16S RIBOSOMAL RNA AND PHYLOGRAMS: CHARACTERIZING STUDENT REASONING TO LEARNING OUTCOMES FROM THE AMERICAN SOCIETY FOR MICROBIOLOGY CURRICULUM

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

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In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Microbiology

June 2016

Fargo, North Dakota

North Dakota State University
Graduate School

Title

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MASTER OF SCIENCE

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ABSTRACT

The American Society for Microbiology (ASM) has established a suggested curriculum for introductory microbiology courses that includes a focus on evolution. However, no data is published to describe how proficiently students address the learning outcomes, in part because validated assessments do not exist. Thus, the goal of this project was to develop assessment prompts that capture student understanding about fundamental statement five under the core concept of evolution. In total, 167 written responses were collected from upper-division microbiology courses, with pre-pharmacy and microbiology majors comprising the majority of students (74.6%). Two coders coded all written responses, and five student interviews were conducted. Results indicate that students have not retained instruction on 16S rRNA, or have not been exposed to it in their classes. Additionally, most students have not been exposed to phylograms, and are unfamiliar with genetic distance being represented on a phylogenetic tree. Emergent reasoning techniques are described.

ACKNOWLEDGEMENTS

I would like to acknowledge my advisor, Dr. Peter Bergholz, for accepting me into his lab and taking on an education project. I would also like to thank Dr. Jennifer Momsen, for guiding Dr. Bergholz and me through the education side of this research, and helping me to grow as a researcher in the field. I'm thankful to Dr. Paul Kelter for his guidance and support when I was transitioning into a new lab and a new field. Also, I need to thank Dr. Lisa Wiltbank for the countless hours she assisted me with coding, and Rachel Salter for helping me practice speeches, edit presentations, and review my thesis throughout my project. Finally, I would like to thank the Department of Microbiological Sciences, specifically Dr. Neil Dyer and Dr. John McEvoy, for supporting me as a graduate student and encouraging my endeavor in microbiology education.

DEDICATION

I would like to dedicate this project to my family and close friends for supporting me unconditionally as I grow both personally and professionally.

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CHAPTER ONE. BACKGROUND AND ASSESSMENT TECHNIQUES

Despite scientific consensus, the general public lacks acceptance of evolution. Ninety-eight percent of scientists within the American Association for the Advancement of Science (AAAS) agree that humans and other living things evolved over time (Pew Research Center, 2014). However, Americans are less likely to accept evolution than Europeans or Japanese citizens, only ranking higher than Turkish citizens in acceptance (Miller et al. 2006). In fact, only 60% of the American general public accepts evolution; and only 32% believe that if evolution occurred, it was independent of divine power (Pew Research Center, 2013). Lack of acceptance extends beyond the general public as well. More than one-third (36%) of high school biology instructors disagree or strongly disagree that "evolution [serves] as the unifying theme for their biology and life science courses" (Berkman, 2008). This is surprising considering 98% of high school biology teachers have taken college level biology to practice their profession (Lyons, 2013). Since acceptance and understanding of evolution is low even in higher education, biology and its subfields should consider standardized measures of assessment to facilitate evolution education of our citizens.

The Value of Evolution in Education

While only 2% of high school biology teachers completely exclude general evolutionary processes from their curriculum, 34% indicate spending five hours or less on the topic in a single course (Berkman, 2008). Minimal incorporation of evolution in coursework has palpable consequences, as biology relies on evolution as a foundation for learning and practice (Dobzhansky, 1973; U.S. National Academy of Sciences, 2008; AAAS, 2011). Evolution is an essential field of study to understand biodiversity, and address growing global challenges such as increasing food production to meet increasing population demands, evading

antibiotic resistance, and curtailing climate change (AAAS, 2011). Thus, educating young generations in evolution is crucial, but post-secondary educators must be aware that incoming students may not have had sufficient exposure to evolution in their high school biology classrooms.

Student Difficulty with Evolution

Research in evolution education has identified key concepts that are important to understanding evolutionary processes, such as understanding the importance of population variation in natural selection (Anderson et al., 2002; Bishop & Anderson, 1990; Nehm & Reilly, 2007). However, research suggests that incoming college-level introductory biology students incorrectly reason about evolution concepts, with one study showing that conceptual proficiency ranges from 0%-31% on key concepts such as origin of traits and population variation (Bishop & Anderson, 1990). It is difficult to speculate why incoming students are deficient in their knowledge about evolution, but recent research suggests that the content of high school biology courses may directly influence students' ability to accept evolution and reason correctly about evolution (Moore & Cotner, 2009).

To further unpack student difficulty with evolutionary concepts in introductory college biology courses, Anderson et al. provide insight into how well student perform on specific key concepts (2002). Collectively, students tend to perform better on concepts such as variation (80.6% correct), biotic potential (69.4% correct), and limited survival (67.2% correct); however, struggle with concepts such as origin of variation (14.5% correct), change in population (18.2% correct), and origin of species (22.3% correct) (Anderson et al., 2002). Thus, student performance varies on different concepts within evolution, and this should be considered when instructors evaluate incoming student conceptions.

Need for Assessments

To better equip post-secondary instructors to evaluate student conceptions, additional assessments are necessary. Some students may enter the classroom with correct ideas on evolution, but research suggests otherwise (Bishop & Anderson, 1990; Anderson et al. 2002; Nehm & Reilly, 2007). Nehm & Reilly found that students incorporate fewer than three natural selection concepts prior to instruction (2007). In addition, students carry a wealth of misconceptions and alternative ideas, with misconceptions being firmly held beliefs and alternative conceptions being loosely held notions (Hammer, 2000). Nehm & Reilly also found that students incorporate an average of more than 2.47 misconceptions when asked to reason about natural selection (2007). Common misconceptions among introductory biology students include the idea that organisms can willfully change phenotypes based on need, and that learned behaviors are heritable (Anderson et al., 2002; Bishop & Anderson, 1990; Perez et al., 2013). Students hold a diverse set of alternative ideas and misconceptions when entering introductory biology courses, and assessments can be attributed for their discovery. It is reasonable to conclude that incorrect ideas may be less persistent if they are identified early in instruction via assessment, because educators will be better equipped to address problem areas through formative instruction.

Assessment Formats

Assessments range from multiple-choice (MC) to open-response (OR), and may even incorporate oral evaluation of ideas. Multiple-choice assessments are considered to be the most convenient to widely distribute and score; however, they require the largest investment of time to develop in a valid and reliable manner (Adams & Wieman, 2011). If time and human resources are not limiting factors, open-response assessments are preferable because they more

accurately diagnose incorrect ideas (Nehm & Reilly, 2007). Nonetheless, although massively time consuming, interviews are considered the gold standard of student evaluation because it is possible to emulate test conditions, but simultaneously uncover underlying student reasoning on assessment prompts (Nehm & Schonfeld, 2008). Thus, assessment methods should be considered based on student population size, time, human resources, and research objectives.

Assessments in Evolution Education

Bishop & Anderson (1990) were among the first discipline-based education researchers (DBERs) to publish a study on evolution, which has since been heavily cited because it provided the first published concept inventory (CI). The Bishop & Anderson CI consists of both open-response prompts and multiple-choice questions (1990). Although the original prompts are not commonly used today due to the availability of more convenient and widely validated CIs, they continue to serve as a building block for assessments on evolution (Anderson et al. 2002; Nehm & Reilly, 2007; Nehm et al. 2012). Notably, the developmental process of the Bishop & Anderson assessment yielded valuable information that we still consider highly relevant. Bishop & Anderson found that: (1) most students do not understand evolution, (2) previous biology exposure does not dictate success in reasoning about evolution, and (3) belief/acceptance of evolution has no correlation with assessment scores (1990). In addition, Bishop & Anderson were the first to establish a comprehensive list of evolution misconceptions in the introductory biology student population. Cotner et al. confirmed that lack of religious affiliation correlated with better evolution understanding (2009). Much of the knowledge we possess about student reasoning in evolution can be attributed to assessment development, and utilization of validated assessments in courses (Table 1).

Table 1. *Current Evolution Assessments.*

Name	Topic of Assessment	Authors
Assessing Contextual Reasoning About Natural Selection (ACORNS)	Natural Selection	Nehm et al., 2012
Bishop and Anderson Diagnostic Test	Natural Selection	Bishop & Anderson, 1990
Concept Inventory of Natural Selection	Natural Selection	Anderson et al., 2002
Knowledge of Evolution Exam	Deep Time and Acceptance of Evolution	Cotner & Moore, 2010
Measure of Understanding of Macroevoltuion	Macroevolution	Nadelson & Southerland, 2009
Open Response Instrument	Natural Selection	Nehm & Reilly, 2007
The EvoDevoCI	Evolutionary Developmental Biology	Perez et al., 2013
The Genetic Drift Inventory	Genetic Drift	Price et al., 2014
The Tree Thinking Challenge	Cladograms	Baum et al., 2005

A list of assessments in evolution education to date.

Subsequent researchers, such as Anderson et al. (2002) and Nehm et al. (2012) have expanded upon Bishop and Anderson by providing an extensive list of misconceptions shown in table 2, including those regarding topics such as population stability, inheritable variation, and differential survival. Anderson et al., like Bishop & Anderson, were able to add valuable information to literature via development of a MC assessment, the Concept Inventory of Natural Selection (CINS). The CINS has gone on to be one of the most convenient, validated, and largely disseminated assessment tools in evolution education research to date, due to several reasons (Nehm & Schonfeld, 2008). As stated before, MC CIs are preferable to free response assessments in cases where the population size is large, because they require no expertise and little time to analyze (Kastner & Stangla, 2011).

Table 2. *Common Misconceptions in Evolution*

Topic	Scientific Concept	Alternative Conception
Biotic Potential	In the absence of environmental limitations, populations have the potential grow exponentially	Not all organisms can achieve exponential population growth[2] Organisms only replace themselves[2] Populations level off[2]
Population Stability	Most populations are stable in size, except for seasonal fluctuations	All populations grow in size over time[2] Populations decrease[2] Populations always fluctuate widely/randomly[2]
Natural Resources	Natural resources are limited in supply at any given time	Organisms can always obtain what they need to survive[2]
Limited Survival	Limited resources leads to limited survival from generation to generation	There is often physical fighting among or between species, the strongest win[2] Organisms cooperate and do not compete[2]
Population Variation	Individuals in a population are not identical at the phenotypic or genotypic level, there is variation among individuals	All organisms in a population are nearly identical[2, 3] Variations only affect outward appearance, and don't influence survival[2] Members of a population share no characteristics with others[2] Species can be partially adapted[3]
Heritability	Much variation is heritable	When a trait (e.g. organ) is no longer beneficial for survival, offspring will not inherit the trait[2,] Traits acquired during an organisms lifetime are heritable[2, 3, 7] Only and/or all environmentally beneficial traits will be inherited[1] Learned traits are heritable[2, 3]

Table 2. *Common Misconceptions in Evolution (continued).*

Topic	Scientific Concept	Alternative Conception
Differential Survival	Survival is not random, and is partially depended on heritable traits. Individuals whose characteristics fit them best to their environment are more likely to reproduce then less fit individuals	Fit equates with dominant alleles, and unfit equates with recessive alleles[1] Fitness as qualities of strength, agility, intelligence, or speed[2, 3] Survival of the fittest applies to the species as a whole, rather than individuals[3] Organisms with many mates are biologically fit[2]
Change in a population	Unequal survival and reproduction among individuals will gradually lead to population change, with the proportion of individuals with favorable characteristics accumulating over time	All members of the population gradually change[2, 3, 7] Evolutionary stasis can occur in the absence of selection[7] Mutations occur to meet the needs of the population[2, 3, 7]
Origin of species	An isolated population may change so much over time that it becomes a new species	Organisms can intentionally become new species over time (tries, wants, or needs to become a new species)[2, 3] New species are created by interbreeding between species (e.g. horses and zebra)[4] Speciation is a hypothetical idea[2]
Origin of Variation	Random mutations and sexual recombination produce individual variations; while most are harmful or neutral, a few are beneficial in some environments	Mutations are adaptive responses to specific environmental factors[2, 3, 4, 7] If one trait is lost, an organism receives compensation via another trait (e.g. "super" hearing in blind individuals)[6] Traits are retained or lost by use or disuse[3, 5, 6, 7] Mutations are intentional: an organism needs, or wants genetic change[2, 3, 5] Selection acts on genes and is independent of phenotypes[7] Change only occurs when entire genes are gained or lost from the genome[7]

Table 2. *Common Misconceptions in Evolution (continued).*

Topic	Scientific Concept	Alternative Conception
Genetic Drift	The relative frequency of alleles which change over time by random events	Genetic drift is gene flow between different species[8]
		Genetic drift is unpredictable due to the influence of random events[8]
		Genetic drift is random mutation[8]
		Genetic drift is not evolution because it does not cause a directional improvement of fitness[8]
		Natural selection is more powerful than genetic drift, or other evolutionary processes[7, 8]
Evolution is Continuous	Evolution is always occurring	Climate change is required for evolution to occur[4]
		Traits continually improve, or species continuously progress[3, 7, 8]
		Humans evolve more slowly or not at all[4]
		Evolving to be similar or more advanced than a human is the ultimate goal of evolution[4]

Common misconceptions in evolution as identified by (1) Abraham et al. 2014 (2) Anderson et al., 2002; (3) Bishop & Anderson, 1990; and (4) Nadelson & Southerland, 2010 (5) Nehm et al. 2012 (6) Nehm & Reilly, 2007 (7) Perez et al. 2013 (8) Price et al., 2013

In addition, wrong answers, known as distractors, in each MC prompt were informed by alternative ideas and misconceptions found in the student population. Thus, the CINS allows instructors to easily discern the prevalence of accurate and inaccurate ideas in their classroom. This is valuable because the instructor can construct learning tools to challenge pervasive alternative ideas and misconceptions in the student population.

Despite the convenience of the CINS, researchers have since deviated from the MC format in exchange for deeper understanding of student reasoning. In 2007, Nehm & Reilly published the Open Response Inventory (ORI). As indicated in the name, the ORI is an OR assessment. Although OR style is less convenient to administer to large masses of students,

Nehm & Reilly demonstrated that the ORI more effective than the CINS at evaluating alternative student conceptions (Nehm & Schonfeld, 2008). Reasonably, because MC assessments are a forced-answer format, students may recognize correct answers on MC assessments but still accept alternative conceptions presented in the distractor choices. Thus, MC assessments may not provide a full description of student conceptions on any one construct.

To further assist instructors in creating open response prompts to evaluate student reasoning, Nehm et al. later developed the ACORNS instrument (Assessing COntextual Reasoning about Natural Selection) (2012). Development of diverse prompts for assessments is important to test for alternative concepts, because evidence shows that students reason differently based on item context (Hudspeth, 2015). A study on introductory biology students found that students were able to more frequently answer questions correctly when animals were used as the item context instead of plants; and even more so when familiar organism names were used (Hudspeth, 2015). Thus, ACORNS is a tool for instructors and researchers to construct isomorphic natural selection prompts using arbitrary contexts. Furthermore, ACORNS provides a unique tool for researchers in new disciplines or sub-disciplines to test evolutionary item contexts in their field.

Microbiology: A Sub-Discipline Lacking Evolution Assessment

A largely untested sub-discipline of biology in evolution education is microbiology. Microbiology offers highly versatile and repeatable models of evolution via microbes (Meyer et al., 2010). The Long-Term Evolution Experiment by Lenski et al. has provided a great model for the evolutionary processes; and there are extensive resources of studies involving evolutionary theory as it relates to the effects of environment, mutation rates, and antibiotic

resistance (Cooper & Lenski, 2010; Elena et al. 2007; Lenski & Hattingh, 1986). Additionally, to fully understand evolutionary theory students should have an idea of how evolution occurs in asexual organisms. However, current popular assessments focus on multicellular organisms and largely exclude microorganisms.

Exclusion of microorganisms means that microbiology lacks validated assessments and/or prompts with respect to evolution theory, despite it being a fundamental concept in the field. Evolutionary relationships explain how microbiologists identify and classify microbes into their many species, which is essential to explore ecological diversity and organismal relationships (Barberan et al. 2016). Of particular importance is pathogen emergence, which is an essential topic of study for students pursuing medicine, nursing, pharmacy, and allied health fields (Greub, 2013). Much of pathogen research is happening via genomics technology. Genomics is a rapidly growing sector of microbiology because of its ability to give us great insight into the evolutionary relationships we've used to define and describe microbes, and to make medical advances in infectious disease (Fournier et al. 2007). In addition, genomics technology has caused one of the greatest debates in current microbiology, that of speciation (Achtman & Wagner, 2008). Finally, evolution is essential for solving applied problems in industry. For instance, directed evolution of microbes holds great power in biofuel production and food quality, such as stress tolerance and fermentation efficiency of yeast in wine industries (Perez-Torrado et al. 2015). Thus, to be a well-informed modern microbiologist, students must understand evolution theory.

Microbiology in Evolution Education Literature

The only insight we have into students' knowledge of evolution in microbiology is via one prompt in the ORI assessment, which reads: "Explain why some bacteria have evolved a

resistance to antibiotics (that is, antibiotics no longer kill the bacteria)" (Nehm & Reilly, 2007). The authors found that on average, introductory biology students employ two misconceptions when providing free response answers to this question (Nehm & Reilly, 2007). Thus, we can expect that introductory microbiology students may employ misconceptions as well. Interestingly, microbes are mentioned in one other CI, the Tree Thinking Challenge (Baum et al, 2005). However, Baum et al. chose microorganisms as species for their phylogenetic trees to avoid preconceptions about trees. In short, microorganisms were used because students know very little of them, or the evolutionary relationships between species.

Assessment Development

Baum et al.'s use of microbes speaks to the need for evolution assessments in microbiology, with appropriate prompts using bacteria, viruses, fungi, and other microorganisms. However, assessment development is a lengthy, arduous process because it must be fully informed by student conceptions over an extended length of time, and across multiple populations (Adam & Wieman, 2011). In addition, there is no agreed-upon prescriptive process for developing assessments. Assessment developers have taken varied iterative paths, although with significant overlap in strategies. Accordingly, a researcher considering assessment development should consider the following iterative phases, but not necessarily in the following order:

(1) Identify the purpose and content of the assessment
    a. Consult experts and instructors for important content
    b. Delineate parameters of the assessment
(2) Develop and pilot open-ended response prompts reflecting content
(3) Interview low and high performing students on the open response prompts

a. Utilize think-aloud protocol

b. Probe deeper for thought processes and difficulties

(4) Develop a rubric by coding responses to the pilot and interviews

a. Establish a framework

b. Use emergent coding

c. Determine interrater reliability

(5) Construct a forced response assessment (multiple-choice or likert-scale)

a. Use common alternative ideas as distractors

b. Perform a readability study

(6) Dispense test (6) to a large sample size of students in multiple courses

(7) Perform validation interviews on experts and students

a. Ensure experts agree on correct and incorrect answers

b. Adhere to a strict think-aloud protocol for students to emulate test conditions

(8) Analyze data

a. Item analysis

b. Factor analysis

(9) Determine reliability across multiple populations

a. Identify final revisions via interviews on new populations

Identify the Purpose and Content of the Assessment

The first step to beginning assessment development is to determine the demand for validated questions in the discipline (Adams & Wieman, 2011; Anderson et al. 2002; Bishop & Anderson, 1990). This can be done through personal experience as an instructor, or by talking to faculty and experts in the field (Adams & Wieman, 2011). For example, experts may

consider evolution an important concept for any biological field, because it provides a framework for how we study diverse living systems and apply research to various problems in medicine, agriculture, and conservation (Understanding Evolution, 2016). Typically, interviewing 6-10 faculty or experts in the field is considered suitable to determine gaps in assessment (Adams & Wieman, 2011).

Another important consideration before constructing questions is to determine what type of assessment will be used. Multiple-choice assessments are ideal for large-scale implementation, because scoring is quick and requires no expertise (Kastner & Stangla, 2011). On the other hand, open response instruments are better at identifying alternative student conceptions (Nehm & Reilly, 2007). In addition, the researcher must determine whether they will create a single- or multiple-construct assessment. In other words, whether they will test a breadth of concepts to accommodate an entire course, or a single concept to accommodate a key concept within a course. In general, it is best to test the concepts within a course that are most difficult for students because high performance on those concepts typically correlate to overall success in a course (Adams & Wieman, 2011).

Develop and Pilot Open-Ended Prompts

To begin prompt development, researchers should consult existing assessments and literature in the field, because alternative ideas direct the content and wording of prompts (Adams & Wieman, 2011; Anderson et al. 2002). Open response prompts are required before forced response prompts can be constructed, because they do not require complete knowledge of student ideas (Nehm & Reilly, 2007). Rather, they serve as a survey of student ideas, and allow the researcher to collect incorrect ideas for distractor choices to later develop alternative assessment formats.

Once open response prompts are formed, the researcher can survey the student population to collect ideas elicited by the prompts. For maximum feedback, it is ideal to capture responses from as diverse a student population as possible, including students of different age, gender, ethnicity, preparation, GPA, etc. (Adams & Wieman, 2011). In addition, including students showing wide range of in-class performance is desirable. High-performing students allow the researcher to gauge the best possible response, while low-performing students inform alternative ideas and misconceptions (Anderson et al. 2002). The ideal response will translate to the correct response for forced answer assessments, and incorrect ideas will become distractor choices (Anderson et al., 2002). Piloting also provides insight into further revisions for the prompts. It is quite possible that the researcher will receive unexpected responses because they are testing a novel population, or the wording of their prompts may not be ideal (Adams & Wieman, 2011).

Interview on Open Response Prompts

To understand students' thought processes beyond written responses, interviews are ideal (Adams & Wieman, 2011; Anderson et al. 2002; Bishop & Anderson, 1990; Nehm & Schonfeld, 2008). Written responses can often be difficult to interpret, and may be limited by time and effort (Adams & Wieman, 2011). Interviews at this stage should follow a think-aloud protocol to emulate test conditions, where students are asked to review the questions and reason about their answer choice aloud (Adams & Wieman, 2011). However, since the goal at this stage of assessment development is also to understand where students find difficulties and incorporate incorrect ideas, it is acceptable to probe further into their thought process by asking 'how' or 'why' they chose their answer (Adams & Wieman, 2011). However, it is important to

keep in mind that probing deeper into thought often changes the students' thinking, and their assessment score will likely improve (Ericsson & Simon, 1998).

Twenty interviews are considered sufficient to uncover patterns of reasoning, and strategies should become apparent by about 12 interviews (Adams & Wieman, 2011). It is advisable to video record each interview, and spend at least 30 minutes following each session recording observations (Adams & Wieman, 2011). Transcription of the video recording is ideal for coding and analysis of the interview.

Develop a Rubric via Coding

For response analysis, the researcher must consider various frameworks to analyze the data. Essentially, frameworks are lenses that the researcher examines data through. It is important to establish the framework, because different lenses will result in differing interpretations. For instance, a grounded theory framework is often used when a researcher is studying a novel field or research question, where data must be collected before formulating hypotheses, and conclusions emerge from the data as analysis occurs rather than from models beforehand (Glaser & Strauss, 1967; Heath & Cowley, 2004). Alternatively, a resources framework has a pre-established lens, and is popular in physics education research (PER), where researchers predict outcomes based on everyday resources that students tend to rely on to reason about problems (Hammer, 2000). For example, students often have a hard time retaining Newton's first law of motion because when they observe tangible objects in motion (e.g. a ball rolling), eventually they come to a stop by an intangible force (i.e. friction).

With a framework to analyze results, rubric development can begin using a subset of responses. Typically, researchers will choose a representative subset of responses, and allow rubric categories to emerge from answers according to the selected framework (Bray Speth,

2009). For instance, if a researcher is using a grounded theory framework, they may notice that "mutation" is a term commonly used by students when addressing natural selection prompts and create a code for mutation (Bray Speth, 2009). Two or more researchers must work on rubric development to establish informal agreement about patterns of responses.

When the researcher(s) is confident that the rubric captures the majority of responses, and continual revision of the rubric approaches limited returns, interrater reliability (IRR) should be determined. IRR quantifies the consensus between two or more coders, returning a value between -1 and +1 (Hallgren, 2012). A value of -1 indicates perfect disagreement, and a value of +1 indicates perfect agreement; while, a value of 0 indicates random agreement (Hallgren, 2012). Values above +0.67 are considered suitable for qualitative data (Krippendorff, 1980), although IRR cutoffs are somewhat arbitrary. For example, Landis and Koch consider IRR at or above 0.61 to be acceptable (1977). For studies using two coders, Cohen's kappa is most commonly used to calculate IRR, and various versions exist to account for multiple data types. For example, Cohen's weighted kappa allows researchers to determine coder agreement using nominal data with an ordinal structure. Alternatively, for three or more coders, the arithmetic mean of kappa is useful (Light, 1971; Davies & Fleiss, 1982). IRR should be calculated on approximately 20% of responses. Low IRR indicates that the rubric needs revision. However, once coders are in agreement, a single coder can independently code the remaining responses.

Construct a Forced Response Assessment

Although more time consuming to develop, multiple-choice assessments are the most convenient form of assessment because analysis of student responses requires no education research expertise (i.e., easy for practitioners to use) and very little time commitment (Adams

& Wieman, 2011). However, if the researcher has already invested time in interviews, they will have the information they need to transform their open response assessment into a multiple-choice assessment. Interviews are the gold standard for gaining alternative student ideas, and common alternative ideas are ideal distractors for multiple-choice assessments (Nehm & Schonfeld, 2007; Adams & Wieman, 2011). By using common student responses derived from interviews, researchers are more likely to gain distractor responses that are appealing to students (Adams & Wieman, 2011). Ultimately, the assessment will be capable of diagnosing the fraction of students who hold correct ideas, and alternative ideas, with minimal investment of time.

Before fully employing the forced response assessment, a readability study should be considered. As wording of prompts becomes more scientifically accurate, so does reading difficulty (Anderson et al. 2002). Thus, to ensure that the audience (students) can comprehend the prompt and answer accordingly, a readability study is advisable to determine the reading level of the assessment. Common measures for assessing readability are outlined by Ricci et al. (2015). For general populations, a sixth grade reading level is recommended (Ricci et al. 2015). However, reading levels for content specific prompts may vary. In instruction, a cloze test score of approximately 60% is ideal for first year college students (Anderson et al. 2002).

Dispense Test to a Large Population

Once the researcher is confident that their prompts are readable and elicit the intended responses from students, they are ready to collect a large dataset of responses from a large population of students. Conventions on sample size for education research studies vary. For example, McMillan recommends at least 30 students for correlational studies, or 1%-5% of the target population (1996). However, Adams & Wieman recommend several hundred responses

across multiple iterations of a course to make statistically significant conclusions (2011). Although, they add that statistics may be used to compensate for sample populations with fewer students (Adams & Wieman, 2011).

Perform Validation Interviews on Experts and Students

To validate the MC assessment, experts and students are once again sought for interviews. Experts should perform better on the assessment, and agree on the correct answer for each question, confirming that distractor questions are unambiguously incorrect (Shavelson & Ruiz-Primo, 2000; Adams & Wieman, 2011). Interviews with students must confirm that students chose the correct answer for the correct reason, and incorrect answers for consistent reasons (Adams & Wieman, 2011). Interviewers should utilize a think-aloud protocol to emulate thinking during the assessment (Durning et al., 2013). It is entirely possible that students are able to recognize correct answers using reasoning unrelated to the content despite expert approval of the question, through tactics such avoiding answers using definite words such as ‘never’ and ‘always’ (Smith et al. 2008).

It should be kept in mind that interviews only validate the test for the population that was interviewed. To ensure reliability of the assessment, various populations must be interviewed, and it is common to revise prompts several times before reliability is established, as discussed below (Adams & Wieman, 2011).

Item and Factor Analysis

Item analysis is an important aspect of interpreting collected data. Notable item analyses include item difficulty and item discrimination (Gugiu & Gugiu, 2013). Item difficulty describes how many students were able to answer a question, while item discrimination

describes how well certain questions correlate to a student's overall test score (Anderson et al. 2002; Gugiu & Gugiu, 2013).

An ideal item difficulty is the value halfway between 100% and the percent that indicates the random chance of students choosing the correct answer (i.e. if there are four possible answers, then there is a 25% chance that students randomly chose the correct answer; thus, ideal item difficulty would be 62.5%) (Anderson et al. 2002). Item discriminability should be at least 0.30, with 1.00 being the maximum discriminatory power (Anderson et al. 2002). However, these measurements may not be suitable for researchers with the goal of using the assessment as formative feedback for teaching, rather than student performance (Adams & Wieman, 2011).

To determine whether students are moving towards an expert-like state of thinking, factor analysis may be appropriate. Factor analysis can provide information about questions that couple together, meaning that students tend to do well on both questions simultaneously rather than just one or the other (Adams & Wieman, 2011). If students consistently answer questions of the same concept at an expert-like level, this indicates their migration towards mastery of the material. If students demonstrate inconsistent organization of ideas within the same concept, instruction may be tailored accordingly to target problematic concepts.

Item Response Theory (IRT) is an additional analysis that can be performed to test the correlation between test scores and "latent" traits, such as math ability or attitude (Adams & Wieman, 2011). IRT becomes important if the researcher wants to search for predictive factors in student success. If IRT reveals that certain traits correlate with lower success on the assessment, course structure can be organized in a fashion that targets at-risk populations more specifically. For assessments aimed at improving instruction, it may be more worthwhile to

look the predictive power of the assessment with course homework, standardized tests, or final grades (Adams & Wieman, 2011).

Determine Reliability across Multiple Populations

Lastly, a reliability study is required before an assessment can be considered suitable for widespread use. If an assessment is reliable, it means that it is applicable across various populations. Thus, the assessment should be given to students in multiple regions and cover a diverse set of demographic factors, such as age, ethnicity, or income (Adams & Wieman, 2011). A Pearson Correlation Coefficient can be used to determine whether there is agreement on multiple-construct assessments between pre- and post- test results across different semesters and/or populations of students (Adams & Wieman, 2011). A correlation coefficient of 0.90 or higher is considered reliable for these assessments. Alternatively, if one is testing a single construct item test, Cronbach's alpha or Kuder-Richardson reliability index (KR-20) can be used to test for internal reliability.

CHAPTER TWO. CHARACTERIZING WRITTEN RESPONSES TO 16S RIBOSOMAL RNA AND PHYLOGRAMS

Evolution is a priority in the American Society for Microbiology (ASM) curriculum, listed as the number one core concept. The ASM curriculum was revised in 2012 following the release of *Vision and Change in Undergraduate Biology Education,* and borrows the five core concepts, adding a sixth to accommodate microbiology:

1. Evolution
2. Cell structure and function
3. Metabolic pathways
4. Information flow and genetics
5. Microbial systems
6. The impact of microorganisms

The curriculum defines important concepts for general microbiology courses, and provides examples for instructors to assess students' understanding. Within each core concept are fundamental statements, which are big picture ideas. Each fundamental statement is supported by lower- and higher-order learning outcome examples, which can be used to assess understanding of the fundamental statement. For example, fundamental statement five under the core concept of evolution reads: "The evolutionary relatedness of organisms is best reflected in phylogenetic trees", and a lower-order learning outcome is: "List the three Domains of the phylogenetic tree of life. State a unique characteristic of each Domain" (ASM, 2014). There are 27 fundamental statements and 184 learning outcomes total.

