Development And Implementation Of Genetics Modules For Young Learners

Michelle V. Ezeoke

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doi: https://doi.org/10.57709/12506222

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ABSTRACT

In recent years, there has been a nation-wide effort to increase the application of genetics-based solutions to problems of medical, agricultural, and environmental importance. Before high school, the only exposure that Georgia students will have regarding genetics will not encompass the applications of DNA that exist in the world around them. Implicit in this delay is the assumption that genetic concepts are simply too complex for younger children to understand. Under the auspices of Georgia State University’s (GSU) Bio-Bus program, I proposed to determine whether the apparent ease with which young children appear to master second
languages can be harnessed to teach them about DNA and genetics. To accomplish this, a set of entertaining and informative learning modules (to be called *DNA is Elementary*) that present genetic concepts to young students (ages 5 through 12) was designed. Whenever possible, the activities associated with these modules focused on the parallels between our 26-letter alphabet and the four-letter alphabet used by DNA in its role as the instructional manual for the cell. The Bio-Bus and Bio-Bus personnel traveled to participating schools and presented the activities to K-5 students. The effectiveness of these modules was measured through the use of feedback forms designed to measure changes in content knowledge and attitude before and after the presentations. The primary objective of this project was to identify the most effective ways to inspire an interest in and an understanding of molecular genetics among young students, with the ultimate goal of making a contribution toward the establishment of a scientifically literate society.

INDEX WORDS: Science education, Genetics education, Elementary science, Likert-scale, attitudes survey design
DEVELOPMENT AND IMPLEMENTATION OF GENETICS MODULES FOR YOUNG LEARNERS

by

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Committee: George Pierce
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Electronic Version Approved:

Office of Graduate Studies
College of Arts and Sciences
Georgia State University
July 2018
DEDICATION

The sweat and tears that came from creating this body of work and the final product is dedicated to mi familia, my husband Captain Chisom B. Ezeoke and to the next generation of 1st generation Latino doctoral students, “¡SI SE PUEDE!”
ACKNOWLEDGEMENTS

I will begin by acknowledging that I am nothing without Him and I am everything because of Him. I would like to proceed by thanking my committee chair, PI and mentor, Dr. Barbara Ruth Baumstark, for being tremendously generous with her wisdom as a scientist, leader, educator and friend. Thank you for your guidance and support. Thank you for always being present and for allowing me to pursue my passion as a scientist and science educator. I will forever cherish my time with you. I would like to thank Dr. George Pierce for all his guidance and support during my graduate career. I am extremely grateful to Dr. Sidney Crow for being a part of my dissertation committee. I would like to thank my committee for believing in the importance of science education. I will be forever indebted to my second family, my Bio-Bus family. In particular, I would like to thank Dr. Chandan Robbins for being an example of what it means to be fierce. I would like to express my gratitude to Dr. Brandi Villa because your knowledge and expertise of educational assessment and evaluation were significant in the preparation of this dissertation however, more importantly, thank you for your friendship and example of what is means to be a “superwoman”. I would like to express my appreciation to the Bio-Bus staff past and present, in particular Shue Casillas, Lorna Gitari-Mugambi, Verdy Jocelyn, Mary Hall and Isela Rodriguez-Bussey, thank you for all the hard work that has been invested into DNA is Elementary and for allowing me the time to write this dissertation. Additionally, I would like to say thank you to Isela Guadalupe Rodriguez-Bussey for the support and friendship during this endeavor where we found ourselves in the same boat and in a field where we didn’t come across a lot of individuals who look like us. I am humbled by your friendship and support. I would like to acknowledge all the Bio-Bus fellows who worked tirelessly to help create and implement the various modules of DNA is Elementary. Last but not
least, I would like to thank my family for without their unwavering love, support and belief in my abilities, I would not be where I am today. I would like to specifically thank my mother, Ms. Ana M. Ventura, for being the best example of strength and perseverance and my husband Captain Chisom B. Ezeoke, you have been my rock and my foundation and I pray that one day I can tell our children how blessed they are to have a father like you.

This research was supported by the National Institute of Health’s Science Education Program Award (SEPA), The Bio-Bus Program, GSU’s College of Arts & Science and Tuition Assistance Program (TAP).
# DEVELOPMENT AND IMPLEMENTATION OF GENETICS MODULES

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<th>Full Form</th>
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<tbody>
<tr>
<td>GSU</td>
<td>Georgia State University</td>
</tr>
<tr>
<td>STEM</td>
<td>Science, Technology, Engineering &amp; Math</td>
</tr>
<tr>
<td>CKA</td>
<td>Content Knowledge Assessment</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
</tr>
<tr>
<td>CRISPR</td>
<td>Clustered Regularly Interspaced Short Palindromic Repeats</td>
</tr>
<tr>
<td>SEPA</td>
<td>Science Education Program Award</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institute of Health</td>
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<tr>
<td>URM</td>
<td>Underrepresented Minority</td>
</tr>
<tr>
<td>SES</td>
<td>Socioeconomic Status</td>
</tr>
<tr>
<td>FRL</td>
<td>Free and Reduced Lunch</td>
</tr>
<tr>
<td>GMO</td>
<td>Genetically Modified Organism</td>
</tr>
<tr>
<td>GADOE</td>
<td>Georgia Department of Education</td>
</tr>
<tr>
<td>NGSS</td>
<td>Next Generation Science Standards</td>
</tr>
<tr>
<td>GSE</td>
<td>Georgia Standards of Excellence</td>
</tr>
<tr>
<td>SAL</td>
<td>Spatial Analysis Lab</td>
</tr>
<tr>
<td>GPS</td>
<td>Georgia Performance Standards</td>
</tr>
<tr>
<td>PPE</td>
<td>Personal Protective Equipment</td>
</tr>
<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
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<tr>
<td>CITI</td>
<td>Collaborative Institutional Training Initiative</td>
</tr>
<tr>
<td>NSF</td>
<td>National Science Foundation</td>
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<tr>
<td>PD</td>
<td>Professional Development</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>qPCR</td>
<td>Quantitative Polymerase Chain Reaction</td>
</tr>
<tr>
<td>UNC-CH</td>
<td>University of North Carolina-Chapel Hill</td>
</tr>
<tr>
<td>UNFSW</td>
<td>Unfiltered Sea Water</td>
</tr>
<tr>
<td>FSW</td>
<td>Filtered Sea Water</td>
</tr>
<tr>
<td>Ct</td>
<td>Cycle Threshold</td>
</tr>
<tr>
<td>Cq</td>
<td>Quantification Cycle</td>
</tr>
<tr>
<td>NC</td>
<td>North Carolina</td>
</tr>
<tr>
<td>gDNA</td>
<td>Genomic Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>TAE</td>
<td>Tris Acetate Ethylenediaminetetraacetic Acid</td>
</tr>
<tr>
<td>GADPH</td>
<td>Glyceraldehyde-3-Phosphate Dehydrogenase</td>
</tr>
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1 DEVELOPMENT AND IMPLEMENTATION OF GENETICS MODULES FOR YOUNG LEARNERS

1.1 INTRODUCTION

1.1.1 Statement of the issue

Advances in our understanding of genetics are revolutionizing society. As they reach adulthood, today’s children will be able to get their genomes sequenced for a small sum, order a genetically engineered dog or cat cloned from a beloved pet and, if they get sick, receive care that is optimized for their own genetic constitution. These opportunities in turn present unique challenges for our educational system. The rapid increase in genetics-oriented information requires that our students obtain the knowledge and skills they will need to assess the impact of both current and future genetic discoveries on their lives.

To be considered scientifically literate in today’s world, individuals must have a working knowledge of DNA structure and function, along with an understanding of the ways in which these aspects of DNA contribute to the process of inheritance. This need extends to all individuals, regardless of their gender, race, ethnicity, or socioeconomic status. Underrepresented minorities and females in particular provide a pool of talent, largely untapped so far, from which to draw the new scientists and health professionals who will be making future contributions to our understanding of molecular genetics. Moreover, individuals belonging to specific racial and ethnic groups will be able to determine their own susceptibilities to different health conditions, such as hypertension and diabetes, which are known to exhibit a heritable component. All members of society need to be sufficiently familiar with genetic principles to evaluate the merit of claims made in the name of science as they relate to controversial topics such as stem cell research, GMO foods, and DNA profiling. Despite the influence of modern
genetics and biotechnology on science and society, however, our nation’s K-12 schools have yet to make the study of genetics, and especially molecular genetics, a priority for their students (Dougherty, Pleasants, Solow, Wong, & Zhang, 2011). Mendelian genetics as a topic of study does not generally get incorporated into the K-12 curriculum until middle school or later, and even advanced placement courses give minimal attention to molecular genetics (GADOE, 2016), leaving recent discoveries in exciting areas such as genomics, epigenetics, CRISPR technology and gene silencing largely untouched.

1.1.2 **The Next Generation Science Standards**

For several years, there have been continuing calls from legislators, educators, and education policy makers to improve science education. The implementation of the Science, Technology, Engineering and Math (STEM) Initiative has identified science education as a priority for our nation’s future (NSB, 2016; Wong, 2015). In addition, there have been concerted attempts within the K-12 educational community to develop “performance standards” that would define what students should know and be able to do at each grade level. The results of a nationwide effort culminated in the Next Generation Science Standards (NGSS), a document that identifies sets of topics to be covered in the K-12 schools along with the grade at which these topics should be introduced. The dissemination of the NGSS has generated a significant level of controversy on both sides of the political spectrum. On the one hand, genetics consultants express concern over what they consider to be inadequate coverage of genetics-related topics, including Mendelian genetics and the inheritance of complex traits (Lontok, K.S., Zhang, H., and Dougherty, M.J. 2015). On the other hand, for many parent groups and education activists, there is a perceived over-emphasis on controversial topics such as evolution and climate change. The resulting dissension has caused many states to withdraw from the NGSS movement. At the onset
of the NGSS initiative, Georgia signed on as one of 26 “lead states” that were expected to play a significant role in standards development. In the wake of the subsequent controversy, however, Georgia declined to adopt the NGSS. At the present time, the number of states that have formally adopted the NGSS stands at 19. Like many states that decided not to join the NGSS movement, Georgia subsequently adopted its own set of standards (the Georgia Standard of Excellence, or GSE). In the GSE, the concept of a “gene” is first introduced in Grade 5, while Mendelian genetics is introduced in middle school (Grade 7).

1.1.3 Call for improvements in science education

The quality of science education in Georgia has consistently lagged behind that of other states, as measured by parameters such as students’ performance on standardized tests and their participation in Advanced Placement science courses (Newsweek, 2015). Additionally, there is a significant shortage of science teachers. As a consequence, many of those currently teaching science have little or no formal training in science or science education (Jung & Tonso, 2006). Too often, teachers without a science background who find themselves assigned to teach a science class end up becoming dependent on worksheets and memorization-based assignments to convey science content to their students (Kier & Lee, 2017). It has been well-documented that young students in elementary school display a natural interest in science, but that this interest wanes as they approach middle and high school (Deemer, Lin, & Soto, 2016; Fitzgerald, McKinnon, Danaia, & Deehan, 2015; Gordon, 2011; NGA, 2014). In many cases, students never get to participate in science activities in ways that show them what scientists really do in practice (Herrington, Luxford, & Yezierski, 2012). To maintain their students’ interest, and to motivate them to consider science-based careers, many science educators advocate the use of hands-on, problem-solving activities that inspire students to ask questions about the world around them.
Exposure to realistic science experiences (i.e., those that involve problem-based learning, experimental design, and data analysis) increases the likelihood that students’ interest in science will persist as they continue to scale the academic ladder (Alfieri, Brooks, Aldrich, & Tenenbaum, 2011; Minner, Levy, & Century, 2010; Sadeh & Zion).

Achievements resulting from long-term initiatives such as the Human Genome Project as well as fictionalized accounts of the use and misuse of genetic information are regularly publicized by the popular press. The scientific merit of claims made in the name of genetics can be highly variable, however, and the sense of excitement that these new discoveries engender has not been accompanied by an increased emphasis on the mastery of genetic concepts.

Recent changes proposed by the NGSS initiative, if implemented on a large scale basis, would result in the introduction of genetics-based concepts at a much earlier stage of the curriculum than what is currently being recommended in Georgia. The NGSS recommends that “Heredity: Inheritance and Variation of Traits” be identified as a Disciplinary Core Idea in the third grade (Lontok, Zhang, & Dougherty, 2015; Pruitt, 2014). Disciplinary Core Ideas are defined by the NGSS as those that “have importance within or across science or engineering disciplines, provide a key tool for understanding or investigating complex ideas and solving problems, relate to societal or personal concerns, and can be taught over multiple grade levels at progressive levels of depth and complexity (Lontok et al., 2015; Pruitt, 2014). In contrast, heredity is not mentioned in Georgia’s standards document until Grade 5 (a full two years later than that recommended by NGSS), where the only requirement listed is that students must “ask questions to compare and contrast inherited and acquired physical traits” (GADOE, 2016). The GSE document also makes it clear that tools of genetic analysis such as Punnett squares will not
be incorporated into the curriculum until high school and, in the case of Hardy-Weinberg analysis, need not be introduced at all (GADOE, 2016).

1.1.4 **Loss of student engagement with genetics and other science disciplines**

The way genetics is treated in middle and high school classes tends to decrease rather than increase students’ enthusiasm for the subject. Students are often graded primarily on their ability to memorize genetic terminology. In those cases where they are actually exposed to genetic concepts (rather than to vocabulary alone), they are likely to receive fragmented information about multiple topics, such as Mendelian genetics, meiosis and mitosis, and DNA structure, without ever understanding the fundamental biological associations among these concepts. For these students, it is like trying to become conversant in a foreign language by memorizing long lists of words without ever trying to put them together to form sentences, or to tell a story.

There have been numerous reports suggesting that students receiving instruction in molecular genetics in high school (and at the college level as well) are expected to have more difficulty with this topic than they have with most other aspects of biology (Longden, 1982; Redfield, 2012; Smith & Knight, 2012). In addition, elementary students are often treated as concrete thinkers who are conceptually incapable of understanding something as complex as genetics (Duncan, Rogat, & Yarden, 2009; Graham & Brouillette, 2016; Piaget, 1954; Ross, Lee, Radebaugh, & Stargell, 2012). Although the results of recent research raise questions about this assumption (Duschl, Schweingruber, & Shouse, 2007; Ross et al., 2012), it is clear that determining how large a role modern genetics could, or should, play in the K-12 curriculum is a matter of considerable debate. Incorporation of additional genetics instruction into the K-12 curriculum would need to fulfill at least two criteria:
1) students at targeted grade levels must exhibit enough growth in their understanding of genetic principles to justify making the investment of instructional time, professional development for teachers, and other resources that would be needed to implement its incorporation into the curriculum; and

2) genetics instruction must live up to its potential to capture and maintain the interest of students from diverse backgrounds, preferably doing so before the loss of interest in science that takes place as students approach middle school (Milwaukee, 2008; Osborne, Simon, & Collins, 2003).

