of delinquents. In their study of institutionalized delinquent boys in Massachusetts, Glueck and Glueck (1950) matched a sample of delinquents and nondelinquents based on age, race, neighborhood characteristics, and intelligence. Their method of studying delinquency should be modeled in order to properly distinguish factors that predict offending in Rock Hill, SC specifically. Future research should examine social class as well as factors relating to time of offense committed and delinquency. Current research examined only participation and duration of juvenile delinquency, so future research will examine frequency and seriousness as additional dimensions of a criminal career.

REFERENCES


Contemporary Black Women Artists’ Narratives

Shannon Snelgrove
Laura Gardner, Ph.D. (Mentor)

ABSTRACT

The purpose of this study is to explore contemporary Black women visual artists’ experiences in the arts. Specific experiences studied include: finding support, balancing family responsibilities, and overcoming gender and race barriers. Though there are numerous articles on Black women’s artwork (e.g., Edwards, 2015; Murray, 2014; Wickham, 2015), there are few about their lived experiences, in their own words. To address this gap, semi-structured interviews were conducted with Black women visual artists from the southeast United States. Despite its focus on a particular group, the study speaks to diverse audiences about surmounting challenges in life and work.

Two interviews have been conducted so far, and preliminary results show common themes. These themes include family support for art-making and family participation in art-making, mentorship and helpful connections with other Black professionals, strategic navigation around gender and race barriers, culture as an important theme in their work, and an integrated relationship between their academic professions and their artistic work. These results are clearly not conclusive nor generalizable, but may provide leads for future investigation. The results might also provide helpful information and guidance to emerging Black women artists. Both artists interviewed affirmed the importance of learning from more experienced artists, and this study can serve that end.

BACKGROUND

Black women visual artists have historically faced challenges including the following:

- **Challenge**: Seeing stereotypical images of Black women in art and media (Ritterhouse, 2009)
  - **Response**: Reappropriating stereotypical images (i.e. Aunt Jemima) in their art (Ringgold, 1996)
  - **Response**: Telling new stories about Black women through their art (Hudson, 1995)

- **Challenge**: Being excluded from the high art world (Farris, Kramer, & Wasserman, 1999)
  - **Response**: Creating separate spaces for Black women’s art (Brown, 2011)
  - **Response**: Not participating in these separate spaces; resisting labeling their work as “Black” (Campbell, 2007)
  - **Response**: Protesting the lack of women and/or Black people represented in art galleries (Wallace, 2015)

- **Challenge**: Balancing family life and work life (Stohs, 1992)
  - **Response**: Possibly interrupting their careers to take care of family responsibilities (Stohs, 1992)
METHODS

Interviewing

Semi-structured interviews were conducted with Black women visual artists from the southeast US. Art professors recommended potential participants. The following interview questions were created for contemporary artists based on historical literature review.

1. What led you to your career in the arts?
2. Did anyone help you along the way? Who? In what way(s)? How did you meet them?
3. Were there any gender or race barriers you had to overcome to get to the position you’re in now? If so, what did you do to overcome those barriers?
4. Do you confront stereotypes in your work? How so?
5. How have galleries responded to you and your work?
6. How do you find time to work on your art? How do you balance family responsibilities and your creative work?
7. Is your family supportive of your work? How so?
8. Are you able to make a living as an artist? Do you have another job to support your work? Does your “work” life inform your creative life and vice versa?
9. Have there been any recurring themes in your career?
10. In my historical study, I found that self-definition was an important theme for Black women artists. Is that true for you? If so, how do you integrate this into your work?

Each interview lasted less than one hour. Audio of the interview was recorded if permission was given by the interviewee. If permission was not given, detailed notes were taken during the interview then verified with the interviewee for accuracy at the end of the interview.

Analyzing the Interviews

Grounded theory was used to analyze the interviews. Grounded theory is the “discovery of theory from data” (Glaser & Strauss, 1967, p. 1). In this study, the “data” are the responses to the interview questions.