Although two concept inventories are under development for introductory and allied health microbiology courses, there is no published data characterizing how well students meet learning outcomes from the ASM curriculum (Horak, 2015). This research makes the first attempt to study student reasoning about the curriculum. Given the size of the curriculum, I

chose to focus this project on three specific higher-order learning outcomes from fundamental statement five under the core concept of evolution:

1. Explain what features of the 16S ribosomal RNA (rRNA) make it useful to compare the evolutionary relationship between organisms.
2. Determine the two most related and two least related organisms from a short list of 16S rRNA sequences.
3. Draw inferences about evolutionary relatedness of organisms based on phylogenetic trees.

Although listed as higher-order, the first learning outcome tests comprehension, a lower order cognitive skill (LOCS). The latter two are analysis tasks, which are higher order cognitive skills (HOCS). Inclusion of LOCS and HOCS will allow us to determine how completely students conceptualize about 16S rRNA and phylogenetic trees.

Following the protocol described in Chapter One, open-ended questions were formulated from the three learning outcomes, piloted, and revised. Final prompts were disseminated to upper-division microbiology students, coded, and analyzed. Additionally, interviews were conducted for more detailed insight into students' analysis techniques. Two main questions were pursued to characterize student reasoning on the learning outcomes listed above:

1. What general ideas emerge from student responses to evolution-based learning outcomes derived from the ASM curriculum?
2. What patterns of reasoning do students use when analyzing 16S rRNA sequence data, and a phylogram?

Education Research on 16S rRNA

The prokaryotic ribosome is comprised of a large subunit (50S) and small subunit (30S). Within the 30S subunit are the 16S rRNA, and small ribosomal proteins 1-21 (Holmes, 2005). 16S rRNA is useful to determine species relatedness because it is ubiquitous in prokaryotes and homologous to the 18S rRNA in eukaryotes. These components are an essential, highly conserved part of the translational process, resulting in fewer mutations over time compared to other parts of the genome. Relatedness can be directly quantified via analysis of the nucleotide sequence of the gene, and is a common technique in microbiology. For example, it can be used to characterize environmental or clinical samples, which contain unknown organisms. Less than 1% of known bacteria can be cultured, and genetic analysis such as 16S rRNA sequencing is required. Thus, understanding the utility of 16S rRNA is essential for microbiology majors, medical laboratory students, or any student pursuing proficient knowledge of prokaryotic diversity and taxonomy. However, no educational research exists on student proficiency.

Education Research on Phylogenetic Trees

Although no research exists on students' understanding of 16S rRNA, there are several studies on tree-thinking. Phylogenetic trees are essential to the study of evolution, as they depict the relatedness of organisms over time. Phylogenetic trees are especially important to microbiologists, such as those interested in pathogen emergence, because building ancestry is often a first step in identifying pathogenic changes in bacterial and viral genomes (Smith et al., 2009). However, students often struggle to reason about phylogenetic trees.

Dees et al. provides a table of tree-thinking misconceptions supported in literature, typically regarding students' analysis of relatedness on cladograms (2014). These misconceptions include "branch tip proximity", "contemporary descent", "node counting", and

"external insights" (Baum et al., 2005; Baum & Offner, 2008; Gregory, 2008; Halverson, 2011; Halverson et al., 2011; Meir et al., 2007; Meisel, 2010; Novick & Catley, 2007; Novick et al., 2010; Novick et al., 2011; Novick & Catley, 2013; Novick et al. 2012; Omland et al. 2008; Perry et al., 2008; Sandvik, 2008; Smith et al., 2013).

"Branch tip proximity" describes students' tendency to determine relatedness based on the closeness of taxa. Students often reason that closer taxa on branch tips are more related, ignoring important features of the tree (Dees et al. 2014). For example, in Figure 1, students might reason that organisms B and C are more closely related than organisms A and B because they are closer in proximity, although they belong to different monophyletic groups and most recent common ancestors.

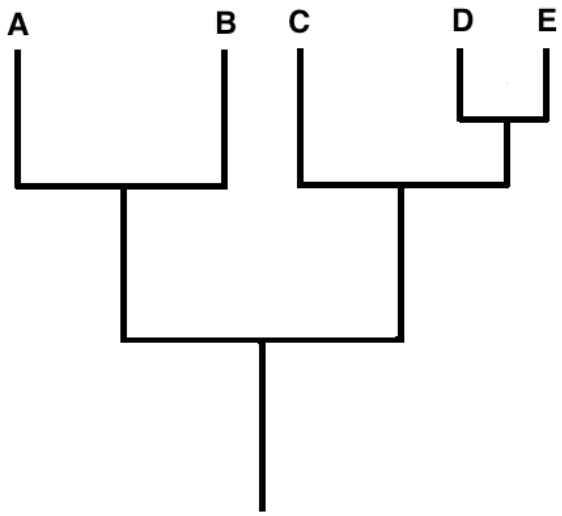

Figure 1. Bracket Style Phylogenetic Tree (modified from Dees et al., 2014).

"Contemporary descent" is the idea that taxa directly descend from other taxa, rather than from common ancestors linking the two (Dees et al. 2014). Using Figure 1 as an example, a student might reason that taxon A and B are closely related because B descended from A. Additionally, students often employ "node counting" to determine species relatedness (Dees et al., 2014). In Figure 1, students may reason that organism A is more closely related to organism

C than organism D, because C and A are separated by three nodes and D and A are separated by four nodes. However, this cladogram suggests that they are equally related. Finally, students sometimes use "external insights", or preconceptions about organisms, to make inferences about species relatedness. For example, a student may reason that mushrooms are more related to plants than animals, but phylogenetic analysis suggests that fungi and animals are more related than fungi and plants (Baldauf & Palmer, 1993). It is expected that students will have fewer preconceptions with microorganisms because students are generally unfamiliar with microbial species, as indicated by Baum et al (2005).

It should be noted that most research findings have been determined by studies testing student knowledge and reasoning on cladograms, where branch length is meaningless. However, the current study focuses on phylograms, where branch length corresponds to the amount of genetic change from the ancestor and is often accompanied by a numerical value representing the genetic change. This study will provide additional insight into student reasoning about phylogenetic trees by testing their reasoning about branch length and genetic change depicted in phylograms.

Methods and Analysis

Pilot Studies

A rough draft of prompts to fundamental statement five and fundamental statement four were created. IRB approval was obtained (Protocol #AG16087) (Appendix E), and the pilot assessment was posted as an online Qualtrix survey for Biology Learning Assistants (LAs) and two upper-division microbiology courses: MICR 452: Microbial Ecology and MICR 486 Capstone Experience in Microbiology (Appendix A). The pilot assessment included questions on 16S rRNA utility and analysis, phylogram analysis, antibiotic resistance (AbR), and horizontal

gene transfer (HGT). The assessment was divided into two sets of questions, and alternated to reduce the assessment length for any one student. Students either received the 16S rRNA and phylogram questions, or the AbR and HGT questions.

In total, 26 survey attempts were made. However, with a 65% completion rate, only 17 complete responses were collected. The average survey completion time was 34 minutes, excluding one student taking four days to complete the survey. Three complete responses were received from Biology LAs, and 14 from the upper-division microbiology courses. Biology LAs did not receive compensation, but microbiology students received a small amount of extra credit. Five students received the AbR and HGT questions, and 12 students received the 16S rRNA and phylogram questions. Additional responses were not sought because the Microbiology Program at North Dakota State University (NDSU) enrolls less than 100 students. It was necessary to limit the respondent pool in order to gain a large population size for the main study.

Pilot Interviews

To facilitate prompt revision, two pilot interviews were conducted. Both were inspired by a think-aloud protocol, in which students read the assessment item and vocalized their reasoning (Appendix B); students were also asked explicitly about prompt revision, since this was the main goal of pilot interviews. Interviews lasted approximately 30 minutes each. Due to operator error, only the first interview was video recorded. Transcription of interview one and notes of interview two can be found in Appendix C. Volunteers received $25 cash in exchange for their time and participation.

Revision of Assessment Prompts

Although it would have been ideal to collect student responses to every question in the initial draft, the average human attention span is 20 minutes; therefore, research assessments

need to be limited, and the assessment was reduced (Burns, 1985; Cornish & Dukette, 2009; D'souza, 2013). AbR and HGT questions were excluded; the final assessment focuses on the 16S ribosomal RNA and a phylogram analysis questions (Appendix D).

Although the 16S rRNA sequence data was unchanged, the phylogram was significantly altered following the pilot study. The original phylogram was constructed from incomplete 16S rRNA data, so the final phylogram is a more accurate depiction of the true species relatedness. Lines and fonts were made larger for readability, and nodes were colored red for ease of reference in student responses. Wording of the majority of prompts was altered as well. Changes made to the prompts as a result of the pilot studies are summarized and justified in Table 3.

Table 3. *Prompt Changes Following Pilot Data*

Original Prompt	Revised Prompt	Justification
While observed traits tell us a lot about whether a species is closely related or not, it is not always obvious. For instance, mitochondria are found in all animal cells, so it is not a reliable method to tell animal species apart. To circumvent the difficulty of identifying species based on observable traits, scientist Carl Woese determined an alternative method: analysis of the 16S subunit of ribosomal RNA (rRNA). The 16S rRNA is a component of the genetic replication machinery found in all living cells. Briefly address the following questions:	While observable traits (e.g. gills, wings) may inform species relatedness, this method can be unreliable, especially with bacteria! As an alternative, Carl Woese proposed analysis of the 16S subunit of ribosomal RNA (rRNA) to compare species relatedness. Briefly address the following questions based on this information:	1. Shortened to reduce length of assessment, and ease of reading 2. Inclusion of macroscopic traits rather than mitochondria, as it may be more tangible to students 3. 16S is only in prokaryotes; the original prompt perpetuates a misconception 4. Ubiquity in prokaryotes is a key concept, the first prompt allows students to restate and gain credit
Explain the features of 16S rRNA that make it useful as a method to determine species relatedness	Why is the 16S rRNA gene useful to determine species relatedness? Provide at least two explanations.	To determine if student responses would improve, the new prompt requests two explanations.
Explain how you determined the two most related and two least related organisms from the 16S rRNA data above.	How did you utilize the sequence data to determine the most and least related species?	Shortened to reduce length of assessment

Table 3. *Prompt Changes Following Pilot Data (continued).*

Original Prompt	Revised Prompt	Justification
16S rRNA data can be used to develop phylogenetic trees to depict genetic relatedness. The phylogenetic tree above represents the species based on the partial 16S RNA gene sequence data from the previous questions; however, this time you can see the names of the genera.	16S rRNA data can be used to develop phylogenetic trees. The phylogenetic tree above represents the 16S RNA gene sequence data from the previous question; however, the names of the genera are included (red dots represent nodes).	1. Genetic relatedness was removed, as it refers to genetic distance depicted by the numerical values; this was a key concept 2. Students are provided the word "node" to facilitate clarity in student explanations 3. Shortened to reduce length of assessment
Based on the phylogram, which two genera are the most related?	What are the **two most related** species according to the phylogenetic tree? Explain in detail how you determined your answer.	1. Reflects new phylogram 2. Explanation merged because method of analysis differs from explaining the *more* related species (below)
Based on the phylogram, which two genera are the least related?	Is *Mycobacterium avium* more closely related to *Campylobacter jejuni* or *Escherichia coli* (or are they equally related)? Explain in detail how you determined your answer.	1. Reflects the new phylogram 2. Due to expert opinion, it was determined that asking the more related species would be a more informative prompt to assess student reasoning 3. Explanation merged because method of analysis differs from explaining the *most* related species (above)
Explain how you determined the most and least related organisms from the phylogenetic tree.	Combined with each prompt as shown above.	1. The second question was revised to ask for the more related species 2. Methods of analysis between *most* and *more* related species differ

A list of changes made to assessment prompts following pilot written responses and pilot interviews.

The pilot study showed that students struggle to recognize nucleotides as the letters (A, C, T, G) in the 16S rRNA sequence data. Some students referred to them as proteins, amino acids, or simply as base pairs. As a result, an additional prompt was added: "What do the individual letters (A, C, T, G) represent?". It was also apparent that students did not recognize 16S rRNA as being unique to prokaryotes, so the final assessment includes a question on the prevalence of 16S rRNA (Appendix D).

Sample Collection

Concepts from the ASM curriculum are introduced in MICR 350: General Microbiology I (for majors) and MICR 202: Introductory Microbiology (for non-majors), and are reiterated in upper-division courses. Therefore, it is assumed that students in upper-division microbiology courses at NDSU have been exposed to the ASM curriculum. A variety upper-division microbiology courses were surveyed, including:

Table 4. *Courses Surveyed for the Assessment.*

Course	Responses Collected (n)
MICR 352: General Microbiology II	22
MICR 445: Animal Cell Culture Techniques	12
MICR 450: Infectious Disease Pathogenesis	18
MICR 460: Pathogenic Microbiology	81
MICR 463: Clinical Parasitology	31
MICR 480: Bacterial Physiology	3

In total, 167 responses were collected. Some responses were discarded due to lack of informed consent or being outside the target demographic population (i.e. graduate students). Some demographic information is excluded for certain students due to transfer status, or students being in their first biology or microbiology course, where class history was recorded as zero.

Table 5 summarizes the demographic factors provided by the University Registrar, and the total students (n) included in each demographic factor for analysis.

Table 5. *Population Size by Demographic Factor.*

Demographic Factor	Population Size (n)
Gender	167
Age	167
Ethnicity	152
College GPA	166
College Credits Completed	167
Classes Taken	153
Major	167
ACT	151
High School GPA	149
Home State	155

Population size of each demographic factor due to availability from the University Registrar.

Ninety-four of the respondents were female (56%) and 73 of the respondents were male (44%). Age ranged from 19 to 40, with a mean age of 21.7 and a standard deviation (SD) of +/- 3.82. Students were predominantly White/Caucasian (90.4%); there was some representation from American Indian/Alaska Native (3.0%), Black/African American (3.0%), Asian (1.8%), and Hispanic/Non-White (1.2%) students. One student (0.6%) did not specify their ethnicity. Collectively, they had an average college GPA of 3.45 (+/- 0.46) and an average ACT score of 26 (+/- 3.64). The population had completed an average of 93 (+/- 27.6) college credits, which is considered senior status, and an average of 7.4 (+/- 4.0) biology and microbiology courses. In addition, students were primarily from North Dakota (47.9%) and Minnesota (44.9%).

The most prevalent major was pre- and declared Pharmaceutical Studies (44.2%), followed by Microbiology majors (30.4%) and Pre-Medical Laboratory Science students (12.2%). The following majors comprised the remaining 13.2% of students:

- Biochemistry & Molecular
- Biology
- Biological Sciences
- Biotechnology
- Chemistry
- Equine Sciences
- English
- Agriculture
- Zoology
- Pre-Respiratory Care
- Pre-Nursing
- Non Degree
- Undeclared

Management and Analysis

Written Responses

Written responses to the finalized assessment (Appendix D) were collected in class. Students took approximately 15-20 minutes on average to complete the survey, although individual completion time varied. Informed consent was collected, and participation was completely voluntary and retractable. No extra credit or incentive for participation was offered.

Interviews

To gain more detailed insight into student reasoning about 16S rRNA and phylograms, five student interviews were conducted. Each interview was recorded and transcribed, and interview volunteers received $20 cash in exchange for their time. The interview began with a free association protocol, and followed with a think-aloud protocol (Appendix F). Interviewees were prompted for reasoning techniques as well. Because only five interviews were conducted, results will be referred to as 'emergent reasoning patterns', and are summarized in the results section below. Full transcriptions of each interview can be found in Appendix G .

Rubric Development and Coding for Written Responses

Grounded theory was used to characterize student responses. To begin rubric development for each assessment item, two independent coders reviewed a subset of ten responses. Emergent patterns of student reasoning were recorded independently, and the coders subsequently merged observations. Several early versions of the rubric were created before the first rubric was ready to apply to student responses, and alternative idea codes were isolated in a separate rubric. In total, 42 responses (25% of all submitted) were reviewed to assess reliability of the first rubric. IRR was calculated (discussed in results section, below), and reliability could not be confirmed. The first rubric was revised, and a second rubric was developed and assessed using 35 new responses, but reliability was still suboptimal. Both coders reviewed all responses due to lack of a reliable rubric. Table 6 outlines the final binary rubric used for characterization of student responses, meaning the code was either present or absent. Table 7 outlines the final rubric used for questions with ordinal codes, meaning the codes could be absent (0), present and incorrect (1), or present and correct (2).

Table 6. *Binary Rubric Codes*

Prompt	Code	Justification
(1.2) Determine the two most related and two least related organisms from the short list (1-4) of aligned 16S rRNA sequences below:	Correct	Species 2 and 4 are most related; species 1 and 3 are least related
	Incorrect	Student only gets one correct, or gets both incorrect
	No Response	The student leaves the answer blank
(1.3) How did you utilize the sequence data to determine the most and least related species?	Sequence	The student stated or demonstrated that they compared sequences 1-4 to determine their answer
	Reference	The student stated or demonstrated that they compared each sequence to the reference sequence
	Unspecified	It is unclear how the student determined their answer, or which sequences they compared
	No Response	The student leaves the answer blank
	Not Addressed	The student indicates that they do not know the answer.
	Letters (AI)	The student refers to A,T,C,G as something other than nucleotides
(1.4) What do the individual letters (A,T,C,G) represent?	Correct	The student states that the letters represent nucleotides, nitrogenous bases, or names each individual nucleotide correctly
	Incorrect	The student refers to the letters as something other than nucleotides, such as “proteins”, “amino acids”, “base pairs”, or “nucleic acids”, or student names individual nucleotides incorrectly
	No Response	The student leaves the answer blank

Table 6. *Binary Rubric Codes (continued).*

Prompt	Code	Justification
(1.5) TRUE or FALSE: all living cells contain 16S rRNA	Correct	False
	Incorrect	True
	No Response	The student leaves the answer blank
(2.6A) What are the two most related species according to the phylogenetic tree?	Correct	*Campylobacter jejuni* and *Escherichia coli*
	Incorrect	Any other combination than *Campylobacter jejuni* and *Escherichia coli*
	No Response	The student leaves the answer blank
(2.7A) Is *Mycobacterium avium* more closely related to *Campylobacter jejuni* or *Escherichia coli* (or are they equally related)?	Correct	*Escherichia coli*
	Incorrect	*Campylobacter jejuni*, or equally related
	No Response	The student leaves the answer blank

Table 7. *Ordinal Rubric Codes*

Prompt	Code	Absent (0)	Present and Incorrect (1)	Present and Correct (2)
(1.1) Why is the 16S rRNA gene useful to determine species relatedness? Provide at least two explanations.	Mutation Rate		The student may state that mutation rates are higher in the 16S rRNA subunit, or that 16S cannot mutate; they do not necessarily mention time	The student recognizes that mutations in the 16S subunit are rare, or they may state that it evolves slowly
	Presence		The student may state that 16S rRNA is in: all living organisms, eukaryotes, or "higher" organisms	The student recognizes that 16S rRNA is present in all prokaryotes or all bacteria, or they may state that 16S rRNA is conserved
	Function		The student may incorrectly analyze the function of 16S/18S	The student states that the 16S/18S rRNA is essential for translation; "ribosomal assembly" would also be an acceptable answer
	Relatedness		The student does not demonstrate an understanding of what is being compared between species, they may refer to something other than nucleotides, including traits	Student states that sequences/genes are unique to species and/or can be compared to draw inferences on relatedness/ phylogeny
	Restatement		The student may give a response that is unclear or unrelated, a restatement of the prompt, or states "I don't know"	
	No Response		Student has no response, or is missing a second key concept as requested in the prompt	

Table 7. *Ordinal Rubric Codes (continued).*

Prompt	Code	Absent (0)	Present and Incorrect (1)	Present and Correct (2)
(2.6B) Explain in detail how you determined your answer (most related)	Numbers		The student wrongly applies numbers to determine the most related species	If mentioned, the student states that numbers do not assist in determining the most common ancestor
	Proximity		The student may attribute vertical proximity of branches to species relatedness, without correctly referring to clades or common ancestry	If mentioned, the student correctly attributes proximity of the two species as important within the same clade or section of the tree
	Branch Length		The student wrongly attributes length of branch to relatedness	If mentioned, the student may correctly attribute length of branches to species relatedness
	Node		The student does not properly recognize nodes or common ancestors; the student may *only* state that the species are in the same "section", "clade", "stem", or "branch" of the tree; or utilize the concept of nodes incorrectly	The student understands that nodes represent common ancestors (or a common point), and utilizes them correctly; the student may *also* state that the species are in the same "section", "clade", or "branch" of the tree
	Not Addressed		Student gives a response that is unclear, or unrelated to the question at hand	
	No Response		The student does not respond to the question	

Table 7. *Ordinal Rubric Codes (continued).*

Prompt	Code	Present and Incorrect (1)	Present and Correct (2)
(2.7B) Explain in detail how you determined your answer (more related).	Numbers	The student mentions numbers, but is unclear if they were used correctly; or the student may mention numbers but they talk about a "gap" or subtract from 0.124	The student understands there is less genetic distance from the common ancestor to *E. coli* than *C. jejnuni*; they may add the numbers to communicate this
	Proximity	The student may attribute vertical proximity of branches to species relatedness, without correctly referring to clades or common ancestry	If mentioned, the student also uses the node to describe proximity
	Branch Length	The student wrongly attributes length of branch to relatedness	The student correctly attributes branch length to species relatedness (i.e. *E. coli* has a shorter branch)
	Node	The student does not properly recognize nodes as common ancestors, they may simply say the "split" or "branch off", incorrectly analyzes the nodes	The student understands that nodes represent common ancestors (or a common point), and utilizes them correctly; the student may also state that the species are in the same "section", "clade", or "branch" of the tree
	Not Addressed	Student gives a response that is unclear, or not related to the question at hand	
	No Response	The student does not respond to the question	

Although all codes were categorical, some questions had binary codes for presence and absence (1.2, 1.3, 1.4, 1.5, 2.6A, 2.7A), while others were ranked from zero to two (1.1, 2.6B, 2.7B) for absent concepts (0), present but incorrect concepts (1), and present and correct concepts (2). In addition to the main rubric, an alternative idea rubric was also used during response analysis. The rubric attempts to characterize possible emergent misconceptions from student responses, and is outlined in Table 8.

Table 8. *Alternative Ideas.*

Prompt	Alternative Idea	Description
Question 1.1	Phenotypes	Student attributes 16S rRNA to phenotypic expression
	Simplicity	Student indicates "ease" or simplicity of analysis
Question 1.3	Letters	The student refers to A, C, T, G as something other than nucleotides
	Base Pairs	The student stated or demonstrated analysis of base pairing
Question 2.7	Equally Related	The student only considers the nodes to determine their answer. They do not consider branch length or the amount of genetic change represented by numbers.

Alternative ideas were recorded separately from the main rubric because they are common but incorrect binary ideas about the content. Identification of alternative ideas may hint at misconceptions in the student population.

Analysis

Interrater Reliability

Interrater reliability (IRR) was calculated for each code in the rubric using Cohen's weighted kappa, which accounts for ranked categorical data (Hallgren, 2012). The equation for Cohen's Weighted Kappa is as follows:

$$\kappa_w = 1 - \frac{\sum_{i,j} w_{ij} p_{ij}}{\sum_{i,j} w_{ij} e_{ij}}$$

Figure 2. Cohen's Weighted Kappa (Zaiontz, 2016)

Where p_{ij} are the observed probabilities, e_{ij} are the expected probabilities, and w_{ij} are the weights (Zaiontz, 2016). A threshold of 0.67 was used, as recommended by Krippendorff (1980).

Characterization of Responses

Simple statistical measures were used to characterize patterns of student responses. For open-response questions, prevalence of each code was calculated, as they often include more than one code and there is no single "correct response". For correct/incorrect responses, percent correct, incorrect, and no response was calculated.

Chi-Square Analysis

Each demographic factor (Table 4) was paired with each code from every question. A chi-square analysis was performed, indicating if the observed distribution of the data was significantly different from expected distribution of the data. Multiplying the categorical total by the nominal total and dividing by the observed total calculated expected values. Subsequently, a Holm's correction (Figure 3) was performed to account for ten demographic factors being tested against one set of data (each code). The equation is as follows:

$$p_{\text{Bonferroni, } i|C} = (C - i + 1) \times p$$

Figure 3. Holm's Bonferonni Equation (Abdi, 2010)

Where *C* is the total number of comparisons, *i* is the rank position, and *p* is unadjusted p-value. Values larger than one are set equal to one (Abdi, 2010). Once p-values were adjusted, a corrected p-value value of 0.05 was used as a threshold for statistical significance. Detailed results of the chi-square analysis and parameters used to assess demographic data (i.e. binary or tertile values) are outlined in Appendix H.

Results

Evaluation of the Assessment Rubric

The results of the first and second iteration of IRR are summarized in Table 9, and show the rubrics were unreliable for independent coding after two iterations because many values fell below the advised threshold of 0.67 (Krippendorf, 1980). Ultimately, both coders coded all responses to accurately characterize student idea

Table 9. *Interrater Reliability*

Question	Code	Iteration One (κ)	Iteration Two (κ)
1.1	Mutation	0.8205	0.7168
	Present	0.7151	0.8444
	Necessary	0.5329	1
	Function	---	0.8188
	Unique	0.5847	0.1258
	Comparisons A	0.8576	0.5764
	Comparisons B	---	0.1667
	No Response/ Restatement A	0.3307	0.2647
	No Response/ Restatement B	---	0.4792
	No Response A	0.7842	0.7960
	No Response B	---	0.6534
	Phenotypes	0.3333	-1
	Simplicity	1	1
1.2	Correct	0.3333	0.8235
	Incorrect	1	0.8235
	No Response	---	*1
1.3	Sequence	0.3940	0.1091
	Reference	0.7670	0.9412
	Unspecified	0.4630	0.5395
	No Response	*1	*1
	Letters	0.8440	*1
	Base Pairs	---	0.3558
	Unchanged Nucleotides	*1	---
1.4	Correct	---	0.8852
	Incorrect	---	0.8852
	No Response	---	*1
	Letters	---	*1
1.5	Correct	---	1
	Incorrect	---	0.9421
	No Response	---	*1
2.6A	Correct	0.952	1
	Incorrect	0.952	0.9278
	No Response	1	*1
	Not Addressed	1	---

Table 9. *Interrater Reliability (continued).*

Question	Code	Iteration One (κ)	Iteration Two (κ)
2.6B	Numbers	0.926	0.9201
	Branching	0.4660	0.5646
	Nodes	0.8090	0.8563
	Not Addressed	0.6410	0.9089
	No Response	0.8290	1
	Distractors	0.7570	0.6648
2.7A	Correct	1	1
	Incorrect	0.9520	0.8852
	No Response	1	*1
2.7B	Numbers	0.8430	0.7010
	Branching	0.7990	0.7871
	Nodes	0.5650	0.7975
	Not Addressed	0.2220	0.8100
	No Response	0.8760	0.5443
	Equally Related	0.9490	0.7464

Table note. *Approximations were made because low utilization of certain codes inhibited accurate calculation of Cohen's weighted kappa.

Characterization of Responses

Student Ideas about the Utility of 16S rRNA

Question 1.1 asked students to report at least two features of 16S rRNA that make it useful to determine species relatedness. Responses are summarized in Figure 4 according to the total and type of response provided by the student population as a whole.

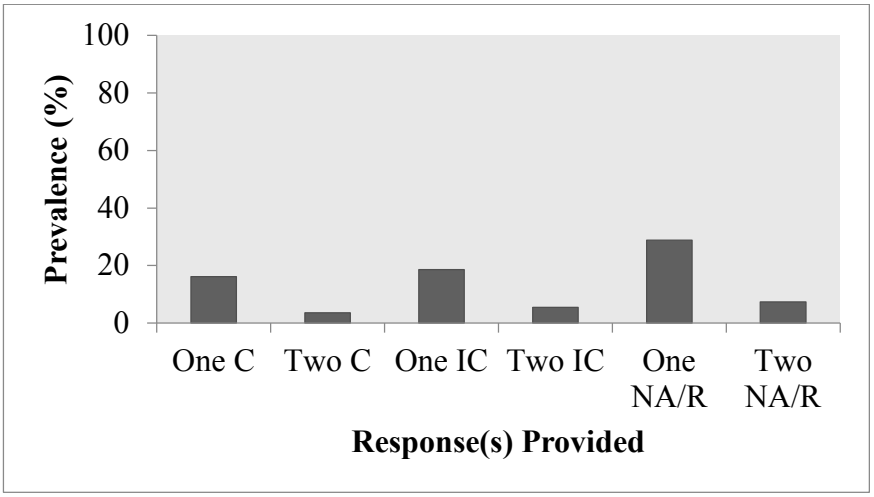

Figure 4. Proportion of students providing one or more correct, incorrect, non-applicable/restatement responses. C=Correct, IC=Incorrect, NA/R=Non-applicable/Restatement, NR=No Response.

While 43.8% of students provided at least one correct or incorrect idea about the utility of 16S rRNA, most students (96.4%) were unable to identify two correct reasons 16S rRNA is useful to determine species relatedness. As a comprehension level question, it seems the majority of students appear to lack long-term retention, which is supported an interviewee recount of 16S rRNA: "I feel like it's a specific part of, like, DNA after it's been transcripted and translated, and then it's just like a certain important piece of gene with an RNA." Only 13 (7.7%) students were not listed as taking the Introductory or General Microbiology course at NDSU at the time this assessment was distributed, indicating that the majority of students have been exposed to 16S rRNA content. Notably, 12 of those students were enrolled in MICR 460: Pathogenic Microbiology, which requires an Introductory or General Microbiology course before enrollment. It is likely that these students took the course at a different institution, leaving only one student who may have not taken an Introductory or General Microbiology course. While it is possible that 16S rRNA was not mentioned at other institutions, it is still apparent that most students have been exposed to the content. This observation is supported by the fact that 24.1% of students identified a feature with incorrect reasoning, suggesting that the students either learned the content in previous instruction but forgot the correct reasoning, or hold an alternative idea about 16S rRNA. It is possible that students guessed or were cued by the prompt to provide a seemingly descriptive idea. It is also apparent that many students could not provide discernable responses, reflected by 36.1% of students providing at least one non-applicable or restatement response, 12.8% of students only providing one response, and 7.3% of students not responding at all. Some of the students providing one or no response may have chosen not to respond despite a working knowledge about 16S rRNA, but their participation in at least part of the assessment

suggests otherwise. To better understand the ideas used by students who provided descriptive ideas (rather than non-applicable ideas, or restatement of the prompt), refer to Table 10.