1.2 IMPETUS FOR THE STUDY.

1.2.1 A Molecular Language

One common way to illustrate the nature of DNA is to place it in the context of language acquisition. The metaphor of DNA as a molecular language can be extended from individual nucleotides (which specify the base pairs) to the sequence variations that distinguish whole populations (Table 1).

<table>
<thead>
<tr>
<th>DNA Components</th>
<th>Vocabulary/Syntax Equivalencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base Pairs (4)</td>
<td>Letters (26)</td>
</tr>
<tr>
<td>Codons</td>
<td>Words</td>
</tr>
<tr>
<td>Genes</td>
<td>Sentences</td>
</tr>
<tr>
<td>Chromosomes (23)</td>
<td>Chapters</td>
</tr>
<tr>
<td>Genome</td>
<td>Book</td>
</tr>
<tr>
<td>Multiple Genomes</td>
<td>Spatial Analysis Lab (SAL) Library</td>
</tr>
</tbody>
</table>

1.2.2 Second Language Acquisition: Easier for the Young?

Second language acquisition refers to any language that is acquired after the native language. Several reports on language acquisition have presented evidence (some anecdotal and some empirical) that mastery of a second language is easier for children than for adults (Sinha,
Banerjee, Sinha, & Shastri, 2009). These observations have led to the suggestion that there is a “critical period” during child development that is optimal for learning a second language. The period when this is proposed to occur may vary, but most investigators set it at some time before puberty. The concept of a “critical period” has come under fire in recent years, but most scientists acknowledge that second languages are mastered more quickly by children than by their parents (Deng & Zou, 2016). If there is indeed a critical period when children can most effectively master a second language, and if DNA obeys the rules of syntax followed by most languages, it may be that delaying the study of molecular genetics until middle school or later misses a window of opportunity for students to become fluent in the “language of DNA”.

The activities described here were conducted to determine whether very young children can learn fundamental concepts in genetics and apply these concepts to new situations. As an initial step, a series of learning modules (termed DNA is Elementary) that are designed to make genetics and DNA accessible and interesting to K-5 students of diverse backgrounds was developed. Subsequently, the following research questions were posed:

1. Are elementary-aged students capable of understanding rudimentary aspects of genetics and solving simple genetics problems?

2. Do demographic or socioeconomic factors affect student gains in content understanding and attitude towards science?

Our observations indicate that children as young as pre-K can learn the fundamentals of genetics and use these fundamentals to answer genetic problems. However, children’s attitudes toward genetics and science in general are highly dependent on gender and socioeconomic status. These results open up the possibility that modules can be designed to be both entertaining and
informative and, as a result, can close the learning gap that may be encountered by students of diverse backgrounds.

1.3 METHODS

1.3.1 Characteristics of the Program

The genetics learning modules designed for this program consist of eight 50-60 minute sessions delivered to elementary school children by “Bio-Bus Fellows,” graduate students and undergraduates enrolled at Georgia State University (GSU). The Bio-Bus is a 30-foot mobile instructional laboratory that travels to schools throughout Georgia, teaching and exposing K-12 students to science experiences that are informative, engaging, and fun. 5300 students from 29 public elementary schools participated in the DNA is Elementary program (Table 2).

Participation in the program was teacher driven, with individual teachers initiating contact to request a visit. Typically, GSU students would remain at a school for an entire school day, during which they would give presentations to 4-5 classes of students at one or two grade levels.

<table>
<thead>
<tr>
<th>Table 2. Elementary School Participants</th>
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<tbody>
<tr>
<td>Schools</td>
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<td>--------------------------</td>
</tr>
<tr>
<td>Atkinson ES</td>
</tr>
<tr>
<td>Atlanta Heights Charter School</td>
</tr>
<tr>
<td>Chapel Hill ES</td>
</tr>
<tr>
<td>Chattahoochee ES</td>
</tr>
<tr>
<td>Cherish Christian Home Educators</td>
</tr>
<tr>
<td>Clairmont ES</td>
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<td>Wynbrooke Elementary School</td>
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<td><strong>TOTAL</strong></td>
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Learning modules consisted of combinations of PowerPoint presentations, problem solving, hands-on activities and group interactions. Guided by principles of three-dimensional learning and using iterative design, modules were developed such that student understanding would be supported with opportunities to solidify concepts with hands-on exploration (National Research Council, 2012). Where possible, activities were designed to conform to state and national standards appropriate for the grade level of the participants.
1.3.2 Meeting standards in non-science disciplines

At the initial stages of this project, Georgia had formulated a preliminary set of standards called the Georgia Performance Standards (GPS), which later became known as the Georgia Standards of Excellence (GSE). Neither the GPS nor the GSE made any significant reference to genetics until middle school or later. Since our DNA is Elementary learning modules were intended to target elementary-aged children, we were concerned that K-5 teachers would be reluctant to devote time and resources to a topic that would not fulfill any of the standards requirements mandated by their administration. Therefore, where possible we sought to include activities in the learning modules that would meet the standards requirements for other disciplines, such as language arts and mathematics, so that teachers would not have to jeopardize their performance evaluations when they offered to host our presentations.

1.3.3 Correlation of biology-based standards with language arts

Considering that the primary language spoken in the Georgia public school system is English, correlations between the English language and the language of DNA are relatively easy to find. In elementary school, for example, students learn that words can sound identical when spoken but have very different spelling and meanings. When the word “genes” is introduced to the students, we compare it to “jeans”, a word that should be very familiar to them. In this way, they learn about homophones, and in doing so are in the position to master one of the language arts standards. This part of the module is accompanied with a visual aid to assist in the teaching of this concept (Figure 1).
As word of our presentations spread, we found that the teachers were happy to host our learning modules without concern for meeting standards requirements. Still, we have retained those activities associated with different disciplines because they reinforce topics that will be useful to students in subsequent years.

1.3.3.1 Module 1. Genetics is All About YOU!

A major goal throughout the genetics learning modules is to introduce topics by relating them to something that students already know and feel comfortable with. Module 1 is focused on the concept of “traits,” a term that describes physical characteristics that can be either inherited or acquired. Bio-Bus Fellows ask the students to participate in a game where they match laminated pictures of dogs with their owners on the basis of appearance (Carcino.gen.nz/images/index.php/00b9a680/131f1c95). Subsequently, they discuss how certain characteristics (i.e. brown fur or an unusual haircut) influenced their choices (Figure 2).
This discussion allows the students to engage in dialogue about their choices. Also, it helps the students to understand that although we may share a particular physical trait (i.e. eyes) we can also differ in the manifestation of this trait (i.e. brown or blue eye color).

In order to demonstrate to students how genetics affects them personally, Bio-Bus Fellows give them a list of heritable (but harmless) traits such as tongue rolling or eye color and asked them to identify which traits they possess (learn.genetics.utah.edu/content/basics). Students receive a handheld mirror to assist them in observing genetically inherited traits including their eye color, ear lobe structure, tongue rolling and hair line (Figure 3).

Furthermore, the students received different colored stickers for their right (red) and left (blue) thumbs to assist in recognizing which thumb naturally is placed on top of the other when they clap their hands. A significant number of students admit that they never associated this last trait as something determined by their DNA.

Students also use pipe cleaners and wooden dowels (Michaels, TX) to construct a double helical model of DNA that they can use to visualize how strands separate and rejoin.

Subsequently, Bio-Bus Fellows use a large model of DNA to illustrate how the base pairs are
incorporated into the helical structure. After making their own model of DNA, students receive a custom made plastic container (ClearTec, MO) to store the DNA model. This activity not only motivates the students by providing them with a souvenir at the end but, more importantly, inspires the students to share with their families and friends their newly acquired knowledge.

1.3.3.2 Module 2. A Pair of Genes for Every Occasion

To ensure that students are comfortable with certain key concepts, these concepts may be reviewed at the beginning of subsequent modules. For example, a series of pictures (animals, plants and non-living items) is presented to the class at the beginning of Module 2, and the students are asked to identify which pictures contain DNA.

Students learn about dominant and recessive traits by constructing a “Bag Baby”, a miniature 6 inch x 6 inch non-woven linen tote bag (Darice, Ohio) that contains five facial features of distinct colors and geometric shapes, based on the specific genes that are passed down to them by the “Bag Parents”.

The activity simulates the random nature of the gene pool where the population represents a certain percentage of dominant traits and recessive traits. Sets of five facial features of different colors and shapes are laminated and cut into figures meant to symbolize genes. A manual hole punch (Michaels, TX) is used to create a hole on which to thread the pipe cleaners. Students randomly select the distinct facial traits from ten different velvet bags (Darice, OH) and then thread the traits onto a pair of pipe cleaners, which are meant to symbolize the DNA backbones (one from Mom and from Dad). Once a student has threaded all of the genes in the right order, s/he will check a genetic key (Appendix A) that identifies each trait as dominant or recessive. Using the key, the student can decide which pair of genes is homozygous dominant, homozygous recessive, or heterozygous. Each student takes a velvet bag of a color that
corresponds to the predicted face color phenotype (red or blue), so that they can create a face based on the phenotypes for the four remaining facial characteristics. Once the student determines the expected phenotypes for each trait, s/he is prepared to assemble the “Bag Baby” (Figure 4). This part of the activity requires different colored foam pieces (Darice, OH) representing the skin of the face (i.e. the linen bag) and the four different facial features (hair, nose, eyes and lips). These foam pieces are cut beforehand utilizing a die cutter machine that was custom made for his project (Ellison, CA). The foam sheets contain a layer of double-sided adhesive (Darice, OH). The student peels off the adhesive backing and places the facial feature on the linen bag, creating a “Bag Baby” that they get to keep. From their results, students can appreciate the ways that genetics simultaneously makes us similar and unique (Figure 4).

![Figure 4. “Bag Baby” Parental DNA](image)

To facilitate the introduction of the concepts of dominant and recessive traits in relatable terms that they understand, we initially showed students the difference between “dominant” and “recessive” genes by referring to them as “loud” and “quiet” respectively. For example, if dominant and recessive genes are defined as “loud” and “quiet”, a “loud” gene is so noisy that it drowns out whatever the “quiet” gene has to say; only in the absence of the loud gene does a quiet gene have an opportunity to be heard or expressed. This activity also contains a kinesthetic component where one student is asked to yell “DOMINANT” while another student simultaneously whispers “recessive”. Through this activity, it is clear to the entire class which trait will prevail (Figure 5).
At this juncture of the module series, students have been exposed to fundamental concepts in Mendelian and molecular genetics. For example, they have learned that genetic information exists in the form of DNA and that DNA is responsible for those physical traits that are inherited. They are able to recognize the shape of DNA, with most being able to identify DNA as a double helix. Additionally, the students have learned that within our DNA we have genes that can be dominant (loud) or recessive (quiet). The final product (Figure 6) is another souvenir that can be shared with their family and friends.

1.3.3.3 Module 3 and 4. Mutations: Nature’s Typographical Errors.

The focus Modules 3 and 4 is on how the language of DNA can be changed and how this change can result in a mutation. At this point we make it clear to the students that although DNA has two strands we only need one to understand the message conveyed by DNA. The concept of
mutation as a change in DNA is also introduced at this time. This approach is accomplished by capitalizing on the first few years of elementary school where students learn how to recite the alphabet and how to organize letters together to make words. The students are taught that the language of DNA adopts a similar approach. For example, during language arts class, students learn how to utilize letters to make words. The same process occurs with DNA, but with the formation of nucleotides instead of letters, and codons instead of sentences.

In the third and fourth modules, students are taught to place DNA sequences into the context of language acquisition. The metaphor of DNA as a molecular language can be extended from individual nucleotides (which specify the base pairs) to the sequences that distinguish whole populations (Table 1).

Several types of activities highlighting the correlation between DNA as a molecular language and the process of language acquisition are brought into play so that we can accommodate students with different learning styles. This can be seen in the array of activities offered in Module 1 (Figure 7):

a. playing the dog/owner matching game (visual)

b. constructing a double helix using a wooden dowel and pipe cleaners (tactile)

c. singing a “DNA song” set to the tune of B-I-N-G-O (auditory/musical)

Figure 7. Correlation between DNA as a molecular language and the process of language acquisition
Other activities in the learning modules make more direct connections between molecular and English languages. Students are asked to read the sentence in the figure below out loud, and they respond with “My tail is black” (Figure 8). This allows students not only to exercise their ability to read but, more importantly, to understand that as with the English language, the mechanisms that scientists have developed for deciphering DNA utilize a linear code. It is also worth noting that, like the printed page, the linear code reads from left to right. Thus it is the sequence that is important for decoding of DNA and not the nucleotide composition. This distinction can be visualized by comparing two triplet base pairs such as CAT and ACT, that have identical base composition but very different meanings.

![Figure 8. Example of the molecular language of DNA using the English language](image)

As they gain experience in deciphering DNA, students realize that a change in a single letter can destroy the meaning of a sentence, either by producing a sentence that has no meaning at all or, less commonly, by producing a sentence with an entirely new meaning (Figure 9).

![Figure 9. Changing one letter in a sentence a comparison to a mutation](image)
Students are asked to read the sentence in the figure above out loud, and respond with “My tail is black.” Then they are asked to read a sentence that contains a single change from a C to an N, and in this way to produce a gene product with a completely different meaning (from “My tail is black” to “My tail is blank”). From examples such as these, students begin to appreciate the effect of mutations in changing the meaning of DNA-encoded instructions. This allows students not only to exercise their ability to read but more importantly to understand that, as with the English language, the mechanisms that scientists have developed for deciphering DNA utilize a linear code that reads from left to right. The students are then given a simple sentence (The cat has one red hat) and asked to change its meaning by making a substitution of one letter.

<table>
<thead>
<tr>
<th>Original Sentence</th>
<th>Modified Sentence</th>
</tr>
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<tbody>
<tr>
<td>The cat has one red hat.</td>
<td>The cat has one red rat.</td>
</tr>
<tr>
<td>The cat has one red mat.</td>
<td>The cat has one red rat.</td>
</tr>
<tr>
<td>The cat was one red rat.</td>
<td>The cat has one red bat.</td>
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</table>

The effect of too many changes: the meaning is lost.

To see the effect of too many mutations on a living organism, students plant squash seeds that have been previously been irradiated with varying amounts of Cobalt 60. Over a 7-10 day period, they tend the plants and monitor their growth (Figure 10). The students then observe the effect of radiation by measuring various parameters of the plant (such as length of stems, length of tap root, etc.). After they graph their results they will find that too many mutations can result in so much damage to the DNA that the organism does not survive.

Figure 10. Effects of gamma radiation on squash seeds.
Once the students have been exposed to the concept of mutations, we remind them that all living organisms, whether animal, plant, or bacterium, have genomic DNA and that the sequence of this DNA can be changed. Plants and animals in particular are likely to be diploid (as were Mendel’s pea plants) and can exhibit dominant and recessive traits. A final reinforcement activity involves making a facsimile of Mendel’s garden. In this example, the trait under consideration is flower color, with purple being dominant over white. The dominant condition is likened to the use of a Magic marker. If the marker is intact and has an adequate amount of ink, it can be used to paint the petals purple. Even if one of the markers is broken, the other marker can fill in for the broken one and produce a purple flower. The flower will only be white if both markers are broken (Figure 11).