Coding is used to develop grounded theory. “Coding means categorizing segments of data with a short name that simultaneously summarizes and accounts for each piece of data” (Charmaz, 2006, p. 43). For example, in this study, the name “family support” was used to categorize pieces of data.

The analysis in grounded theory has two main steps. Step one is initial coding. Charmaz (2006) describes this step as “open” (p. 47). Step two is focused coding. Charmaz (2006) describes this step as “selective” (p. 57).

RESULTS

Two interviews have been conducted so far, and preliminary results are as follows. The results are not conclusive nor generalizable, but may provide leads for further investigation. The following common themes were found:

- The artists come from families who have supported their art and creativity from a young age. Both families also participated in art-making activities with the interviewees.
- The artists emphasized the importance of mentorship and connections, and they were both presented with career opportunities by other Black professionals.
- Both said they have faced gender and race barriers, but have found ways to strategically navigate around them.
• Culture is an important theme in their art. One artist highlights African and African-American culture, and the other uses her art to reach out to cultures other than her own.

• The artists make the majority of their income from teaching. They both said that their academic careers and artistic careers are highly integrated and symbiotic.

**DISCUSSION**

A clear limitation of this study is its small sample size. However, there is much opportunity to expand this research by interviewing Black women artists outside of the southeast US. Results from the study may provide insight and/or encouragement to emerging Black women artists about overcoming career challenges, but the benefit is not limited to this demographic. Further coding will produce “generalizable theoretical statements that transcend times and places” (Charmaz, 2006, p. 43).

**REFERENCES**


Investigating X chromosome non-disjunction in *Drosophila melanogaster* su(var)3-9 mutants

Camerun Washington
Kathryn Kohl, Ph.D. (Mentor)

**ABSTRACT**

Meiotic recombination is a highly regulated process necessary for promoting proper chromosome disjunction during the first meiotic division. Notably, reduced levels of meiotic recombination are observed in heterochromatic regions of the chromosome. This study seeks to investigate the molecular mechanisms underlying this observation by examining the effects of reduced heterochromatin on non-disjunction rates in *Drosophila melanogaster*. To accomplish this, we measured non-disjunction in wild-type and reduced heterochromatin mutant *su(var)3-9* flies. To begin, we confirmed the presence of a mutation within *su(var)3-9* via Sanger sequencing. Next, we created allele-specific primers and designed a PCR protocol to more accurately identify mutant flies at the molecular level. Finally, we assayed non-disjunction in wild-type and *su(var)3-9* mutant flies and discovered that *su(var)3-9* mutants have significantly higher levels of non-disjunction than wild-type flies. We also uncovered a striking sex bias in the non-disjunction progeny of *su(var)3-9* mutants.

**INTRODUCTION**

Chromosomes are thread-like structures of DNA wrapped around histone proteins. This genetic information can be transferred across the generations through meiosis, a type of cellular division resulting in the production of four genetically diverse haploid gametes. During prophase I of meiosis, replicated paternal and maternal homologs pair up and exchange genetic material in a process called meiotic recombination, or crossing over. Notably, this exchange appears to show high levels of regulation. For example, crossover assurance states that every chromosome pair will engage in crossover events during cellular division (Shinohara et al. 2008), resulting in increased genetic variability among offspring. Moreover, crossover events are essential for proper chromosome segregation because they serve as a tether for homologs as they align at the metaphase plate (Youds and Boulton 2011). During normal cellular activity, chromosomes are also subject post-translational modifications to histones. These modifications — referred to as “marks” — regulate gene expression by organizing the genome into two distinct regions: euchromatin and heterochromatin. These distinct regions are characterized by their varying levels of DNA compaction and gene expression. Euchromatin, which comprises a large portion of the genome, functions as the most active region of gene expression. Alternatively, heterochromatin — found in regions proximal to the centromere and telomere — is characterized by tight DNA compaction, low gene density, transcriptional inertness, and strongly reduced levels of meiotic recombination. Studies have also shown that crossovers that occur in heterochromatic regions show strong inhibition of proper chromosome segregation (McKim et al. 2002). Within this study, we seek to examine the role of heterochromatin in the suppression of meiotic recombination and its effect on chromosome segregation.