Table 10. *Percent of Responses for Descriptive Codes.*

	Mutation (%)	Present (%)	Function (%)	Relatedness (%)
Correct	5.4	19.8	7.2	16.2
Incorrect	3.6	12.6	2.4	19.8
Total Responses (%)	9.0%	32.4%	9.6%	36.0%

Most students identified relatedness and presence (i.e. 16S rRNA is present in all prokaryotes) as being useful features to determine species relatedness. As mentioned previously, it is possible that the prompt itself cued students to provide a particular response, because the prompt specifically uses the words "gene" and "species relatedness". Both of these words may account for 'relatedness' being the most stated idea by students. It may also explain the observation that 41% of students responded "true" to question 1.5, which suggested that 16S rRNA is present in all organisms, rather than only prokaryotes. In addition, 7.8% of students suggested the alternative idea that differences in 16S rRNA confer observable phenotypic changes to organisms, providing utility. This may have been induced by the description that preceded the prompt, which reads:

> While observable traits (e.g. gills, wings) may inform species relatedness, this method can be unreliable, especially with bacteria! As an alternative, Carl Woese proposed analysis of the 16S subunit of ribosomal RNA (rRNA) to compare species relatedness. Briefly address the following questions based on this information:

Referring to 'observable traits' may have caused some students to conclude that 16S rRNA can alter observable traits, which is an incorrect idea. Two interviewees recalled using 16S rRNA in

their MICR 352: General Microbiology II course, which may also explain why 10.8% of students suggested the alternative idea that simplicity of 16S rRNA analysis is a useful feature to determine species relatedness. While this is true, it is not a specific feature of the rRNA that makes it reliable to determine evolutionary relatedness.

Analysis and Reasoning about 16S rRNA

After students responded to the comprehension level question about the 16S rRNA, students were asked to analyze a small piece of 16S rRNA sequence data, and determine the two most and two least related species. While more than one-third of students correctly identified the two most and two least related species (35%), it should be noted that due to a design flaw in the question, comparing each organismal sequence to the reference sequence could result in a correct response as well. Thus, to better understand how students reasoned about the 16S rRNA sequence data, self-reported written explanations (Figure 5) and in-person interviews were analyzed.

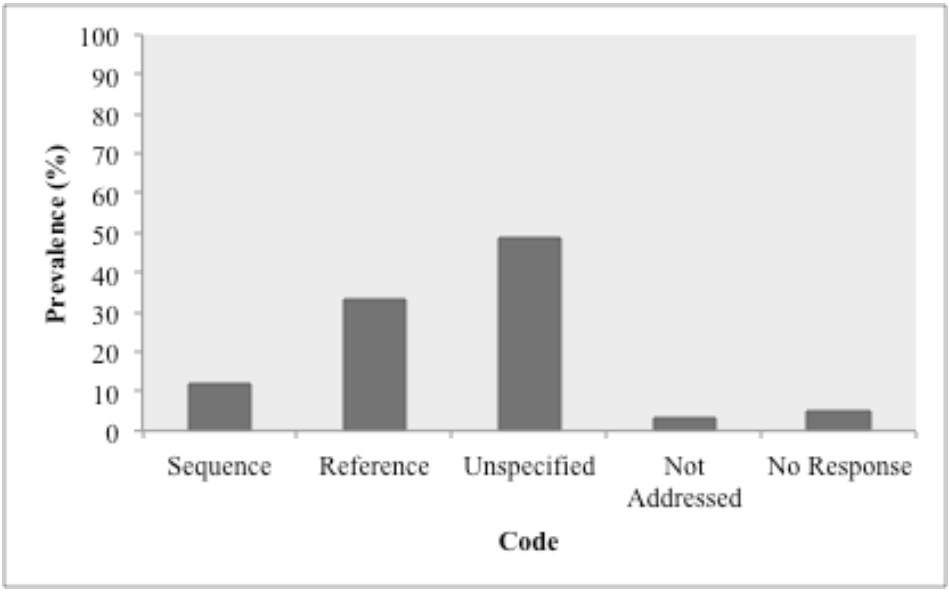

Figure 5. Q.1.3 Prevalence of Explanations. The percent inclusion of key concepts identified by students when asked to describe their analysis technique for 16S rRNA sequence data.

Nearly half of students provided a response that was unspecified (49%), meaning they did not specify, or were unclear, when describing their reasoning technique. Chi-square analysis revealed that low high school GPA and low college GPA corresponded to providing an unspecified response (p_{Holm}= 0.010). Vague responses have been referenced in literature, and are a problem for analysis. When students provide vague responses, interpretation is left fully to the coder. It is suspected that students who do not fully understand the question, or cannot justify their reasoning technique, may provide vague answers because it is often rewarded on exams (Newman et al., 2016).

Of the 45% of students who did provide a specified answer, more students utilized the reference sequence (33%) than compared between species (12%), suggesting that the majority of students did not understand what the reference sequence represents, or were distracted by the term. To support this observation, an analysis of correct response according to reasoning technique was performed. Results show that regardless if students utilized the reference sequence or the organismal sequences to solve the problem, students most frequently provided an incorrect response about the two most and two least related species (Figure 6). The observation that students infrequently employed the correct analysis method (12%), and most often still answered the question incorrectly when using the correct analysis method, indicates that students were unsure how to reason about this problem.

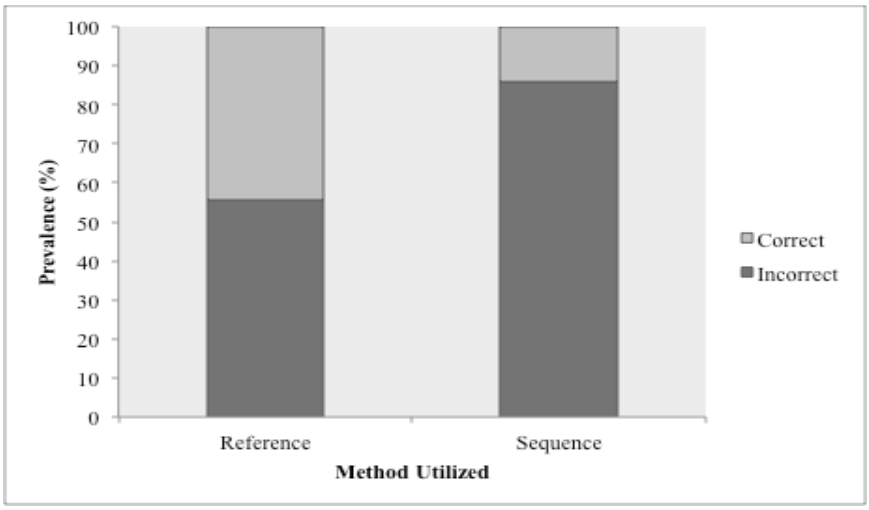

Figure 6. Percent Correct for Students Utilizing Reference or Organismal Sequences.

Interviews confirmed that students were determining the two most and two least related species from the 16S rRNA sequence data by several techniques, and often incorrectly. Each interview subject recognized that more nucleotide differences equate to more distantly related species. It was also observed that students were comparing sequences 1-4 to each other, and sequences 1-4 to the reference sequence. As reflected in written data, fewer students understood that the question asked them to compare sequences 1-4 to each other, with two interviewees employing this method alone. Similar to written data, more interviewees considered the reference sequence to be the most useful piece of information, as demonstrated by three interviewees who used it to determine their final answer. Interestingly, when one interviewee was asked what the sequences represent, her response was: "Well for organism one, it could have a tail for all we know. Then organism two could have legs. And organism three could have wings. And one could be a unicorn, that four. So…". Her response indicates that students may have understood a comparison needed to be made between sequences, but they were unsure of what was being compared.

One new reasoning technique infrequently mentioned in written responses (2.8%) was base pairing. At least two interviewees considered base pairing when analyzing the sequence data, suggesting it may be a more common technique than implied by written responses. To base pair, students used the reference sequence as their primary piece of information, and base paired sequences 1-4 to the reference sequence to determine species relatedness. They integrated the reasoning that fewer differences equal closer relatedness, and applied it to base pairing. Interestingly, interviewee one ignored nucleotides that were not highlighted, which influenced her decision to base pair between the reference sequence and sequences 1-4 during the assessment. She changed her reasoning during the interview and discarded the base-pairing technique, choosing only to use the reference sequence. It is not uncommon for students to alter their reasoning technique once probed about their approach (Adams & Wieman, 2011). Interviewee two stated that the highlighted sequences were her first focal point as well, and considered base pairing between sequences, although she ultimately used the reference sequence during the interview.

While none of the five interviewees referred to the letters as something other than nucleotides, unlike in the pilot study, question 1.4 suggests that many students in the population still reasoned about the tree using incorrect information about the letters in the 16S rRNA sequence. The question asked students to explain what the letters (A, C, T, G) represent in the 16S rRNA sequence data, and many students were unable to answer correctly (42%) (Figure 7).

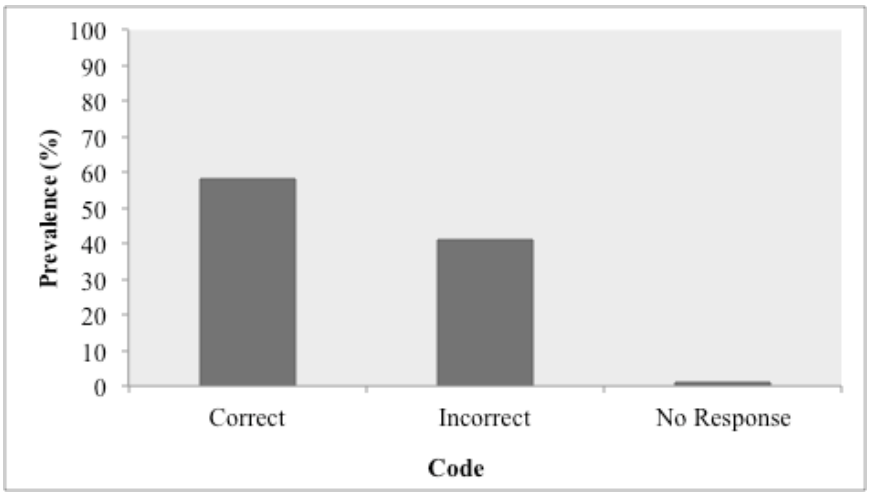

Figure 7. Q.1.4 Prevalence Correct, Incorrect, No Response. The percent correct, incorrect, and no response when students were asked what the letters (A,C,T,G) represent in the 16S rRNA sequence data.

While lack of familiarity with 16S rRNA sequence data analysis could be falsely inflating the proportion of students who incorrectly identified nucleotides, 3.5% of students specifically mentioned that the letters represented something other than nucleotides when they explained their approach, independent of question 1.4. Furthermore, previous studies have shown that some students struggle to identify nucleotides as the building blocks of DNA and RNA (Bowling et al., 2008; Newman et al., 2016; Smith et al., 2008). Thus, it is likely that a proportion of this student population shares the same difficulty.

Student use of Jargon

Results indicate that students may understand some discipline-specific jargon, which would influence the aforementioned results. For example, “conserved” is currently included under the presence code in question one, because it was unclear from student responses whether participants fully understood the meaning of the word. However, interview five suggests that some students may understand what conserved means in a biological context, as shown by the following transcription from interview five when asked about 16S rRNA:

Interviewer: Ok, so I want to go back to the word conserved, what do you mean by that?

Interviewee Five: It changes very little, it's changed very little over time.

Interviewer: Ok, do you have any thoughts for maybe why that is?

Interviewee Five: So for processes that are very um, very essential to the organism, and there's not a whole lot room for error, mutation's, um, a very important process that's very honed in, usually aren't advantageous.

Although the student may have alternative ideas about mutation, it seems she understood the meaning of "conserved". If further research showed that this is common, conserved should be moved to the "Mutation" code. Additionally, interviewee five revealed that she used "base pairs" as an analogous term for nucleotides, as shown via the following piece of transcript:

Interviewer: Ok, and the letters, I think you mentioned it but what do those represent?

Interviewee Five: Um, those are base pairs of the DNA, so guanine, thymine, cysteine, and adenine, cytosine.

This information suggests that students might use "base pairs" as an exchangeable term for nucleotides, which would alter the results of this study.

Analysis and Reasoning about Phylograms

Most Related Species

Most students were able to assess relatedness from the phylogram, and correctly identified the most related species (64%) (Figure 8). Interestingly, students' age ≥ 22 provided fewer correct responses than expected for this question. No other demographic factor was linked

to a correct response, so it is unclear why age corresponded to fewer correct responses. It may be that younger students more recently took General Biology I and General Biology II, and can more easily recall the necessary information to solve this problem.

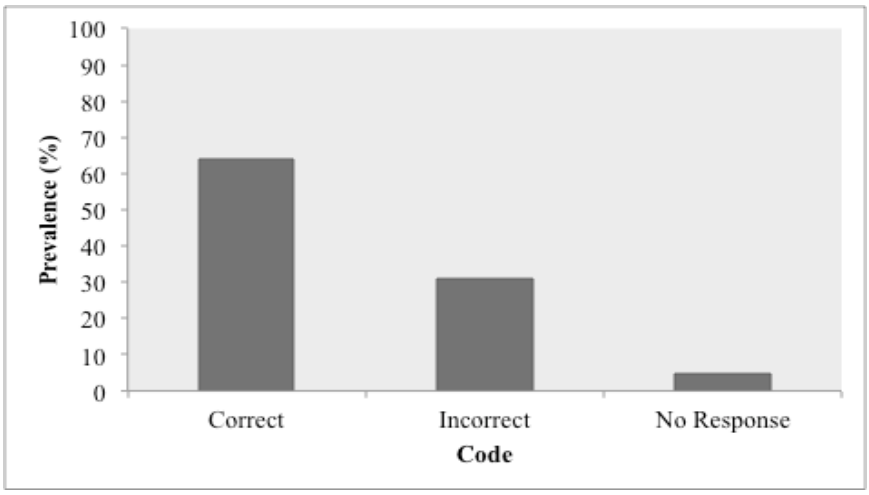

Figure 8. Q.2.6A Prevalence Correct, Incorrect, No Response.

To receive full credit for the question, students had to indicate that *Campylobacter jejuni* and *Escherichia coli* were the most related species. It seems that most students not only answered the question correctly, but also recognized that analysis of the most recent common ancestor was informative of the most related species (Table 11).

Table 11. *Tree Features used to Determine Most Related Species.*

Code	Numbers (%)	Proximity (%)	Branch Length (%)	Node (%)	Not Addressed (%)	No Response (%)
Correct	0	3.6	0.0	37.1		
Incorrect	21.6	6.0	0.6	19.2	9.0	12.0
Total Usage (%)	21.6	9.6	0.6	56.3		

The majority of students utilized the nodes to determine the most related species, which was a correct approach. Students also could have used reasoning of monophyletic grouping, which has been observed in the student population at the same institution, but this was not observed in this particular study (Dees et al., 2014). All other analysis techniques identified in student responses

(31.8%) were not useful to determine the most related species. A large proportion of students reasoned about the tree using numbers (21.6%), but it is likely that few of these students understood what the numbers represent, and may have even had an alternative idea about the genetic distance values. This was confirmed in interviews, where every interviewee explicitly stated that they did not know what the numbers represent. Except for one interviewee who correctly guessed that the numbers represent genetic distance, interviewees thought that the numbers might represent time, and it is likely a reasoning technique used by students who utilized numbers in the written responses. Some students wrote that *Bacillus thuringiensis* was most related to *Escherichia coli* because the numbers were closest in value, confirming that some students thought numbers represent time.

Genetic Distance

Question 2.7 asked students to assess the divergence of species represented in the phylogram. The purpose of this question was to test the features of the tree that are specific to phylograms: genetic change values and branch length. Fewer students correctly reasoned about divergence than relatedness, with 49% of students recognizing that *Escherichia coli* is genetically more similar to *Mycobacterium avium* than *Campylobacter jeju*ni, as shown by the genetic distance values. The descriptive profile of student reasoning is shown in Table 12.

Table 12. *Q.2.7B Tree Features and Correctness.*

Code	Numbers (%)	Proximity (%)	Branch Length (%)	Node (%)	Not Addressed (%)	No Response (%)
Correct	4.2	1.2	2.4	23.4		
Incorrect	38.3	7.8	0.6	12.0	5.4	8.4
Total	42.5	9.0	3.0	35.4		

The majority of students using a discernable reasoning technique (42.5%) recognized that numbers were a primary source of information provided on the phylogram to answer this

particular question. However, most of these students utilized the numbers incorrectly (38.3%) by subtracting genetic distance values, or doing a simple comparison of genetic distance values (i.e. which values are closest). This was reflected in the student interviews, where none of the students were sure what the numbers meant, but three out of five respondents reasoned that *Escherichia coli* was genetically more similar to *Mycobacterium avium*, because the numbers must indicate something important. Each interviewee who guessed *Escherichia coli* reasoned it was because the number values were closer than for *Campylobacter jejuni*. All of the interviewees noticed that branch length reflected the values of the genetic distance, which may explain why some students (3.0%) chose to use branch length as their reasoning technique in the written responses. One interviewee utilized proximity for their final answer, while at least two others considered proximity to be important. For example, interviewee five stated:

> …I that know the spatial relation right here has something to do with it but I don't know what that means… I know that there's a reason that you place this one, that you don't flip it the other way.

This student clearly displayed the common misconception that "branch tip proximity" represents relatedness (Dees et al., 2014; Gregory, 2008; Meir et al., 2007; Novick & Catley, 2013). Interviewee three suggested that the proximity of *Campylobacter jejuni* and *Mycobacterium avium* indicated that *Campylobacter jejuni* evolved "back into" *Mycobacterium avium*, reflecting how students might sometimes use "branch tip proximity" while also incorporating "contemporary descent".

Many students in the written responses applied cladogram analysis to the phylogram. Supporting this observation, interviewee four stated that *Campylobacter jejuni* and *Escherichia coli* were equally similar to *Mycobacterium avium* because most recent common ancestry

suggests so. Many students utilized the node (35.4%) and suggested that the organisms were equally related (28.7%), and students most commonly stated equally-related when using node as a reasoning technique, as shown in Figure 9.

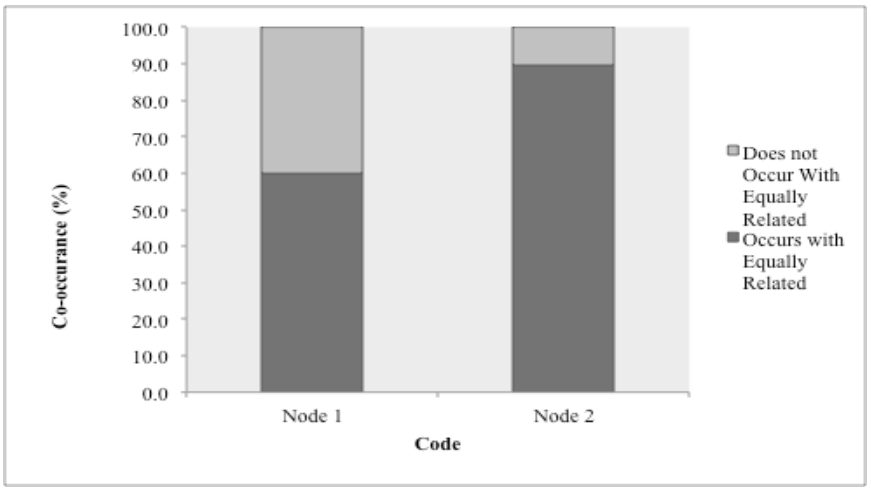

Figure 9. Co-occurrence of Equally Related with Node Explanation.

Chi-square analysis revealed that high GPA corresponded to students concluding that *Campylobacter jejuni* and *Escherichia coli* were equally related (p_{Holm}= 0.001) to *Mycobacterium avium* and using nodes (p_{Holm}= 0.001) as a reasoning technique. This observation is likely a reflection of cladograms being the primary type of phylogenetic tree taught in General Biology I and General Biology II at NDSU. Thus, high-achieving students applied cladogram analysis techniques to the phylogram.

Discussion

Student Knowledge and Analysis of 16S rRNA

Students have not retained 16s rRNA content

No published research exists to characterize student thinking about 16S rRNA. In this study, very few students identified two relevant and correct features of 16S rRNA that make it useful to determine species relatedness, and it seems the most utilized concepts may have been cued by the prompt. These results indicate that most students have not retained instruction on the

topic. Students who provided two correct answers were rare and reflect individuals who retained the information long term, or were able to recall information more quickly than other students, indicating a greater shift from novice to expert thinking in certain students (Dittinger et al., 2016; Rikers et al., 2003; Chi et al., 1988).

It is possible that many students did not recall information on 16S rRNA because it was taught using LOCS, or time-on-task was limited, which decreases understanding and long-term retention (Gijselaers & Schmidt, 1995; Seifert & Beck, 1984; Stalling, 1980; Reynolds and Walberg, 1991; Admiraal et al., 1999). Nonetheless, a study on medical students, who are presumably highly-motivated students with previous content exposure and interest, revealed that students forget as much as 25% to 35% of basic science knowledge after one year, and more than 50% the next year (Custers, 2010). Even if there are few factors inhibiting student learning and retention, the ability to recall comprehension level questions will decline over time, simply because information is eventually lost without application and repetition. In addition, if students do not recall information about 16S rRNA with ease, this likely reduces their confidence about the subject material. Although there is conflicting data about confidence and assessment performance, it cannot be ruled out as a motivation factor for answering the question (Chui et al., 2013; Clayson, 2005; Liaw et al., 2012). Inability to recall content with ease may be due to the nature of the content. Translation happens on a microscopic level, so it may be difficult to conceptualize about 16S rRNA and integrate it into existing resources.

16S rRNA sequence data analysis skills are lacking

A lack of content knowledge about 16S rRNA was demonstrated via the comprehension question, but research in math and chemistry education has shown that students can have procedural knowledge without conceptual knowledge (Heyworth, 1999; Rittle-Johnson, 1999).

For example, a student can memorize an equation but not understand the origins or the reasoning behind it. Approximately one-third of students answered the 16S rRNA sequence analysis question correctly, suggesting they understood the method of comparison. However, detailed explanations were often unspecified or illogical, and when students did provide a discernable reasoning technique, they were most often incorrect. This observation may be due to students rushing through the problem or miscounting, but most likely, students did not fully understand the concept of counting differences between organismal sequences, and there is likely a lack of both procedural and conceptual knowledge about 16S rRNA. Newman and colleagues speculate that students provide vague answers when they are uncertain or unconfident about their reasoning, explaining the frequent occurrence of unspecified responses (2016).

Students struggle with basic genetics concepts

Pilot interviews suggested that some students might not identify nucleotides in a DNA sequence. This observation was corroborated in the main study, as well as student difficulties with the central dogma. Furthermore, research in genetics education supports these findings. Newman et al. found that students struggle to identify the molecules needed for transcription and translation (2016). Additionally, they found that students generally struggle to identify identical chemical groups in DNA and RNA, and often do not recognize nucleotides as the building blocks (Newman et al., 2016). Similar studies have shown that students struggle to describe the molecular makeup of genes and genomes as well (Bowling et al., 2007; Smith et al., 2008). If students struggle with basic genetics concepts, it may be unfair to assume that they are able to properly analyze 16S rRNA sequence data, which may have contributed to incorrect analysis of the sequence data and fewer respondents explaining their reasoning techniques.

Student Knowledge and Analysis of Phylograms

Although phylograms have been described in education research literature, they are not often used to assess student reasoning about phylogenetic trees (Baum & Offner, 2008; Gregory, 2008; Sandvik, 2008). One study found that experts draw upon phylograms to explain evolutionary relationships, but students do not (Halverson et al., 2011). Sandvik notes that phylograms are more difficult than cladograms for students to understand, and argues that phylograms may convey inaccurate information by compressing a multi-dimensional analysis into a "two dimensional compromise", warranting removal from instruction (2008). However, this advice should not be generalized to all fields of study. Although phylograms add an additional feature to the tree, genetic distance, they are important in microbiology. Phylograms are highly useful when comparing two strains in the same species, such as when researchers are tracking pathogen emergence. Changes in bacterial and viral genomes such as transduction and mutation can confer lethal pathogenic traits in very little time with massive consequences to human health, and phylograms facilitate analysis of such changes by mapping genetic distance values (Chin et al., 2011; Feng et al., 1998; Johnson, 2015). Thus, this study attempted to characterize student reasoning about phylograms, because microbiology students interested in clinical microbiology, epidemiology, or related research should understand phylograms to practice in their field.

Students most often utilize nodes to determine most related species

In written responses and in interviews, nodes were the most frequently used feature to reason about the phylogram, although many students utilized nodes incorrectly. Previous research confirms that some students count nodes to analyze phylogenetic trees, as much as 38% of the student population, but this was not apparent in written responses collected in this study

(Dees et al., 2014; Perry et al., 2008). During interviews, however, three participants described *Campylobacter jejuni* and *Escherichia coli* as being at the end of the tree, and one interviewee referred to the tree as having "levels", suggesting that node counting might have been a strategy for determining the most related species. Additionally, while some interviewees recognized that nodes represent common ancestors, at least two did not. Students described nodes as a mutation event, a perspective that suggests the node indicates an exact moment, or does not change along the branch, as described by Gregory (2008). One interviewee went on to say that branch length represented how long the species was "conserved", and another interviewee who acknowledged that nodes represent ancestors still referred to them as "a point of evolution". While, "a point of evolution" is similar to the idea of divergence, it is incorrect because evolution is continuous along branches. Perry and colleagues (2008) found that as many as 40% of the student population believes organisms do not change along branches, and similar ideas have been observed in other studies (Baum et al., 2005; Meir et al., 2007). Baum and colleagues (2005) expand on this idea by indicating that students sometimes think trait change only happens at nodes, an idea that might be suggested in students' drawings of phylogenetic trees in a study by Dees & Momsen, where branch tips are left unlabeled along straight lines on ladder trees (2016).

Alternatively, it is possible that students were simply noting the direction of the tree, which has been linked to student reasoning (Novick et al., 2012; Dees & Momsen, 2016). Evidence of students reasoning about the direction of trees is shown explicitly in a study by Dees & Momsen, where students drew arrowheads at external nodes to indicate the direction of the tree (2016). It is also known that students generally reason more accurately about bracket trees, and trees drawn in a downward right direction (Novick et al., 2012, Dees & Momsen, 2016). One study showed that the number of students able to analyze species relatedness nearly doubled

when questioned using a bracket tree versus a ladder tree (Novick & Catley, 2013). Students in this study tended to read the phylogenetic tree in a downward right direction, concluding *Campylobacter jejuni* and *Escherichia coli* were most related because they were "at the end" of the tree, and the high occurrence of correct answers for species relatedness could be due to the use of a bracket tree. However, research has shown that as much as 31% of the student population interprets the flow of time on trees incorrectly, which may have contributed to incorrect reasoning in other students' answers (Perry, 2008). Four out of five interviewees concluded that branch length represented time, rather than genetic distance. The authors in this study suspect that genetic distance values were a significant distractor, fostered by "partial knowledge" where students applied cladogram thinking to the phylogram despite a lack of knowledge about the numbers represented on the tree (Burton, 2002).

Branch proximity was used to reason about divergence

The use of branch proximity as a reasoning technique was a common occurrence in this study. Some students suggested that *Campylobacter jejuni* was more closely related to *Mycobacterium avium* in the divergence question because they were closer in proximity, despite genetic distance values. Interestingly, these students also ignored most recent common ancestor and monophyletic grouping, which would be also be correct reasoning strategies (Gregory, 2008). Novick & Catley found that students are more often inaccurate when referencing proximity in their explanations (2013). Previous research shows that it is a persistent idea in student populations; two separate studies found that 24% of the student population utilizes proximity to determine species relatedness (Meir et al., 2007; Perry et al., 2008), while Dees et al. observed a similar proportion of students (21%) early in the semester (2014). Interestingly,

Dees et al. found that the idea persistent in student populations, with 9% of students utilizing it as a reasoning technique on final exams (2014).

Additional misconceptions were uncovered in interviews

"Contemporary descent" was suggested by one interviewee who reasoned that *Campylobacter jejuni* was more closely related to *Mycobacterium avium* because it "evolved back" into it. His wording suggests that *Campylobacter jejuni* evolved from *Mycobacterium avium*. Observations of contemporary descent were noted in research early on (Baum et al. 2005; Baum & Offner, 2008). Meir et al. confirmed contemporary descent as a reasoning technique when students changed their reasoning about ancestry depending on rotation of the nodes (2007). One study observed low frequency of "contemporary descent as a reasoning technique, as low as 5% after targeted instruction (Dees et al., 2014). However, an alternate study showed that it could be as high as 23% (Dees & Momsen, 2016).

While it was expected that this phylogram would limit "external insights" by using bacteria, this expectation was challenged. One interview subject initially reasoned that shorter branches on the phylogram might represent extinct species, but knowing that *Escherichia coli* is still a species, she adapted her reasoning so that the branch length represented the amount of time it took for species to evolve. Although both explanations are incorrect, it is a clear example of a student changing their reasoning based on external insights. This corroborates the idea that previous knowledge can be a limiting factor in tree-thinking. Novick & Catley found that "external insights" so extensively interfered with cladogram analysis that 45% of students with a strong background in biology and 73% of students with a weak background in biology incorrectly analyzed a cladogram due to conflicting prior taxa knowledge (2013). It is possible

that microbiology majors reason about microorganisms on a phylogenetic tree similar to the way that biology students reasoning about animals or plants.

Limitations of the Study

Lack of expert and non-major responses

Expert and novice responses are useful for assessment development because they serve as an indication of the level of student reasoning (Adams & Wieman, 2011). Adams & Wieman recommend using topic experts or instructors for expert responses, but advanced students can be used as well (2011). Bowling et al. used graduate students as experts, and psychology undergraduate students as novices; this allowed them to gain an informative continuum of student responses (2007). This work did not include the use of expert responses or non-major responses. Future work should include expert-like responses, and possibly non-science undergraduate student responses, to identify a wider spectrum of student responses, as well as uncover additional alternative ideas.

Prompt Wording

A five-year-long study with 6,100 student participants conducted by Schurmeier et al. found that small changes in wording has a significant impact on student assessment performance (2010). For example, by asking students to "state" rather than "suggest" an answer, correct responses increased significantly from 40% to 54% on an assessment question (Crisp et al., 2008). Removing the direction to describe "features" of 16S rRNA in the comprehension question may have caused the prompt to lose specificity, and performance to be underestimated. Prompt rewording should be considered for the 16S rRNA sequence data and phylogram as well. The "reference sequence" label on the 16S rRNA sequence should have been "consensus sequence" because "reference sequence" suggests all of the sequences are the same species,

while the phylogram prompt should consider asking about divergence rather than the "more related" species. Future work should explore labeling effects on student responses.