Students plant a garden by picking a gene that specifies flower color from each of two velvet bags (one for each parent). They are then asked to choose a flower of the color predicted by the genes that were pulled out of the bag (homozygous dominant = purple; heterozygous = purple; and homozygous recessive = white). The garden can be kept in the classroom, where it will serve as a reminder of the contribution of Mendel’s groundbreaking work.

1.3.3.4 Summary of Modules 1-4

Depending on how quickly they respond to questions posed by Bio-Bus Fellows, students may be given additional activities to try. At a minimum, students should 1) understand that some
traits are inherited while others are acquired, 2) recognize DNA as the genetic material, 3) recognize the double helical structure of DNA, 4) make a connection between English and molecular language, 5) predict inheritance patterns involving dominant and recessive traits, and 6) learn how mutations change the instructions that are encoded in our DNA. The remaining four learning modules illustrate ways that students can use their new knowledge to address specific genetic questions and to learn about other components, like mRNA and ribosomes, that affect gene expression.

1.3.3.5 Module 5: Differences in Animal DNA

The fifth module is dedicated to showing students how DNA gives the instructions for all inherited traits exhibited by a given organism. Module 5 features one of the most essential rules of DNA replication: the purine/pyrimidine base pairing that ensures faithful copying of DNA during each replication cycle. To illustrate this, we introduce the vast diversity in the animal kingdom, a diversity that may have been initiated by mutation, but is maintained by the fidelity of DNA replication.

Students are introduced to traditional taxonomy as a method of grouping organisms according to their similarities and differences. Teams of 3-5 students engage in a hands-on activity where they are given a set of cards with fantasy creatures (Figure 12).

![Figure 12. Taxonomy Activity](image)
Each team is asked to divide the creatures into groups based on characteristics shared by every member of the group. After completing this task, each team submits its groups to the other teams to see whether they too had chosen the same criteria for dividing the groups. For example, one team might place #4 with other creatures containing wheels instead of feet, while another team might decide to include it in a group that contains antennae. By participating in this activity, students become aware of the difficulties faced by scientists in determining what animals are more closely related than others. To illustrate the conventions adopted by taxonomists to show relatedness, we give each student a family tree derived from the television series SpongeBob Squarepants (Figure 13). In their discussion of the SpongeBob tree, students learn that multiple criteria have been used for grouping SpongeBob and his friends. As with their attempts to group the fantasy creatures in the previous activity, students gain an appreciation for the difficulties experienced by taxonomists to come up with a consistent set of parameters for determining relatedness. At this stage, they are introduced to DNA as a criterion that can be used to compare any set of organisms.

Figure 13. SpongeBob Squarepants Survey of the Animal Family Tree
Once again, the analogy is made between the English language and the language of DNA. The students learn that the alphabet of DNA consists of four letters (A, T, C, and G). They are given examples of short sentences that can be made using only four letters of the English language (Figure 14).

Students are then told that, unlike the 26 letters of the English alphabet, the four letters of the molecular alphabet serve a dual role. Not only do they encode the instructions for making an organism, but they also interact with each other through base pairing. The use of base pairing ensures that the instructions encoded in the DNA sequence are passed onto subsequent generations (Figure 15).

The students learn a fun rap-like song that helps them to remember the base pairing rule (“A to the T and C to the G”), all while clapping along with the instructor. Following this exercise, the students participate in a complementary base-pairing activity where they are given a simulated
strand of DNA. The simulated strand of DNA was created using a wooden dowel (Michaels, TX) and differently shaped foam pieces (Darice, OH) that come together like puzzle pieces.

The students are asked to take their simulated strand of DNA, armed with the knowledge that A always pairs with T and G always pairs with C, and walk around the classroom looking for the person with a complementary sequence. At this stage, students’ experiences with base pairing should make it possible for them to use DNA sequence analysis to determine relatedness. For example, one source of considerable controversy among scientists has historically been the relationship between pandas, bears, and raccoons. Some taxonomists chose to group the two pandas together, while others claimed that the giant panda is more closely related to the bear, while the red panda is more closely related to the raccoon. A look at their diet reveals that both the giant panda and the red panda (formerly called the greater and the lesser panda, respectively) subsist almost entirely on bamboo. Furthermore, they both have an unusual sigmoidal bone extension off their wrist that too many scientists appears to be a false thumb. However, the facial features of the red panda appear to be much more similar to those of a raccoon. Now that scientists are able to obtain thousands of DNA sequences in a single small-scale sequencing run, it is possible to generate reliable sequence data that resolve this issue. Using the movie “Kung Fu Panda” as a frame of reference, students conduct DNA sequence comparisons on four sets of DNA (Figure 16).
Students working in teams are given magnetic boards (Fisher Scientific) and laminated strips of DNA strands containing sequences for each of the animals under consideration (giant panda, red panda, brown bear and raccoon). The students are charged with taking each of the DNA strands and comparing them to the giant panda DNA. Before they begin the activity, the students are taught that the greater the differences in the DNA sequences of two organisms, the less closely related they are. Once they have completed the exercise and determined the differences in DNA between the giant panda, brown bear, raccoon and red panda the students find that, based on DNA typing, the two pandas are most closely related to each other.

1.3.3.6  Module 6. Digging Deep with DNA.

Investigation of the properties of DNA is greatly facilitated if microscopic analysis can be undertaken. For this purpose, we give each student the opportunity to use a MiScope (Zarbeco, NJ) a small hand-held microscope with a 40x –140x magnification. Students become aware of the world we normally cannot see, a world inhabited by planaria, daphnia, paramecia, and tardigrades (water bears). We stress that each of these microorganisms contains within its cells the DNA-based instructions necessary to thrive in the environment where it lives.

The students then carry out a DNA extraction, using split peas, bananas, or strawberries as the source of the DNA. At the end of the extraction procedure, students precipitate the DNA by floating ethanol gently at the meniscus. With this approach, the DNA becomes easily visible as a flocculent material which, when dry, pulls apart in a way characteristic of the long narrow molecule that DNA has become. Students also learn the importance of protocols and proper personal protective equipment (PPE) such as goggles in a laboratory setting. Once the DNA is dry, students are encouraged to take it home and share it with family members.
1.3.3.7 Module 7. Phenotype vs Genotype.

For the seventh learning module, we once again return to heredity. This module is dedicated to building the scientific vocabulary of the student. Although we have had many examples of phenotype versus genotype, this is the first time where the specific science vocabulary is taught. In order to relate to the students, we use the familiar theme of “Monster’s Inc.” In this case, students use their DNA sequence knowledge to construct a monster with defined traits. For example, a student is given a DNA sequence and a key where a series of 4 nucleotide bases (e.g., TTCT=trait) represents a physical characteristic or phenotype (i.e. purple spots). Each sequence has approximately three traits which enable the student to correctly select their monster. [Note: we recognize that nearly all traits would require more than four base pairs to exist, but we limit the sequence to simplify the activity (Figure 18).

The emphasis of the seventh module is heredity. This module is focused on building the scientific vocabulary of the student. Although the other modules provided many examples of phenotype versus genotype, for instance, this module would be the first time where the specific science vocabulary was taught. In order to relate to the students, the familiar theme of “Monster’s Inc.” was co-opted for this module. The first activity asked the students to find the correct monster from a collection of monsters given the information that most of the students had previously acquired. For example, the student was given a DNA sequence and a key where a series of 4 nucleotide bases (i.e. TTCT=trait) represented a physical characteristic or phenotype.
(i.e. purple spots). Each sequence had approximately three traits which enabled the student to correctly select their monster (Figure 24).

1. Pick a DNA strand

   | C | C | G | T | C | T | A | G | T | A | C | C |

2. Find the traits that belong with DNA code

   - I have blue skin
   - I have two eyes
   - I have three fingers

3. Pick the correct monster from the bag

   ![Monsters]

   Figure 18. Find the correct monster activity

In the second activity, students construct their own monster from a bag of “monster parts”. The monster parts were created designing monster trait templates, e.g., body color and shape as well as feet, arm, eye, mouth, ear and wing type (Figure 19).

   ![Monsters and Traits]

   Figure 19. Create your monster Punnett square activity

After creating a monster, the students determined the possible genotypes based on the monster’s phenotype (Figure 20).

   ![Punnett Square]

   Figure 20. Determine your monster’s possible genotype
Up until this time in the learning series, we de-emphasized vocabulary terminology in favor of general concepts that are essential foundations for genetic processes. This activity introduces the students to scientific vocabulary such as heredity, genotype, phenotype, homozygous and heterozygous genes. Subsequently, the students learn how to design and utilize a Punnett square.

1.3.3.8 Module 8. Decoding DNA

This module created the platform to bring the information from the first seven modules together via protein synthesis utilizing the concept of jewelry making. The activities begin with a discussion of cell anatomy and physiology, using the concept of a house as an analogy. For example, the house/cell has different rooms (representing organelles) where different events occur (Figure 21). The students will be introduced to some of the different components of the cell leading to the main focus of molecular genetics - the central dogma theory.

The students ascertain that the nucleus is similar to a bedroom in a house because this is where the DNA rests. Additionally, they learn that the lysosomes are like the restroom because this is
here all the waste is broken down and discarded. Most importantly, the students learn that the kitchen is comparable to the ribosome because this is where the proteins are made (Figure 22).

![Figure 22. Visual aid for nucleus and ribosome](image)

At this juncture, the students review the correlation between language acquisition and the molecular language of DNA. The students are now able to understand that the letters of the English alphabet are similar to the nucleotide base pairs in DNA in that four letters can come together in different ways to form words and sentences. Furthermore, the students are also able to compare other parts of language to the language of DNA (Figure 23). For example, in genetics, codons are similar to words and genes are similar to sentences. Moreover, paragraphs are similar to the DNA coming together to form a story or a book. The students come to know that the book is us because all our DNA compiled are the instructions that make us who are.

![Figure 23. Comparison between the English language and the language of DNA](image)
The students are asked the question, “How do we get from DNA to us?” and then the concept of the central dogma theory is introduced. The students are given the following scenario:

We have this chef who has many delicious recipes for baked goods such as cakes and cupcakes. He would be happy to share his recipes; however, he only speaks Japanese (the language can be changed dependent upon the class). Fortunately, there is one little boy who can speak both Japanese and English. Not only can he speak both languages, but he also volunteers to write down the recipe (i.e., transcribe) in English so that everyone can read it. That’s right! We can change the writing to English. We realize that this is a cupcake recipe. Now we have to change the form of the writing to the actual ingredients (translation) we can use to make the cupcake because we can’t take the written recipe and simply place it into the oven, can we? Perfect! We are now going to mix up the ingredients, put it in the oven and voila! We get our final product! A scrumptious cupcake!

The students learn that the central dogma theory works in a similar fashion to the cupcake scenario. At the start of the process, the DNA is sequestered within the nucleus. The source of the information needed to make the cupcake resides in the nucleus, where it can be copied into a message that can cross the nuclear membrane and travel to the cytoplasm. At that point, that wonderful translating machine (the ribosome) decodes the message and makes the protein. This process is called translation and the entire process as it occurs in an organism represents the central dogma theory (Figure 24).
Figure 24. Central dogma theory analogy to baking

In the first activity of this module, students learned to differentiate between DNA and RNA (i.e. differences in their alphabet). Equipped with the fact that one of the main differences between DNA and RNA is that one of the letters in the alphabet is different (i.e. RNA has “U” instead of “T”), the students participated in a kinesthetic activity where the class formed a double stranded DNA. This was accomplished by assigning each student a nucleotide base (a laminated card attached to a wooden dowel). They were then tasked with finding their partner. After the human double strand was created, they were tasked with verbally expressing what needed to happen in order to make one of the DNA strands look like RNA. The class rearranged themselves to replace the students within the strand that were “T” with students that were assigned a “U”.

During the activity, the students were asked to identify whether transcription or translation was occurring (Figure 25).

Figure 25. Transcription Kinesthetic Activity
Additionally, the students were shown the role of amino acids and codons during a simulation of protein synthesis utilizing a jewelry making activity. Just as in the previous activity, the entire classroom became a part of the kinesthetic activity where the students moved from one station (i.e. nucleus or ribosome) to the next depending on whether they were undergoing transcription (nucleus) or translation (cytoplasm) simulating protein synthesis. Students constructed a string of different colored jewelry beads which represented different amino acids. The students either made a ring or bracelet, their choice being determined by the type of protein that is produced which depended on what the DNA dictated. A bracelet represented an extracellular protein while a ring represented an intracellular protein; in this way students were able to understand that different types of proteins have different functions similar to the house/cell analogy where some chores are done inside the house (i.e. washing dishes) and some are done outside the house (i.e. mowing the lawn).

The protein synthesis simulation consisted of different colored jewelry string and beads (Michaels, Texas). Students were given a strand of DNA and they determined what the RNA and in turn the codon would be. Utilizing a key with only a few amino acids represented (Figure 26), they constructed the protein moving from station to station simulating the functions of protein synthesis in the cell.

![Figure 26. Protein Synthesis Kinesthetic Activity](image)
The first four beads determined what type of protein the student was making (Figure 27). For example, if the student had green, orange, black and blue as their first four beads they would be making an extracellular protein or ring.

1.3.4 Utilizing feedback to refine the genetics learning modules.

To determine how module participation affected both students’ content knowledge about genetics and their attitudes toward science, a pictorial Likert scale was used to design the pre and post-feedback forms (Likert, 1932; Mantzicopoulos, French, & Maller, 2004). These feedback forms were administered at the beginning of the first module and at the end of the second or last module. Due to the young age of the students, the feedback forms were limited to six items. Three of the items were defined as “content” questions:

1. a DNA structure question (“Which of the following looks like DNA?”)
2. a dominant/recessive question (“Billy Smiley Face has one dominant gene coding for a red nose and one recessive gene coding for a blue nose. Which picture below looks like Billy?”)
3. a mutation question (“Which difference between Billy Big Ears and the other faces is most likely the result of mutation?”)

In each case, the students had the option to circle “I don’t know” (Appendix C).
In the initial design of the survey instrument, in order to ensure that correct answers were not dependent on memorizing vocabulary, the dominant/recessive statement was phrased as: “Billy Smiley Face has a dominant (loud) gene coding for a blue nose and one recessive (quiet) gene coding for a green nose. Which picture below looks most like Billy?” We subsequently found that the removal of the “loud” and “quiet” descriptors from this question made no difference in the accuracy of student responses to this content question, so those were no longer used. The other three items on the feedback form consisted of statements designed to measure students’ opinion of science as a subject of study. We chose to ask for their impression of science in general rather than genetics specifically because, for many of them, the term “genetics” was an entirely new concept. Students were asked if they agree or disagree with statements examining interest in science (“I like science”), self-efficacy (“I am good at science”) and long-term interest or an intent to persist in science (“I would like to be a scientist”). The attitude items had three emoji-response options due to the limited reading capacity of some of the younger participants initially. The response options had both a written and visual component, with the visual component consisting of yellow facial icons with smiling, neutral or unhappy faces (Reynolds-Keefer & Johnson, 2011). The evaluation tool was later modified to contain a five point Likert-scale with emoji-response options in order to increase the evaluation tool’s variability and item homogeneity (Mellor & Moore, 2014).