For this study, we used *Drosophila melanogaster* as a model system. *Drosophila* is an ideal organism for genetic studies because of their fast generation times, large brood sizes, and fully sequenced genome. *Drosophila* is also particularly useful for meiotic studies because flies are able to survive some forms of aneuploidy and moreover, meiotic crossing over was first observed in *Drosophila*. The *Drosophila* genome consists of 4 chromosome pairs including its sex chromosomes. With respect to its composition, chromosome 4 is >70% heterochromatic compared to the other
autosomes comprising ~30% (Hoskins et al. 2002). However, the absence of recombination on chromosome 4 is of primary interest because of its direct contention with crossover assurance. Absence of crossover events on chromosome 4 seems to suggest the activity of strict regulation in place to prevent unfavorable recombination events. Interestingly, one genetic mutant background has recently been discovered, which allows crossing over on chromosome 4 (Hatkevich, Kohl, et al. 2017). This suggests that suppression of recombination events are the result of strict regulatory mechanisms governing chromosome 4. These findings also suggest that chromosome 4 serves as an ideal model for studies involving meiotic recombination control mechanisms collectively.

This study seeks to elucidate the meiotic regulatory mechanisms functioning on Drosophila chromosome 4 by genetically reducing heterochromatin levels using suppression of variegation (su(var)) mutants. Within this family, the gene su(var)3-9 was chosen because it encodes a protein responsible for histone H3 lysine 9 (H3K9) methylation — a key agent in the production and maintenance of heterochromatin (Schotta et al. 2002). In a previous study, rates of recombination in Drosophila su(var) mutants were assayed in limited centromere proximal intervals not including chromosome 4 (Westphal and Reuter 2002). Our lab seeks to extend this work by investigating the effect of su(var)3-9 mutation on meiotic recombination and non-disjunction on multiple chromosomes, including chromosome 4. This study aims to elucidate the molecular mechanisms and epigenetic factors affecting meiotic recombination on chromosome 4 and across the genome. These results may provide us with a molecular explanation of the 80-year old paradigm in Drosophila genetics that recombination does not occur on chromosome 4.

METHODS

Fly stocks
Fly stocks were obtained from the Bloomington Drosophila Stock Center (Bloomington, IN) and included: ci sv (wild-type control), In(1)ma4 / su(var)3-92 / TM3 (experimental stock) and y w v f / Dp(1;Y)B (indicator of non-disjunction).

Verification through DNA sequencing
The su(var)3-92 mutation is a G to A single nucleotide polymorphism (SNP) (G15260859A) causing a missense mutation (C427Y) (Krauss et al. 2006). To verify the presence of the su(var)3-92 mutant SNP, the region flanking the mutation was amplified from a standard fly prep, excised from a 2.0% agarose gel, and purified using a QIA QuickGel Extraction Kit (Qiagen). Following quantification using a Nanodrop Spectrophotometer (ThermoFisher Scientific), the sample was sent for Sanger sequencing (Eurofins). A heterozygous peak at the predicted location confirmed the presence of the su(var)3-92 mutation (Figure 1).

![Figure 1. Sanger sequencing chromatogram of su(var)3-92 mutant fly. Low heterozygous peak (overlapping green and black peaks) at the predicted location (indicated by an arrow) confirms the presence of the su(var)3-92 SNP.](image)

Identification through allele-specific PCR
To quickly identify su(var)3-92 mutants, which do not produce an outward phenotype, we designed allele-specific primers and optimized an allele-specific PCR protocol. Primers were as follows: asf2 (AGGAGTCTACTGCTTCCTACGAATT) and 2858r (GCTGCATCGATCCCTCTACGAATT) Reagent concentrations were as follows (Table 1):