Interpretation of Jargon used by Students

Jargon is often a concern when assessing students. For example, Smith et al. were unsure how students interpreted the term "parsimony" when describing tree-thinking reasoning, and took this into consideration for data analysis (2013). In this study, jargon was a concern as well, and results may change after rubric revision due to student comprehension of terms. Students may understand the meaning of "conserved", which would increase the prevalence of the mutation code. Similarly, students may be able to identify nucleotides if they refer to them as base pairs. However, further research would be necessary to determine how frequently students correctly name nucleotides after using the term "base pairs", as well as determining if students can distinguish why "base pairs" is incorrect. Also, the nucleotide names sound similar to other organic molecules, such as cysteine (as used above) and thiamine (pronounced similar to thymine), so questions targeting similar-sounding words should be considered.

Applications of the Study

The results of this study should be used to inform teaching in science. Diagrams are common to any field of science, from flow charts to gene maps (Novick & Catley, 2013). The ability for students to read diagrams is important for their professional development, as it teaches students to organize abstract topics using conventional rules, and practice problem solving skills (Novick & Catley, 2013). In addition, analysis skills are considered HOCS, which increases the likelihood of LTR (Halpern & Hakel, 2003).

Halpern & Hakel refer to long-term retention (LTR) as "the first and only goal of" education (2003). LTR requires many factors controlled by the instructor such as repetition, use

of HOCS, and inclusion of diverse contexts (Halpern & Hakel, 2003; Nehm et al., 2012; Nehm & Ha, 2011). For example, Young & Anderson found that by using a personal narrative in clinical case studies, where the patient's personal concerns, family situation, treatment goals etc. are used to construct prompts rather than using dense jargon—LTR increased from 42% correct to 90% correct on multiple choice questions (2010). This study emphasizes the importance of teaching beyond a single, traditional context. Since the prokaryotic 16S rRNA is homologous to eukaryotic 18S rRNA, and provides an essential function to the ribosome, there is ample opportunity for instruction using diverse contexts, and should be considered for targeted microbiology instruction in the future.

More specifically, this study provides additional context for instruction in natural selection, genetics, and tree-thinking. Using 16S rRNA as a natural selection question would provide a unique prompt that requires unconventional application of the key concepts of natural selection identified by Bray Speth et al. (2009). Some key concepts would be applied the same as any other context, such as inheritance. 16S rRNA is inheritable. However, the other key concepts require more complex reasoning. Reasoning about origin of variation in the context of 16S rRNA requires multiple factors. While 16S rRNA mutates, mutations are often not tolerated due to its highly conserved function, which is opposite of the conventional idea that mutation happens at a reasonably constant rate. Also, bacteria are asexual organisms, so sexual recombination does not contribute to origin of variation. Furthermore, because of conserved function and asexual reproduction of bacteria, 16S rRNA varies very little within species, and changes very little on the population level over time. Lastly, relating 16S rRNA to fitness is more abstract because observable traits are more often associated with fitness. Translation is a microscopic process, but it would obliterate the fitness of any organism if it were dysfunctional, because the organism

would not be viable. For these reasons, 16S rRNA would likely be a challenging context for students to reason about natural selection, but would provide a unique context nonetheless. Future work should include correlational data between descriptive responses for question one and biology coursework, and revisions of the rubric should consider the natural selection key concepts (Brey Speth et al., 2009).

Phylograms have been largely left out of literature due to their perceived difficulty and limitations (Sandvik, 2008). However, phylograms are increasingly important in microbiology, where pathogen emergence studies rely on the ability to depict small genetic changes within species. The origin of the *Vibrio cholerae* strain of the Haitian cholera outbreak in 2010, where 470,000 cases and 6,631 deaths occurred in a single year, was revealed by phylogram construction (Chin et al., 2011). The results of this study suggest that more focus may be necessary to expose microbiology students to phylograms, but these findings are applicable to fields outside of microbiology as well. The purpose of a phylogram is to map the degree of genetic divergence, while cladograms map overall relatedness, with no quantification of similarity. Furthermore, it seems phylograms might be useful to uncover misconceptions found previously in literature, such as those related to branch length, time axes, and the concept of "higher" and "lower" organisms (Gregory, 2008). Since branch length is different for each organism on a phylogram, it is an appropriate construct to probe for misconceptions. The variation in branch length also misled students to reason incorrectly about time represented on a phylogenetic tree, which is an issue with cladograms as well. Here, at least one student reasoned that short branches represent extinct species, and long branches represent present day species. Future work to uncover detailed explanations of time related to branch length may provide additional insight into student reasoning about time axes on phylograms. Lastly, microorganisms

are an appropriate context to probe for the idea of "higher" and "lower" organisms. Students tend to think that "lower" organisms evolved first, and that evolution moved in a direction that results in "higher organisms" (Gregory, 2008; Omland et al., 2008). Since bacteria are the oldest organisms on the tree of life, they are useful to test for this idea. Although not often, the idea that bacteria are "lower" organisms was mentioned in written responses here, and is indicated in the coding rubric (Table 6). In summary, these questions have the potential to become a useful assessment tool to test students' understanding of evolution-related learning outcomes in microbiology, and for instruction in natural selection and tree-thinking.

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APPENDIX A. PROMPT DRAFTS

Common Data Set

1. What is evolution? Briefly explain in your own words.

2. Briefly describe the following terms in your own words, and circle if they're random or non-random processes:

   Natural Selection (Random/Non-Random)

   Genetic Drift (Random/Non-Random)

   Fitness (Random/Non-Random)

Question Data Set #1

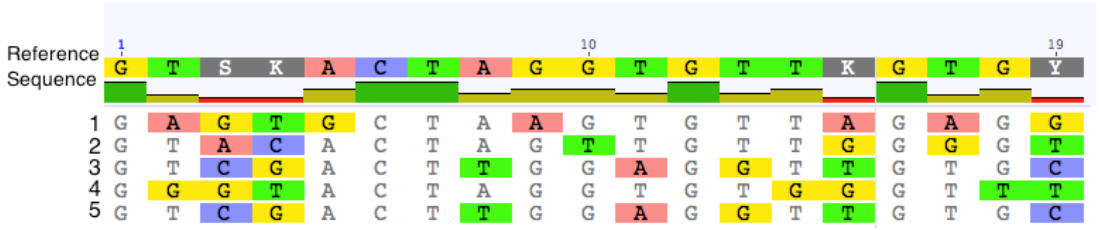
To determine genetic relatedness of organisms biologists typically observe shared and disparate traits. One observation is that metabolic pathways are universal among all living species, such as glycolysis and the Kreb's cycle. However, membrane bound organelles are found only in eukaryotic species, such as the mitochondria and chloroplast. Briefly answer the following questions based off of this observation:

1. Explain why metabolic pathways such as glycolysis and the Kreb's cycle are so highly conserved in all living cells.

2. Why are membrane bound organelles such as the mitochondria and chloroplast found in eukaryotic cells, but not prokaryotic cells?

While observed traits tell us a lot about whether a species is closely related or not, it is not always obvious. For instance, mitochondria are found in all animal cells, so it is not a reliable method to tell animal species apart. To circumvent the difficulty of identifying species based on observable traits, scientist Carl Woese determined an alternative method: analysis of the 16S subunit of ribosomal RNA (rRNA). The 16S rRNA is a component of the genetic replication machinery found in all living cells. Briefly address the following questions:

3. Determine the two most related and two least related organisms from the short list (1-5) of aligned 16S rRNA sequences below:

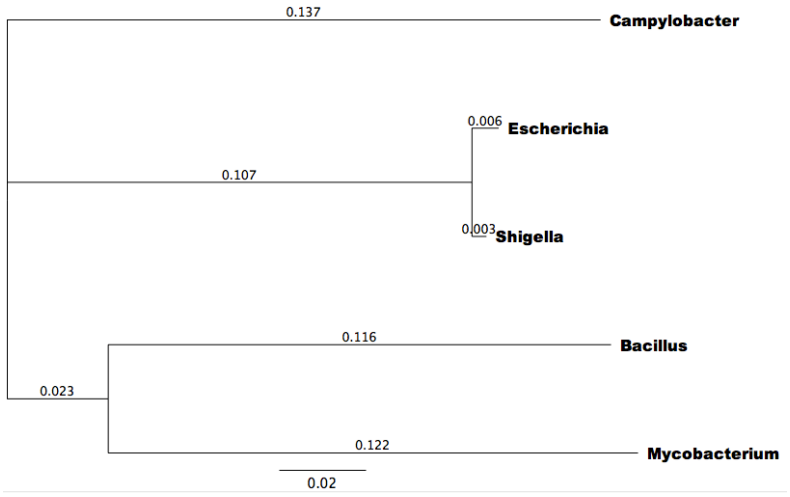


Two Most Related: 1 2 3 4 5

Two Least Related: 1 2 3 4 5

4. Explain the features of 16S rRNA that make it useful to determine species relatedness?

16S rRNA data can be used to determine phylogenetic trees to depict genetic relatedness. The phylogenetic tree below represents the species in the previous 16S RNA data; however, this time you can see the names of the genera.



5. Which two genera are most related, and which two are least related according to the phylogenetic tree above?

Question Data Set #2

Food pathogen outbreaks are quite common in the United States and around the world. Common food pathogens include *Pseudomonas aeruginosa*, *Escherichia coli*, and *Yersinia enterocolitica*. All three of these pathogens are different species, yet they encode and conserve the same type III secretion system (T3SS) in their genome. T3SS is considered a pathogenicity island, a type of genomic island that confers virulence on its host. Briefly address the following question based on this observation:

[**Note**: a genomic island is a linked cluster of genes.]

1. How would a microbiologist explain how the T3SS came to be found in these diverse bacteria?

Interestingly, *Salmonella*, another common food pathogen with the T3SS, shares approximately 2,040 genes, and has 96% 16S rRNA sequence identity to *E. coli*. Yet, they are considered different species. Briefly address the following questions explaining this observation:

2. Describe the concept of a species with regard to a core genome and genomic islands.

3. Explain why the traditional definitions of species using reproductive isolation do not apply to bacteria like *E. coli* and *Salmonella*?

Typically, antimicrobials are used to clear infections from humans and animals, such as those infected by foodborne illness. However, this is not their only use. Briefly address the following questions:

4. Describe at least two human practices in agriculture that may have led to an increase of antimicrobial resistance in *E. coli*.

5. Explain how not completing a full treatment of antibiotics can lead to an eventual increase in resistance in a bacterial population over time.

6. Why would the T3SS be an attractive antibiotic target to evade antimicrobial resistance?

Synthesis Question #1:

Biological molecules in *E. coli* function well around 37^{o}C (body temperature). However when temperature rises (e.g. when we get fevers) the heat-shock chaperone system must kick in to prevent denaturation of proteins, which is their primary function.

Suppose you have two colonies of E. coli adapted to growing at 37^{o}C:

1. A clonal (i.e. identical) colony with genetically identical heat-shock chaperone systems and
2. A diverse colony of *E. coli* with genetically variable heat-shock chaperone systems

Propose an experiment to measure the survival of the clonal colony vs. the diverse colony when they are moved to a 65°C environment; state your hypothesis and explain why you chose the higher-surviving population to do better, and the lower-surviving population to do worse.

Hypothesis:

Experiment:

Explain:

Synthesis Question #2:

Dr. Richard E. Lenski is famous for his long-term evolution experiments where he performs daily passages of mixed population *E. coli* in glucose limiting media (0.5% citrate) in a constant environment. *E. coli* preferentially metabolize glucose, and will not metabolize citrate unless they are in anaerobic conditions.

However, after 31,500 generations this aerobic population of *E. coli* evolved to metabolize citrate in addition to glucose.

[**Note**: *E. coli* also produces citrate as a metabolic byproduct, so there are significant citrate concentrations in culture.

1. Hypothesize why a population of aerobic *E. coli* evolved this trait when glucose supplies were refreshed daily.

2. Explain the process by which traits originate and become the dominant trait in a population under constant environmental conditions.

APPENDIX B. PILOT INTERVIEW PROTOCOL

Interview Protocol

Directions

Interviews will last approximately one hour. The interviewer will ask the interviewee to repeat the question written on the paper, so that they can collect the answer and request justification as to why the interviewee believes that is the best answer. The interviewee is allowed to take as much time as possible, and can write on the paper as needed. Interviewer is to cut off answers when they become non-productive.

Explain to interviewee

While the interviewer is ultimately responsible for making sure that all questions in the interview guide are addressed during the interview, explain that you are there to gain from the participant's own knowledge about the research topic, not to correct ideas or dispense advice. Assure the participant that there are no right or wrong answers; it is his or her personal knowledge and perspective that are of interest to the study.

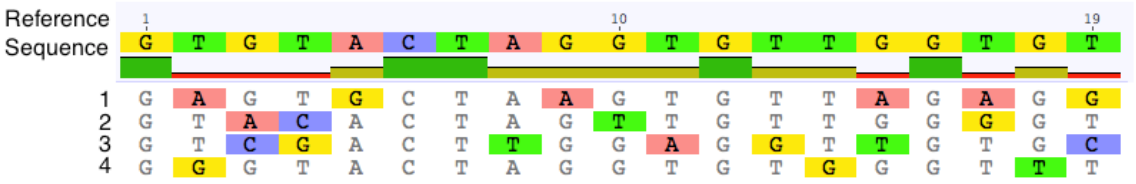
[Review informed consent document and obtain signature]

Question 00. Begin by asking where they're from, about classes they're currently enrolled in, when they're graduating etc.

Question One

While observed traits tell us a lot about whether a species is closely related or not, it is not always obvious. For instance, mitochondria are found in all animal cells, so it is not a reliable method to tell animal species apart. To circumvent the difficulty of identifying species based on observable traits, scientist Carl Woese determined an alternative method: analysis of the 16S subunit of ribosomal RNA (rRNA). The 16S rRNA is a component of the genetic replication machinery found in all living cells. Briefly address the following questions:

6. Determine the two most related and two least related organisms from the short list (1-4) of aligned 16S rRNA sequences below:



Two Most Related: 1 2 3 4

Two Least Related: 1 2 3 4

- Ask participant to demonstrate how they would determine the most and least related species by writing it out on the page.
- Did participant compare to each other or reference sequence?

7. What features of the 16S rRNA sequence data allowed you to determine the most and least related species?

   If Confusing:

   - How did you determine your answer?
   - What are the most important features of the data that allowed you to determine your answer?

   Possible Replies:

- Count Single Nucleotide Polymorphisms (SNPs) or Mutations
  - What are SNPs (or mutations)?
  - How do SNPs (or mutations) arise in a genome?
  - Mutations can be good, bad, or neutral. Does this apply to SNPs? Are SNPs generally more good, bad, or neutral?
- Compared to the reference sequence
  - If someone didn't know what a reference sequence was, how would you describe it?
  - Related Questions
- What do the individual letters in the data represent?
- Why do letters differ from sequence to sequence? What causes the change?
- If someone didn't know what a reference sequence was, and how to use it, what would you tell them?
- What types of limitations of the 16S rRNA have you learned about?
- Counting nucleotide differences is a common way to analyze 16S rRNA data, what other types sequence analysis methods have you learned about?

8. <u>Explain the features of the 16S rRNA gene that make it useful to determine species relatedness.</u>

If Confusing:

- *Why is 16S rRNA useful to determine species relatedness?*

Possible Replies:

- Differences/Similarities in Sequence Inform Species Relatedness
  - How do these differences arise?
  - Why do more differences mean they're more distantly related?
  - How do we determine when a species has diverged?
- Unique to species
  - Why is every sequence unique to a species?
    - If every sequence is unique, how do we determine relatedness?
  - How do we tell when a compared sequence represents a new species?
- Compare
  - What elements do we look for in a comparison that provide us with information on relatedness?
- Conserved
  - Every organism: restate answer for confirmation

- All microorganisms: restate answer for confirmation
- In bacteria: do animals and plants have something different, or do they also contain 16s rRNA?
- In prokaryotes: how does 16s rRNA sequencing apply to eukaryotes?

Related Questions:

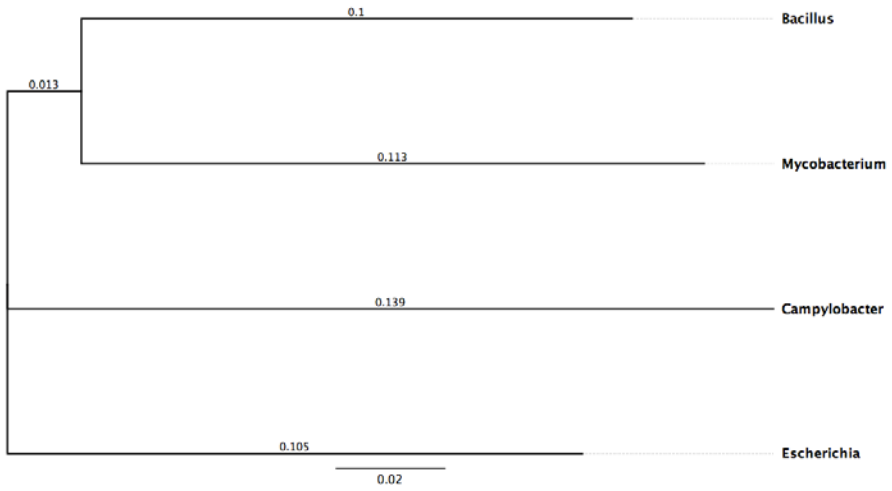
- So it seems like 16S rRNA is pretty useful. If we wanted to use the 16s rRNA of bacteria and perform a comparison with yeast (a eukaryote), how would we do that?

Follow up Questions:

9. *Did the wording of these questions confuse you at all?*
10. *Do you have suggestions for how we could ask this question more clearly or concisely, while still getting the information you need to answer the question?*

Question Two

16S rRNA data can be used to develop phylogenetic trees to depict genetic relatedness. The phylogenetic tree above represents the species based on the partial 16S RNA gene sequence data from the previous questions; however, this time you can see the names of the genera.



1. **<u>Based on this phylogram, which two genera are most related, and which two are least related according to the phylogenetic tree above?</u>**

2. **<u>Explain which features of the phylogenetic tree allowed you to determine the most and least related organisms.</u>**

If Confusing:

- *How did you use the tree to make your decision? Which parts were the most important?*

Possible Replies:

- Numbers
    - How did you utilize the numbers on the tree to determine distance?
- Nodes
    - What do the nodes tell you?
    - How does this help you determine relatedness?
- Branches
    - What are the branches?
    - Why are they different lengths?
- Distance
    - What do you mean by distance?
    - How did you determine distance?

Related Questions:

- Do the order of the branches provide us information?
- What types of phylogenetic trees have you seen? (Provide examples)

Question Three: Definitions

Paraphrase the answers that respondents give and allow them to elaborate. If answers are completely wrong and you cannot get valuable information about the definitions, move on. This symbol: ❖ denotes questions to ask every participant. Below are key words to probe if they mention them:

Evolution:

- *Phenotype:* Ask for clarification. How is it different from genotype?
- *Genotype*: Ask for clarification. How is it different from phenotype?
- *Allele:* Ask for clarification. How does it relate to phenotype and genotype?
- *Adaptation:* Ask for clarification. How does environment influence it?
- *Selective advantage:* what is an example of a selective advantage?
- *Change:* what drives change?
- A popular response has been that evolution applies to biotic and abiotic things in the universe. What do you think?
- Does evolution happen on the individual level, or population level?
- How do we define new species?
- How do changes arise that eventually lead to new species?
- Are we evolving right now?

Natural Selection:

- *Environment:* what is an environment? How does it influence selection?
- *Thrive/Grow:* ask for clarification.
- *Selective advantage:* what is an example of a selective advantage?
- Does selection happen on the individual level, or on the population level?
- Adaptation is a term that comes up quite frequently when discussing natural selection, what is adaptation?
- Is natural selection random or nonrandom? Why?

Fitness:

- How does fitness in evolutionary terms differ from fitness in everyday terms?
- What did Darwin mean by the phrase "survival of the fittest"?
- Is fitness random or nonrandom? Why?
- Is fitness observed at the individual or population level?
- How do we measure fitness?

Genetic Drift:

- *Population:* does genetic drift happen on the individual level, or just the population level?
- *Changes in genome:* why do changes in the genome that contribute to genetic drift occur?
- *Codons:* explain what codons are.
- Does genetic drift happen on the individual or population level?
- Is genetic drift random or non-random? Why?

Genetic Polymorphism:

- *Genetic Code:* what specifically in the genetic code changes during mutation?
- *Variation of Alleles:* how does variation arise in alleles? What is the consequence?
- Are genetic polymorphisms observable?
- Are genetic polymorphisms generally beneficial, neutral, or detrimental?
- Is genetic polymorphism random or non-random? Why?

APPENDIX C. PILOT INTERVIEWS

Transcription of Pilot Interview One

Interviewer: So I'm going to ask you these questions and you're just going to explain them to me as you would explain them to anybody to the best of your knowledge as completely as you can. So we'll do that as the first pass, and then the second pass like I said we'll go through and do a revision and you can tell me, like oh it was maybe a little wordy, it was a little confusing.

Interviewee One: [agrees]

Interviewer: We should go through this again. So yeah, this is not about me trying to get correct ideas or incorrect ideas, this is about me just gaining your ideas and listening and if you do have any questions about the content at the end, I will answer them to the best of my ability and so if you have questions I will take notes and if I hear little key words, I might just write little key words. Other than that I'm going to try my best just to listen to you and let that do the work of everything.

Interviewee One: [agrees]

Interviewer: Alright, so let's get started for each question I want you to read it which will help us understand later whether it was just kind of too much and then answer the question how you would normally go about it. So let's start with the 16s rRNA question one. You can go whenever you're ready and if you do want to do some writing or thinking you're free to do that--

Interviewee One: Ok

Interviewer: At your own pace.

Interviewee One: Ok so the most related would be one and four, and least related would be two and three.

Interviewer: Cool, so how did you come to that conclusion?

Interviewee One: Looked at the different um allele sequences between the two species.

Interviewer: Perfect

Interviewee One: So one and four had I don't know, 5 or 6 differences, where two and three and 9, 10, 11 differences stuff like that.

Interviewer: Yeah. A bunch of differences, right? So for these differences, what exactly are they, do you know how they come about?

Interviewee One: Like mutations.

Interviewer: Yup, cool.

Interviewee One: Would be one of them. Um I can't think of what the word would be; not just mutations. Um, like allele um like allele switching so like if something is better recognized by a certain primer or something like that um…

Interviewer: Awesome, so a lot of the time mutations can be good, bad, neutral. Do the ones that typically arise in 16S rRNA typically fall in a certain category, are they just kind of, there?

Interviewee One: depends if they change the amino acid or not. So the amino acid to go from DNA, RNA, to protein, if that was changed, then it could cause harm to specific cells things like that. Or if it needed that mutation to better itself it could be good too as well. It kind of depends on the situation.

Interviewer: So it can be, it's just different based on different circumstances. Cool. So, looking at this data too there's a reference sequence there. If someone didn't know what a reference sequence was what would you tell them?

Interviewee One: A reference sequence is basically what you look at so if you have four different types of species and you want to know what species this, the reference sequence would be what you the one you compare it to. So the most similar it is to that reference sequence is the most related to that species.

Interviewer: Is the reference sequence just out there or something we create from

Interviewee One: It's something that's we've actually gone through and sequenced and then put it in a database and then we actually compare to that known sequence

Interviewer: So the letters represent, we were talking about alleles, so the letters represent alleles?

Interviewee One: Yes.

Interviewer: So counting the nucleotide differences. You were completely on with that. That's a really common way to analyze 16S rRNA data. Are there any other methods we that we could possibly use?

Interviewee One: Well there's PCR. Um, I don't know, it's another gel electrophoresis.

Interviewer: If you can't think of it don't worry. It's not a big deal.

Interviewee One: We just did it in the lab the other day, oh the stacking gels.

Interviewer: Cool.

Interviewee One: I guess that's really all I can think of right now.

Interviewer: Yeah, no problem. Let's move on to the second part of the question. So we already talked about what about the data allowed you to determine the most and least related species. Let's go on to explaining the general features of 16S rRNA that makes it so useful to determine species relatedness

Interviewee One: It's unique to every species.

Interviewer: So what makes it unique?

Interviewee One: No sequence within the 16S is the same between each species. So humans have very, well, I guess humans wouldn't be a very good example, every human person has a different 16S species, or 16S, um, sequence, so each species has a 16S species and may have one or two differentiations between each, I don't know, I don't know what to call them, each animal in that species. So, you can similarize it, or, the 16S that you know of you can actually similarize it to that species and you can actually tell if it's related to that species or not.

Interviewer: Awesome, so when, so we were talking about difference. So why is it that more differences equals, um, least related, like less related than if it were more similar of a sequence

Interviewee One: Basically it's not even the same species, so if you have one allele that's the same, it's in the same spot of the 16S, it's um, it's not going to be related, basically, like there's a 1% chance, if you have 100 alleles there's a 1% chance that it's related, versus if you have 99 of those alleles

matching that then there's 99% alleles, there's a 99% chance that it's related to that.

Interviewer: Cool, awesome. So we're kind of getting into it a little bit. Is there a way to tell if a species has diverged from this type of data?

Interviewee One: Well, basically, if it's different allele changes. So, um basically it can be related. But if it has two, three, or four different alleles, it can actually have, become a subset of that species.

Interviewer: So basically, I think we're kind of getting at, the number of differences, so there might be a certain cutoff or something?

Interviewee One: Yeah.

Interviewer: So, also there are snacks here if you want snacks. I brought some, I didn't know what to bring so I just brought a variety of things. Alright, awesome. Ok so, it seems like 16S RNA is pretty useful. If we wanted to build a tree we could basically take 16S of different organisms and kind of compare them. Um, cool. We can move on to the phylogenetic tree. So I'll have you read that.

Interviewee One: Ok, so the most related would be the *Bacillus* and *Mycobacterium*. And the least related would be, um well it's hard to tell because they're all semi related because they all come off the same branch. Um, probably would be like, the *Bacillus* and *Mycobacterium* and then like *E. coli* or *Campylobacter* like those probably would be least related compared to *Bacillus* and *Mycobacterium*.

Interviewer: So, Um.

Interviewee One: Either *Bacilli Mycobacterium* or, least related compared to *E. coli*, or *Escherichia*.

Interviewer: Ok, so *E. coli* compared to *Bacillus* and the *Myco*?

Interviewee One: Yeah.

Interviewer: Cool. Alright, so what features of the tree did you use to help you inform that decision?

Interviewee One: Branching. So looking at the tree you have this one branch and then the *Bacillus* and *Mycobacterium* both come off that same branch so that means they're more related than they are to *Campylobacter* or *E. coli*, so looking at that. For least related I guess it's, it's hard to tell because you have you're your these are all off of one branch over here somewhere and then so these are all somewhat related but they're not as related as the *Bacillus* and *Mycobacteriu*m would be.

Interviewer: Yeah, so we have all of these different parts. So you're talking about branching so there would be a common ancestor kind of somewhere over here. So then how are you, how are you informing your decision of where the common ancestor would be? Like what are, like where would the common ancestor of *Bacillus* and *Mycobacterium* fall on this phylogenetic tree?

Interviewee One: Ummm.

Interviewer: Seems like you're a little familiar with them.

Interviewee One: Yeah, right here would be a common ancestor for these two.

Interviewer: And then do we have, so there are numbers on this tree, um what do those numbers represent?

Interviewee One: No, idea, I've never used numbers on a phylo.

Interviewer: No problem, it can be a little different and that's really common. So and then also these are different lengths…

Interviewee One: Ok, yup.

Interviewer: Do these, does that inform us of anything?

Interviewee One: How far away they are from the similarities of the ancestor. [Amount of differences?]

Interviewer: Cool, perfect. Alright, Ok so we can move on to definitions. So these are a little more ambiguous, obviously, because I just had people do free responses of what they think about these different topics. So we can just go through these and you can give me a definition as much as you can about each of the topics and then I'll just try to get a little clarification on each your response, so.

Interviewee One: So evolution is basically the change in the either the genotype or phenotype of a species so you can either see physical appearances or changes or you'll, um, or if you look at their DNA, um so you if you look at their different alleles different base pairs things like that they would be different because the genome so you might not actually see the differences but they could be there. Um, it usually happens over time so people are always evolving for reasons that, so that they can better survive and things like that.

Interviewer: Awesome, that was pretty good. A common response I've gotten is that evolution applies to biotic and abiotic things in the universe. What do you think about that response?

Interviewee One: Biotic and abiotic? Um I guess kind of yes, and no. Um, So biotic species, yeah they have, they're able to, um evolve, so are abiotic species because the thing is that we thing that we all think that we came from some sort of small little microorganism that had been there forever, um so it had to of evolved somehow, but maybe unsure of maybe how it evolves is different because of different structures different things being there present because you can't actually see physical changes in the bacteria but you can see like different organisms within the bacteria changing, maybe, but you can't see like. It's always going to be rod shaped, always going to be cocci shaped, things like that so.

Interviewer: Yeah, sure, so um. How should I word this? So, those species diverged at one point, right?

Interviewee One: Mmhmm.

Interviewer: Um so, is that just basically along that trajectory, that's why it will kind of never change? So since they diverged in this little path, right, is that why it would never, go, like a bacilli wouldn't become a cocci. You know what I mean?

Interviewee One: Yes and no, I mean they can change somewhat but I mean, they might not necessarily, it might not be physically seen right away, um so like I know like there are like really really really tiny bacilli that may have at one point

kind of been a cocci that eventually evolved into a rod shaped um so, it I mean it could potentially become different shapes but basically it would take a long long time for you to even notice it.

Interviewer: Yeah, cool, so what do you think about the abiotic part of that answer, that I've gotten pretty commonly?

Interviewee One: I guess I don't really, abiotic is, what do you mean by abiotic?

Interviewer: Non-living things, Evolution applying to non-living things.

Interviewee One: Probably doesn't happen. {shakes head} Can't really change something that's not alive.

Interviewer: Perfect, alright, so does evolution, so that's kind of a process right, does that generally happen to individuals or is that something we see more on the population level?

Interviewee One: Um, so basically, it will start with individuals so that, and then it will spread throughout the population so that the population can better survive. So, if um, if one individual starts changing the other, the rest of the species is going to start noticing that they're going to need to start changing something and that, and then um, so their bodies would eventually evolve to better help them survive whereas if the ones that don't evolve won't survive as long as the ones that do.

Interviewer: Perfect, and how do we generally define species? Have you heard that definition?

Interviewee One: The definition of a species?