Demographic and socioeconomic data were determined at the school level rather than reported by students on the instrument. Due to the young age of the students, self-reporting of this information did not provide us with reliable data. In accordance with survey development best practices, we revised earlier iterations of the survey to remove this information. Thus, we were limited in our ability to collect individual demographic data; instead, we relied upon the
state-database, queried in January of 2016) to determine this information at the school level
(https://oraapp.doe.k12.ga.us). These school-level numbers were used in our analyses of
underrepresented minority and socioeconomic factors. According to Harwell et al. 2010, the use
of Free and Reduced Lunch (FRL), based on the National School Lunch Program, continues to
be a link to a student’s socioeconomic status (SES) in quantitative education research (Harwell &
LeBeau, 2010). The schools represented in this study were divided into low and high SES
Statistical analyses of data drew upon unpaired samples t-tests to determine significance of
differences in various student samples. P values of less than 0.05 were reported as significant; p-
values of less than 0.01 were reported as highly significant.

Current Institutional Review Board (IRB) standards require a four-hour Collaborative
Institutional Training Initiative (CITI) course on how to administer surveys and gather data
without bias when evaluating human subjects. Survey forms were administered by GSU graduate
students who took the 4-hour CITI course training. The graduate students read the statements on
the evaluation tool aloud and asked students to circle the response that represents what they
believe to be the correct answer (questions 5, 6 and 8) or that reflects their opinion (statements 2-
4 and 7). An identical form (post feedback form) was administered at the end of the final session.

1.3.5 Inclusion of K-5 teachers in the implementation of a genetics learning community.

According to the National Science Foundation (NSF) an estimated 80 percent of the jobs
created within the next decade will require some form of math and science skills. In order to
meet this challenge and prepare a workforce with adequate competencies and knowledge of how
the STEM initiative affects our daily lives, we need to invest in teacher quality. In order to
effectively teach science, a teacher must be confident in his/her ability to understand science
content and know how students learn science. Although there is an abundance of research that
indicates the importance of quality teaching in maximizing student achievement (Grigg, Kimberle, Adam, & Geoffrey, 2013; Tseng, Tuan, & Chin, 2013), many K-5 science educators are not confident in their abilities to teach science compared to other subject matters (BayerCorp, 2004). If the students are experiencing science from an educator who lacks content knowledge of the subject matter, the quality of the experience will decline (Herrington et al., 2012; Payne, 2004). This may happen during a critical point in a student’s life, especially if this experience is the only exposure to science that they have. One of the challenges observed in the K-12 science classroom is the ability to mimic the professional scientific community and the work that is being performed by researchers. On one hand, it may be difficult to convert a classroom into a research laboratory. However, it may be possible to turn the research laboratory into a classroom. This approach has many advantages such as allowing teachers who may not have an extensive science background the opportunity to enter a research lab as a student. This would better prepare the teacher to implement scientific inquiry through hands-on, student-centered and inquiry based approaches within their classrooms (Jones et al., 2016; Jung & Tonso, 2006).

To empower the Georgia K-5 educators to create a quality experience for their students, a professional development experience was created where instead of being subjected to yet another science lecture, teachers were a part of an active learning community where they interfaced with colleagues from different districts and together rediscovered the subject matter that inspired them to become teachers. This learning community was utilized to pilot the new genetics modules (DNA is Elementary), creating a collaboration between the scientists and the pedagogical experts. Consequently, this collaboration gave rise to a product that not only consists of creative activities conveying science concepts but also aligns with the Georgia Standards of Excellence (GSE) for Science that are required to be taught (www.georgiastandards.org/Georgia-
Standards/Pages/Science.aspx). The professional development experience can empower science educators to rethink their science classroom and give them the confidence to inspire and educate the next generation of scientists (Schneider, Krajcik, Marx, & Soloway, 2002). Additionally, the teachers assisted the Bio-Bus program with the implementation of the series in their classrooms. The teachers participated in a pre and post survey that asked them to rate their experience based upon the how well the learning objectives were met as well as content knowledge assessments (CKA). The teacher participants received a “DNA is Elementary” kit with materials and protocols in order to enable them to reproduce the activities in their science classrooms. Additionally, they received a stipend for participating in the professional development which was funded by the National Institute of Health Science Education Program Award (NIH SEPA).
1.4 RESULTS

1.4.1 Evaluating the classroom intervention after module implementation.

Approximately 3500 students at 29 public elementary schools took part in learning modules 1-4 of this program. Pre- and post-tests (each containing the same three content questions and three attitude statements) were administered to every participant immediately before the program began and immediately after it ended. Note: because we excluded from our analyses all responses from those students who indicated that they did not participate in the full set of modules (the numbers of participants whose responses were included in the data set used for this study was less than the number who actually responded to the surveys), analysis of the results revealed a significant increase in the number of students who answered the content questions correctly at the conclusion of the 4-module program (Figure 28). These results led us to conclude that students can grasp rudimentary genetic concepts if these concepts are presented in an engaging and entertaining way.

Figure 28. Growth in understanding of genetics concepts.
Error bars represent SE. Numbers in parentheses indicate N values for respondents on the indicated items (pre- and post-, respectively). DNA structure (3673, 2381); Mutation (3694, 2373); Dominant/recessive (3214, 2000). *** indicates a high level of significance with p value <0.0001.

When we disaggregated the content question responses by grade level, we found that the post-test accuracy of respondents exceeded that of the pre-test in nearly all cases (Figure 29 A-C).

![Figure 29A-C. Percent of respondents answering content questions correctly by grade level.](image-url)

Numbers in parentheses indicate N values for respondents of the indicated grade levels (pre- and post-, respectively). (A) DNA structure. Kindergarten (248, 147); Grade 1 (398, 195); Grade 2 (310, 202); Grade 3 (721, 536); Grade 4 (294, 172); Grade 5 (1494, 1084). (B) Mutation. Error bars represent SE. ** indicates significance with p value <0.01; *** indicates significance with p value <0.0001.
Due to scheduling constraints, many students saw only modules 1 and 2; mutation is presented in modules 3 and 4. Therefore, we concluded that the number of students who received the information necessary to respond correctly to the question was too low for analysis in grades K and 2.

We noted, however, that the pre-test accuracy in response to the content questions involving DNA and mutations (and to a lesser extent the dominant/recessive question) was much higher than one would expect on the basis of random chance. A large number of participants were able to identify DNA and predict the effects of mutations on the pre-test even before they took part in the learning modules. This was particularly true for the content question involving DNA structure, where nearly half of the participants in all grades were able to recognize the double
helical structure of DNA before they participated in any of the learning modules. The percentage of correct responses on the pre-test increased steadily with increases in grade level: approximately 40% of those in kindergarten identified the correct structure while that number rose to nearly 100% for those in grade 5. By the time of the post-test, over 90% of the children, regardless of age, could correctly identify DNA as a double helix.

These results provide support for the assumption that young learners can acquire the knowledge and skills necessary to understand general genetic concepts such as DNA structure and mutations. A sizable number of students also correctly predicted inheritance patterns based on dominant and recessive traits, an ability that forms the cornerstone of classical genetic theory. Additionally, we wanted to determine if students were able to retain their newly acquired information hence a second post survey was administered after approximately 60 days following the last presentation. For example, at Sharon elementary school, we administered the first post-test immediately after module 4 and we later administered a second post-test after 60 days following module 4 and found that the students retained most of the information regarding dominant and recessive traits (Figure 30). However, the mere fact that the participants are capable of learning about DNA and genetics (and in some cases have already absorbed a significant amount of information from the mass media) does not mean that they will be attracted to genetics as a discipline of study. Students, no matter how knowledgeable, will turn their backs on genetics as a potential career choice if they regard science as dull, intimidating, and irrelevant to their lives.
Our project operates under the auspices of the Georgia State University (GSU) Bio-Bus program. The Bio-Bus is a 30-foot mobile instructional laboratory that travels throughout the state of Georgia. Graduate students and undergraduates ride with the Bus and present activities designed to generate enthusiasm for science among children and their families. These “Bio-Bus Fellows” constitute the core of our program, bringing with them a fascination for science that is contagious. They are trained to give presentations in a wide variety of topics, in the process sharpening their communication skills and increasing their own understanding of fundamental science concepts. Bio-Bus Fellows are valued not only for their excellence in instruction, but also for the key role they play as GSU’s ambassadors to the K-12 schools we visit. Our Fellows are drawn from many disciplines, including biology, chemistry, geology, physics, anthropology, and education. Like the rest of the student population at GSU, they come from diverse racial and ethnic backgrounds. The percentage of underrepresented minorities within our group of Fellows (55% Black, 10% Latino, and 3% Native American) mirrors or exceeds that of the school systems in the state of Georgia (38% Black and 9% Hispanic).
To investigate students’ attitudes toward science at this stage in their lives, we presented them with statements designed to measure their interest (‘‘I like science’’), their self-efficacy (‘‘I am good at science’’), and their consideration of science as a lifelong pursuit (‘‘I would like to be a scientist’’). More than 2/3 of the students responded ‘‘yes’’ to the first two statements, indicating that, as a group, they are favorably inclined toward science, and feel that they are good at it (Figure 31).

Figure 31. Percent of students responding ‘‘Yes’’ to attitude items.
Numbers in parentheses indicate N values for respondents to the indicated items (pre- and post-, respectively) (A) Grades K-2; ‘‘I am good at science’’ (999, 543); ‘‘I would like to be a scientist’’ (991, 530); ‘‘I like science’’ (991, 530). (B) Grades 3-5; ‘‘I am good at science’’ (1512, 1069); ‘‘I would like to be a scientist’’ (557, 539); ‘‘I like science’’ (1870, 1221). Error bars represent SE. * indicates significance with p value <0.05. ** indicates significance with p value <0.01.

Fewer students expressed an interest in becoming a scientist, especially at the higher grade levels (Figure 32A-C). When the ‘‘yes’’ responses and the ‘‘maybe’’ responses were pooled, however, the number of students who were at least open to the idea of becoming a scientist jumped to nearly 70% (data not shown). Participation in the genetics learning modules produced a slight but in some cases significant increase in students who expressed a positive attitude toward science.
I would like to be a scientist (% yes)

I am good at science (% yes).

pre  post

pre  post
In general, students’ perception of science remained steady as they became older. The one exception to this trend was students’ responses to the statement, “I would like to be a scientist.” This decrease was especially pronounced among girls (Figure 33).

Girls also exhibited a decrease in the number of correct answers they obtained on the content questions as they became older, resulting in an increasingly large disparity between the accuracy of girls’ responses and that of the boys. Particularly at the higher grade levels, the percentage of girls who circled the correct answer on the content questions was considerably lower than the percentage obtained by the boys at the same grade level. Also noteworthy, when we looked at the
data for the percent of students who responded “I don’t know” when asked to identify the structure of DNA before the presentation, we found that for most grade levels girls were more inclined to answer “I don’t know” when compared to the boys (Figure 34).

![Graph showing percent of students responding “I don’t know” to DNA structure question](image)

*Figure 34. Percent of students responding “I don’t know” to DNA structure question*

The 29 schools who hosted our DNA is Elementary program represent a cross section of elementary schools in terms of race, ethnicity, and socioeconomic status (SES). Direct comparison of students based on school demographics were compromised by the fact that comparable schools did not necessarily host visits to the same grade. Moreover, some of the schools combined grade levels when they organized their schedules. Since student responses varied widely as a function of grade level, the numbers of responses that met the criteria for a direct comparison were in most cases too low to obtain meaningful data.

A category for which we could gather usable data was in the area of SES. Schools were divided into two categories based on the percentage of students who qualified for free or reduced lunch: low SES (>80%) and high SES (<20%). In most (though not all) cases, there was a positive correlation between low SES status and high enrollment of underrepresented minorities.
We found that children attending schools with a high proportion of economically disadvantaged students were far more likely to express dissatisfaction or discomfort with science than those attending schools where the students were more economically privileged. Nearly half of the children attending the low SES schools gave a negative response to all three of the attitude statements on the pre-test. Ironically, this same cohort of children was the most likely to change their attitudes after participating in the DNA is Elementary series (Figure 35 A-C). In most cases, this increase exceeded 15%. In contrast, those from high SES backgrounds showed virtually no change in their responses to the attitude statements. At the completion of the 4-module series, the number of positive responses to the survey statements by students from low SES schools approached those of students attending high SES schools.

Figure 35. Percent of student “No” responses disaggregated by socioeconomic group and grade level.

Numbers in parentheses indicate N values for respondents to indicated items (pre- and post-, respectively). (A) “I am good at science” Grades K-2, Low SES (171, 87); Grades 3-5, Low SES (412, 225); Grades K-2, High SES (374, 188); Grades 3-5, High SES (886, 615). Error bars represent SE. * indicates significance with p value <0.05. ** indicates significance with p value <0.01. *** indicates significance with p value <0.0001.
(B) “I like science.” Grades K-2, Low SES (163, 81); Grades 3-5, Low SES (411, 232); Grades K-2, High SES (376, 186); Grades 3-5, High SES (895, 619). Error bars represent SE. * indicates significance with p value <0.05. ** indicates significance with p value <0.01. *** indicates significance with p value <0.0001.

(C) “I would like to be a scientist.” Grades K-2, Low SES (170, 86); Grades 3-5, Low SES (411, 231); Grades K-2, High SES (375, 190); Grades 3-5, High SES (890, 619). Error bars represent SE. * indicates significance with p value <0.05. ** indicates significance with p value <0.01. *** indicates significance with p value <0.0001.
1.4.2 The impact of the PD on K-5 science educator participants.