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Amount</th>
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<tbody>
<tr>
<td>dH2O</td>
<td>13.825 μL</td>
</tr>
<tr>
<td>10x Standard Taq (Mg2+ Free) Reaction Buffer (NEB)</td>
<td>2.0 μL</td>
</tr>
<tr>
<td>25 mM MgCl2</td>
<td>1.575 μL</td>
</tr>
<tr>
<td>10 mM dNTPs</td>
<td>0.5 μL</td>
</tr>
<tr>
<td>50 pmol/μl Primer asf2</td>
<td>0.5 μL</td>
</tr>
</tbody>
</table>
Allele-specific PCR reactions were run on a Mastercycler Thermacycler (Eppendorf) using the following conditions (Table 2):

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Cycle Time</th>
<th>Number of Cycles Repeated</th>
</tr>
</thead>
<tbody>
<tr>
<td>95</td>
<td>3 min.</td>
<td>N/A</td>
</tr>
<tr>
<td>95</td>
<td>30 sec.</td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>30 sec.</td>
<td>16 cycles</td>
</tr>
<tr>
<td>72</td>
<td>30 sec.</td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>30 sec.</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>30 sec.</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>5 min.</td>
<td>N/A</td>
</tr>
<tr>
<td>10</td>
<td>∞</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*Indicates the annealing temperature decreased by 0.5 °C each cycle for 16 cycles in a “touchdown” procedure.

A 2.0 % agarose gel was run at 120 volts for approximately 45 to 50 minutes to visualize PCR products.

**Experimental crosses**

A standard *Drosophila* crossing scheme was used for this experiment (Figure 3). All crosses were conducted at 25° C on standard fly media (Genesee Scientific, Bloomington recipe) under a 12:12 light/dark cycle. First, virgin *su(var)3-92* females were mated to *ci sv* wild-type males in bottles to remove the TM3 balancer. Approximately 16 males and 16 females were mated in 8 individual bottles. Parents were then cleared from these bottles after 3 days. From these bottles, virgin *su(var)3-92 / +* females (lacking the TM3 balancer chromosome) were collected. Next, 3 *su(var)3-92* females were crossed to 3 *y cv v f / Dp(1;Y)B* flies per vial for a total of 25 vials. After 3 days, parents were cleared. Progeny were then scored for sex and non-disjunction phenotypes for 8 days post-eclosion (Figure 3). Non-disjunction phenotypes or “exceptionals” include: males with wild-type eyes (XXY) and females with bar-eyes (OX), normal disjunction phenotypes include: males with bar-eyes (XY) and females with wild-type eyes (XX) and two resulting lethal classes include: XXX and ØY (Figure 4).
non-disjunction will produce XX or ∅ gametes. Normal chromosome segregation in males will produce gametes with either an X or Y chromosome. Progeny indicative of female non-disjunction include X∅ males and XXY females (exceptionals; circled). Two lethal classes can also result from female non-disjunction (∅Y and XXX, crossbones).

Data analysis
Results of non-disjunction scoring were analyzed using a standard percent of non-disjunction equation. The number of exceptional progeny observed was multiplied by 2 (to account for lethal classes) and divided by total number of flies scored plus the number of observed exceptional. This number was then multiplied by 100 to compute percent non-disjunction. Wild-type non-disjunction rates and su(var)3-92 non-disjunction rates were compared via Fisher’s exact test (GraphPad QuickCalcs). A chi-square goodness of fit test was used to analyze sex bias in the exceptional male progeny of su(var)3-9 mutants (GraphPad QuickCalcs).

RESULTS
Su(var)3-9 mutants show statistically higher rates of non-disjunction

Percent non-disjunction was compared between ci sv (wild-type) and su(var)3-92 mutants. A Fisher’s exact test strikingly rejects the null hypothesis that a decrease in the production of heterochromatin in su(var)3-92 mutants does not increase rates of non-disjunction (p = 0.001). A total of 719 wild-type progeny were scored with no observed non-disjunction events (Bar eyed females and wild-type eyed males). Thus, frequency of non-disjunction for this class was 0.00%. In the su(var)3-92 progeny, a total of 4,792 flies were scored including 20 exceptional. For su(var)3-92 mutants, a non-disjunction frequency of 1.13% was calculated (Figure 5).