Interviewer: Yeah. Like how do we say, oh this is one species, this is another species,

Interviewee One: Well, everyone's different.

Interviewer: That is true, that is very true.

Interviewee One: Everybody's always like, some teachers are like species, um, is an organism that has difference than something else. But then other people are like species can have different subsets of each other. So, um, so you might have birds, and birds are a species, but then you have different subspecies within that species. So like, but then, some people are like a finch is one species and an oriole is another species, they're not necessarily the same, not necessarily from the same ancestor, but they could potentially be somewhere.

Interviewer: Yeah.

Interviewee One: So I guess it kind of depends on who you talk to.

Interviewer: That could be. And how do changes arise that eventually lead to new species. So we see, so have you heard of the, the cave salamander? It's really common in evolution you know tests and stuff, where they're like how does this, um, salamander come from a salamander that could see but now it's blind? So do those, how does something go form, how does it make those changes over time?

Interviewee One: Well basically they adapt to their environment. So, like the cave one they want, they want it to be dark because, if they're, if they're able to see and then all of a sudden they go out to the light and they're from the dark all the time and then they could potentially be blinded and not be able to

know when to be able to protect themselves. Whereas, so like, they eventually evolved to help live and adapt in their environment.

Interviewer: Cool. So, um, environment kind of influences the adaptation.

Interviewee One: [Agrees]

Interviewer: Perfect. And so, for these alleles, how do we get, so how do we get these different alleles that maybe you can see, maybe we can't see.

Interviewee One: Mutations.

Interviewer: Perfect. So we can move on to natural selection, that was really great.

Interviewee One: Well natural selection is basically how you evolve, um, so basically natural selection is, um, selecting a specific trait so that species is better able to survive so like they're not outcompeted from another species, they can, um, adapt to their environment better, things like that.

Interviewer: Cool, so what is, this probably seems really basic, but what is an environment?

Interviewee One: An environment is basically the niche which, which in they live, so if their natural niche is to live within the dirt that would be their environment, or in a tree, or water, things like that.

Interviewer: Cool, and does selection happen on the individual level or is that a population level event?

Interviewee One: It starts with the individual level and could eventually evolve to the population level.

Interviewer: Perfect. And do you think it's random or nonrandom?

Interviewee One: Nonrandom.

Interviewer: Cool. Do you have a reason for why you think it's nonrandom?

Interviewee One: Well if it was random, it might randomly select for a trait that might not actually help them. So nonrandom would make it more reasonable to select for a trait that would actually help them.

Interviewer: Perfect, awesome. So um, in the environment, so we kind of got into this whole part about adapting. So say, so let's, so say you have a really hot environment. That's an example of a selection pressure. Can you give me an example of selection pressures that you can think of and environments that might influence certain types of phenotypes?

Interviewee One: So living in the arctic, really thick skin and fur keep them warm would be one. Living in like caves, um having, being able to, being able to see well in the dark, things like that. Um, living way deep in the ocean, not having color is another one, so you're basically see through like a lot of fish are. Um, yeah, things like that.

Interviewer: Cool, we can go on to fitness.

Interviewee One: Um, Basically it's how well you're able to survive within a species or within a population or in an area of a certain, certain environment things like that

Interviewer: Cool, so when we talk about fitness how does that differ from everyday terms you know we have this fitness that people just kind of throw around but how would we contrast that to fitness like we would talk about it in evolution?

Interviewee One: Well basically fitness in evolution is how well you're able to survive. So if you have a great, like a great fitness standard you're more likely able to survive than those that have lower fitness.

Interviewer: So then what did Darwin mean by survival of the fittest? That gets thrown around a lot too.

Interviewee One: Survival of the fittest basically means that like, how well you're able to adapt to your environment, and changing environments, things like that.

Interviewer: So is fitness random or nonrandom?

Interviewee One: More likely nonrandom but they're might be some random possibilities in there, if like you're environment suddenly changes or you have an increase in numbers predators that are actually involved and things like that. So mostly nonrandom but there can be random aspects to it.

Interviewer: Sure, so is that on the individual or population level?

Interviewee One: Mostly on the population level.

Interviewer: And then I'm not sure if you've talked about this in any of your class but, classes, how do we measure fitness? So that's actually something we actually can measure and--

Interviewee One: Never have I talked about that.

Interviewer: Ok, no problem! So we'll just say no, we don't know. That's ok. So genetic drift, what is that?

Interviewee One: I always get this confused with shift.

Interviewer: Totally, well this is a confusing one.

Interviewee One: Drift is between species, I think. I always get the two confused. Um I'm pretty sure that it's how um changes, um so like if you have influenza it can um infect one animal species and within that single species it can actually change and evolve itself and then it can be transmitted to another species and then it can keep evolving like that, I think that's what drift is.

Interviewer: Sure, awesome. So is that uh random or nonrandom?

Interviewee One: Both?

Interviewer: Both, so do you have a reason why you think it's both?

Interviewee One: Well basically it's like for influenza as an example. If it, me, if it wants to infect another species or if it needs to change, it could be nonrandom. But then if it, um, all of a sudden is in contact with another type influenza strain or things like that then it can be random at the fact that it randomly bumped into this next sort of strain of influenza.

Interviewer: Cool, and so is that more in an individual things or a population thing?

Interviewee One: Individual.

Interviewer: Cool. And then last one! Genetic polymorphism.

Interviewee One: K. I think, I really don't remember talking about this much.

Interviewer: Sure, that's fine.

Interviewee One: Basically, it's how your, um, basically your genetics change, so how they change over time is what I think genetic polymorphism is.

Interviewer: Yeah, cool. So what would these changes be represented by do you think?

Interviewee One: Um, changes in the DNA structure, would could eventually lead to different changes in the phenotype, um, so like, the appendix for example

used to be used and now it's not so that could be a form of genetic polymporphism.

Interviewer: Cool, so then they're observable.

Interviewee One: Mhmm.

Interviewer: Cool. So do you think this is a random or nonrandom thing?

Interviewee One: Random.

Interviewer: Random, cool. Do you have a reason for that?

Interviewee One: No, I really have no idea.

Interviewer: No? Just a gut feeling? Cool. So genetic polymorphisms can be random changes, we're going with. So are they beneficial, neutral, detrimental?

Interviewee One: Can be all of the above.

Interviewer: Sure. Alright. Awesome. Do you have anything to add to any of the definitions?

Interviewee: No.

Notes from Pilot Interview Two

- Interviewee two is from Fargo, North Dakota
- At the time, interviewee two was enrolled in Capstone (for microbiology), Animal Virology, Sociology, and Psychology
- He is graduating May 2016
- He is applying to medical schools, and had his first interview December 17, 2015
- Overall, interviewee two seemed hesitant to answer questions unless he was confident about his knowledge. He admitted that he had not thought much about these topics for a very long time and wasn't very comfortable with the material.

- No suggestions of how to change wording or structure of questions; felt they were fine
- Interviewee two said they spoke about 16S rRNA in Capstone but they never looked at the data
    - He had nothing to say about how to interpret the data, nor the general utility of 16S rRNA
    - He did mention that the letters are amino acids, and that he figured you would count changes
- Interviewee two asked if the nature of the changes should be taken into account when determining genetic relatedness, indicating he understands that nucleotide changes are not equal; I explained Kimura 2 Parameter
- For the phylogenetic tree, Interviewee two admitted that he had no idea what the numbers indicate
- Interviewee two said that most related were *Bacillus thuringiensis* and *Mycobacterium avium* because they're in the same "box"
- Interviewee two said that the least related were *Bacillus thuringiensis* and *Escherichia coli* because they are the farthest apart in the tree
- He recognized nodes as place of divergence
- He thought that length of the branch was due to the number of genetic changes in the 16S rRNA
- Interviewee two said he had not been exposed to phylogenetic trees in a very long time, and didn't feel comfortable with them
- Interviewee two defined evolution as movement from single cell organisms to multicellular organisms over time due to endocytosis, beneficial relationships, and environment

    - This response suggests that he believes evolution applies to the origin of life
- He mentioned environmental influences, provided climate change and air pollution as examples
- He recognized that evolution is occurring right now
- He recognized that changes are due to mutation
- He defined natural selection as driven by survival of the fittest, and being the most "capable" to survive
- He defined adaptation as genetic change, provided chameleons' ability to camouflage as an example because it would help hide them from predators
- He defined natural selection beginning on the individual level and eventually moving to the population level
    - He identified natural selection as random and nonrandom; random due to changes but nonrandom due to environment
- He defined fitness as the ability to reproduce
- He stated that fitness is measured based on viable progeny
- He felt fitness is nonrandom because it is based on the fitness of parents, so it is pre-determined
- He stated that fitness begins on the individual level and ends on the population level because populations would eventually trend towards the individuals that survive
- He defined genetic drift as small mutations in DNA
- He stated that mutation is random, so genetic drift is random
- He recognized that traits are directly influenced by genetics, provided the central dogma

- He indicated that genetic drift happens on the individual level because of mutation, and that happens in individuals
- He defined genetic polymorphism as observable traits such as hair color, eye color, etc.
- He stated that genetic polymorphisms can be detrimental because if the trait is not beneficial in a particular environment, then it is detrimental
    - Provided example that if a camouflage was the wrong color, it wouldn't work well, and that would be a detrimental polymorphism
- He indicated that genetic polymorphism is random because of recombination of traits and random gametes
- He mentioned he was not sure how genetic polymorphism works in bacteria because traits are not observable

APPENDIX D. FINAL ASSESSMENT PROMPTS

Please address the following statements for each concept, by writing a **T** or **F** for true or false, respectively.

Evolution

1. Evolution explains how organisms change over time.
2. Evolution is descent with modification via genetic inheritance.
3. Evolution is the interplay between mutation and selection.
4. Evolution is observed on the individual level.
5. Species arise via small genetic and phenotypic changes that accumulate over time.

Mutation

6. Mutation gives rise to the variation that leads to new traits.
7. Organisms mutate in order to gain the changes they need to adapt to the environment.
8. Mutation is not random.

Natural Selection

9. Selection tends to retain deleterious mutations.
10. Selection is random.
11. Selection can be biotic (interactions with other organisms) or abiotic (interactions with the non-living environment) in nature.

Genetic Drift

12. Genetic drift is the change in genetic composition of a population, and occurs when genes are randomly fixed.
13. Selection influences genetic drift.
14. Genetic drift is non-random.
15. Migration/gene flow ameliorates genetic drift by averaging out allele frequencies among populations.

Genetic Polymorphism

16. Polymorphisms are genetic changes between one or more individuals.

17. Observed polymorphisms are never neutral.

18. Deleterious polymorphisms are not observed because they are purged by selection.

19. Adaptive polymorphisms are common.

20. Genetic polymorphism can only be measured via genetic sequencing.

Fitness

21. Fitness is the ability of an organism to adapt to its surroundings.

22. Fitness depends on the environment.

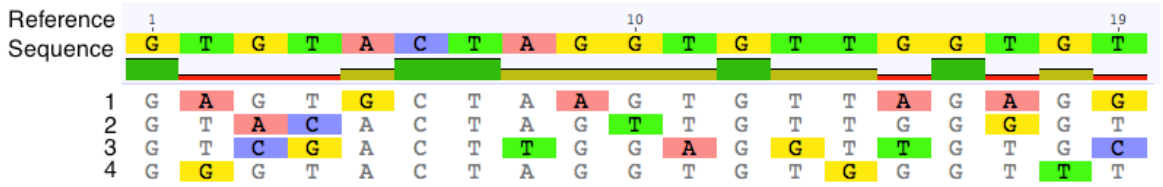
23. Fitness is measured via reproductive success

Question One

While observable traits (e.g. gills, wings) may inform species relatedness, this method can be unreliable, especially with bacteria! As an alternative, Carl Woese proposed analysis of the 16S subunit of ribosomal RNA (rRNA) to compare species relatedness. Briefly address the following questions based on this information:

11. Why is the 16S rRNA gene useful to determine species relatedness? Provide at least two explanations.

12. Determine the two most related and two least related organisms from the short list (1-4) of aligned 16S rRNA sequences below:



Two Most Related: 1 2 3 4

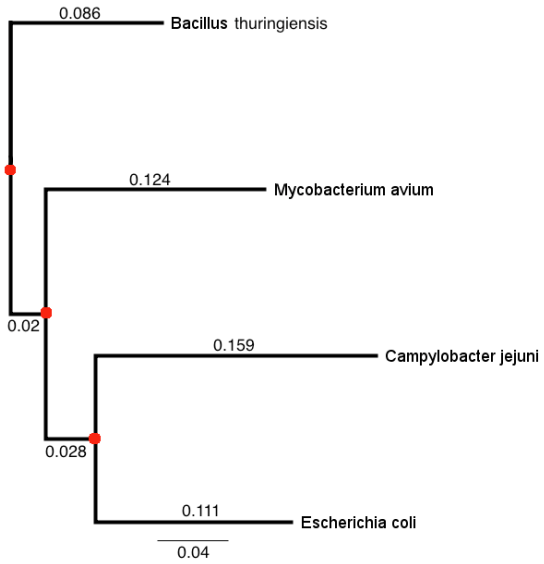
Two Least Related: 1 2 3 4

13. How did you utilize the sequence data to determine the most and least related species?

14. What do the individual letters (A,G,T,C) represent?

15. **TRUE or FALSE**: all living cells contain 16S rRNA.

Question Two



16S rRNA data can be used to develop phylogenetic trees. The phylogenetic tree above represents the 16S RNA gene sequence data from the previous question; however, the names of the genera are included (red dots represent nodes).

16. What are the **two most related** species according to the phylogenetic tree? Explain in detail how you determined your answer.

17. Is *Mycobacterium avium* more closely related to *Campylobacter jejuni* or *Escherichia coli* (or are they equally related)? Explain in detail how you determined your answer.

APPENDIX E. IRB APPROVAL

NDSU NORTH DAKOTA STATE UNIVERSITY

October 22, 2015

Dr. Peter Bergholz
Veterinary & Microbiological Sciences

Re: IRB Certification of Exempt Human Subjects Research:
Protocol #AG16087 , "A research-based approach to improve microbial evaluation and ecology education"

Co-investigator(s) and research team: Chelsey Grassie, Jennifer Momsen, Kaycie Schmidt

Certification Date: 10/22/2015 Expiration Date: 10/21/2018
Study site(s): NDSU
Sponsor: n/a

The above referenced human subjects research project has been certified as exempt (category # 1) in accordance with federal regulations (Code of Federal Regulations, Title 45, Part 46, Protection of Human Subjects). This determination is based on the original protocol and email narrative (received 10/22/2015).

Please also note the following:
- If you wish to continue the research after the expiration, submit a request for recertification several weeks prior to the expiration.
- The study must be conducted as described in the approved protocol. Changes to this protocol must be approved prior to initiating, unless the changes are necessary to eliminate an immediate hazard to subjects.
- Notify the IRB promptly of any adverse events, complaints, or unanticipated problems involving risks to subjects or others related to this project.
- Report any significant new findings that may affect the risks and benefits to the participants and the IRB.

Research records may be subject to a random or directed audit at any time to verify compliance with IRB standard operating procedures.

Thank you for your cooperation with NDSU IRB procedures. Best wishes for a successful study.
Sincerely,

Kristy Shirley

Digitally signed by Kristy Shirley
DN: cn=Kristy Shirley, o=NDSU, ou=Institutional Review Board, email=kristy.shirley@ndsu.edu, c=US
Date: 2015.10.22 13:43:48 -05'00'

Kristy Shirley, CIP, Research Compliance Administrator

For more information regarding IRB Office submissions and guidelines, please consult http://www.ndsu.edu/research/integrity_compliance/irb/. This Institution has an approved FederalWide Assurance with the Department of Health and Human Services: FWA00002439.

INSTITUTIONAL REVIEW BOARD
NDSU Dept 4000 | PO Box 6050 | Fargo ND 58108-6050 | 701.231.8995 | Fax 701.231.8098 | ndsu.edu/irb

Shipping address: Research 1, 1735 NDSU Research Park Drive, Fargo ND 58102

APPENDIX F. FINAL INTERVIEW PROTOCOL

Interview Protocol
Spring 2016

Directions:

Interviews will last approximately one hour. The interview will start with basic ideas on evolution, and terms that fit with evolution by asking for free association responses. For the remaining questions, the interviewer will ask the interviewee to review the question written on the paper, so that they can collect the answer and request justification as to why the interviewee believes that is the best answer. The interviewee is allowed to take as much time as possible, and can write on the paper as needed. Interviewer is to move on to the next question if answers become non-productive.

To explain to interviewee:

The interviewer is ultimately responsible for making sure that all questions in the interview guide are addressed during the interview. Explain that you are there to gain from the participant's own knowledge about the research topic, not to correct ideas or dispense advice. Assure the participant that there are no right or wrong answers; it is his or her personal knowledge and perspective that are of interest to the study.

[Review informed consent document and obtain signature]

Question 00. Begin by asking where they're from, about classes they're currently enrolled in, when they're graduating etc.

To get students to become more comfortable with talking to the interviewer, we will first cover these basic questions to get at the interviewee's ideas on these topics. Paraphrase the answers that respondents give, and allow them to elaborate.

Evolution:

- What comes to your mind when you hear the word: evolution?
- A popular response has been that evolution applies to biotic and abiotic things in the universe. What do you think?
- Do we typically observe evolution the individual level, or population level?

Possible key words to probe further:

- Phenotype: Ask for clarification. How is it different from genotype?
- Genotype: Ask for clarification. How is it different from phenotype?
- Allele: Ask for clarification. How does it relate to phenotype and genotype?
- Adaptation: Ask for clarification. Explain how the adaptation and the environment relate.
- Selective advantage: Provide an example of a selective advantage.

- Change: Explain why changes occur.

Natural Selection:

- What comes to your mind when you hear: natural selection?
- Scenario question:
    - How would biologists explain how Chlamydomonas nivalis ("snow algae") evolved with an optimal growth temperature of 1ºC from ancestral "lake algae" with an optimal growth temperature of 28ºC? [Provide Subject Sheet 1]

If student does not mention variation within a population, mutation, inheritance, fitness, or population change as the corresponding question:

- Variation within a Population: are all of the animals exactly the same at the beginning? How might variation be beneficial?
- Mutation: How do changes happen that allow the animals to be more "fit"?
- Inheritance: How are beneficial traits retained in a population?
- Fitness: What did Darwin mean by the phrase "survival of the fittest"? How does survival of the fittest apply to this situation?
- Population Change: Why do we end up with many snow algae able to survive at 1C? Why not just a couple?

Possible key words to probe further:

- Environment: what is an environment? How does it influence selection?
- Thrive/Grow: ask for clarification.
- Selective advantage: what is an example of a selective advantage?
- Genetic Code: what specifically in the genetic code changes during mutation?
- Variation of Alleles: how does variation arise in alleles? What is the consequence?

Genetic Drift:

- What comes to your mind when you hear: genetic drift?
- Is genetic drift observed at the population level or the individual level?
- Is genetic drift random or non-random? Why?

Possible keywords probe further:

- Population: does genetic drift happen on the individual level, or just the population level?
- Changes in genome: why do changes in the genome that contribute to genetic drift occur?
- Codons: explain what codons are.

Question One
While observable traits (e.g. gills, wings) may inform species relatedness, this method can be unreliable, especially with bacteria! As an alternative, Carl Woese proposed analysis of the 16S subunit of ribosomal RNA (rRNA) to compare species relatedness. Briefly address the following questions based on this information:

1. Explain to me your current understanding of the 16S rRNA gene.
2. Explain how the 16S rRNA gene is useful to determine species relatedness.
3. Is 16S rRNA in all organisms?

Possible Replies:

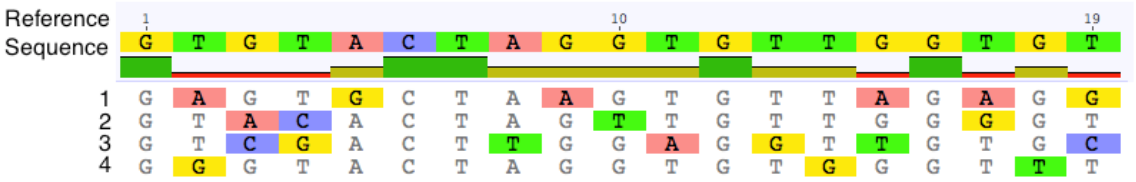
- Differences/Similarities in Sequence Inform Species Relatedness
    - Explain how differences arise.
    - Explain why more distantly related species are represented by more differences in the sequence.
- Unique to species
- If every sequence is unique, explain how it's possible to map relatedness.
- Compare
- Explain what elements we look for in a comparison that provides us with information on relatedness.
- Conserved
    - Every organism: [restate with added clarification] "so all organisms, bacteria, animals, plants, all contain 16S rRNA".
    - All microorganisms: [restate with added clarification] "so all microorganisms, bacteria, viruses, fungi, all contain 16S rRNA."
    - In bacteria: do fungi, animals, and plants have something different, or do they also contain 16s rRNA?
    - In prokaryotes: what do eukaryotes have?

1. Are there any additional reasons we haven't discussed?

**Direct the student to complete the problem by writing on the projected problem on the board. Ask them how they would conduct each step, and gain reasoning at each step. Begin with:*

2. Explain what the reference sequence represents.
3. Explain what the letters represent.
4. Explain why the sequences are different for each organism.
5. What would you do first to solve this problem?
6. Why would you do that first?
7. Show me.
8. What would you do next to solve this problem?
9. Why would you do that first?
10. Show me.
11. Repeat steps until you have completed the entire problem, and ask them to answer the most/least related questions

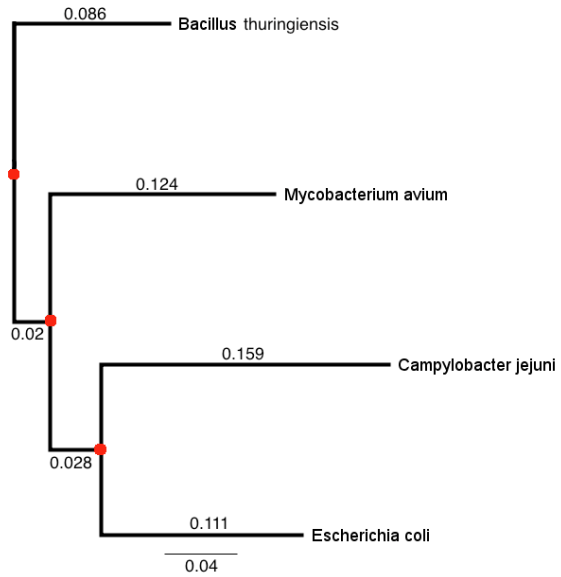
Determine the two most related and two least related organisms from the short list (1-4) of aligned 16S rRNA sequences below:



Two Most Related: 1 2 3 4

Two Least Related: 1 2 3 4

Question Two



16S rRNA data can be used to develop phylogenetic trees. The phylogenetic tree above represents the 16S RNA gene sequence data from the previous question; however, the names of the genera are included (red dots represent nodes).

1. What are the two most related species according to the phylogenetic tree? Circle your answer with the red pen.
2. Explain in detail how you determined your answer.

Possible Replies:

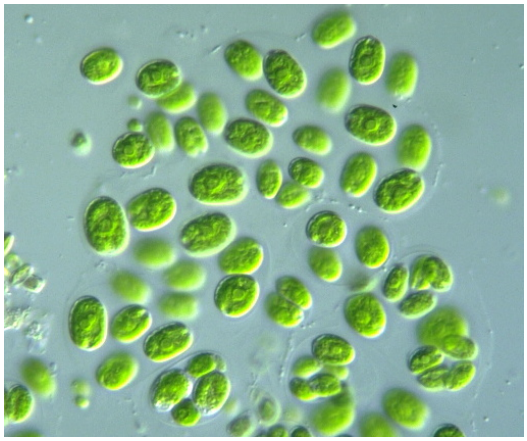
- Numbers
- Explain how you utilized the numbers on the tree to determine distance.
- Nodes
- Explain what nodes represent.
- Explain how nodes help you determine relatedness.
- Branches
    - Explain what branches represent.
    - Explain why branches are different lengths.
    - Does the order of the branches provide information?
- Distance
- Clarify what you mean by distance.
- How did you determine distance?

3. Is *Mycobacterium avium* more closely related to *Campylobacter jejuni* or *Escherichia coli* (or are they equally related)? Circle your answer in the black pen.
4. Explain in detail how you determined your answer.

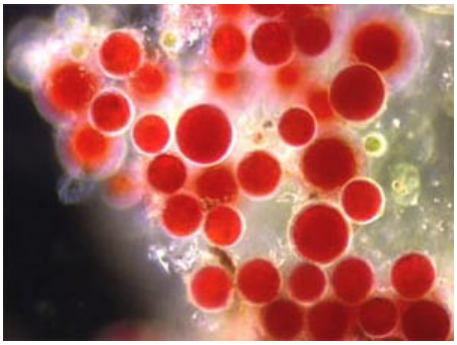
Same as above

How would biologists explain how *Chlamydomonas nivalis* ("snow algae") evolved with an optimal growth temperature of 1°C from ancestral "lake algae" with an optimal growth temperature of 28°C?

Optimal Growth Temperature: 28°C



Optimal Growth Temperature: 1°C



APPENDIX G. INTERVIEW TRANSCRIPTS

Interview One

Interviewer: The 16S, do you remember—

Interviewee One: Yup.

Interviewer: Recall this question?

Interviewee One: [Agrees]

Interviewer: Cool. So 16S is one of the concepts I'm studying as part of the ASM curriculum. So I want to get insight into how students analyze the data. So here, if you want to read the question again just so you have a reference. Um, but I would love if you could explain to me your current understanding of 16S.

Interviewee One: 16S is found in the, [refers to prompt] ok it says here that it's in the 16S ribosomal RNA with species relatedness. And I think it's in, ok, I think there's like a 18S or something like that. For, because I don't think all organisms, organisms have 16S. But I can't remember. I'm not 100% confident. But the 16S, the more related it is, it shows the closer on your phylogenetic tree. The more relative it is the, the more recently they would have diverged on the tree of species the phylogenetic tree.

Interviewer: So in that respect, why is 16S, like why was this such a great discovery, why is it so useful to us?

Interviewee One: Because we can use it like carbon dating, like a source to track the times. When we know what times certain organisms were around, we can use that, use that sequencing as a time point to figure out when the species,

um, came up or kind of diverged- how related two are. Because the information is most useful when you're comparing things.

Interviewer: Yeah, how do we make a decision when um things are maybe related or not related if we're looking at 16S.

Interviewee One: Well, when I was looking at it, I figured that you kind of want the groups. Because if you have like groups, if you were to find a chunk here and a chunk here that would mean more than just finding random one, two three. Because if they move as chunks, that would be like during meiosis and rearranging. It would show that they're really similar. Whereas, if you just get individual ones, the um, that could be just random, um, random recombination that comes up with individual base pair matching.

Interviewer: So the pattern in how it's changing informs us how related they are.

Interviewee One: [nods]

Interviewer: Yeah? Ok. So, how do I phrase this? Sorry. Um. So, since every single organism has some sort of ribosomal subunit, we established that perhaps some have 16, some have 18, um how do we compare between that? Or why, why do all organisms even have this?

Interviewee One: Um, it's used for, the ribosomal subunits are used for replication of um DNA. So, it's there because, it's there, we can find it in the organisms because in order to produce more daughter cells you have to have it. But then based on natural, uh, based on the um divergent evolution, the um, the units changed through, I don't know, I would guess through uh recombination, or something like that, I would say recombination for that

to get it different sequences and then the more recombination and manipulations you have, the less related you'll see that the 16S is.

Interviewer: Ok, cool. Um, are there any other important things you want to note about 16S before we move on to trying and solve the problem?

Interviewee One: Um, I do know that there are companies now that you can send that off. When you purify DNA, send it off and they'll give you, they'll spit you out a sequence and then you can run in through the different aligner. Something like that, there's a software that you can get, free software, and then it will figure some of that out for you.

Interviewer: Yeah, exactly, that was how I made this sample we have right here.

Interviewee One: I was with uh Janice and Ms. Teresa Bergholz, Dr. Bergholz. And um we did in 352, we were trying to work with this. I think I, I don't know. I'm going to look that up now. I was wondering when I took the test if there was an 18S but I can't remember.

Interviewer: At the very end of our interview if you do have questions, I would be happy to answer them. But during our interview…

Interviewee One: Right.

Interviewer: I don't want to influence.

Interviewee One: Right, don't want to sway it. Um so yeah, we did this for, for um, 352 lab.

Interviewer: How'd that go?

Interviewee One: Pretty good, we had some setbacks but. We ran it through like gel electrophoresis and all that stuff too so. And we got to play around with PCR.

Interviewer: Very cool, definitely useful skills.

Interviewee One: A little frustrating.

Interviewer: Lab work can be very frustrating, I suppose that's a part of getting the research, so. We all feel it, don't worry.

Interviewee One: It was a lot of down time too.

Interviewer: Well it's nice if you have to write a paper or do something else but yeah, for I suppose for a class, when you're--

Interviewee One: We sat around talking.

Interviewer: You can't do much else huh? Cool, um. So if you don't mind, we can go on to solving it.

Interviewee One: Yeah.

Interviewer: And so I do want to take this process a little slow so that I can an idea of how you're thinking through the problem. So, I have yeah, this big one, you can write on it, scribble on it, take any notes you want, and then I'll just keep that for after the interview or whatever.

Interviewee One: For the...

Interviewer: Yep, so, and actually it's, it's really helpful if you write down as much as you can to think through it so that I better understand what's happening.

Interviewee One: Um right off the bat I looked at we only have the three matches on these ones, and then I compared them to the base pairing. So with G we want um C, and with T we want A, and T we want A. And so we don't have any matches in 4. So I said that one would be of the least related. Um…

Interviewer: Ok so let me slow you down for a second, so initially, before. So how did you decide that you were going to look at four as the first one?

Interviewee One: I looked at how many highlights we've got here.

Interviewer: Ok.

Interviewee One: Because I assumed the highlights were the important parts.

Interviewer: Sure.

Interviewee One: And then, and then with those, I looked, I compared it. And one might guess that with only three matching, or um, three highlights, whereas the rest of them have more than that, that these ones would be the only important parts out of this genome in relation to this one.

Interviewer: Ok

Interviewee One: So that, and then these ones didn't match up. So then, um, that kind of tells me that they've split, or they've um gone their own path so much that they don't match. Oh wait. Looking at this again, let me see this. These are the highlighted ones are the ones that, these are the ones that don't have [draws]… these are the only three that don't match. That's what that is. I didn't pay enough attention to that when I was looking at it before.

Interviewer: That's ok. Does that make you change your answer?