1.4.2.1 Content Knowledge Assessment.

Between 2010 and 2017 during the summer semesters approximately 100 teachers from the metro-Atlanta area (mainly) convened for the DNA is Elementary Professional Development. It was hypothesized that participation in the professional development experience would enable teachers to feel more confident in assisting with activities and facilitating dialogue among their students. The teachers participated in a pre and post survey which aimed to gauge their reactions to each component of the workshop, their sense of whether learning objectives had been met, and their content knowledge at the end of the workshop. Overall, participants’ average rating of the entire workshop was 4.76 on a 5-point scale. In general, teachers responded positively to all components of the workshop, finding them informative, useful, engaging, and applicable. Teachers felt that some sessions were more applicable than others. Based on feedback collected from open ended items, this is likely a result of the fact that much of the higher-level genetics content is not covered in the elementary science standards. The higher-level genetics content was covered to give the educators knowledge that they could use to feel more confident in their science teaching ability. Nonetheless, the professional development workshop had a significant impact on the teacher’s content knowledge. The pre and post survey (Appendix C) asked three CKA questions pertaining to the central dogma theory, simple monohybrid Punnett squares and DNA sequencing (identifying which animal is more closely related to another if given a short genomic DNA sequence for each animal being compared). For the item relating to sequencing, 68.1% of the teachers correctly answered this question before the PD however, after the intervention 94.3% were able to answer correctly. For the monohybrid Punnett square item, 68.8% of the teachers answered this item correctly before the PD compared to 78.8% who
answered correctly after the intervention. The most significant was the item asking them to apply knowledge on the Central Dogma Theory. Before the PD, 36.7% of teachers answered this item correctly and after the intervention, 94% were able to correctly answer this item (Figure 36).

![Bar chart showing growth in understanding of genetics concepts after a professional development.](image)

*Figure 36. Growth in understanding of genetics concepts after a professional development.*

### 1.4.2.2 Teaching Self Efficacy.

Using a pre and post administration of a modified version of a previously validated teaching self-efficacy evaluation tool (Yoon Yoon, Evans, & Strobel, 2014), we measured the effect of the workshop on teaching self-efficacy. Teachers demonstrated significant growth in self-efficacy related to teaching both science and genetics. We used a paired t-test for significance, and found that the difference between the pre and post tests were highly significant (Figure 37).
1.5 DISCUSSION

1.5.1 Impact on girls and underrepresented minorities.

The large numbers of women and underrepresented minorities (URM) in this country represent a highly promising pool of untapped potential from which to draw new STEM professionals. Although the push for gender equity among college science majors is now beginning to reach fruition (thereby effectively doubling the pool of potential scientists to include the 50+ percent of the population that is female), the proportion of women scientists continues to decrease as they attempt to climb the employment ladder. While women constitute 46% of the potential STEM workforce, they account for only 28% of those who are actually working in STEM-related fields (NSF Science and Engineering Ind. 2014). Moreover, African-Americans and Hispanics in particular have been and continue to be significantly underrepresented in graduate schools and other academic programs that are responsible for training new scientists. The percentage of Caucasians in this country is decreasing steadily, with Census Bureau projections predicting that by 2050 groups that are now considered to be minorities will collectively account for more than half of the U.S. population.

Genetics is often singled out as one of the most challenging disciplines in the biosciences. The fear of genetics has been ascribed to many factors, including the fact that 1) success in
mastering genetics principles cannot be achieved solely through rote memorization; and 2) the field of genetics, and particularly molecular genetics, is advancing so rapidly that high school teachers (and to a certain extent even college instructors) must struggle to stay current. Based on our work with elementary students, however, we conclude that even very young children can understand and work with genetic concepts as long as they are presented in an entertaining, age-appropriate fashion. In fact, we have found that most of the K-5 students who take part in our program are already familiar with some genetic terminology, especially as it relates to DNA structure and mutations. When asked on our pre-test to select from a set of diagrams the one that looks like DNA, almost half of the kindergarten students and greater than 90% of students in grades 4-5 correctly chose a double helix. To determine how the students obtained this information (since they had clearly gotten it prior to any interaction with us), we met with about 100 children in small focus groups, and talked about DNA. Nearly all of them told us that they had learned about DNA by watching cartoons and games that featured superheroes and genetically engineered monsters. They also were aware that changes in DNA occur through mutation, and that scientists can manipulate DNA to change its sequence. Interestingly, not one of the students we talked with said that they had learned about DNA at school.

The controversy over the proposed adoption of the NGSS illustrates how difficult it is for educators to decide how much weight to give to the many topics that make up the life sciences. Based on our results to date, we would agree with the NGSS recommendations that genetics should be introduced into the curriculum by grade 3.
Our post-test results indicate that very young children enjoy genetics-oriented activities and that they retain what they learn for a reasonable period of time (Figure 30). We recognize that although the school (Sharon Elementary School) that showed high retention is a high SES school and gifted class, any retention would be accepted as a positive result. Given the multidisciplinary nature of genetics and its potential to apply to any number of academic areas, genetic components could be incorporated into subjects that already have a place in the curriculum if a large commitment to genetics is considered unfeasible. Traditional lessons in taxonomy, for example, could use comparative DNA sequence relationships to address questions of species relatedness. In fact, establishing a genetics correlation need not be restricted to STEM disciplines. The preoccupation of Tsar Nicolas II with the health of his hemophiliac son, for example, could be used as a way to illustrate the relevance of genetics to historical events, as could the subsequent identification of the bones of his family members through DNA testing. Even the concept of the quintessential caveman could be re-visited, now that scientists are becoming aware of the close genetic relationship between modern humans and Neanderthals. Clearly, an understanding of genetics and its application to many different disciplines will enhance the scientific literacy of our nation.
There have been numerous reports that girls and boys exhibit approximately equal engagement with science when they are very young, but that girls’ interest begins to weaken as they approach middle school (Cavanagh, 2005; Myra & David, 1986). Boys lose interest in science as well, although their loss seems to be delayed relative to that of the girls. Our results also show this trend, with girls’ interest declining somewhat earlier than boys’. The proportion of girls who responded correctly to the content questions also decreased with an increase in grade level. This could be due to their self-reported loss of interest in science; girls as a group may be less knowledgeable about DNA and genetics because they pay less attention to shows and games that incorporate them to their story line. Alternatively, they might simply be less confident in their choice of an answer. During the administration of the pre- and post-tests, the students were told that “I don’t know” was as acceptable response. When answering questions on the pre-tests, girls were much more likely to select the “I don’t know” option than boys (Figure 34) and thus as a group scored fewer “correct” responses. If the “I don’t know” responses are removed from the surveys, and only the “correct” and the “incorrect” responses are tabulated, then the percentage of girls who choose the correct answer is indistinguishable from that of the boys.

The attitude survey responses from students who are socioeconomically disadvantaged suggest that a major obstacle to minority representation in STEM fields is not so much a deficiency in the students’ knowledge base as it is a general lack of self-confidence in their own ability to navigate a purportedly difficult field like genetics. On the positive side, our experiences suggest that interventions such as the DNA is Elementary program can counterbalance this insecurity and provide students with the self-efficacy they need to succeed. We have not determined what accounts for this reversal in self-perception, but we do note that our presenters are themselves from predominantly underrepresented minorities and socioeconomically
disadvantaged backgrounds. As vibrant young adults who just happen to love science, they help to counteract the negative stereotypes of scientists that are often presented by the media. Moreover, they are compelling role models for young learners who share their racial and ethnic heritage, illustrating by their own example that the life of a scientist can be a fulfilling, and obtainable, career choice.

1.5.2 Current and Future Work for the DNA is Elementary Program

1.5.2.1 Modification of the Evaluation Feedback Form

It was noted that the 3 pt. Likert-scale evaluation tool utilized in this study did not provide sufficient variability for the attitudinal component. We learned from the 3 pt. Likert-scale and modified the tool to increase the variability by using a 5 pt. Likert scale (see below) to determine if this will provide a clearer picture regarding the students’ attitudes towards science.

_A five-point Likert scale was used to measure students’ agreement with the following statements._

1. Scientists are cool.
2. I like science.
3. I am good at science.
4. I would like to be a scientist.

Initially, the surveys administered used the 3pt. Likert-scale and there was approximately 3500 students evaluated with this tool up until 2016 when we modified the tool to have a 5pt. Likert scale as well as a modified dominant/recessive question and mutation question. Thus far, we have evaluated approximately 2200 students using this tool. The only item that has remained consistent throughout the project was the ability to recognize the structure of DNA item. There has been approximately 6100 pre surveys and 5700 post surveys collected for this question.
1.5.2.2 Transition from Formal Science Education to Informal Science Education

Initially, the DNA is Elementary project and activities were directed toward serving students in the K-5 school system, with the bulk of activities concentrated in the academic year. The relatively uncrowded summer months offered us the opportunity to reach out to public libraries and other community groups with the modules that had been used successfully with the K-5 students. Participants in summer activities tend to be members of family groups, and thus exhibit a wide range of age and experience. The Bio-Bus summer programs thus provided us with a unique opportunity to measure the reaction of participants representing a wide range of experience, interest, and backgrounds to our modules.

These previously established partnerships with libraries and librarians have enabled the program to organize activities within the libraries during the school year. Over the past few years we have established partnerships with numerous libraries and community organizations. During the summer months, we present activities at libraries that are typically seen by approximately 2100 - 2400 children and their family members. In last year’s summer period, for example, we presented activities at 19 libraries, averaging 3-5 visits.

1.5.2.3 DNA Runs in the Family

In 2014, we started DNA Runs in the Family, a project that targets whole families for participation in genetics learning modules. We have created a total of 10 DNA modules of differing levels of complexity. Sample topics include a discussion of traits (introductory level); dominant/recessive relationships (intermediate) and the polymerase chain reaction (high). DNA modules are scheduled at one of several participating libraries on weekends during the school year however, the goal is to have the visits scheduled for families year round. Families can sign up for the topics that are at a level of complexity that is comfortable to them. All modules
contain a variety of hands-on activities to ensure that the experience is both entertaining and informative.

Since the summer of 2014, when we first began offering the DNA Runs in the Family modules, we have had a highly positive response to our new program, as indicated by the numbers of libraries and other organizations who have booked visits. We have visited over 100 libraries/community groups where over 4,000 participants have experienced the DNA is Elementary project as an informal science education program. Those who complete a specified set of modules will have the opportunity to participate in an authentic scientific experience involving metagenomics (the search for novel organisms in the environment). Additionally, in the Spring 2016 semester, we hosted a DNA Runs in the Family workshop for 16 librarians and currently have over 100 librarians who have participated in a professional development with this program.

With our DNA Runs in the Family series, we bring together an extended age range and tailor the genetic presentations to appeal to a wide variety of participants. This puts us in the unique position where we can prepare activities that we believe will be enjoyed by all members of a family, paying close attention to the children as they make the journey from pre-K to adulthood, but especially as they transition from late elementary and subsequently into middle
and high school. In a recent review of outreach programs that serve immigrant populations (Brewster & Railsback, 2017), the authors identified the 3 attributes that are consistently associated with positive outcomes.

1. **Programs that target families**: The first criterion is met through the use of the *DNA Runs in the Family* program itself.

2. **Programs that feature a bilingual translator**: The second criterion takes advantage of the characteristics of the Bio-Bus fellows, who come from a wide range of racial and ethnic backgrounds, and are already giving presentations in Spanish.

3. **Culturally enriched programs that promote a sense of self-worth in their participants**: To meet the third criterion, we are currently designing and pilot testing new genetics modules with the following characteristics.
   a. illustrate the science behind the subject being presented.
   b. contain a genetics and/or DNA component.
   c. incorporate the rich cultural traditions surrounding the subject.
   d. present the topic using an instructional style that sets it apart from the traditional ways that the topic is usually taught.

1.6 **CONCLUSION**

Because genetics is regarded as a hard subject to learn, its incorporation into the science curriculum is typically postponed until relatively late in students’ academic careers. Indeed, students may not even encounter genetics until they are well on their way to losing their interest in science. By waiting until middle school or beyond, we are missing the chance to introduce our
students to a field of science that is exciting, stimulating and topical enough to forestall their flagging interest. In fact, learning about genetics and the impact it has on their own lives may actually re-ignite their enthusiasm for science.

The activities described here were designed to determine whether young learners can grasp the fundamental principles of genetics if these principles are presented to them in an entertaining and non-intimidating way. Our primary objective is for students, as they continue with their schooling, to use their understanding of these principles as a foundation on which to incorporate increasingly complex aspects of classical and molecular genetics. In this way, they will be able to keep pace with genetic breakthroughs that will raise new ethical issues for discussion while simultaneously improving their overall quality of life.

1.6.1 *Correlation between Dissertation Chapter 1 and 2*

The first chapter of this dissertation is focused on activities designed to determine whether K-5 students can learn fundamentals of genetics through hands-on and problem-based experimentation. It is acknowledged that creation of such activities is more likely to resemble participation in a true research experience if the designer has received training in designing experiments, carrying out experimental protocols, and analyzing results. The following chapter describes my research experience as a doctoral student. This experience (which is concentrated on educational research) provided me with the expertise that I could use to ensure that the genetics learning modules described in this document are accurate reflections of the experimentation process that occurs in a research laboratory.
2 QUANTITATIVE ANALYSIS OF “CANDIDATUS ENDOBULA SERTULA”

2.1 Abstract

A series of experiments were carried out to investigate the relationship between the host *Bugula neritina* and its endosymbiont, “*Candidatus Endobugula sertula,*** throughout the different stages of metamorphosis. Various molecular techniques were utilized to analyze *B. neritina* samples acquired from Beaufort and Morehead City, North Carolina. One of the specific aims for this project was to document the developmental stages of the *B. neritina* population in North Carolina. Additionally, this study focused on the quantification of *E. sertula* at the different developmental stages of *B. neritina*. The main techniques used were Trizol reagent for DNA/RNA extractions, Polymerase Chain Reactions (PCR), Quantitative Polymerase Chain Reactions (qPCR) and GeneJet PCR Purification.

2.2 Introduction

*B. neritina* of Family Bugulidae, Order Cheilostomatida and Class Gymnolaemata (Linnaeus, 1758) are sessile, arborescent bryozoans that form colonies on various types of substrata. This organism is found in warm temperate water in areas around the world (Keough, 1989). *B. neritina* is most commonly known as a fouling organism as it is often found on ship hulls. Although the *B. neritina* are sessile as adults (Figure 35), they have a free swimming larval stage (like a poppy seed).

![Figure 38: Adult and Ovicell bearing zooid of Bugula neritina from Morehead City, North Carolina](image-url)
Larvae of the *B. neritina* are protected from predators by bryostatins, a complex group of polyketides (Lindquist & Hay, 1996; Lopanik, Lindquist, & Targett, 2004). It has been suggested that the source of bryostatin production is the symbiotic relationship between the bacterial symbiont of *B. neritina*, known as “*Candidatus* Endobugula sertula” (Davidson, Allen, Lim, Anderson, & Haygood, 2001; Sharp, Davidson, & Haygood, 2007). *E. sertula* is an uncultivated, rod-shaped, gamma proteobacterium which is transmitted vertically prior to the release of larvae from the ovicell (R. M. Woollacott, 1981). Since the late 1960’s, it has been suggested that these bryostatins also have anti-cancer properties. More recently, it has also been suggested that bryostatins properties may be used as drug therapy to improve memory in Alzheimer’s patients (Kraft, Smith, & Berkow, 1986; Pettit, 1982; Sun & Alkon, 2005). One of the interesting observations, made in 2001, was that the concentration of bryostatins decrease when the host is exposed to antibiotic treatments, suggesting that there is a direct correlation between the production of bryostatins and the unculturable endosymbiont (Davidson et al., 2001).