Su(var)3-9 mutants show altered sex ratio of non-disjunction offspring

Whereas the Mendelian expectation is an equal proportion of male and female exceptional progeny (Figure 6), the total exceptional mutant progeny from the su(var)3-92 heterozygote cross included: 1 female with Bar eyes compared to 19 males with wild-type eyes. A chi-square test rejects the null hypothesis that there exists no difference in the distribution of sex within scored progeny (p < 0.0001).

DISCUSSION
The molecular function of SU(VAR)3-9 as a H3K9 methyltransferase is well characterized (Schotta et al. 2002), however much less is known about how mutations in su(var)3-9 affect meiotic recombination. One
previous study tested effects on recombination, but only in centromere-proximal intervals on chromosome 2 in su(var)3-92 mutants (Westphal and Reuter 2001). Thus, this project goes further by investigating the role of su(var)3-92 on X chromosome disjunction using Drosophila melanogaster. Flies were scored phenotypically based on sex and eye shape. Amongst progeny, there was a statistically significant difference in the percent of non-disjunction between wild-type flies and su(var)3-92 mutants (p = 0.001). Progeny of su(var)3-92 mutants also exhibited a sex bias towards male exceptional offspring (p < 0.0001). This result, combined with the knowledge that SU(VAR)3-9 is a H3K9 methyltransferase necessary for heterochromatin formation, suggests that heterochromatin plays an important role in meiotic chromosome disjunction. However, important data from recombination proclivity (i.e. frequency and location of crossovers) studies are needed to better elucidate the relationship between recombination and chromosome disjunction.

Mutations to H3K9 methyltransferases make heterochromatic DNA sequences more prone to spontaneous or induced damages. This damage may include deletions, insertion of extrachromosomal DNA, or chromosome rearrangements (Peng and Karpen 2008) that may result in recombination errors leading to non-disjunction. Along with the idea that mutations to epigenetic agents may contribute to downstream changes, this observation serves to highlight how changes in chromosome integrity may affect molecular recombination mechanisms that result in increased non-disjunction. This increased frequency of missegregation may also explain the observed sex bias in exceptional progeny. The Y chromosome of Drosophila is composed almost entirely of heterochromatin (Wang et al. 2014). Therefore, we hypothesize that XXY females inheriting a heterochromatically “handicapped” paternal Y chromosome may be less viable than those inheriting a highly heterochromatic Y chromosome. Since su(var)3-92 is a dominant allele, this may be influencing the occurrence of female su(var)3-92 flies within parental stock without any phenotypic indices. With this, it can be assumed that the bias in su(var)3-92 exceptional progeny may be due to a compounded inheritance of mutated sex chromosomes or compromised recombination proclivity. Interestingly, XO males can also arise through spontaneous loss of an X chromosome during development. This suggests that normal chromosome segregation that would have resulted in a female (XX) may freely produce a male exceptional (XØ), providing another pathway to the increased observation of male exceptions. In order to understand this bias more precisely, molecular recombination assays are needed to determine whether weakened X chromosome integrity has caused a shift in the loci of crossovers. Furthermore, since the frequency of primary X chromosome non-disjunction is usually highest in the first eggs laid by a female (Tokunaga et al. 1970), measuring hatch rates of fertilized eggs may be another option for identifying premature sex biases in su(var)3-92 mutants. In this way, we can determine in which stages of development exceptional female progeny are incurring speculated chromosome loss.

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re-fission from a functionally unrelated gene. BMC Evol Biol. 6:18.
The following Scholars do not have work included here because they have published their work in peer-reviewed professional journals or are in the process of preparing a manuscript to submit to a journal for peer review.

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Mentor: Dr. Matthew Heard, Biology

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Mentors: Dr. Victoria Frost, Biology & Dr. Matthew Heard, Biology

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Alexis Williamson
Mentor: Dr. Bradley Witzel, Special Education

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