Interviewee One: Yeah, I would put that then at the most related one. Because that one out of the, I guess I looked too fast at it. The ones with the highlight, that's highlighting the ones that are incorrect. Yeah. So that means that there are only three spots where it doesn't make sense. So it's the most adapted to, or the most related, I would say.

Interviewer: So what would be your next step? So you've looked at one with three changes. Where would you go next?

Interviewee One: Um, then, I would look at the number two maybe and go through. Number two has one, two, three, four changes. [Counts changes for each sequence in comparison to the reference sequence]. Yeah, so then I would put the two that are most similar, 3 and 4, but so, which would make 2 and 4 would be the most related, because they have the least amount of changes. And then number 1 and 3 because they have 6 and 7, respectively, for amount of changes. Because you want them to match up. If they match up completely you've got the same species. So, the fact that these are less, these ones only have three changes gives you the idea that they're pretty similar.

Interviewer: Yeah, that makes sense. Thank you for slowing down and explaining it.

Interviewee One: Yep. I didn't notice that before, I was kind of wondering why some were highlighted and some weren't.

Interviewer: Well if you aren't familiar with the data I could see how that might be confusing. When you first looked at the data had you, did you recognize what you were supposed to do?

Interviewee One: I recognized when I looked at it right away that you were looking for ones that are matched. That you wanted the most, the two, the less change, the less different they are the more similar they are, is the most related. So, but, I guess when I looked at the software it pretty much, we didn't

compare things quite like this or the software did it for us or something. Because I never, I don't think I ever looked at a printout like this.

Interviewer: Often software will have that ability.

Interviewee One: Yeah, the um, I kind of assumed with the highlights I was looking for um, the more important parts of the gene were highlighted. But the whole strip of section of gene, of base pairs um are here, and then we're just looking to see which ones are matching.

Interviewer: So before you were just looking at the highlighted areas?

Interviewee One: At the highlighted areas and I saw that the highlighted areas didn't match but, but like this highlighted area, T and A base pair together. Yeah, no A and U. Either way, I think, I don't know. I thought the A looked ok there so that might have given me more of a... because when you're replicating DNA based on RNA, you should have U's. Maybe that's it. I'm not sure. That would be my guess. These are highlighted because they have. No, because there are A's here that aren't highlighted. Oh, but it's not going by base pairing, it's going just by. Ok. Because the G's, all the G's here are the same so there's nothing highlighted. But in this one, all of them that aren't A's are highlighted. So even though this one would hypothetically would base pair, it's not what you would want, you want T. That's that I guess.

Interviewer: Sure, that makes sense. So taking a step back, let's go over um, what do the letters represent. You hinted at it, but.

Interviewee One: The um, base pairs. And they stand for: guanine, thymine, adenine, and cytosine.

Interviewer: Ok. And what are--

Interviewee One: Then there's the U one for, uracil I think, I'm going to say, uracil. And then when you get the three, it codes for a, um, a, the three code for a, um, the little pieces and then the, all the little pieces make up a protein. But they go for like methionine. What are they called? Mrs. Offerdahl would be pretty upset. Dr. Offerdahl. What, oh shoot. Amino acids. Yup. So your codes of, three code for amino acids and they help, they give you like the start codon and stop codon and the rearranging of these give you different amino acids which will change how your protein functions.

Interviewer: Ok.

Interviewee One: So then the more similar their DNA is the more similar they function. So the more closely related they turn out to be.

Interviewer: That makes sense. Yeah, that makes sense. And so looking at this too we did have the component of the reference sequence which we utilized, um, what is a reference sequence?

Interviewee One: You know, at first when I looked at this and I was taking the test, I didn't even know that was the reference sequence. I was trying to compare them against each other, the highlighted parts.

Interviewer: Sure.

Interviewee One: But the reference sequence is the sequence that we, I think that might be the sequence that we have compared it, comparing it against four sequences that are known in the database.

Interviewer: Ok

Interviewee One: And then it will tell you, um the software it will say like 99% match with *E. coli*. And then you can assume, it, this one would probably would say the 99% here because that's probably the closest. And then you would probably assume that you have, you either have, *E. coli* or something very similar to *E. coli* in function.

Interviewer: So you would pull a known sequence? Is that what I'm hearing?

Interviewee One: Yeah, I would, I assume that these four are known, and then this one that you're putting in is unknown?

Interviewer: Ok

Interviewee One: If you're using it to try and figure out, if you're using it to try and figure out what this is. If you were to culture something that you don't know what it is. But if you do know that is say *Serratia marcescens*, then it you're just looking to see what the closes thing to *Serratia marcescens* is given these four. So you could put four strains in here and then it will tell you which is most phylogenetically related to *Serratia marcescens*. So there are a lot of different applications for it.

Interviewer: So this could be known or unknown, is that what I'm hearing?

Interviewee One: Yeah, but I think these ones are known.

Interviewer: Ok

Interviewee One: Because, you wouldn't want to compare four unknowns to a known because then, well you could, but you would just have to run that through software. Here you'd have to take, I think it's just more feasible to assume that you're comparing four known things, either to something that's known to see how they're related, or to something that's unknown in the hopes that you figure out what it is.

Interviewer: That makes sense. Cool, so we're running short on time, so I'm going to move us to the next, um, question. That was really great, thank you. Um, ok I'll just take that. So, on the next page we can move there. And if, I will, I'll state the questions for you either way, but in case you do understand reading a little better, I think I am more comfortable with having something to read--

Interviewee One: Yeah.

Interviewer: But, so, based on this phylogenetic tree, what would you say are the two most related species?

Interviewee One: These two because they diverged the farthest apar--, the farthest down the line. But I'll tell you straight up I have no idea what those numbers are for.

Interviewer: Ok

Interviewee One: I would assume that's probably either how, uh maybe like uh, to show how far apart they are, but I don't know. Because these numbers are closer than these two numbers, but I would say since these two diverged off that, that these two are the, share the most recent common ancestor. I would say those two are the most similar.

Interviewer: Ok, what do the red dots represent?

Interviewee One: A common ancestor that they had that they both went different directions from, from the common ancestor.

Interviewer: Ok, so could you explain that maybe, so what happened right here?

Interviewee One: Oh yeah, like right here we have one species, and then, and then like with that, my--, with this bacterium here [refers to free association natural selection question], we would end up like, here's your stud, the 28, and then you would end up with the 28 degree and a 1 degree strain, and then they're no longer the same species, they're two different species, so, but they would share a common ancestor. Typical of something like that right there [points to node].

Interviewer: Ok, yeah. So then for the second question, um, is *Mycobacterium,* based on your interpretation of this tree, is *Mycobacterium* more related to *Campy, Campylobacter,* or *E. coli*?

Interviewee One: Um, I don't think the placement of these has importance, I think the, but the lengths here are more similar than like here. But I really, am not brushed up on this. But I would assume to go by the number being the most closely related to say *E. coli*. But I don't know what the numbers mean so that's a blind guess.

Interviewer: Sure, and that's helpful to know if you aren't sure how to utilize the numbers.

Interviewee One: Because the numbers would, yup, the numbers would tell you something, I'm sure. But, there's probably something that has to do with the lengths here, but I don't know.

Interviewer: Yeah, do you have any ideas for maybe why um these branches are different lengths?

Interviewee One: I would have, well, initially I was thinking like, if this was the present day, we would see that maybe these ones are gone. But I know that E. coli is still in existence, but, um, maybe the amount of time that it took to, for this species to evolve? Because like E. coli has been around for a while, and maybe this is a newer species.

Interviewer: That makes sense.

Interviewee One: That would be my educated guess.

Interviewer: Sure.

Interviewee One: I don't know.

Interviewer: That's good. Alright… so you were mentioning that the placement of these doesn't necessarily matter. Can you explain that a little further, what you meant by that?

Interviewee One: Um…

Interviewer: I think it was when we were talking about the uh, this [*Mycobacterium*] being related to these two [*Campylobacter* and *E. coli*].

Interviewee One: I think if you were to compare to this one, I think that. I don't know. I think that maybe the numbers here have something to do with the direction that it's placed. But, the fact that these two, that this one's more in

proximity, that might have a little influence. But I don't think that it's the most, it shouldn't be the only thing to look at when looking at these two. I think if you flipped them around… [VIDEO CUT OUT, REFER TO NOTES]

From Notes:

- When the video cut out, the interviewee was debating whether the numbers or the orders branches (vertical proximity) were more important. She concluded that the numbers were likely more informative than branch order, but that vertical arrangement may still matter
- She drew an additional branch below E. coli, indicating that if we placed a branch there, it may mean they're more related than to other organisms on the tree, although she was not 100% certain

Interview Two

Interviewer: So let's go to this first question for the 16S. So just explain to me your current understanding of 16S, the subunit, what that is.

Interviewee Two: I have absolutely no idea.

Interviewer: And that's ok. Um, have, do you recall, so do you recall learning about it in any of your classes?

Interviewee Two: I'm sure it was mentioned at some point but like I just don't have any recollection of what 16S is. Like, I know what RNA is, like I know a little about how genes work, well I did struggle with that part in classes.

Interviewer: Sure, yeah. Yeah, so maybe reading this prompt. I suppose from this prompt, what, I mean does it give you an impression maybe of what 16S is?

Interviewee Two: I feel like it's a specific part of like DNA after it's been transcripted and translated, and then it's just like a certain important piece of gene with an RNA.

Interviewer: Ok.

Interviewee Two: Like, that's what I get from it. It's just like I don't know what the 16S is.

Interviewer: Yeah.

Interviewee Two: Other than that.

Interviewer: So if we have this piece, how could that be useful for determining species relatedness?

Interviewee Two: Well, part of it you could take that little segment and compare, cross-compare it with other species. I'm not sure if it looks like anything in particular or has certain traits. But I just don't know what I'm looking at necessarily. So, it can be used as a base to compare different species.

Interviewer: Ok

Interviewee Two: And if certain things are similar in comparison then they may be more related than others.

Interviewer: Sure, that makes sense. So is it something in all organisms, some organisms… some species, all species?

Interviewee Two: It could be, it might be, I really don't know.

Interviewer: That's ok. But yeah, I don't want to make you feel uncomfortable, if you don't know I'm not going to push you on it. Um, ok cool. So, well we can go onto how, I'm actually going to have you kind of show me how you went through this problem. So I have this projector behind you and we're

going to kind of go through this together. So here's this marker, just in case you, it will be helpful. The more you can kind of show me what you did, um, the better. So that I understand probably how other people are doing it too.

Interviewee Two: I will first start off by saying I guessed on this question.

Interviewer: Ok.

Interviewee Two: Because I did not know what to do, and I will try to walk through like my thought processes.

Interviewer: I can guarantee you you're not alone in that, so this is still useful to me.

Interviewee Two: Ok so I think the first thing I started doing, was I just went across this row and like looked to see what's bolded here [first sequence] because I just didn't know what to compare, and cross comparing it between that [reference sequence]. And then I just went down that [sequence 2-4 to reference], I could not find any patterns, so I moved on to the next trick. I started comparing these rows [sequence 1-4] and I noticed very quickly that the ones are not highlighted are all the same. And I'm like, I don't know what that means or if that means anything important so I was like, well this was kind of an unproductive path to take. And then at one point I started comparing like different lines to each other instead of just this reference one. And then a couple times I tried to compare to see if any of the opposites matched up like the pairings from A to T…

Interviewer: Sure.

Interviewee Two: Or C to G. And I couldn't really find anything. And I'm not sure if I was supposed to be look at this more [reference sequence] [touches smartboard]—

Interviewer: That's ok, it's going to follow you if you touch it but don't worry.

Interviewee Two: So, like I feel had I been looking more at this [reference], because I was totally focusing on all this stuff [sequence 1-4], and not on this [reference], so I'm sure if I was supposed to be focusing on this more [reference], but I was just comparing this more [sequence 1-4].

Interviewer: Ok

Interviewee Two: And ultimately I couldn't figure out what to do so I'm like, "well, I'm going to guess".

Interviewer: Sure. Um, so reference sequence, what, what would you say that might be?

Interviewee Two: Some information that could be important but not necessarily something you could use to solve a problem.

Interviewer: Sure. And what do the individual letters represent?

Interviewee Two: Um adenine, I think. Guanine, cytosine maybe, and then thymine.

Interviewer: Yup.

Interviewee Two: I might be off on some of these maybe but.

Interviewer: I think you got it. And so why might these sequences be different for one, for organisms 1-4? That might help us understand what, what we're doing.

Interviewee Two: Well for organism one, it could have a tail for all we know. Then organism two could have legs. And organism three could have wings. And one could be a unicorn, that four. So… [shrugs shoulders]

Interviewer: Ok, so what if I told you that um, it was based, so the 16S, it's present in these four species and so it represents the same thing. Does that, does that change your answer at all?

Interviewee Two: Could you say that one more time?

Interviewer: So 16S is in all these four species. Um so this is the same piece of information just coming from different organisms.

Interviewee Two: Ok

Interviewer: So why might there be these differences? Considering if we know this is something that they all have, but for some reason it's different.

Interviewee Two: Ok, before I answer your question let me just throw out some of my thoughts.

Interviewer: Yeah, go for it.

Interviewee Two: So I can follow what you want. So, one thing I could possibly consider is that maybe this is the 16S like reference gene, so full what the gene is. And then, I'm not sure if in the four organisms you would need the full gene of it to be like the 16S gene like, or if you're just taking parts of it and looking at it or if it has to be the whole thing for reference, within these different organisms. And then I think what I would maybe do next is just pick some segments from here [reference] to then see if each of these have.

Interviewer: So segments, what do you mean by segments?

Interviewee Two: So like from say A through T is a segment.

Interviewer: Ok, so then you would compare A through T with the other four sequences.

Interviewee Two: Yeah. And see if that's in there somewhere.

Interviewer: Ok, so what would you say about organism one, where that G is different from the A.

Interviewee Two: Wait, which one?

Interviewer: So from the A to the C, or A to the T, whatever, so if we're looking at all of these four organisms, see how number one um, the A is different.

Interviewee Two: Oh, ok yeah that right there.

Interviewer: So what might we conclude about that?

Interviewee Two: Possibly, that this one is a little more different from these ones.

Interviewer: Ok.

Interviewee Two: Because these two through four follow the reference sequence.

Interviewer: Ok.

Interviewee Two: Whereas, one strays a little bit from it.

Interviewer: Cool, so why might one have strayed from it a little bit?

Interviewee Two: Could have been a mutation or it could just be a very different species from the rest. So like some could be more closely related species, and then one is just not necessarily a closely related species at all.

Interviewer: Ok. Cool. So, I'll let you. Ok so we kind of went through that part. Does that change how maybe you would approach this problem at all? Since I

know you guess before, there wasn't really an approach. So knowing the information you have now, do you think you would still guess? Or do you think you have a game plan? And you can think about it, for what you would kind of do. And we can even say, first step, what would you do?

Interviewee Two: Well, I would probably, hmm, for finding things that are more in common, I would probably look for ones that have less of the same ones highlighted.

Interviewer: Ok

Interviewee Two: If that makes sense.

Interviewer: Yup, I think so. So you would look at the sequences that have less highlights.

Interviewee Two: Yeah.

Interviewer: And what would you, what would you say about the ones that have less highlights?

Interviewee Two: That they don't stray as far from the reference sequence.

Interviewer: Ok.

Interviewee Two: And as a result they're more similar to the reference sequence. And if they're more similar to the reference sequences the two that I'm looking at are more similar to each other as well.

Interviewer: Ok, so how about you go through your process and try to answer the question as you would with your new game plan.

Interviewee Two: Ok.

Interviewer: And feel free to draw on there, that curser will follow you when you draw but don't worry about it.

Interviewee Two: Ok, let's see. Well, at this point I'd probably say two and four are most related because they have the fewest changed genes from the reference sequence.

Interviewer: Ok. Cool.

Interviewee Two: And then, one and three would probably be the least related because they vary so much more.

Interviewer: Ok. Cool. So I think I understand that. So basically we're going, we're taking the reference sequence, we're comparing which has the least highlighted for most related, and the most highlighted for least related.

Interviewee Two: [agrees].

Interviewer: Ok.

Interviewee Two: Because there's going to be the most variance in the ones that have the more highlighted, so they're going to vary more.

Interviewer: Ok. Cool. Well then.

Interviewee Two: I mean I suppose I could sit down and like go through each of them, and see which ones match up the most. But on a test I wouldn't likely be doing that, I'd probably be looking for a simple look through like that.

Interviewer: So for, when you saw this um when it was in front of you in the classroom, did it kind of, did it overwhelm you at all?

Interviewee Two: Oh, yeah.

Interviewer: Yeah? Because just, you didn't really know what to do with it?

Interviewee Two: No. I had no idea. I'm like "well I have to finish the rest of this, so I better pick something".

Interviewer: Alright, that's fair. Well I can, hopefully this comes up real nice, mostly nice [projects phylogenetic tree on board]. I think I can, ok cool so it's at least fitting. Alright.

Interviewee Two: [mumbles something inaudible]

Interviewer: Yeah. So going through here.

Interviewee Two: I'm going to start out by saying I had no idea, I have never seen this before.

Interviewer: Phylo trees?

Interviewee Two: Yup.

Interviewer: Ok.

Interviewee Two: Never seen this before.

Interviewer: Ok, so. So, then let's talk about what maybe grabbed your attention. When you were looking at this and you knew you had to answer the question, "what's the two most related species", what went through your mind? Where did you go first?

Interviewee Two: I looked at the numbers, because that's the only thing I could think of doing because the bacteria names were not the same.

Interviewer: Yup.

Interviewee Two: So they weren't of the same species.

Interviewer: Sure.

Interviewee Two: And so I just assumed that maybe that this chart would have some mathematical like, consistency or logic behind it. So I assumed that the ones that were closest the numbers that were on these top lines here [points to branch numbers], I used those to compare each other.

Interviewer: Ok, so--

Interviewee Two: So the ones that were closest, assuming that I didn't do my math wrong on the test, because I just glanced at the numbers and didn't actually like subtract to see what the differences were, but I said um the *Mycobacterium avium* and the *E. coli* were more closely related than the other two.

Interviewer: Ok, that makes sense. So you, did you, did you kind of subtract them from each other, or you just kind of glanced like "oh, these two are close".

Interviewee Two: Yeah.

Interviewer: Ok

Interviewee Two: At least closer in comparison than the other numbers.

Interviewer: Yeah. So for the next question, how did you utilize the numbers to determine how, if *Mycobacterium* was more related to *Campylobacter* or *E. coli*?

Interviewee Two: Uh let's see, then I would assume that the ones that have the larger difference in number would be more related.

Interviewer: The larger difference in number?

Interviewee Two: Yeah, if the *avium* or, ok question on the question, is it comparing the *avium* to the *campyl*---

Interviewer: *Campy*, we can call it *Campy*.

Interviewee Two: *Campy*, ok. And then comparing the *mycobacterium* to *E. coli*?

Interviewer: Yup, and so based off of that, you, which one would you say is more related?

Interviewee Two: The *E. coli*.

Interviewer: The *E. coli*. And why would you say that?

Interviewee Two: Because that's following my first thought that closer in numbers, the more related they are.

Interviewer: Ok, that's good. So um, let's talk about some other features of the tree just to maybe get ideas. I know you might not exactly be confident on what they are, but we can maybe think about it anyway.

Interviewee Two: Ok.

Interviewer: So for the nodes, those are those red dots, what, what might those represent?

Interviewee Two: Hmm probably the doorway where pathways of different species changed. Maybe.

Interviewer: Can you explain that a little bit?

Interviewee Two: Like, I kind of want to look at this like, three, as if these little squares are related. So like, I feel like there should be more branching out this way [from first node] that it's starting with this species and branched off into these species this way, and goes along that path, if that makes sense.

Interviewer: That does make sense; generally, yeah we might have more organisms on a tree.

Interviewee Two: So, it just feels like it's missing some of the origins.

Interviewer: Ok, if, so pretend we did have some origin over here [points to first node], what's, can you explain the order of this tree then? So what, if we, say we had a line coming from over here [draws from first node], and we had a bunch of ancestors over here…

Interviewee Two: Well, so, started here with the ancestors and when down this pathway. But then, a mutation or something occurred, across a couple different things. So then one went this way towards the [*Bacillus*] and then one went the other way towards the first red dot at the 0.02. And then there was another mutation, and then it broke off again, except for this time it went to *Mycobacterium avium* and then to the next red dot at .028…

Interviewer: Ok

Interviewee Two: and then another mutation, and then goes out towards the last two, the *Campy* and *E. coli*.

Interviewer: Ok, so--

Interviewee Two: That's logically how I want to like look at this, but I have no idea how it works.

Interviewer: That's fair. And so thinking about what you just explained, how might, um, the numbers fit into that?

Interviewee Two: Yeah. And, like while I'd like to say the ones right next to each other are the more closely related, I don't necessarily know that, because I don't know what happened at the red dot that caused them to change, and how much they changed and the mutations that they've had.

Interviewer: Ok.

Interviewee Two: So like I do want to stick with my original thing, that the numbers that are closer are more related.

Interviewer: Ok, yeah, and so the branches seem to be different lengths too. Do you have any ideas for why that may be?

Interviewee Two: I supposed I didn't really notice that before.

Interviewer: That's ok. So like Campy--

Interviewee Two: Maybe how long it took for it to actually grow to become a species?

Interviewer: Ok.

Interviewee Two: But. Hmm. Or maybe the line is representing the number that they have. Because that's really the only pattern I can see. But I don't know what the numbers they have means either, so.

Interviewer: Ok, that's fair.

Interviewee Two: But yeah, my best guess is maybe that's associated with the number or how long it took to develop into a species, or become that thing.

Interviewer: Ok, cool. Well any, any last thoughts on the tree or 16S? I know this might have been a little bit of foreign information to you.

Interviewee Two: Oh yeah! Definitely. Because I still have only taken like, technically I'm on my second microbiology course that I've taken. Because I've taken Intro to Microbiology, and now I'm taking, um, Animal Cell Culture Techniques.

Interviewer: Yup.

Interviewee Two: So, it's a lot of new information being thrown my way.

Interviewer: Yeah, and Animal Cell Culture Techniques I don't think gets into any of this.

Interviewee Two: No.

Interviewer: No. So I mean if you take more micro classes you might.

Interviewee Two: Yeah, which I will be getting to.

Interviewer: Yeah, and so part of me collecting um the demographic data, so I'll know that, I'll be able to see that well she's taken Animal Cell Culturing, and she's taken, what? Intro.

Interviewee Two: Yeah.

Interviewer: Which one was it?

Interviewee Two: Um, 202?

Interviewer: 202? Yeah, so that will, I mean that's very telling right?

Interviewee Two: And then just Bio 150.

Interviewer: Yeah, and so you haven't taken as many classes as other people so that's understandable.

Interview Three

Interviewer: So the first one that I had on the assessment was the 16S RNA, um so that was the actual problem if you do recall it. Before we get to that though I just want to know, what is your current understanding of 16S ribosomal RNA subunit, or gene?

Interviewee Three: I would say it's very, it's very base knowledge. I know it exists and I know in general what we utilize it for, and you know um, things like that.

But as far as like more detailed stuff there's no way I could like sit and teach a class on it or even like talk for 10 minutes on it, really.

Interviewer: Sure, um, do you have any ideas for maybe why it's useful to determine species relatedness?

Interviewee Three: Well, going back to my animal science background, um, it's important to avoid inbreeding, because, especially like, especially nowadays with so much line breeding with dogs and cats and all of our domesticated animals the last thing you want to do is inbreed and start bringing out more defects because of that. Um, on a more human standpoint, um if I remember right, I believe this is the one you use for like paternity tests and stuff? And so it's used to um check that, you can use it to detect genetic diseases if you know the specific strain that, um causes a specific, um, genetic disorder in people you could find—you could compare the traits side by side and see the chances of it occurring.

Interviewer: Ok, cool. So kind of getting to that. So is 16S, is that in all organisms? Is that something everybody has?

Interviewee Three: Um, I mean, I, just from the problems and what little I know it makes sense that even like the smallest microorganisms would have it because, I mean we're able to differentiate between two different strains of E. coli by looking at the genetics, so that leads me to believe that this can be found in all. If it's not found in any, I would say maybe it's not found in certain, um, prokaryotes because they don't have as complex of a genetic structure and cell structure as other things and…

Interviewer: So for prokaryotes can you give me an example of maybe what, what that might look like?

Interviewee Three: Um, I don't know if I would be able to because the main thing in prokaryotes is the fact that they don't have a nucleus and organelles and set structures to help kind of define their genetic structure. They're just kind of have, you know, free-floating DNA, so um, that's why I'm kind of on the fence about whether or not they it; because they do have DNA, but it's not as structured as other organisms.

Interviewer: Sure.

Interviewee Three: And that's why I'm like kind of on the fence about viruses too. Because they're not, you know, the whole argument are they living or not because they technically have some form of DNA but.

Interviewer: Yup.

Interviewee Three: So if it's not found in anything I would say it's not in prokaryotes or viruses but I kind of think they have at least some semblance of that because we're able still to genetically differentiate them.

Interviewer: Sure. Ok, cool. Well let's get into this actual problem. So I do have a marker, and you can feel free, so there is paper on that board and so we're basically going to go through this step by step just to see how went through it when you were doing it by yourself.

Interviewee Three: Ok.

Interviewer: So the first thing I'm wondering is the reference sequence, what, what is that?

Interviewee Three: Uh that is the specific um pattern that is um, that all the other uh strains, strains, I'm not sure if I'm using the correct terminology, all the other strains are compared to basically it's just kind of like the baseline for what is being sought after.

Interviewer: Ok. And so, what do the letters represent?

Interviewee Three: They represent the different uh, nucleotides in the DNA/RNA sequence.

Interviewer: Ok and so why are these sequences different for organisms 1-4? Why might they have different letters?

Interviewee Three: Um it could be anything as simple as uh it was just the genetics they received from their parents. Um, it could be um a simple um, mutation where just a nucleotide got altered. Or like in two and three where there's the two side by side the two are kind of different, it could have just been a flip of the nucleotides. Um, you know, it could be anything from a freak accident to actually um you know just naturally happening from parents. And I guess you could argue that it could be, um this could be an instance where, um, they have the baseline line of, the base reference sequence of an organism and then all of these are different alterations that have been made in a laboratory, and then to compare how the alterations um, are to compare and contrast the alterations of the genetic sequences from the reference sequence.

Interviewer: Ok, that makes sense. So first step, when you looked at this problem, um what were your thoughts.

Interviewee Three: Well my first thought was, I've never really seen a problem like this before, thank goodness this isn't graded. But um, I knew immediately the reference sequence was going to be the key to this. And once I recognized the fact that the colored nucleotides in 1-4 represent variations from the reference sequence, I was able to start piecing together, um, things that were similar and different, and so I knew that the more white I saw in a single strain, the more like the reference sequence it would be. So I was able to find ones that were very different from the reference sequence, and ones that were very similar to the reference sequence.

Interviewer: And then what did you do when you found the most similar or the most different?

Interviewee Three: Um, basically what I did was, um I immediately noticed that number four had the least amount of change among all of them. So I knew that when it came to the least related um, nucleotides, the genetic sequences, that it would probably be whichever one furthest from number four, and I could, and I knew that number three was the furthest from the reference sequence because of how many, uh, nucleotides had changed. Um, yeah, if I counted right it was three.

Interviewer: Yup.

Interviewee Three: And so I was able to kind of piece together that, um, three and four seemed to me to be the least related.

Interviewer: Ok.

Interviewee Three: And then when it came to finding the most related ones um, basically, I kind of took it as, obviously the, I immediately took notice of the ones that shared the most um non-colored nucleotides. The ones that were closest to the reference sequence. And then I guess the way I kind of thought about it, um I kind of did this with the least related one too, is I basically, um, imagined if I took, you know like I took three and four, and I kind imagined if I overlaid them on top, how many of the letters would actually overlap. And so like three and four, if I overlapped those, that sequences would be almost completely colored with how different they are and so I tried to find ones where if I overlapped them, um, you wouldn't see too much of a change in it. So um like, my first thought was one and three might be the most related because they have about the same amount of mutations in them.

Interviewer: Yup.

Interviewee Three: Um but as I looked a little closer I kind of leaned between one and two, um, just because of going through the non-colored ones, um, almost all their nucleotides that are the same as the reference sequence, um, they all seem to line up a lot for the most part. I mean outside of four, if I'm counting right they all generally seem to line up.

Interviewer: So would it be possible for you to kind of, um, I think, I think I'm understanding, but would it be possible for you to draw it and show me what you mean I guess?

Interviewee Three: Yeah, so like if I, so like when I was looking for least related I took uh these two like that.

Interviewer: Sorry, yeah that's going to follow you.

Interviewee Three: That's fine. Um and if brought this down there, this down there, that, and just kind of overlaid them…

Interviewer: Ok.

Interviewee Three: This sequence, um, you know there wasn't very, like all in all there weren't very many that you know, lined up. Like, they lined up here, but these three didn't line up, these three didn't line up, so it felt like those two had the most variation to me…

Interviewer: Ok.

Interviewee Three: Because they didn't share that much in common.

Interviewer: Sure.

Interviewee Three: But if looked at one and two, which is what I put my answer for the most related. Um, is just immediately looking, like it matches up here, here, here, here, [crossing off white spaces between sequences one and two]. Like, there's just so many that it legitimately matches up with.

Interviewer: Yeah.

Interviewee Three: And I just kind of ignored this one [pointing to nucleotides changed in both sequence one and two], I kind of ignored this one when I was looking at it because, um, I kept everything in relation to the reference sequence, so since both of these variated from the reference, I just kind of, in my head they kind of cancelled out.

Interviewer: Ok

Interviewee Three: And so that only left a few that were, um, avariant.

Interviewer: Ok, that makes sense. And so then, so then you put one and two for most related. What did you have for least related?

Interviewee Three: Uh, three and four.

Interviewer: Ok, cool. Sorry, just moving that. Alright, and so then for three and four we said that it was least related because they have more of the overlaps that don't match together.

Interviewee Three: Right.

Interviewer: Yup. Ok, good, I think I understand that. So, we can go on to our next, next one. And perhaps I will change the paper, if that's too distracting.

Interviewee Three: Oh, it's not too distracting.

Interviewer: Ok, well I think we have to zoom out a little bit. Alright. So for this phylo trees. How do you feel about phylo trees?

Interviewee Three: I really had no idea what they were going into it, so I just kind of related it to any other information I could possibly think of, and tried to common sense it out a little bit.

Interviewer: Sure, yeah. So going through it, the first question asked you what are the two most related species. How did you, how did you approach that problem?

Interviewee Three: Um, basically how I kind of approached it, was um, I basically looked, in my head, and I know this is not even close to what if I took the right class what they would tell me to do, I looked at each red points as kind of, as if

these were, as if these were complete evolutionary branches. And so, um, well I can just get up and do this right off the bat.

Interviewer: That would be great.