Most marine bryozoans possess a chamber used for brooding the embryos termed an “ovicell” which is composed of a calcified ooecial fold and a membranous ooecial vesicle (R. Woollacott & Zimmer, 1972). After embryogenesis is complete, the larvae released are non-feeding and will have a free swimming period before they attach to a substratum (R. Woollacott & Zimmer, 1972). During the host’s larval stage, it is exceptionally vulnerable due to factors such as size, ability to avoid predators, and lack of morphological features to deter predators; thus, utilizing a secondary metabolite like the bryostatins is a necessary defense mechanism (Lopanik et al., 2004). Metamorphosis and development into the ancestrula (primary zooid) of *B. neritina* takes place within the first four days after being released from the oovicell. The
ancestrula stage is identified by the appearance of the first feeding apparatus of the colony, known as the lophophore (R. Woollacott & Zimmer, 1972).

New zooids continue to develop forming a mature colony via asexual reproduction. However, the host is also able to undergo sexual reproduction. It has been suggested that the adult colonies are not defended by bryostatins (Lopanik et al., 2004). Additionally, it is not understood how the endosymbiont progresses throughout the colony and, ultimately, is vertically transmitted to the new generation. According to Sharp et al 2007, the bacteria are possibly transferred from zooid to zooid during development through the funicular cords. To our knowledge this hypothesis has not been studied in great detail. This study attempted to quantify the endosymbiont within the host as well as track the progression of the endosymbiont throughout the host during the various developmental stages of its metamorphosis.

2.3 Methods

2.3.1 Collection and larval harvesting of B. neritina

During November of 2012 and 2013, adult colonies of B. neritina were collected by hand from floating docks in Beaufort and Morehead City, North Carolina (Figure 36).
These adult colonies were transferred and maintained in flowing sea water tables within a wet lab at the UNC-CH Institute of Marine Sciences in Morehead City, North Carolina. Following the placement of the colonies within the water tables, the tables were covered with dark colored trash bags in order to simulate the dark cycle. This was done to prepare the colonies for the release of larvae the following morning. Larvae were collected every morning shortly after dawn for five days (Figure 3). The adult colonies were transferred from the water tables to large glass jars with unfiltered seawater (UNFSW) and placed outside in order to expose the host to sunlight which induces the release of the brooded larvae. Once the larvae were released, a glass Pasteur pipette was used to transfer the larvae into a glass dish with chilled filtered sea water (FSW).

![Image](image_url)

*Figure 40. Collection of free swimming larvae*

At a temperature of 8°C, larval movement stops and the larvae tend to sink to the bottom of the glass dish, which allows for easier decanting of the FSW and larval collection. The larvae were then transferred into a volumetric Wheaton vial (Millville, NJ) in order to determine the total volume of larvae collected. On average, this collection resulted in 0.1-0.3 mL (~100 µL) of larvae to be used for the various experiments throughout the day.
In order to have enough samples for the experiments, the collections for each developmental stage was performed in triplicate. For example, each stage of development consisted of approximately 250 larvae. These larvae were counted using a Wheaton long-tip glass Pasteur pipette (Millville, NJ) and placed into a Thermo Scientific six-well plate (Grand Island, NY) which contained 10 mL of FSW per well. Once the larvae were transferred to a well, they were exposed to one hour of the dark cycle in order to allow attachment. Following attachment, (which approximately occurred within one hour), the FSW was decanted using a long tip Pasteur pipette and any unattached larvae were placed into a clean glass dish and counted in order to determine how many larvae would develop in each well. This method was performed twice for each well to ensure that all unattached larvae had been accounted for. Subsequently, 10 mL of FSW was placed in each well and a final decanting was performed to collect any remaining unattached larvae. Finally, after observations were made, juveniles at different developmental stages were collected in 1.5 mL Eppendorf tubes and preserved in 500 µL of the Trizol® reagent (Grand Island, NY).

2.3.2 DNA/RNA extractions of B. neritina at various developmental stages

After collecting the various developmental stages and preserving the samples in RNAlater (a tissue preservative reagent), RNA and DNA extractions were performed using the Trizol® Reagent (Carlsbad, CA) protocol. Trizol is a ready-to-use reagent for the isolation of total RNA and DNA. The reagent is a mono-phasic solution of phenol and guanidine isothiocyanate. During sample homogenization, Trizol maintains the integrity of the RNA while disrupting cells and dissolving cell components. Addition of chloroform followed by centrifugation separates the solution into an aqueous phase and an organic phase. RNA is extracted from the aqueous phase and precipitated by using isopropyl. After the transfer of the
aqueous phase, DNA and proteins can be precipitated by using ethanol and isopropyl. This technique is ideal for these samples because it performs well with small quantities of tissue.

2.3.3 Polymerase Chain Reactions performed on B. neritina samples

Polymerase Chain Reactions (PCR) allow for many copies of a gene from a sample of DNA to be created and analyzed using gel electrophoresis. PCR also allows the detection of a specific gene or the ability to study a gene whose sequence is unknown from the genomic DNA (gDNA). When the band of interest has been amplified, further analysis can be done utilizing quantitative PCR (qPCR) techniques. The PCR experiments were performed using the DyNAzyme EXT (FINNZYMES, Finland). The specific primers used were the symbiont-specific primers EBn16s_223f and EBn16s_446r as well as EBn16s_254f and EBn16s_643r. All PCRs were performed using a bench top Eppendorf thermocycler (Hauppauge, NY).

2.3.4 Quantitative Polymerase Chain Reaction (qPCR)

In this study, real-time quantitative (q) PCR (TaqMan®) of the 16S and 18S molecule from the endosymbiont, ‘Candidatus Endobugula sertula’ and the host B. neritina were used to study the ratio between the host and symbiont. This technique is ideal due to the fact that the endosymbiont is a member of the microbial community that is un-culturable. In qPCR, the amount of amplicon is linked to a fluorescent reporter molecule. It is more sensitive compared to traditional PCR because data are collected during the exponential growth (log) phase of PCR when the quantity of the product is directly proportional to the amount of template nucleic acid. This method allows for the most precise and accurate quantification of a sample. During the exponential phase of qPCR, two values are collected. The first value, the threshold line, is the level of detection where a reaction reaches a fluorescent intensity above background. The PCR cycle at which a sample reaches this intensity is referred to as the cycle threshold (Ct). The Ct
value can be directly correlated to the starting target concentration of the sample. By comparing the Ct values of the *B. neritina* developmental stages (samples of unknown concentration) with a series of standards, the amount of template DNA in an unknown reaction can be accurately determined. The qPCR studies were performed utilizing the DyNAmo Probe qPCR kit (Thermo Fisher Scientific, Grand Island, NY). This kit is based on a hot-start *Thermus brockianus* (*Tbr*) DNA polymerase. *Tbr* DNA polymerase is chemically engineered to be inactive at room temperature in order to prevent the extension of non-specific primers during reaction setup which in turn will increase PCR specificity. This allows for reactions to be prepared at room temperature and the initial denaturation step will reactivate the hot-start polymerase. Careful primer design is essential in order to minimize nonspecific primer binding as well as primer-dimer formation. Primers that were used for qPCR were designed in the Lopanik lab by Fahmina Akhter during her research under the MS program at Georgia State University. Using these previously designed primers (16S_434f and 16S_589r), the first step in performing qPCR was to design the standard curve. The standard curve is used for absolute quantitation and for analyzing the efficiency of the qPCR reaction. The standards should consist of DNA sequences similar those of the template of interest. Therefore, the standards utilized were *B. neritina* gDNA acquired from North Carolina by a fellow lab member, Meril Mathew. A 10ng-0.1pg series of dilutions were prepared using the purified PCR product of the aforementioned *B. neritina* samples. After qPCR, the resulting plots of fluorescence versus cycle number for the standard curve were analyzed for efficiency. PCR efficiency is usually calculated by using a plot of the quantification cycle (Cq) against the logarithm of the amount of DNA. The slope of the equation is correlated to the efficiency of the PCR reaction. Hence, the efficiency can be determined using the following equation: PCR efficiency = \((10^{-1/slope} - 1) \times 100\%\)
The 16S standard curve resulted in a PCR efficiency of 95% and will be utilized for future qPCR studies on the different developmental stages of *B. neritina*.

### 2.4 RESULTS

#### 2.4.1 Comparison of Developmental Stages of *B. neritina* acquired in California versus North Carolina.

The following is a comparison of the development of *B. neritina* according to Sharp et al. (2007) and the observations made during collection of the various stages of metamorphosis. Sharp et al. (2007) suggested that the host *B. neritina* developed in the following manner (Table 3).

![Diagram of developmental stages of *B. neritina*](#)

**Table 3.** Adapted from Sharp et al. (2007) description of *B. neritina* developmental stages

<table>
<thead>
<tr>
<th>Stage #</th>
<th>Time(h)</th>
<th>Morphological Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>--</td>
<td>Adult colony with ovicells and rhizoids</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>Ciliated, free-swimming larvae</td>
</tr>
<tr>
<td>3</td>
<td>1.25</td>
<td>Attached larva; pallial epithelium evagination toward oral surface</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>Elongation into upright pillar</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
<td>Further Elongation</td>
</tr>
<tr>
<td>6</td>
<td>96</td>
<td>Lophophore functional; bud for second autozoid starting to develop</td>
</tr>
</tbody>
</table>

After observing the developmental stages of the *B. neritina* samples from North Carolina, they appear to develop on a different time scale (Table 4).
Table 4. Stages of B. neritina collected in Beaufort and Morehead City, NC

<table>
<thead>
<tr>
<th>Stage #</th>
<th>Time(h)</th>
<th>Morphological Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>Free-swimming larvae</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Attached larva</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Post attachment: Elongation into upright pillar</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>Post attachment: Pre-ancestrula</td>
</tr>
<tr>
<td>5</td>
<td>48-51</td>
<td>Ancestrula: Appearance of functional lophophore</td>
</tr>
<tr>
<td>6</td>
<td>72</td>
<td>Lophophore functional; bud for second autozoid starting to develop</td>
</tr>
<tr>
<td>7</td>
<td>89-92</td>
<td>Two zooids per individual</td>
</tr>
<tr>
<td>8</td>
<td>124-169</td>
<td>Third zoid budding</td>
</tr>
</tbody>
</table>

According to Sharp et al. (2007) the functional lophophore was apparent at 96 hours whereas the samples collected in NC appear to develop their functional lophophore between 48-51 hours. Additionally, the bud for the second autozoid began to develop sooner than previously suggested for sibling species. All developmental stages from samples acquired in NC had on average an 80% or higher rate of attachment.

2.4.2 DNA/RNA extractions of B. neritina at various developmental stages

The concentration of the genomic DNA (gDNA) was measured using ThermoScientific NanoDrop 1000 Spectrophotometer (Wilmington, DE). In the first set of extractions performed, the RNA concentrations were much lower than the DNA concentrations (Table 5). However, the second set of samples showed higher concentrations of gDNA (Table 6).

Table 5. DNA/RNA concentrations from B. neritina samples collected in NC in 2011

<table>
<thead>
<tr>
<th>Sample</th>
<th>[RNA]ng/ul</th>
<th>260/280</th>
<th># of zooids</th>
<th>Date Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bnl FS</td>
<td>14.67</td>
<td>1.72</td>
<td>200</td>
<td>11/22/2011</td>
</tr>
<tr>
<td>Bn 3h</td>
<td>50.47</td>
<td>1.67</td>
<td>200</td>
<td>11/22/2011</td>
</tr>
<tr>
<td>Bn 8h</td>
<td>97.97</td>
<td>1.88</td>
<td>125</td>
<td>11/21/2011</td>
</tr>
<tr>
<td>Bn 24h</td>
<td>40.23</td>
<td>1.78</td>
<td>183</td>
<td>11/21/2011</td>
</tr>
</tbody>
</table>
### Table 6. DNA/RNA concentrations from *B. neritina* samples collected in NC in 2011 (2nd set)

<table>
<thead>
<tr>
<th>Sample</th>
<th>[gDNA] ng/ul</th>
<th>260/280</th>
<th># of zooids</th>
<th>Date Collected</th>
<th>Date Extracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bn 1h@att</td>
<td>88.97</td>
<td>1.57</td>
<td>200</td>
<td>11/22/2011</td>
<td></td>
</tr>
<tr>
<td>Bn 3h</td>
<td>67.7</td>
<td>1.46</td>
<td>200</td>
<td>11/22/2011</td>
<td></td>
</tr>
<tr>
<td>Bn 8h</td>
<td>90.27</td>
<td>1.53</td>
<td>125</td>
<td>11/21/2011</td>
<td></td>
</tr>
<tr>
<td>Bn 24h</td>
<td>93.47</td>
<td>1.49</td>
<td>183</td>
<td>11/21/2011</td>
<td></td>
</tr>
<tr>
<td>Bn 48h</td>
<td>89.07</td>
<td>1.48</td>
<td>140</td>
<td>11/23/2011</td>
<td></td>
</tr>
<tr>
<td>Bn 72h</td>
<td>97.53</td>
<td>1.4</td>
<td>155</td>
<td>11/22/2011</td>
<td></td>
</tr>
<tr>
<td>Bn 81h</td>
<td>67.2</td>
<td>1.37</td>
<td>120</td>
<td>11/23/2011</td>
<td></td>
</tr>
</tbody>
</table>

Samples were then diluted to various concentrations in preparation for PCR amplification. Initially, the Zymo mini-prep extraction kit (Irvine, CA) used resulted in low concentrations of gDNA. Use of the Zymo micro-prep extraction kit increased the concentrations of gDNA but its use also resulted in less volume of the final product. Performing extractions with the Trizol reagent resulted in better gDNA concentrations as well as the ability to extract...
RNA which would be used for qPCR. The concentration of gDNA was much higher than previously reported. Considering that a limited amount of larvae was used, the larval sample size collected was increased from 200 to 250 larvae. In the spring of 2013, RNA and DNA extraction from *B. neritina* samples collected from Morehead City, North Carolina in November 2012 was begun. Preserving the samples in Trizol versus RINAlater yielded in higher concentrations of RNA and DNA from the samples (Table 7).