Interviewee Three: Um so like here in my head [points to first node], is like a point of evolution, and I know I said it's over a period of time but I just kind of thought about it that way. And so whatever this was branched off into this [Bacillus] and then into new, this one [second node]. So in my head, this, um, organism here [Bacillus] and this red point [second node] branched into two different microorganisms.

Interviewer: Ok

Interviewee Three: And so then when it hit this red point [second node] and it branches this uh microorganism [Mycobacterium] and this red point [third node] were two branches of evolution again. And so we it hit here, you know, these both [Campy and E. coli] came directly to that same point [third node], in my head like the same branch of evolution, and so that, um, and so I put these two as most closely related.

Interviewer: Ok, that makes sense. And so when, the next problem asks for Mycobacterium versus Campylobacter and E. coli, asking which one is more related to Mycobacterium. How did you approach that?

Interviewee Three: Um, I see, it was between these two and then these two, right? Or is it…

Interviewer: Um between Mycobacterium, and then Campy and E. coli.

Interviewee Three: Ok, so um, basically the way I looked at it is, um, you know I still did the same trick where this is just the same branch. So, from my logic I know

that these two come back to the same ancestor, which goes back to this ancestor [second node] and back to *Mycobacterium*. Um, the way I kind of thought about it is um, whenever I see a branched evolutionary tree, which is the way I was approaching this, um typically ones that are closer related will end up closer to each other on the spectrum. And so in my mind, since this one evolved to here [third node] and then I guess for lack of better term it kind of evolved back into that one [tracing up], this one [Campy] um kept the most genetics closer to that one [Mycobacterium] which is why they're closer together. I mean, I know that you know these numbers are probably an indicator of, you know, something, but I never took a class yet that showed me this. So I just kind of when off evolutionary trees since that was what I was able to relate to.

Interviewer: Sure, that makes sense. Um, so some people didn't notice this, but did you notice that the branches are different lengths?

Interviewee Three: I do now.

Interviewer: Do you have any ideas for maybe what that, why that might be?

Interviewee Three: Um it's probably the amount of, I guess the best way to say this, the amount of variation from whatever point. So…

Interviewer: From the red node?

Interviewee Three: Yeah so, you know, like so this one is I think that's 66 or 68, .068 or .066, I can't really tell from here.

Interviewer: Yeah it's a little fuzzy.

Interviewee Three: Yeah, but you know so, maybe the amount of distance from this point [first node] to this point [Mycobacterium] now is the .02 plus the .124.

Interviewer: That makes sense.

Interviewee Three: Yeah, I was really not sure how to approach this one so I just went with what it looked like to me.

Interviewer: Yeah, that's totally cool. I mean I think, I actually think we pretty much covered it all. I think one thing I noticed, so you were talking about genetic distance. What do you mean by that?

Interviewee Three: Um, I don't' really know how to put it in specific terms but genetic distance in my head is the amount of variation you have from an organisms along the same genetic line. So, um, I mean I guess you could argue otherwise, like for example, um, our genetic distance from chimpanzees, in my mind, is not that far because what is it 98% of our genetics we share. So the genetic distance is, you know, fairly short there. But if we were to go back 500,000 years to earlier primates, we've evolved significantly more in, you know, in relation. And so we have a lot more genetic distance. And you could probably even bring that into specific traits of people as well. I don't specifically know how to word it, but it would be kind of similar to um how maybe something skips a generation so the genetic distance from you know the last occurrence could be measured that way too, like an actual set distance.

Interviewer: So for like the 98%, how do we determine, "oh, 98% different".

Interviewee Three: Yeah, I, in my mind it was always, they probably just took the, uh, chromosome profile of a chimpanzee and the chromosome profile of your general human on things that, um, generally would stay the same. Obviously they wouldn't look at, they probably wouldn't look at genetics that factor into say mental health or cholesterol levels, but maybe just basic genetics, you know, like muscle development, um, body structure, um, hormone production. Just base functions of the body and see how they compare on the genetic level.

Interviewer: That makes sense. Ok, well I think we've covered everything I want to cover. Um, so I can stop this.

Interviewee Three: Ok.

Interview Four

Interviewer: What is your current knowledge of 16S ribosomal RNA?

Interviewee Four: Um, not very much, really. I just taken like general micro, so then like my general biology courses, that's not that much. I think you have like 16S and 18S where you differentiate like eukaryotes from prokaryotes?

Interviewer: Ok.

Interviewee Four: And I think that's it.

Interviewer: Ok have you, do you have any thoughts on maybe you know, so why is the 16S is so useful? What about it makes it so useful to make comparisons?

Interviewee Four: Oh well I guess because it reduces like the amount of organisms that you're dealing with, it makes up for separation, so you can kind of follow

that pattern of separation that it's given to you. I don't know if I explained well myself.

Interviewer: So by separation, how do you determine that they're kind of separated?

Interviewee Four: Oh I'm thinking of phylogenetic tree, actually. So that's like the separation that it provides.

Interviewer: Ok, well, we can, I mean we can move into this problem. So I kind of want to take this slow, and let you, I mean write draw do whatever you need to do. The more you write the more I'll probably be able to follow you, so that is really helpful for me. Um, and so I just have some quick starting questions about the tree itself. So first off, what do you think the reference sequence might be?

Interviewee Four: Hmm. This one right here?

Interviewer: Yup!

Interviewee Four: So this is standard and you have four different sequences that you're comparing it to this one. So that's um, the reference point.

Interviewer: Sure. Um, and what do the different letters represent?

Interviewee Four: Bases, DNA. Bases.

Interviewer: And how come some of them are different, why they aren't all the same?

Interviewee Four: On the reference sequence?

Interviewer: Or the individual ones, why do, why do some have an A, and some a G, and some a T in the same spot?

Interviewee Four: Because these are not the same organism, probably. Or they have variations, so these are the variations that you see in the population when you have different like traits in the same population.

Interviewer: What causes the variation?

Interviewee Four: It's random. You have replication events, DNA gets copied and everything, and sometimes it is not on line in the same way, you know, for every cell division so it just happens randomly.

Interviewer: Yeah that makes sense, so when you first looked at this problem, um, I guess what did you think? What was your first step into trying to solve which two were the most related and which two were the least related?

Interviewee Four: The more bases that are the same in a sequence, the more related they are going to be because if you think that one letter change can make a change in a protein and can make a change in the whole organisms function, uh, the more similar the letters the more similar the proteins and traits are going to be.

Interviewer: Ok, so can you give me an example of how you went through the sequences, or show me how you went through it?

Interviewee Four: Um, so I didn't took that much attention to the reference sequences because I first like looked between them or among them [sequence 1-4], which ones were like most similar and then I took all of the reference sequence. So, first I just count, so you know that these colored ones are different. So I just count the numbers. So here, you have like one, two,

three, four, five, six. Here you have one, two… I'm just going to write here.

Interviewer: Yeah, perfect.

Interviewee Four: Six. Here you have four, here you have three, four, five, six, seven. And here you have seven, and here you have three. So, from this number we can say that number four is the most related to the reference sequence. Because you have three differences. And then you can go on and compare among the sequences, which one are more related and which ones are more distant.

Interviewer: So how did you handle um, deciding, for deciding your answer, how did you handle combining that information? So we have these numbers, that are the differences, and then we're also comparing with each other, so how did you combine all that information to determine your answer?

Interviewee Four: I guess just adding, like, compare like one to two, one to three, one to four, and then two to three, and two to four, and going like that. So for example here, you have six differences, but with these two you would have like one, two, you basically just add them, because each color is different for each, unless they would be the same color in the column, but for most of them they aren't, so I just add them.

Interviewer: Can we go through that?

Interviewee Four: Ok, so for example for one to two you will have one, two, three, four, five, six, seven, eight, nine, ten, and then you go one to three, and you have one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, and

then, so you have ten, twelve, then here you have two, three, four, five, six, eight, nine. So one and four would be more related than one to two to three.

Interviewer: Ok.

Interviewee Four: Then you can the same for two and three and one.

Interviewer: So did you ultimately decide to use um the comparisons between one through four to make your comparisons? Or did you use these? I suppose I'm, I'm just wondering how all the information is coming together. So you have, so for instance, I mean what was it for here, you had one, two, three, four, five, six, seven, eight, nine differences for one and four and then, four is three away from this and one is six away from this. So how did all that information come together for a determination? Or did you decide to go with one over the other?

Interviewee Four: Um, I guess I just decided to go with just a comparison between these four. I didn't use too much the reference sequence. I don't know if you can actually use it, probably yeah, but I just used the differences in the accumulation, so yeah. And how I did it, it was that, I got all the comparisons and the one with the highest number were like in the same group, and then with the, uh, more and more differences were in like other groups.

Interviewer: Can you explain that a little? So more differences were other groups?

Interviewee Four: Oh, for example like you have one and four so these ones are the more related. And then, one and three will be the least related because of the number of differences. And then one and two, will be the middle one.

Interviewer: So what was your, um, final answer for this question?

Interviewee Four: The two were most related were one and four, and the least related were one and three.

Interviewer: Least related one and four…

Interviewee Four: So I'll put it here [circles answers]

Interviewer: Ok and that was because when you counted these two together there were one, two, like, did you go through this again?

Interviewee Four: Yeah.

Interviewer: Like, one, two, three, four, five, six, seven, eight, nine, ten, eleven, yeah. Twelve?

Interviewee Four: [agrees]

Interviewer: Thirteen?

Interviewee Four: Yeah.

Interviewer: Ok and then when you counted those together that was nine.

Interviewee Four: Yeah. And the reference sequence I guess just makes it easy, because if you count both, it's the same as counting, it's just, I don't know if it's a coincidence or not but it's the same number as counting this. Like oh, the difference between one and four, it's nine bases. And two and three it will be eleven bases.

Interviewer: Oh, sure.

Interviewee Four: It's the same. So, the reference sequence actually helps you to make it faster.

Interviewer: Yeah, to count it. That makes sense. Ok, alright awesome. Do you have any additions or anything you maybe want to say about ribosomal RNA?

Interviewee Four: Probably not, but, just another thing is that, you can use the reference sequence because if you see the columns where there are changes, you have no repeated changes, so you don't have G and G, so that's why you can use these numbers. If you would have like for example T and T here you have to count them as one.

Interviewer: Ok, awesome. Well we can go for the phylogenetic tree, which is always fun. If I can find it, ok alright, so this is the tree that was on the assessment. Are you familiar with trees at all, are you comfortable with trees? Yeah? Ok, so the first question asks what are the two most related species? How did you determine that?

Interviewee Four: These two, *E. coli* and *Campylobacter*.

Interviewer: Ok, um, so what is your reasoning behind that? How did you rationalize that?

Interviewee Four: So you have the tree here like this, and these are all like common ancestors [points at all nodes]. So you have the first common ancestor [first node] that is for the whole group, and then you have [inaudible, points to second node] and all of these are related, and all of this in time are more recently related.

Interviewer: That makes sense. Ok.

Interviewee Four: Just got four levels.

Interviewer: Yeah, and so the second question is was *Mycobacterium* more related to *Campylobacter* or *E. coli*? What did you say for that one?

Interviewee Four: *Mycobacterium with…*

Interviewer: More related to *Campylobacter* or *E. coli*.

Interviewee Four: Equally.

Interviewer: Equally?

Interviewee Four: Yeah.

Interviewer: Can you go a little more into that?

Interviewee Four: Yeah, because you have this common ancestor which is for both groups. So, that this is this could be up or down [*Campy* and *E. coli* branches], it doesn't matter. And this actually is more related to time so it doesn't matter also [branch length]. So there's nothing that is indicating that. Because it's closer it's going to be like more closely um evolutionary [inaudible]. So it's just the same.

Interviewer: Yeah, so I want to go into something you just mentioned. So the time, what might those numbers represent, or branch length? Both I suppose.

Interviewee Four: The numbers I'm not sure, but the length is just time.

Interviewer: Time from?

Interviewee Four: From, this separation [third node].

Interviewer: Ok, that makes sense. Alright. Great. Um. I mean I think that was pretty clear. Um, do you have any other thoughts about the tree that you could share?

Interviewee Four: Um, I don't know, maybe I'm just thinking what I know about those bacteria. But, besides that, I think it's just showing you the relationships, that's it.

Interviewer: Alright.

Interview Five

Interviewer: So first I want to start by asking, what's your current understanding of 16S ribosomal RNA?

Interviewee Five: Um 16S r--, 16S r?

Interviewer: Ribosomal RNA

Interviewee Five: Ribosomal RNA is a very conserved, um, segment that is used to, um, to compare two species or three species and figure out how they fit together. You look at the, um, the change between them and the theory is that if two, if two species are more closely related than a third species they'll have more in common than the third sequence.

Interviewer: Ok, so I want to go back to the word conserved, what do you mean by that?

Interviewee Five: It changes very little, it's changed very little over time.

Interviewer: Ok, do you have any thoughts for maybe why that is?

Interviewee Five: So for processes that are very um, very essential to the organism, and there's not a whole lot room for error, mutation's, um, a very important process that's very honed in, usually aren't advantageous.

Interviewer: Sure, and what function does 16S provide?

Interviewee Five: Well it's part of the ribosome, so I would say that it helps with, um, translation.

Interviewer: Yeah, sure.

Interviewee Five: I'm not sure, and the packaging aspect.

Interviewer: That's ok, that's good. Um, so you went into this a little bit, let me make sure I understand. So for using 16S ribosomal RNA to compare species, we're looking, so at differences on what level? So you're saying that if they're more similar, they're more related. Um, so how do we determine those similarities or those differences?

Interviewee Five: You use, um, RNA, so you'd get the um, you can have sequencing done and that will print out the, the bases and then you just line them up from start to finish and you just look at how many times they differ--

Interviewer: Ok

Interviewee Five: and then the more times like if one and two differ only on two base pairs but one and three differ on like six different base pairs, then one and three will be less related than one and two, and one and three would have diverged before one and two diverged.

Interviewer: Ok, that makes sense. So that's a good um, transition. Last question though, is 16S, is that in all organisms or is that in some organisms?

Interviewee Five: Some, I believe it's not in fungus. Which is why when you do soil you can use 16S just for bacteria because the, the fungus doesn't have it.

Interviewer: Ok, does the fungus have anything?

Interviewee Five: I think it has an 18S.

Interviewer: Ok, alright cool. So, I mean you started to explain it, but I'm hoping you can show me what you did to go through this problem. So, I have a giant marker, I will turn this, and you can go through this together. [inaudible, movement].

Interviewee Five: Sure.

Interviewer: Ok so marker, so first thing I want to ask, um, what is the reference sequence? [video cut out] [new video] Ok, we're good.

Interviewee Five: Um the reference sequence is um, the reference sequence is the one that the rest of them were compared to, I believe that that is the um, if you look at like a bunch of, a bunch of one species of bacteria and you find like the s--, the one that's most common and you're able to rule out like mutations by just averaging the sequence among a bunch of individuals of one species.

Interviewer: Ok, and the letters, I think you mentioned it but what do those represent?

Interviewee Five: Um, those are base pairs of the DNA, so guanine, thymine, cysteine, and adenine, cytosine.

Interviewer: Cool, um great. So what, when you first looked at this, what your first step, what your first approach, first thought?

Interviewee Five: Well, I looked at the um highlighted differences and, I just looked at, um, how, I noted how many of them were different from the reference sequence, so four had like three differences, and three had [counting] seven, and two had [counting] four, and one had [counting] six.

Interviewer: Ok.

Interviewee Five: They're all different so then I just went by the number of differences.

Interviewer: Ok so, going by the number of differences, so you went from here and then you answered the question, how, that's what I'm, is that what you did?

Interviewee Five: That's how I started looking at the question.

Interviewer: Ok! So what was the second step?

Interviewee Five: What was the question again for this?

Interviewer: Oh, so, right down here you see the two most related and the two least related, so we were trying to determine, um, which species were most related and which were least related.

Interviewee Five: Oh. Um, I looked…

Interviewer: And do you want, I can show--

Interviewee Five: That's not exactly helpful if you're trying to compare to the reference sequence. So um, what I did for that I guess was, I looked at the ones that had the um, the most base pairs in common so then like, if you're comparing one and four, that'd be one, two, three, four, five, six, seven of them don't match up. And then if you… So then one and four would be seven. And then, um, then you would have one and three would be one, two, three, four, five, six, seven, eight, nine, and then ten.

Interviewer: Ok.

Interviewee Five: [Counting]. Then, one and two is, one, two, three, four, five, six, seven, eight, nine. Then you have, two and three, and that's one, two, three, four, five, six seven, eight, nine. And then two and four is one, two, three, four,

five, six, seven. And then three and four is one, two, three, four, five, six, seven, eight, nine, and ten. Ok that's all of them. So then I would just go with, um, one and four, and two and… [re-counts]. I thought that one of them was less than the others.

Interviewer: Yeah, take your time.

Interviewee Five: Oh um, maybe [counting]. I'm going to change my number one and four, because I count one two three four five six seven, and eight. I think I missed that one. So if I change that one to eight. I think I missed that one the first try. So if I change that one to eight then, there's one that's smaller than the rest of them. So then I would pick, um, two and four because um, they have the smallest number. Wonder if I messed up one and three [re-counts]. I think one and three is… so if that one's eleven and it's higher than the rest of them so that would mean that one and three have the most mutations compared to each other, so I would say that the least related are one and three. That's how I went about it.

Interviewer: Cool.

Interviewee Five: Just counting the numbers, and then going by the fewest mutations and the most mutations.

Interviewer: Yeah. Do you have any last thoughts about 16S before we go on to the phylogenetic tree?

Interviewee Five: No.

Interviewer: Ok, sounds good. So I'll set this down and we can change everything up. Just scroll [inaudible]… Ok so I can change this [changes paper on

board]… just grab a new one… Ok, cool. So, and [inaudible], there it is, ok, so basically we're just going to do the same thing, going through and seeing how you solved the problem, so the first question was the two most related species. How did you determine what the two most related species on this tree were?

Interviewee Five: Um, I didn't take my biology class here, in high school, and we didn't really talk about this a whole lot back home, so I, what I know is that um the closer they diverge, that means that uh, or, the sooner to the reference point up here [third node] that they diverge, that means the more different that they are. So the—you asked for the closest ones?

Interviewer: Yup.

Interviewee Five: So the closest ones they survived this mutation here [second node], and they survived there—they survived this mutation here [third node], and they survived this mutation here [second node] and so I said that these two were the most similar [*Campylobacter* and *E. coli*], I don't know what the numbers are though.

Interviewer: Ok, that's fair.

Interviewee Five: That hasn't come up in my classes… that I remember.

Interviewer: That's totally fine, um so, the red dots, what do those represent?

Interviewee Five: That represents um, the, where the two, in the case of the species where two, the point in time which those species diverged into each other.

Interviewer: Ok, so the other question was, is *Mycobacterium avium* more related to *Campylobacter jejuni* or *E. coli*? How did you determine that answer?

Interviewee Five: I believe that I said *Mycobacterium* is… I this part is was a part I wasn't sure on. Because… I was, I remember I was trying to debate if you want the numbers closer together or farther apart. Since I didn't know, I believe… I that know the spatial relation right here has something to do with it but I don't know what that means. I told myself I was going to look it up after I looked at it but I didn't.

Interviewer: Can you elaborate on that, the spatial relation?

Interviewee Five: So there's, a, I know that there's a reason that you place this one, that you don't flip it the other way. And I think that's because you're going down and looking at something more specific. And so the ones on the top and the ones on bottom are usually the least related, and I think that's what I went with in the paper, was that these are the least related [*Mycobacterium* and *E. coli*] because they're farther apart than these two [*Mycobacterium* and *Campylobacter*], but their numbers are also closer together so I was confused by that.

Interviewer: Sure.

Interviewee Five: Last time I took an actual biology class was my senior of high school, I took it dual credit, so I got college credit for it, but it was my high school teacher teaching it and we didn't really discuss phylogenetics other than like can you pick out, and like, we didn't have numbers on ours when we learned about it and so we just did which ones are closer here and which ones are farther apart.

Interviewer: Yeah, and I'm learning that that's unfamiliar so you're not alone. Um, ok so do we want to pick one, or do we want to go with we're unsure because the numbers, we don't know what that means? And either is totally fine.

Interviewee Five: I think one of the, oh no, I think the line length is directly related to the number. Oh yeah, because you get 111, and 23, and 124.

Interviewer: Do you have any thoughts on maybe--

Interviewee Five: I think that's a good, that's a good um, I would, if this were on a test I think I would go with the one with 111, just because 111 and that makes 124, and I'm not sure if that's a good way to go about it but I think that would be my guess.

Interviewer: Sure.

Interviewee Five: Oh wait that's not 124, that's 134. Is that, wait what's this number?

Interviewer: Uh, 111.

Interviewee Five: Oh it is 111.

Interviewer: Yeah, it's a little fuzzy.

Interviewee Five: That'd be 134, so that would be, that'd still be 10 points difference versus 35 on here.

Interviewer: [agrees]

Interviewee Five: I would pick *E. coli* and *Mycobacterium*.

Interviewer: Ok, great. Yeah and I think we hit all the spots. Um, do you have any thoughts on maybe why those branches are all different lengths? And you noticed that they were corresponding with the numbers.

Interviewee Five: I believe that this would with the next branch point. And this is just how long this specific, um the length of time before um, this one branched off, or um, um, evolved into a different branch, I think that's just how long it was conserved. Like um, *Bacillus*, is that *thuringiensis*?

Interviewer: [Agrees}

Interviewee Five: Would have, um, I don't know if this is directly related to years or not, or if it's like a diversity index or something but, I would say that this is the length of time that it, before it branched off next. I'm not sure on that though.

Interviewer: Ok.

Interviewee Five: I should probably look at that.

Interviewer: That's, and if you have questions when we're done I will answer any questions too. So you don't have to leave feeling totally, like you got nothing from this. Cool, well any other thoughts on phylogenetic trees? Or how this, did this overwhelm you, did this question, was it a little overwhelming or what it just like eh, tree?

Interviewee Five: I don't think it was overwhelming. I think if it was on a test and it was worth a lot of points and I didn't know this that would be overwhelming because I because I get overwhelmed by not knowing things sometimes…

Interviewer: Yeah.

Interviewee Five: Phylogenetic trees as a whole really don't bother me.

Interviewer: Sure, ok cool. Alright, well I think that's all I have so I can end this.

APPENDIX H. CHI SQUARE ANALYSIS AGAINST DEMOGRAPHIC FACTORS

Table H1. *Parameters for Chi-Square analysis.*

Demographic Factor	Parameter	
Gender	Female	
	Male	
Age	Low	≤20
	Middle	21
	High	≥22
Ethnicity	White	
	Non-White	
College GPA	Low	≤3.255
	Middle	3.778
	High	≥4.000
College Credits Complete	Low	≤80
	Middle	99
	High	≥194
Classes Taken	Low	≤6
	Middle	8
	High	≥22
Major	Pre-Pharmacy	
	Microbiology	
	(Pre) Medical Laboratory Science	
	Other	
ACT	Low	≤25
	Middle	28
	High	≥35
High School GPA	Low	≤3.665
	Middle	3.940
	High	≥4.000
Home State	North Dakota	
	Minnesota	

Table H2. *Significant Demographic-Code P-Values*

Prompt	Code	Demographic Factor	Holm's P-Value	Individual Contribution to Chi									Interpretation
				High			Middle			Low			
				0	1	2	0	1	2	0	1	2	
1.3	Unspecified	H.S. GPA	0.010	2.47	2.71		0.20	0.22		3.96	4.34		High fewer than expected; Low more frequently than expected
1.3	Unspecified	College GPA	0.045	3.44	3.61		0.73	0.76		1.00	1.05		High fewer than expected; Middle fewer than expected; Low more frequently than expected
2.6A	Correct	Age	0.033	5.34	3.00		0.49	0.27		1.48	0.83		High fewer than expected; Middle as expected; Low higher than expected
2.7A	Incorrect	College GPA	0.010	4.12	5.00		0.79	0.96		1.31	1.58		High more frequently than expected; Low fewer than expected, Middle fewer than expected

Table H2. *Significant Demographic Code P-Values (continued).*

Prompt	Code	Demographic Factor	Holm's P-Value	Individual Contribution to Chi									Interpretation
				High			Middle			Low			
				0	1	2	0	1	2	0	1	2	
2.7B	Node	College GPA	0.001	4.57	1.72	7.03	0.40	1.44	0.04	2.34	0.00	6.45	High more frequently 1, 2 & less frequently 0; Low more frequently 0 & less frequently 2; Middle less frequently 1
2.7B	Equally Related	College GPA	0.001	3.74	9.20	---	0.13	0.32	---	2.62	6.44	---	High more frequently than expected; Low fewer than expected; Middle as expected

APPENDIX I. RAW DATA

Table I1. *Q.1.1 Raw Data*

Response	Mutation	Present	Function	Relatedness	Not Applicable/ Restatement A	Not Applicable/ Restatement B	No Response A	No Response B
1	0	0	0	0	1	0	1	0
2	0	1	0	2	0	0	0	0
3	0	1	0	1	0	0	0	0
4	0	0	0	0	0	0	1	1
5	2	0	0	2	0	0	0	0
6	0	2	0	2	0	0	0	0
7	0	0	0	2	1	0	0	0
8	0	2	0	0	1	0	0	0
9	0	2	0	0	1	0	0	0
10	0	0	1	0	1	0	0	0
11	0	0	1	0	0	0	1	0
12	0	1	0	2	0	0	0	0
13	0	2	0	2	1	0	0	0
14	0	0	0	0	1	1	0	0
15	0	0	0	2	1	0	0	0
16	0	0	2	0	0	0	1	0
17	0	2	0	0	1	0	0	0
18	0	0	0	0	1	1	0	0
19	0	0	0	2	1	0	0	0
20	---	---	---	---	---	---	---	---
21	0	2	0	2	0	0	0	0

Table I1. *Q.1.1 Raw Data (continued).*

Response	Mutation	Present	Function	Relatedness	Not Applicable/ Restatement A	Not Applicable/ Restatement B	No Response A	No Response B
22	0	0	0	0	1	0	1	0
23	0	0	0	0	1	0	1	0
24	0	0	0	2	1	0	0	0
25	0	0	0	0	1	1	0	0
26	0	0	0	0	1	0	1	0
27	0	2	0	1	0	0	0	0
28	0	0	0	0	1	0	1	0
29	0	0	0	0	1	0	1	0
30	0	0	0	0	1	1	0	0
31	0	0	0	2	1	0	0	0
32	0	0	0	0	0	0	1	1
33	0	1	1	0	1	0	0	0
34	0	2	0	1	0	0	0	0
35	0	1	0	2	0	0	0	0
36	0	0	0	0	0	0	1	1
37	0	0	0	0	0	0	1	1
38	0	0	0	0	0	0	1	1
39	0	1	0	0	0	0	1	0
40	0	0	0	1	0	0	1	0
41	---	---	---	---	---	---	---	---
42	0	0	0	0	1	0	1	0
43	0	0	0	0	1	0	1	0
44	0	0	0	0	1	1	0	0

Table I1. *Q.1.1 Raw Data (continued).*

Response	Mutation	Present	Function	Relatedness	Not Applicable/ Restatement A	Not Applicable/ Restatement B	No Response A	No Response B
45	---	---	---	---	---	---	---	---
46	0	0	0	1	1	0	0	0
47	0	1	0	2	0	0	0	0
48	---	---	---	---	---	---	---	---
49	0	0	0	0	0	0	1	1
50	0	0	0	0	1	1	0	0
51	0	0	0	0	1	1	0	0
52	0	0	0	0	1	1	0	0
53	0	0	0	1	1	0	0	0
54	2	0	0	0	1	0	0	0
55	0	1	0	2	0	0	0	0
56	0	1	0	0	0	0	1	0
57	2	0	2	0	0	0	0	0
58	0	0	0	0	1	1	0	0
59	0	0	0	2	0	0	1	0
60	0	0	0	2	0	0	1	0
61	0	0	0	0	1	0	1	0
62	0	0	0	0	1	0	1	0
63	0	0	0	1	0	0	1	0
64	0	0	0	0	1	0	1	0
65	0	0	0	0	1	0	1	0
66	1	0	0	0	1	0	0	0

Table I1. *Q.1.1 Raw Data (continued).*

Response	Mutation	Present	Function	Relatedness	Not Applicable/ Restatement A	Not Applicable/ Restatement B	No Response A	No Response B
67	0	1	0	0	1	0	0	0
68	0	1	0	0	1	0	0	0
69	0	0	0	0	1	1	0	0
70	0	0	0	0	0	0	1	1
71	2	0	0	0	1	0	0	0
72								
72	1	0	0	1	1	0	0	0
73	0	1	0	1	0	0	0	0
74	0	1	0	0	1	0	0	0
75	1	1	0	2	0	0	0	0
76	0	0	0	1	1	0	0	0
77	---	---	---	---	---	---	---	---
78	1	1	0	0	0	0	0	0
79	0	0	0	0	1	1	0	0
80	0	0	0	1	0	0	1	0
81	0	1	0	0	1	0	0	0
82	0	0	1	0	1	0	0	0
83	0	0	0	2	1	0	0	0
84	0	0	0	0	1	1	0	0
85	0	0	0	0	1	1	0	0
86	0	0	2	1	0	0	0	0
87	0	0	0	0	1	0	1	0

Table I1. *Q.1.1 Raw Data (continued).*

Response	Mutation	Present	Function	Relatedness	Not Applicable/ Restatement A	Not Applicable/ Restatement B	No Response A	No Response B
88	0	0	0	0	0	0	1	1
89	0	0	0	2	1	0	0	0
90	1	0	0	1	0	0	0	0
91	0	2	0	2	0	0	0	0
92	0	2	0	0	1	0	0	0
93	0	0	0	0	0	0	1	1
94	0	0	0	0	1	1	0	0
95	0	1	0	0	1	0	0	0
96	0	0	0	0	0	0	1	1
97	0	0	0	0	1	0	1	0
98	0	0	0	1	1	0	0	0
99	0	1	0	0	1	0	0	0
100	0	0	0	1	1	0	0	0
101	0	0	0	2	1	0	0	0
102	0	1	0	1	0	0	0	0
103	0	1	0	0	1	0	0	0
104	0	0	0	1	1	0	0	0
105	0	0	0	0	1	1	0	0
106	0	0	1	1	0	0	0	0
107	0	0	0	2	0	0	1	0
108	0	0	0	0	1	0	1	0
109	---	---	---	---	---	---	---	---