**Table 7. 1st set of samples extracted from November 2012 collection**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Developmental Stage</th>
<th># of Zooids</th>
<th>Date Collected</th>
<th>Date Extracted</th>
<th>[RNA] ng/ul</th>
<th>260/280</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Free Swimming Larvae #3</td>
<td>250</td>
<td>11/2/2012</td>
<td>04/22/2013</td>
<td>267.70</td>
<td>1.90</td>
</tr>
<tr>
<td>2</td>
<td>1h at attachment</td>
<td>242</td>
<td>11/3/2012</td>
<td>04/22/2013</td>
<td>282.93</td>
<td>1.86</td>
</tr>
<tr>
<td>3</td>
<td>3h post-attachment</td>
<td>244</td>
<td>11/3/2012</td>
<td>04/22/2013</td>
<td>239.17</td>
<td>1.89</td>
</tr>
<tr>
<td>4</td>
<td>24h post-attachment</td>
<td>222</td>
<td>11/4/2012</td>
<td>04/22/2013</td>
<td>297.70</td>
<td>1.87</td>
</tr>
<tr>
<td>5</td>
<td>48h post-attachment</td>
<td>221</td>
<td>11/4/2012</td>
<td>04/22/2013</td>
<td>216.20</td>
<td>1.88</td>
</tr>
<tr>
<td>6</td>
<td>72h post-attachment</td>
<td>148</td>
<td>11/4/2012</td>
<td>04/22/2013</td>
<td>131.83</td>
<td>1.74</td>
</tr>
<tr>
<td>7</td>
<td>124h post-attachment</td>
<td>202</td>
<td>11/6/2012</td>
<td>04/22/2013</td>
<td>150.90</td>
<td>1.81</td>
</tr>
<tr>
<td>8</td>
<td>168h post-attachment</td>
<td>219</td>
<td>11/8/2012</td>
<td>04/22/2013</td>
<td>158.93</td>
<td>1.81</td>
</tr>
</tbody>
</table>

2.4.3  *Polymerase Chain Reactions performed on B. neritina samples*

The success and quality of the PCR products was verified using agarose with 1x Tris-acetate EDTA (TAE) gel electrophoresis. After analyzing the gels, it was evident that the DyNAzyme EXT did not work well with the aforementioned primers as the bands were faint (Appendix G). The PhireII Hot Start DNA polymerase (FINNZYMES, Finland) worked much better with the samples and was subsequently used to verify the ability of the samples to be
amplified and to ensure the absence of inhibitors (Appendix G). However, the protocol for qPCR would not work with the Phire II Hot Start DNA polymerase therefore the PCR reactions were performed using the DyNAzyme II Hot Start DNA polymerase.

After analyzing the various agarose gel electrophoresis images acquired from the PCR products using the DyNAzyme II Hot Start DNA polymerase, and the aforementioned symbiont specific primers (at 10 µM), the endosymbiont in the various stages of development was identified by amplification of bands which suggests that these samples have the EBn254f/EBn643r gene (Appendix G). A second PCR was performed on the EBn16s_223f/EBn16s_446r and the endosymbiont was also observed in all the developmental stages (Appendix G). The template (gDNA) concentration for all the samples was 40 ng/µL. In order to determine the efficacy of the PCR, a positive control, a sample which had previously resulted in a gel with the appropriate size, was included in all PCR reactions. Using samples with higher concentrations compared to previous PCR reactions resulted in a stronger banding pattern and hence a better amplification of the symbiont specific primers in the stages of metamorphosis. All samples collected in November 2011 and 2012 had RNA and DNA extracted. Considering PCR was performed on all collected samples, future work will include performing PCR using the Thermo Scientific Maxima Hot Start Taq DNA Polymerase. During the fall of 2012, the previous PCR reactions using the EBn254f/EBn643r and EBn16s_223f/EBn16s_446r primers were re-accomplished using the Maxima Hot Start Taq DNA Polymerase (Appendix G). The PCR reaction was performed on the different stages of development for B. neritina. Gel analysis seemed to show that the EBn254f/EBn643r primers worked best. A PCR reaction using the qPCR primers, Ebn 434f and Ebn 589r was then conducted (Appendix G). For this particular PCR reaction, only a few of the developmental stages were used in order to observe the
efficiency of the qPCR primers on the samples. At the conclusion of all PCR work, a qPCR template was designed for the 16S and 18S molecule in preparation for the qPCR standard curve (Figure 48). Ultimately, this analysis will allow for quantification of the endosymbiont. The RNA will also be used to determine the expression of bry genes during the development of B. neritina.

2.4.4 Design of Quantitative Polymerase Chain Reaction (qPCR) primers

In order to design the 16S and 18S standard curve, a PCR was performed with samples that had previously been observed to have the appropriate sized product that could serve as a standard. This sample was taken from a control plate that was collected by Meril Mathew during the November 2012 collection trip. The PCR product (Figure 41) was purified via column purification and verified by a 1.5% TAE gel electrophoresis. Dilutions were prepared for the 16S and 18S standard curve using this purified product at a concentration of 18.67 ng/µL and a 260/280 of 1.5. The dilutions ranged from 10ng to 0.001pg and after performing the standard curve qPCR the efficiency was calculated to be approximately 92% for the 16S molecule. However, the 18S standard curve was unable to detect a value for the concentration threshold. This result could be due to one of two reasons: either the value was out of range thus preventing the observation of the threshold, or the primers for the 18S molecule were not designed properly. Therefore, another reference gene had to be selected in order to develop a standard curve for the 18S molecule. During a transcriptome data analysis that was performed on B. neritina, the reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which is used as an intermediate in metabolic pathways, was found. This allowed for
GAPDH to be utilized as the new target gene and new primers and qPCR primers were developed. New primers were processed using 1X TE buffer. After performing the gradient PCR, instead of the ~900 bps anticipated size, the size observed for the amplicon was 4,000 bps (Figure 39).

Lane 1: 50°C
Lane 2: 52°C
Lane 3: 54°C
Lane 4: 56°C
Lane 5: 58°C
Lane 6: 60°C
Lane 7: NTC (52°C)

*OneTaq

~4000 bp’s

Figure 42. New 18S target gradient PCR on BnG3P_59f and BnG3P_865r

Considering that the gDNA was used to perform the gradient PCRs, the primers could have been hitting at the 4,000 base pair mark due to the presence of introns. The product should have been observed at approximately 800 base pairs.

Lane 1: 50°C
Lane 2: 52°C
Lane 3: 54°C
Lane 4: 56°C
Lane 5: 58°C
Lane 6: 60°C
Lane 7: NTC (52°C)

*OneTaq

~4000

Figure 43. New 18S target gradient PCR on BnG3P_59f and BnG3P_987r
Similar observations were made after using primers BnG3P_59f and BnG3P_987R where the product should have been observed at approximately 900 base pairs, BnG3P_164f/865r and BnG3P_164f/987r (Figure 41) where the product should have been observed at approximately 700 and 800 base pairs.

Lane 1: 50°C
Lane 2: 52°C
Lane 3: 54°C
Lane 4: 56°C
Lane 5: 58°C
Lane 6: 60°C
Lane 7: NTC (52°C)

*One Taq

Figure 44. New 18S target gradient PCR on BnG3P_164f/865r and BnG3P_164f/987r

The following diagram is a sketch of the introns found in the sections between the forward and reverse primers designed.
After determining that introns were playing a role in the primer design process, it was determined that primer walking would be needed. Primer walking is a method used to determine the sequence of DNA up to the 1.3-7.0 kb range. Custom primers are designed to be complementary to the known sequence and can be used to extend the DNA sequence iteratively from the distal ends of known regions into the unknown regions of the same molecule. Additionally, after performing this PCR on the QPCR primers, it was observed that the product was amplifying at 1000 base pairs and 700 base pairs which was contrary to the anticipated range of 293 base pairs and 182 base pairs (Figure 42). This result was consistent with the possible intron activity observed in between the beginning and end primers.

![Figure 45. New qPCR primer design gradient PCR on BnG3P_QPCR_72f/364r and BnG3P_72f/511r](image_url)
In order to confirm the hypothesis that the introns were interfering with primer design, a PCR was performed utilizing the primers BnG3P_59f/865r (Figure 43).

Additionally, a PCR was performed using qPCR primers BnG3P_QPCR_72f/364r on B. neritina cDNA (Figure 47).

These purified products were sent off for sequencing (GSU core facility and Yale sequencing facility). These sequences allowed us to determine where the introns and exons were located as well as places within the gene where primers could be designed for primer walking (Table 8).
After the sequencing was received, they were assembled in mega align and it was observed that the areas that were the cDNA (complementary DNA) aligned with gDNA. This allowed for determination of where the introns and exons were located. From this mega align file, a Seqman Pro (DNASTAR, WI) software was used to create a builder file. Seqman Pro software is used for sequence editing and annotation, automated virtual Cloning, and Primer Design which enabled us to annotate the intron and exon fragments. This also allowed for qPCR primers for both the outer and inner regions to be designed.

2.4.5 Quantitative Polymerase Chain Reaction (qPCR) after primer walking

Once the PCRs confirmed the target product via gel electrophoresis, the G3P_QPCR primers were ready to design a standard curve for qPCR. To begin designing the standard curve, a few dilutions needed for the standard curve were used. After confirming that the primers worked properly, a qPCR was performed using G3P_qPCR_1f and 1r. The qPCR efficiency was calculated at 83% and all of the sample dilutions (10ng-0.001pg) reported a concentration threshold (Ct) value (Figure 45).

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>BnG3P_1375f</td>
<td>GATTCTACTGCATACCATG</td>
</tr>
<tr>
<td>BnG3P_1267f</td>
<td>GAGGCTGTATCAAGCATT</td>
</tr>
<tr>
<td>BnG3P_REV1Ar</td>
<td>AGATAACACTGCACTCCAAC (first set of reverse primers</td>
</tr>
<tr>
<td></td>
<td>from 865r)</td>
</tr>
<tr>
<td>BnG3P_REV1Br</td>
<td>CAACACAATCTAGTAGCCAG (first set of reverse primers</td>
</tr>
<tr>
<td></td>
<td>from 865r)</td>
</tr>
<tr>
<td>BnG3P_ENDf</td>
<td>ATAGGCCATATTCCTTTTC (to complete F seq to 865)</td>
</tr>
<tr>
<td>BnG3P_ENDr</td>
<td>TACAGTAGATGAGCTTTAGC (to finish)</td>
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</table>
The qPCR consisted of duplicates of each of the sample dilutions. The SyBr green maxima hot start kit was used to perform the experiment. After a 2% gel was performed the samples all displayed the 100 base pair product and all the samples fluoresced which was observed via the amplification plot.

### 2.5 DISCUSSION

The observations of this project have shown that the *B. neritina* from NC develops differently than other sibling species that have been observed. This record can serve as protocol for the collection of the North Carolina *B. neritina*. It will also serve as a protocol to collect the host at its different developmental stages. A series of polymerase chain experiments were performed in order to develop primers that allowed observation of both the host and the symbiont during metamorphosis. Primer walking was used to determine the Glyceraldehyde-3-
phosphate dehydrogenase (GAPDH), which is more commonly known as an intermediate in metabolic pathways however, it is also considered a multifunctional protein (Sirover, 2014). Despite its ability to have multifunctional activities, GAPDH is encoded by a single structural gene in somatic cells (Gail A. P. Bruns & Park S. Gerald, 1976) where the protein itself is highly conserved across the phylogenetic scale. The GAPDH was utilized as the reference gene for the 18S molecule and qPCR primers. The primers that were designed as a result of this study were used in several experiments in the lab including projects on Symbiont Dependent Host Reproduction in the Marine Bryozoan, *Bugula neritina* (Mathew, 2016) and the Distribution and Dynamics of Defensive Symbiosis in the *Bugula neritina* (Bryozoa) Sibling Species Complex (Linneman, 2016). The next direction for the study is to utilize the primers that have been designed in order to ascertain if the symbiont is transferred from zooid to zooid during the developmental stages of the free-swimming larvae into the ancestrula stage (adult *B. neritina*).

### 2.6 CONCLUSION

After concluding the preliminary PCR and qPCR experiments, a template was designed for the 16S and 18S molecule in preparation for the qPCR experiments. Ultimately, this analysis will allow for quantification of the endosymbiont. Additionally, the RNA can be utilized to determine the expression of *bry* genes during each of the developmental stages of *B. neritina*. The results from this study were utilized in a variety of experiments where a more comprehensive understanding was ascertained as it pertains to the role of the symbiont, “*Candidatus* Endobugula sertula” in the mutualistic association with the bryozoan host.
REFERENCES


Gordon, K. (2011). Middle School Transition: How It Affects The Achievement of Hispanic Students Relative to ELL Status, Socioeconomic Status, Gender, and Previous Test Scores University of South Florida Scholar Commons.


NSB. (2016). Science & Engineering Indicators. Retrieved from National Science Foundation


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APPENDICES

Appendix A. Dominant and Recessive “Bag Baby” Guide.

Dear Parents/Guardians – Today, Georgia State University’s Bio-Bus Program visited your child’s school to lead Day 2 of a 4-Day series of activities called “DNA is Elementary.” On the first day, your student learned about how the DNA which a person gets from his parents determines some of their physical TRAITS. Today, he or she made a “Bag Baby.” The color of the body (bag), eyes, hair, nose and lips were determined by pulling GENES (paper tags) out of a bag, one gene from each parent. The students strung their paper genes on two pipe cleaners, just as real genes are strung on two strands of DNA. The chart on the other side of this sheet shows which genes are DOMINANT and which are RECESSIVE. Get your child to explain his Bag Baby to you, along with the new science words he has learned so far.

The Program

This program was designed by Dr. Barbara Baumbach of Georgia State University, along with the Bio-Bus Fellows (GSU graduate students) who taught your child today. It is part of a pilot program funded by a Science Education Partnership Award (SEPA) from National Center for Research Resources (NCRR) of the National Institutes of Health (NIH). GSU will be following the progress of these City Schools of Decatur students, to see if they have less difficulty understanding DNA in middle school and high school, because they were first introduced to the “language” of DNA at a language-exceptive age.
Appendix B. Module 3 and 4: “Zap” Log example

**QUESTIONS (day of planting)**

How many seeds did you plant in each pot? (Hint: Think of pot numbers 1-5)?
______________________________

What about pot number 0? ________________

Which pot has the seeds that have been zapped the most? ________________________

Which pot has the seeds that have been zapped the least? ________________________

Which plant do you think will grow first?
______________________________

Which plant do you think will grow the tallest? Why?
______________________________

**QUESTIONS (1st observation)**

Did you add water to your plants today? ________________
How much? __________________

Did any of the plants grow? ________________

How do you know that the plants grew?
______________________________

Which plants did not grow at all?
______________________________

Why do you think some plants did not grow?
______________________________
Appendix C. Evaluation Tools for Attitudinal and Content Knowledge Assessment

Appendix C.1. 3-point Likert scale: Pre Survey

Bio-Bus Pre-Visit Feedback Form

Date of Module: 
Grade: 1 2 3 4 5 
School: 
Teacher: 

1. I am a: 

2. The Bio-Bus people visited my class last year. 

3. I like science. 

4. I would like to be a scientist. 

5. Circle the picture that looks like DNA. 

6. Billy Smiley-face has one dominant [loud] gene for a red nose. He has one recessive [quiet] gene for a green nose. Circle the picture that looks like Billy. 

7. I am good at science. 

8. Which difference between Billy Big-Ears and the other three faces is MOST likely to be caused by a mutation? 
Appendix C.2. 3-point Likert scale: Post Survey

Bio-Bus Post-Visit Feedback Form

Select your answer by coloring in the bubble below the picture.

1. I am a:
   - [ ] 1
   - [ ] 2

2. The Bio-Bus people visited my class last year.
   - [ ] Y
   - [ ] N
   - [ ] ?

3. I like science.
   - [ ] Y
   - [ ] N
   - [ ] M

4. I would like to be a scientist.
   - [ ] Y
   - [ ] N
   - [ ] M

5. Which picture looks like DNA?
   - [ ] 1
   - [ ] 2
   - [ ] 3
   - [ ] 4

6. Billy Smiley-face has one dominant (loud) gene for a red nose. He has one recessive (quiet) gene for a green nose. Fill in the bubble below the picture that looks like Billy.
   - [ ] 1
   - [ ] 2
   - [ ] 3
   - [ ] 4

7. I am good at science.
   - [ ] Y
   - [ ] N
   - [ ] M

8. Which difference between Billy Big-Ears and the other three faces is MOST likely to be caused by a mutation?
   - [ ] 1
   - [ ] 2
   - [ ] 3

I'm Billy Big-Ears.
Appendix C.3. 5-point Likert scale: Pre Survey

Bio-Bus DNA Runs in Families: Pre-Module Survey

Select your answer by circling the picture.

School:  
Date:  
Module(s):  
Grade: ⬤ 1 2 3 4 5

1. I am a:  
  ![boy] ![girl]  

2. Scientists are cool.  
  ![very] ![yes] ![maybe] ![no] ![no way]  

3. I like science.  
  ![lots] ![yes] ![maybe] ![no] ![no way]  

4. I would like to be a scientist.  
  ![lots] ![yes] ![maybe] ![no] ![no way]  

5. Which picture looks like DNA?  
  ![picture]  

6. Billy Smiley-face has one dominant gene for a red nose. He has one recessive gene for a blue nose. Circle picture that looks like Billy.  
  ![blue nose] ![purple nose] ![red nose]  

7. I am good at science.  
  ![very] ![yes] ![maybe] ![no] ![no way]  

8. Which difference between Lucy Cat and the other three cats is MOST likely to be caused by a mutation?  
  ![difference]  

I'm Lucy Cat.  

I don't know!
## Appendix C.4. 5-point Likert scale: Post Survey

### Bio-Bus DNA Runs in Families: Post-Module Survey

**Select your answer by circling the picture.**

I am a:  
- boy  
- girl  

<table>
<thead>
<tr>
<th>School:</th>
<th>Date:</th>
<th>Module(s):</th>
</tr>
</thead>
</table>

**Grade:**  
- 1  
- 2  
- 3  
- 4  
- 5

---

1. Scientists are cool  
   - VERY!!!  
   - YES  
   - Maybe  
   - No  
   - NO WAY!!!

---

2. I like science.  
   - LOTS!!!  
   - Yes  
   - Maybe  
   - No  
   - NO WAY!!!

---

3. I would like to be a scientist.  
   - LOTS!!!  
   - YES  
   - Maybe  
   - No  
   - NO WAY!!!

---

4. Which picture looks like DNA?  
   - ![DNA](image1)  
   - ![DNA](image2)  
   - ![DNA](image3)  
   - ?  
   - I don't know!

---

5. Billy Smiley-face has one dominant gene for a red nose. He has one recessive gene for a blue nose. Circle picture that looks like Billy.  
   - Blue nose  
   - Purple nose  
   - Red nose  
   - ?  
   - I don't know!

---

6. I am good at science.  
   - VERY!!!  
   - Yes  
   - Maybe  
   - No  
   - NO WAY!!!

---

7. Which difference between Lucy Cat and the other three cats is MOST likely to be caused by a mutation?  
   - I'm Lucy Cat.  
   - ![Cat1](image4)  
   - ![Cat2](image5)  
   - ![Cat3](image6)  
   - ?  
   - I don't know!
Appendix D. DNA is Elementary Logic Model

Georgia State University’s Bio-Bus DNA is Elementary Program

- Inputs
  - Staff create teaching modules (tied to general elementary standards) to be delivered during school visits
  - 8 teaching modules
  - Staff fellows become science (possibly K-12) educators.
  - K-5 Students: Perceive science as fun and something they can do
  - Increase content knowledge of science

- Activities
  - Staff members travel to schools where they:
    - Deliver hands-on DNA modules
    - Serve as role models to students
    - Provide materials
    - Provide instruction for 6 days (1 hour of instructional time per student)
  - Up to 8 modules delivered to schools/year (target: 31)
  - Visited school have X% students on free/reduced lunch, X% under-represented minorities

- Outputs
  - # training events provided to teachers annually with a satisfaction rating > 3.5
  - # teachers present and engaged during student learning. High teacher satisfaction ratings.
  - Offer hands-on science to their students
  - Students express greater content knowledge, interest, and confidence in biology (scientific literacy).

- Dissemination
  - Conference Presentations
  - Online Kit Orders
  - NSTA, MLCC, SEPA, EHS, LAA
  - If Bio-Bus does disseminate, what is the intended outcome of that effort? To fulfill a funding requirement? To serve as a successful model?
Appendix E. Documents from the Teacher Professional Development

Appendix E.1. Flyer for the teacher’s workshop

The Bio-Bus Program of Georgia State University is sponsoring a Teachers’ Workshop on its K-5 teaching module “DNA is Elementary”

The Bio-Bus Program has been funded by the National Institutes of Health’s SEPA Program to develop activities that make DNA fun and exciting for young learners. We find that children as young as five pick up fundamental concepts of DNA, genes, and inheritance with ease. This workshop is designed to:

- provide teachers with the appropriate fundamental concepts in genetics
- show teachers how to use biotechnology to turn students into microbe hunters
- showcase the activities we have developed and obtain your input on ways to make them even better!

Teachers will engage in the same hands-on activities which help children learn:

- how our DNA makes us unique
- that parents pass genes down to their children
- how changes in DNA can change the way plants and animals grow
- how to extract DNA from split peas
- how to multiply DNA from a small sample

**Dates:** Monday-Wednesday, June 4-6, 2018

**Times:** 9:00 am – 4:00 pm

**Location:** GEORGIA STATE UNIVERSITY
Petit Science Center – 100 Piedmont Ave. ATL
(Easily accessible by MARTA, or you can pay to park in GSU’s G lot.)

**Instructors:**
Barbara Baumstark, Ph.D., Professor of Biology, Georgia State University
Chandan Robbins, Ph.D., Academic Professional, Georgia State University
Michelle Ezeoke, M.S., Bio-Bus Program Manager, Georgia State University
Other members of the Bio-Bus Program staff

**Participants:** up to 15 Teachers; K-5 Teachers will be given preference, others are encouraged to apply

**Costs and Incentives:**
- Stipends of $100/day will be offered to those who complete the three-day, 18-hour course.
- The course is free.
- Application online: biobus.gsu.edu

DNA is Elementary 2011 Teacher Workshop

Participants working on making “bog babies”, an activity used to simulate the expression of dominant and recessive traits.
Appendix E.2. Application for the teacher’s workshop

2018 Teacher Workshop: “DNA Is Elementary”
Monday-Wednesday, June 4-6, 2018 * Petit Science Center, Georgia State University

Last Name: ________________________________

First Name: ___________________ Middle Initial: ____________________________

Name as you wish it to appear on your name tag: ____________________________

Preferred Email: _______________________________________________________

Home Address: _________________________________________________________

_____________________________________________________________________

Cell Phone: ____________

Educational Background:

<table>
<thead>
<tr>
<th>Degree</th>
<th>Major / Minor</th>
</tr>
</thead>
</table>

University / College | Dates of Attendance

School Name: ____________________________

School System (or “private” + county, if applicable): _________________________

2017-2018 Teaching Assignment: ___________________________________________

Anticipated 2018-2019 Teaching Assignment: _________________________________

Total Years Teaching: _____ Y/N _ Do you wish to apply for a stipend of $300.00?

*ON AN ATTACHED PIECE OF PAPER, please submit a paragraph on each of the following:
1) How you see this workshop impacting your teaching effectiveness.
2) Where you hope to be five years from now in your educational and career goals.

Please answer the following questions if you wish to receive a stipend; Georgia State’s Office of Disbursements needs the answers to fill out the Service Provider Classification Worksheet (SPCW) for you:

Please circle Yes or No

Is Service Provider (you) a GSU employee / student employee? Yes / No

Does the Service Provider have a pending contract for employment? Yes / No

Is Service Provider an employee of any other University System of Georgia (USG) institution? Yes / No

Is Service Provider a US citizen or holder of a Green Card? Yes / No

Do GSU employees have a relationship, financial or otherwise, with a party involved in this transaction (including an employee, representative, or agent of a party involved in this transaction)? Yes / No

SIGNATURE _______________________________ DATE __________

APPLICATION DEADLINE: MAY 18, 2018

Please return application (including essay sheet) to the Bio-Bus Program by fax to 404 413-5301, or by e-mail to biobus@gsu.edu, or by US mail to Bio-Bus Program, Dept. of Biology, Georgia State University, P O Box 4010, Atlanta, GA 30303-4010

Telephone 404 413-5421
Appendix E.3. Schedule for the teacher’s workshop

2016 Bio-Bus Program Teacher Workshop  
“DNA IS ELEMENTARY”  
June 4th-7th, 2016  
Petit Science Center, Georgia State University

Host:  
Dr. Barbara Baumstark, Director, The Bio-Bus Program  
Dr. Chandan Robbins, Associate Director  
Instructions:  
Michelle Ezioke, Program Manager  
Lorna Gitari-Mugambi, Laboratory Coordinator  
Verdy Jocelyn, Activities Coordinator  
Isela Rodriguez-Bussey  
Bio-Bus Fellows & Volunteers

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<tr>
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<td>Registration &amp; Breakfast</td>
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<tr>
<td>10:00am – 10:15am</td>
<td>Decoding Ice Breaker Game</td>
</tr>
<tr>
<td>10:15am – 10:30am</td>
<td>Introduction</td>
</tr>
<tr>
<td>10:30am – 11:30am</td>
<td>DNA and You!</td>
</tr>
<tr>
<td>11:30am – 12:30pm</td>
<td>Genetics is ALL about You!</td>
</tr>
<tr>
<td>12:30pm – 1:30pm</td>
<td>Lunch</td>
</tr>
<tr>
<td>1:30pm – 3:30pm</td>
<td>Mutations! &amp; Mendelian Genetics</td>
</tr>
<tr>
<td>3:30pm – 4:30pm</td>
<td>Wrap-up</td>
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<td>10:00am – 11:00am</td>
<td>Differences in Animal DNA</td>
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<td>11:00am – 12:00pm</td>
<td>Genotype vs. Phenotype</td>
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<tr>
<td>12:00pm – 1:15pm</td>
<td>Lunch</td>
</tr>
<tr>
<td>1:15pm – 2:15pm</td>
<td>Decoding DNA! / Digging Deep with DNA</td>
</tr>
<tr>
<td>2:15pm – 3:15pm</td>
<td>Decoding DNA! / Digging Deep with DNA</td>
</tr>
<tr>
<td>3:15pm – 4:00pm</td>
<td>Materials/Resources &amp; Wrap-up</td>
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Appendix F. Publications featuring DNA is Elementary project.

Imagine your elementary school student collecting information that could help Georgia’s top researchers better understand pollen and asthma in the state. Under a new project rolling out this year, that image could become a reality.

As part of the project, students in public schools throughout Georgia will conduct pollen counts in their local neighborhoods and schools. They’ll look at pollen under a microscope and then upload the data to a website, which will create a map showing the densest areas for certain allergens. Then public school students, college students and professors alike will be able to mine the health care data to ask questions.

For example, professors at Emory University in Atlanta want to know whether high pollen counts can predict a higher number of visits to the emergency room.

“Allergies and asthma are a big health care problem in Georgia, and this new project gives us the ability to contribute to real science in the health care arena,” says Adam Marcus, an associate professor of hematology and medical oncology at Emory University. Marcus and fellow cancer researcher Theresa Gillespie are creating the statewide program as part of a $1.2 million grant they received from the National Institutes of Health (NIH) last June. The grant will create the new Center for Advancing Health and Diversity Through Citizen Science.

“We’re focusing on this idea of ‘citizen science’ because it gives the general public the ability to contribute to real science,” Marcus says. “We want Georgians to be part of the end result of improving health in the state.”

The grant focuses on the entire state, prioritizing questions about the high rates of cancer, chronic disease and health disparities in urban counties and rural Title I schools where residents have less access to preventive health care and other resources. Other projects will map food deserts (urban...
Emory University cancer researchers Adam Marcus and Theresa Gillespie are launching the Center for Advancing Health and Diversity Through Citizen Science, which will enlist K-12 students as well as college students in investigating health concerns such as allergies and asthma.

“STEM participation is lagging across the country, especially in Georgia and especially in middle school,” Marcus says. “Data show that if we don’t introduce STEM experiences by middle school, especially with middle school girls, they lose interest in it later in life.”

Next month, Marcus and Gillespie will open registration for a new two-week summer workshop for coding skills. Designed for middle school girls, the boot camp will be led by coding experts who teach classes for adults at Emory University. For information about the summer workshop, go to citizen scienceeds.com/girls-for-science.

“We want Georgia girls to experience the college setting and startup culture,” Marcus says. “They need female mentors who are some of the leading coding gurus in the state.”

Building the STEM mindset

By 2024, STEM careers are expected to grow by 16 percent, while non-STEM careers will grow by 9 percent, according to the U.S. Bureau of Labor Statistics (BLS). The STEM careers with the most job openings include software developers, civil engineers, mechanical engineers, electrical engineers and sales representatives for scientific products, and the fastest-growing STEM careers include information security analysts, statisticians, environmental science technicians and petroleum engineers. The annual salaries for all of these fields are between $40,000 and $130,000, and all of them require bachelor’s or master’s degrees for entry-level positions, according to the BLS.

For Georgia to be prepared for this growth, higher education institutions must boost the conversations about STEM in a way that will draw more students, not only during college but also before it. At Georgia State University in Atlanta, for example, more than 125 college students learn how to communicate science to younger students through the Bio-Bus program, which takes hands-on science activities and demonstrations to elementary schools around the state in a bus.

“It’s a science field trip that travels to the school,” says Barbara Baumstark, a biology professor at Georgia State who created the Bio-Bus program. “The activities get students excited about science and make them want to learn more.”

As part of one Bio-Bus program, Georgia State students teach elementary school students the basics of genetics as a language. Because young children pick up languages quickly, students as young as 4 have begun learning about DNA.

“We try to help students across Georgia in ways that aren’t available to many teachers and parents, and we try to do it in a way that gets students interested,” Baumstark says. “The genetics program works like a charm.”
Appendix G. PCR on *B. neritina* samples at different developmental stages collected from North Carolina 2011 using the symbiont specific primers.

**Lane assignments:**

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**PCR on *B. neritina* samples using qPCR primers Ebn 434f and Ebn 589r**

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<tr>
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<td>+ control (BnS)</td>
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*PhireII HS*
qPCR Template Design for 16s_Bn240f and Bn 1253r