Table I1. *Q.1.1 Raw Data (continued).*

Response	Mutation	Present	Function	Relatedness	Not Applicable/ Restatement A	Not Applicable/ Restatement B	No Response A	No Response B
110	0	0	1	0	1	0	0	0
111	0	0	0	2	1	0	0	0
112	0	0	0	0	1	1	0	0
113	0	0	1	2	0	0	0	0
114	0	0	0	2	0	0	1	0
115	---	---	---	---	---	---	---	---
116	---	---	---	---	---	---	---	---
117	---	---	---	---	---	---	---	---
118	---	---	---	---	---	---	---	---
119	0	0	0	0	1	0	1	0
120	0	0	0	0	1	0	1	0
121	0	1	1	0	0	0	0	0
122	0	1	0	0	1	0	0	0
123	---	---	---	---	---	---	---	---
124	---	---	---	---	---	---	---	---
125	2	0	0	2	0	0	0	0
126	0	1	0	0	1	0	0	0
127	0	2	0	0	1	0	0	0
128	0	0	0	0	1	0	1	0
129	0	0	0	1	1	0	0	0
130	---	---	---	---	---	---	---	---
131	0	0	0	1	1	0	0	0

Table I1. *Q.1.1 Raw Data (continued).*

Response	Mutation	Present	Function	Relatedness	Not Applicable/ Restatement A	Not Applicable/ Restatement B	No Response A	No Response B
132	0	0	1	0	1	0	0	0
133	1	0	0	0	1	0	0	0
134	0	2	0	1	0	0	0	0
135	0	0	0	0	1	0	1	0
136	0	1	0	0	1	0	0	0
137	2	2	0	0	0	0	0	0
138	0	0	0	0	1	0	1	0
139	0	0	0	0	0	0	1	1
140	0	0	0	0	1	1	0	0
141	0	0	0	1	1	0	0	0
142	0	2	0	0	1	0	0	0
143	0	0	1	1	0	0	0	0
144	0	1	0	0	1	0	0	0
145	0	0	0	1	1	0	0	0
146	0	0	0	2	0	0	1	0
147	---	---	---	---		---	---	---
148	0	0	0	0	0	0	1	1
149	0	1	0	2	0	0	0	0
150	0	0	0	1	1	0	0	0
151	0	0	0	0	0	0	1	1
152	0	0	0	0	1	0	1	0
153	0	2	0	2	0	0	0	0

Table I1. *Q.1.1 Raw Data (continued).*

Response	Mutation	Present	Function	Relatedness	Not Applicable/ Restatement A	Not Applicable/ Restatement B	No Response A	No Response B
154	0	1	0	0	1	0	0	0
155	0	2	0	0	1	0	0	0
156	0	1	0	0	1	0	0	0
157	0	1	0	0	0	0	1	0
158	0	0	0	0	1	1	0	0
159	1	1	0	2	0	0	0	0
160	0	2	0	0	1	0	0	0
161	0	2	0	0	1	0	0	0
162	0	0	0	0	0	0	1	1
163	0	0	0	0	0	0	1	1
164	0	0	0	0	1	1	0	0
165	0	1	0	0	1	0	0	0
166	0	0	0	0	0	0	1	1
167	0	2	0	1	0	0	0	0
168	0	0	0	0	0	0	1	1
169	0	0	0	0	0	0	1	1
170	0	0	0	0	1	0	1	0
171	1	0	0	0	1	0	0	0
172	0	2	0	2	0	0	0	0
173	0	0	1	1	0	0	0	0
174	0	0	0	2	1	0	0	0
175	0	0	0	0	1	1	0	0

Table I1. *Q.1.1 Raw Data (continued).*

Response	Mutation	Present	Function	Relatedness	Not Applicable/ Restatement A	Not Applicable/ Restatement B	No Response A	No Response B
176	1	1	0	0	0	0	0	0
177	0	2	0	0	1	0	0	0
178	0	0	0	0	0	0	1	1
179	0	0	0	0	0	0	1	1
180	0	1	2	0	0	0	0	0
181	0	0	1	2	0	0	0	0

Table I2. *Q.1.1 Alternative Idea Raw Data*

Response	Phenotypes	Simplicity
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	1
9	0	1
10	0	0
11	0	0
12	0	0
13	1	0
14	0	0
15	1	0
16	0	0
17	0	0
18	1	0
19	0	0
20	---	---
21	0	0
22	0	0
23	0	0
24	0	1
25	0	0
26	0	1
27	0	0
28	0	0
29	0	0
30	0	0
31	0	0
32	0	0
33	0	1
34	0	0
35	0	0
36	0	0
37	0	0
38	0	0

Table I2. *Q.1.1 Alternative Idea Raw Data (continued).*

Response	Phenotypes	Simplicity
39	0	0
40	0	0
41	---	---
42	0	0
43	0	0
44	0	0
45	---	---
46	0	0
47	0	0
48	---	---
49	0	0
50	0	0
51	0	0
52	0	0
53	0	0
54	0	1
55	0	0
56	0	0
57	0	0
58	1	0
59	0	0
60	0	0
61	0	0
62	0	0
63	0	0
64	0	0
65	0	0
66	0	0
67	0	0
68	1	0
69	0	0
70	0	0
71	0	0
72	0	0
73	0	0
74	0	0
75	0	0
76	1	0
77	---	---
78	0	0

Table I2. *Q.1.1 Alternative Idea Raw Data (continued).*

Response	Phenotypes	Simplicity
79	0	0
80	0	0
81	0	0
82	0	0
83	1	0
84	0	0
85	0	0
86	0	0
87	0	0
88	0	0
89	0	0
90	0	0
91	0	0
92	0	0
93	0	0
94	0	0
95	0	0
96	0	0
97	0	0
98	0	0
99	0	0
100	1	0
101	1	0
102	0	0
103	0	0
104	0	0
105	0	0
106	0	0
107	0	0
108	0	0
109	---	---
110	0	0
111	0	0
112	0	0
113	0	0
114	0	0
115	---	---
116	---	---
117	---	---
118	---	---

Table I2. *Q.1.1 Alternative Idea Raw Data (continued).*

Response	Phenotypes	Simplicity
119	0	0
120	0	0
121	0	0
122	0	0
123	---	---
124	---	---
125	0	1
126	1	0
127	0	0
128	0	0
129	0	0
130	---	---
131	0	1
132	1	0
133	0	0
134	0	1
135	0	0
136	0	1
137	0	0
138	0	0
139	0	0
140	0	0
141	0	1
142	0	1
143	1	0
144	0	1
145	0	0
146	0	0
147	---	---
148	0	0
149	0	0
150	0	0
151	0	0
152	0	0
153	0	0
154	0	1
155	0	1
156	0	0
157	0	0
158	1	0

Table I2. *Q.1.1 Alternative Idea Raw Data (continued).*

Response	Phenotypes	Simplicity
159	0	0
160	0	1
161	0	0
162	0	0
163	0	0
164	0	1
165	0	0
166	0	0
167	0	0
168	0	0
169	0	0
170	0	0
171	0	0
172	0	0
173	0	0
174	0	0
175	0	1
176	0	0
177	0	0
178	0	0
179	0	0
180	0	0
181	0	0

Table I3. *Q.1.2 Raw Data*

Response	Correct	Incorrect	No Response
1	0	1	0
2	0	1	0
3	0	1	0
4	1	0	0
5	1	0	0
6	0	1	0
7	1	0	0
8	1	0	0
9	0	1	0
10	0	1	0
11	1	0	0
12	0	1	0
13	1	0	0
14	1	0	0
15	1	0	0
16	1	0	0
17	0	1	0
18	1	0	0
19	1	0	0
20	---	---	---
21	1	0	0
22	0	0	1
23	0	1	0
24	0	1	0
25	1	0	0
26	0	1	0
27	0	1	0
28	0	1	0
29	0	1	0
30	0	1	0
31	0	1	0
32	1	0	0
33	0	1	0
34	0	1	0
35	1	0	0
36	0	1	0
37	0	1	0
38	1	0	0
39	1	0	0

Table I3. *Q.1.2 Raw Data (continued).*

Response	Correct	Incorrect	No Response
40	0	1	0
41	---	---	---
42	1	0	0
43	0	1	0
44	0	1	0
45	---	---	---
46	1	0	0
47	1	0	0
48	---	---	---
49	0	1	0
50	1	0	0
51	1	0	0
52	1	0	0
53	0	1	0
54	1	0	0
55	0	1	0
56	0	1	0
57	0	1	0
58	0	1	0
59	0	1	0
60	0	1	0
61	1	0	0
62	1	0	0
63	1	0	0
64	0	1	0
65	0	1	0
66	0	1	0
67	1	0	0
68	0	1	0
69	0	1	0
70	0	1	0
71	1	0	0
72	1	0	0
73	0	1	0
74	0	1	0
75	0	1	0
76	0	1	0
77	---	---	---
78	0	1	0
79	0	1	0

Table I3. *Q.1.2 Raw Data (continued).*

Response	Correct	Incorrect	No Response
80	0	1	0
81	1	0	0
82	1	0	0
83	0	1	0
84	1	0	0
85	0	1	0
86	0	1	0
87	0	1	0
88	1	0	0
89	1	0	0
90	0	1	0
91	1	0	0
92	0	1	0
93	0	1	0
94	0	1	0
95	0	1	0
96	0	1	0
97	1	0	0
98	0	1	0
99	0	1	0
100	1	0	0
101	0	0	1
102	0	1	0
103	0	1	0
104	0	1	0
105	0	1	0
106	1	0	0
107	0	1	0
108	0	1	0
109	---	---	---
110	0	1	0
111	1	0	0
112	0	1	0
113	0	1	0
114	0	1	0
115	---	---	---
116	---	---	---
117	---	---	---
118	---	---	---
119	0	1	0

Table I3. *Q.1.2 Raw Data (continued).*

Response	Correct	Incorrect	No Response
120	0	1	0
121	0	1	0
122	1	0	0
123	---	---	---
124	---	---	---
125	0	1	0
126	0	1	0
127	1	0	0
128	0	1	0
129	1	0	0
130	---	---	---
131	0	1	0
132	0	1	0
133	0	1	0
134	0	1	0
135	0	1	0
136	0	1	0
137	1	0	0
138	0	1	0
139	1	0	0
140	1	0	0
141	1	0	0
142	1	0	0
143	0	1	0
144	0	1	0
145	1	0	0
146	0	1	0
147	---	---	---
148	0	1	0
149	0	1	0
150	0	1	0
151	0	1	0
152	1	0	0
153	0	1	0
154	0	1	0
155	1	0	0
156	1	0	0
157	1	0	0
158	0	1	0
159	1	0	0

Table I3. *Q.1.2 Raw Data (continued).*

Response	Correct	Incorrect	No Response
160	0	1	0
161	1	0	0
162	0	1	0
163	0	1	0
164	0	1	0
165	0	1	0
166	1	0	0
167	1	0	0
168	0	1	0
169	0	1	0
170	0	1	0
171	0	1	0
172	1	0	0
173	0	1	0
174	0	1	0
175	0	1	0
176	1	0	0
177	0	1	0
178	0	1	0
179	0	1	0
180	0	1	0
181	0	1	0

Table I4. *Q.1.3 Raw Data*

Response	Sequence	Reference	Unspecified	Not Addressed	No Response
1	0	0	1	0	0
2	0	0	1	0	0
3	0	0	1	0	0
4	0	1	0	0	0
5	0	0	1	0	0
6	1	0	0	0	0
7	0	0	1	0	0
8	0	0	1	0	0
9	0	0	1	0	0
10	0	0	1	0	0
11	0	1	0	0	0
12	0	0	1	0	0
13	1	0	0	0	0
14	1	0	0	0	0
15	0	1	0	0	0
16	0	1	0	0	0
17	0	1	0	0	0
18	1	0	0	0	0
19	0	1	0	0	0
20	---	---	---	---	---
21	0	1	0	0	0
22	0	0	0	1	0
23	0	1	0	0	0
24	0	0	1	0	0
25	0	1	0	0	0
26	0	0	1	0	0
27	0	0	1	0	0
28	0	0	1	0	0
29	0	0	1	0	0
30	0	0	1	0	0
31	0	1	0	0	0
32	0	1	0	0	0
33	0	0	1	0	0
34	0	1	0	0	0
35	0	1	0	0	0
36	0	1	0	0	0
37	0	0	1	0	0
38	0	0	1	0	0
39	0	0	0	0	1

Table I4. *Q.1.3 Raw Data (continued).*

Response	Sequence	Reference	Unspecified	Not Addressed	No Response
40	0	0	1	0	0
41	---	---	---	---	---
42	0	0	1	0	0
43	0	0	1	0	0
44	0	0	1	0	0
45	---	---	---	---	---
46	0	1	0	0	0
47	0	1	0	0	0
48	---	---	---	---	---
49	0	0	1	0	0
50	0	1	0	0	0
51	0	0	1	0	0
52	0	1	0	0	0
53	0	0	1	0	0
54	1	1	0	0	0
55	0	0	1	0	0
56	0	0	1	0	0
57	0	0	0	0	1
58	0	0	1	0	0
59	0	0	1	0	0
60	0	0	1	0	0
61	0	1	0	0	0
62	0	1	0	0	0
63	0	0	1	0	0
64	0	0	1	0	0
65	0	0	1	0	0
66	0	0	1	0	0
67	0	1	0	0	0
68	0	0	1	0	0
69	0	0	1	0	0
70	0	0	1	0	0
71	0	1	0	0	0
72	0	1	0	0	0
73	0	1	0	0	0
74	0	0	1	0	0
75	0	1	0	0	0
76	0	1	0	0	0
77	---	---	---	---	---
78	0	0	1	0	0
79	0	0	1	0	0

Table I4. *Q.1.3 Raw Data (continued).*

Response	Sequence	Reference	Unspecified	Not Addressed	No Response
80	0	0	1	0	0
81	0	1	0	0	0
82	1	0	0	0	0
83	0	1	0	0	0
84	0	0	1	0	0
85	0	0	1	0	0
86	0	0	1	0	0
87	0	0	1	0	0
88	0	0	0	0	1
89	0	1	0	0	0
90	0	0	1	0	0
91	0	1	0	0	0
92	0	0	1	0	0
93	0	0	1	0	0
94	1	0	0	0	0
95	1	0	0	0	0
96	0	0	1	0	0
97	0	0	1	0	0
98	0	0	1	0	0
99	0	1	0	0	0
100	0	0	1	0	0
101	0	0	1	0	0
102	0	0	1	0	0
103	0	0	1	0	0
104	0	0	1	0	0
105	0	0	1	0	0
106	1	0	0	0	0
107	0	0	1	0	0
108	0	1	0	0	0
109	---	---	---	---	---
110	1	0	0	0	0
111	0	1	0	0	0
112	0	0	1	0	0
113	0	1	0	0	0
114	0	0	1	0	0
115	---	---	---	---	---
116	---	---	---	---	---
117	---	---	---	---	---
118	---	---	---	---	---
119	1	0	0	0	0

Table I4. *Q.1.3 Raw Data (continued).*

Response	Sequence	Reference	Unspecified	Not Addressed	No Response
120	0	0	0	1	0
121	0	0	1	0	0
122	0	1	0	0	0
123	---	---	---	---	---
124	---	---	---	---	---
125	0	0	1	0	0
126	0	1	0	0	0
127	1	0	0	0	0
128	0	0	0	0	1
129	0	1	0	0	0
130	---	---	---	---	---
131	0	0	1	0	0
132	0	0	1	0	0
133	0	0	1	0	0
134	0	0	1	0	0
135	0	0	1	0	0
136	0	0	1	0	0
137	0	1	0	0	0
138	0	0	1	0	0
139	0	1	0	0	0
140	0	0	1	0	0
141	1	0	0	0	0
142	1	0	0	0	0
143	1	0	0	0	0
144	1	0	0	0	0
145	0	1	0	0	0
146	0	0	1	0	0
147	---	---	---	---	---
148	0	0	1	0	0
149	0	0	1	0	0
150	1	0	0	0	0
151	0	1	0	0	0
152	0	1	0	0	0
153	0	1	0	0	0
154	0	0	1	0	0
155	1	0	0	0	0
156	0	0	1	0	0
157	0	1	0	0	0
158	0	0	1	0	0
159	0	1	0	0	0

Table I4. *Q.1.3 Raw Data (continued).*

Response	Sequence	Reference	Unspecified	Not Addressed	No Response
160	1	1	0	0	0
161	0	1	0	0	0
162	0	0	1	0	0
163	0	0	1	0	0
164	1	1	0	0	0
165	0	1	0	0	0
166	0	1	0	0	0
167	0	0	1	0	0
168	0	0	1	0	0
169	1	0	0	0	0
170	0	0	1	0	0
171	0	0	1	0	0
172	0	1	0	0	0
173	0	0	1	0	0
174	0	1	0	0	0
175	0	1	0	0	0
176	0	1	0	0	0
177	0	0	1	0	0
178	0	0	0	0	1
179	0	0	0	0	1
180	0	0	0	0	1
181	0	0	0	0	1

Table I5. *Q.1.3 Alternative Idea Raw Data*

Response	Letters	Base Pairs
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	1	0
7	0	0
8	0	0
9	0	0
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
15	0	0
16	0	0
17	0	0
18	0	0
19	0	0
20	---	---
21	0	0
22	0	0
23	0	0
24	0	0
25	0	0
26	1	0
27	0	1
28	0	0
29	0	0
30	0	0
31	0	0
32	0	0
33	0	0
34	0	0
35	0	0
36	0	0
37	0	0
38	0	0
39	0	0

Table I5. *Q.1.3 Alternative Idea Raw Data (continued).*

Response	Letters	Base Pairs
40	0	0
41	---	---
42	0	0
43	0	1
44	0	0
45	---	---
46	0	0
47	0	0
48	---	---
49	0	0
50	0	0
51	0	0
52	0	0
53	0	0
54	0	0
55	0	0
56	0	0
57	0	0
58	1	0
59	1	0
60	0	1
61	0	0
62	1	0
63	0	0
64	0	0
65	0	0
66	0	0
67	0	0
68	0	1
69	0	1
70	0	0
71	0	0
72	0	0
73	0	0
74	0	0
75	0	0
76	1	0
77	---	---
78	0	0

Table I5. *Q.1.3 Alternative Idea Raw Data (continued).*

Response	Letters	Base Pairs
79	0	0
80	0	0
81	0	0
82	0	0
83	0	0
84	0	0
85	0	0
86	0	0
87	1	0
88	0	0
89	0	0
90	0	0
91	0	0
92	0	0
93	1	0
94	0	0
95	0	0
96	0	0
97	0	0
98	0	0
99	0	0
100	0	0
101	0	0
102	0	0
103	0	0
104	0	1
105	0	0
106	0	0
107	0	0
108	0	0
109	---	---
110	0	0
111	0	0
112	0	0
113	0	0
114	0	0
115	---	---
116	---	---
117	---	---

Table I5. *Q.1.3 Alternative Idea Raw Data (continued).*

Response	Letters	Base Pairs
118	---	---
119	0	0
120	0	0
121	0	0
122	1	0
123	---	---
124	---	---
125	0	0
126	0	0
127	0	0
128	0	0
129	0	0
130	---	---
131	0	0
132	1	0
133	0	1
134	0	0
135	0	0
136	0	0
137	0	0
138	0	0
139	0	0
140	0	0
141	0	0
142	0	0
143	0	0
144	0	0
145	0	0
146	0	0
147	---	---
148	0	0
149	0	0
150	0	0
151	0	0
152	0	0
153	0	0
154	0	0
155	0	0
156	0	0

Table I5. *Q.1.3 Alternative Idea Raw Data (continued).*

Response	Letters	Base Pairs
157	0	0
158	0	0
159	0	0
160	0	0
161	0	0
162	0	0
163	0	1
164	0	0
165	0	0
166	0	0
167	0	0
168	0	0
169	0	0
170	0	0
171	0	0
172	0	0
173	0	0
174	0	0
175	0	0
176	0	0
177	0	0
178	0	0
179	0	0
180	0	0
181	0	0

Table I6. *Q.1.4 Raw Data*

Response	Correct	Incorrect	No Response
1	1	0	0
2	1	0	0
3	0	1	0
4	0	1	0
5	1	0	0
6	0	1	0
7	0	1	0
8	1	0	0
9	0	1	0
10	0	1	0
11	1	0	0
12	0	1	0
13	1	0	0
14	1	0	0
15	0	1	0
16	1	0	0
17	1	0	0
18	1	0	0
19	0	1	0
20	---	---	---
21	1	0	0
22	0	1	0
23	1	0	0
24	1	0	0
25	1	0	0
26	1	0	0
27	1	0	0
28	0	1	0
29	0	1	0
30	1	0	0
31	1	0	0
32	0	1	0
33	1	0	0
34	1	0	0
35	1	0	0
36	0	1	0
37	1	0	0
38	1	0	0
39	0	1	0

Table I6. *Q.1.4 Raw Data (continued).*

Response	Correct	Incorrect	No Response
40	0	1	0
41	---	---	---
42	1	0	0
43	0	1	0
44	1	0	0
46	0	1	0
47	1	0	0
48	---	---	---
49	0	1	0
50	0	1	0
51	0	1	0
52	1	0	0
53	1	0	0
54	1	0	0
55	1	0	0
56	1	0	0
57	0	1	0
58	0	1	0
59	0	1	0
60	0	1	0
61	0	1	0
62	0	1	0
63	0	1	0
64	1	0	0
65	1	0	0
66	1	0	0
67	1	0	0
68	1	0	0
69	0	1	0
70	0	1	0
71	1	0	0
72	1	0	0
73	1	0	0
74	1	0	0
75	0	1	0
76	0	1	0
77	---	---	---
78	0	0	1
79	1	0	0

Table I6. *Q.1.4 Raw Data (continued).*

Response	Correct	Incorrect	No Response
80	0	1	0
81	0	1	0
82	1	0	0
83	0	1	0
84	1	0	0
85	1	0	0
86	1	0	0
87	0	1	0
88	1	0	0
89	0	1	0
90	0	1	0
91	0	1	0
92	0	1	0
93	1	0	0
94	1	0	0
95	0	1	0
96	0	1	0
97	1	0	0
98	0	1	0
99	0	1	0
100	1	0	0
101	0	1	0
102	1	0	0
103	1	0	0
104	0	1	0
105	1	0	0
106	1	0	0
107	0	1	0
108	0	1	0
109	---	---	---
110	1	0	0
111	1	0	0
112	0	1	0
113	1	0	0
114	0	1	0
115	---	---	---
116	---	---	---
117	---	---	---
118	---	---	---
119	1	0	0

Table I6. *Q.1.4 Raw Data (continued).*

Response	Correct	Incorrect	No Response
120	0	1	0
121	1	0	0
122	0	1	0
123	---	---	---
124	---	---	---
125	0	1	0
126	1	0	0
127	1	0	0
128	0	1	0
129	1	0	0
130	---	---	---
131	1	0	0
132	1	0	0
133	1	0	0
134	1	0	0
135	0	1	0
136	1	0	0
137	1	0	0
138	1	0	0
139	1	0	0
140	1	0	0
142	1	0	0
143	1	0	0
144	1	0	0
145	1	0	0
146	1	0	0
147	---	---	---
148	0	1	0
149	1	0	0
150	0	1	0
151	1	0	0
152	0	1	0
153	0	1	0
154	0	1	0
155	0	1	0
156	0	1	0
157	0	1	0
158	0	1	0
159	1	0	0

Table I6. *Q.1.4 Raw Data (continued).*

Response	Correct	Incorrect	No Response
160	1	0	0
161	1	0	0
162	1	0	0
163	1	0	0
164	0	1	0
165	0	1	0
166	1	0	0
167	1	0	0
168	0	1	0
169	1	0	0
170	1	0	0
171	1	0	0
172	1	0	0
173	0	1	0
174	1	0	0
175	0	1	0
176	1	0	0
177	1	0	0
178	1	0	0
179	1	0	0
180	1	0	0
181	1	0	0

Table I7. *Q.1.5 Raw Data*

Response	Correct	Incorrect	No Response
1	1	0	0
2	0	1	0
3	0	1	0
4	0	1	0
5	1	0	0
6	1	0	0
7	0	1	0
8	1	0	0
9	1	0	0
10	0	1	0
11	0	0	1
12	1	0	0
13	1	0	0
14	1	0	0
15	0	1	0
16	0	1	0
17	1	0	0
18	1	0	0
19	1	0	0
20	---	---	---
21	1	0	0
22	0	1	0
23	0	1	0
24	1	0	0
25	1	0	0
26	1	0	0
27	1	0	0
28	0	1	0
29	0	1	0
30	1	0	0
31	1	0	0
32	0	1	0
33	0	1	0
34	1	0	0
35	0	0	1
36	1	0	0
37	1	0	0
38	0	1	0
39	1	0	0

Table I7. *Q.1.5 Raw Data (continued).*

Response	Correct	Incorrect	No Response
40	1	0	0
41	---	---	---
42	1	0	0
43	1	0	0
44	1	0	0
46	0	1	0
47	0	1	0
48	---	---	---
49	0	0	1
50	1	0	0
51	1	0	0
52	1	0	0
53	1	0	0
54	0	1	0
55	0	1	0
56	0	1	0
57	1	0	0
58	0	1	0
59	1	0	0
60	0	1	0
61	1	0	0
62	0	1	0
63	1	0	0
64	0	1	0
65	1	0	0
66	1	0	0
67	0	1	0
68	0	1	0
69	1	0	0
70	1	0	0
71	1	0	0
72	1	0	0
73	0	1	0
74	1	0	0
75	0	1	0
76	0	1	0
77	---	---	---
78	1	0	0
79	0	1	0

Table I7. *Q.1.5 Raw Data (continued).*

Response	Correct	Incorrect	No Response
80	1	0	0
81	1	0	0
82	0	1	0
83	0	1	0
84	0	1	0
85	0	1	0
86	0	1	0
87	1	0	0
88	1	0	0
89	0	1	0
90	0	1	0
91	0	1	0
92	0	1	0
93	1	0	0
94	1	0	0
95	1	0	0
96	1	0	0
97	0	1	0
98	0	1	0
99	0	1	0
100	0	1	0
101	1	0	0
102	0	1	0
103	0	1	0
104	1	0	0
105	1	0	0
106	1	0	0
107	1	0	0
108	1	0	0
109	---	---	---
110	0	1	0
111	1	0	0
112	1	0	0
113	1	0	0
114	1	0	0
115	---	---	---
116	---	---	---
117	---	---	---
118	---	---	---

Table I7. *Q.1.5 Raw Data (continued).*

Response	Correct	Incorrect	No Response
119	0	1	0
120	0	1	0
121	0	1	0
122	0	1	0
123	---	---	---
124	---	---	---
125	1	0	0
126	0	1	0
127	1	0	0
128	1	0	0
129	0	1	0
130	---	---	---
131	1	0	0
132	0	1	0
133	1	0	0
134	1	0	0
135	0	1	0
136	0	1	0
137	1	0	0
138	1	0	0
139	1	0	0
140	0	1	0
141	1	0	0
142	1	0	0
143	1	0	0
144	0	1	0
145	0	1	0
146	1	0	0
147	---	---	---
148	1	0	0
149	0	1	0
150	1	0	0
151	0	1	0
152	1	0	0
153	1	0	0
154	1	0	0
155	0	1	0
156	0	1	0
157	0	1	0

Table I7. *Q.1.5 Raw Data (continued).*

Response	Correct	Incorrect	No Response
158	1	0	0
159	0	1	0
160	1	0	0
161	1	0	0
162	1	0	0
163	1	0	0
164	1	0	0
165	0	1	0
166	1	0	0
167	1	0	0
168	1	0	0
169	0	1	0
170	0	1	0
171	1	0	0
172	1	0	0
173	1	0	0
174	1	0	0
175	0	1	0
176	0	1	0
177	1	0	0
178	1	0	0
179	1	0	0
180	1	0	0
181	1	0	0

Table I8. *Q.2.6A Raw Data*

Response	Correct	Incorrect	No Response
1	1	0	0
2	1	0	0
3	1	0	0
4	1	0	0
5	0	0	1
6	0	1	0
7	0	1	0
8	1	0	0
9	1	0	0
10	0	1	0
11	0	1	0
12	1	0	0
13	1	0	0
14	0	1	0
15	1	0	0
16	0	1	0
17	1	0	0
18	1	0	0
19	1	0	0
20	---	---	---
21	1	0	0
22	0	0	1
23	1	0	0
24	0	1	0
25	0	1	0
26	0	1	0
27	1	0	0
28	0	0	1
29	1	0	0
30	0	1	0
31	1	0	0
32	0	1	0
33	1	0	0
34	1	0	0
35	1	0	0
36	1	0	0
37	0	1	0
38	0	0	1
39	0	1	0

Table I8. *Q.2.6A Raw Data (continued).*

Response	Correct	Incorrect	No Response
40	0	1	0
41	---	---	---
42	1	0	0
43	1	0	0
44	1	0	0
46	1	0	0
47	1	0	0
48	---	---	---
49	0	0	1
50	1	0	0
51	1	0	0
52	1	0	0
53	0	1	0
54	1	0	0
55	1	0	0
56	0	1	0
57	1	0	0
58	0	1	0
59	0	1	0
60	0	1	0
61	1	0	0
62	0	1	0
63	1	0	0
64	1	0	0
65	1	0	0
66	0	1	0
67	1	0	0
68	0	1	0
69	0	1	0
70	0	1	0
71	0	1	0
72	0	1	0
73	1	0	0
74	1	0	0
75	1	0	0
76	1	0	0
77	---	---	---
78	0	1	0
79	1	0	0

Table I8. *Q.2.6A Raw Data (continued).*

Response	Correct	Incorrect	No Response
80	1	0	0
81	1	0	0
82	0	1	0
83	1	0	0
84	0	1	0
85	1	0	0
86	1	0	0
87	1	0	0
88	0	1	0
89	1	0	0
90	0	1	0
91	1	0	0
92	1	0	0
93	0	1	0
94	1	0	0
95	1	0	0
96	1	0	0
97	1	0	0
98	0	0	1
99	1	0	0
100	1	0	0
101	1	0	0
102	1	0	0
103	1	0	0
104	0	1	0
105	1	0	0
106	1	0	0
107	1	0	0
108	0	1	0
109	---	---	---
110	0	1	0
111	0	1	0
112	0	1	0
113	1	0	0
114	1	0	0
115	---	---	---
116	---	---	---
117	---	---	---
118	---	---	---
119	1	0	0

Table I8. *Q.2.6A Raw Data (continued).*

Response	Correct	Incorrect	No Response
120	0	1	0
121	1	0	0
122	1	0	0
123	---	---	---
124	---	---	---
125	1	0	0
126	1	0	0
127	1	0	0
128	0	1	0
129	0	1	0
130	---	---	---
131	1	0	0
132	0	1	0
133	0	0	1
134	1	0	0
135	0	1	0
136	1	0	0
137	1	0	0
138	1	0	0
139	1	0	0
140	1	0	0
141	1	0	0
142	1	0	0
143	1	0	0
144	0	1	0
145	0	1	0
146	0	1	0
147	---	---	---
148	0	1	0
149	0	1	0
150	1	0	0
151	1	0	0
152	0	0	1
153	1	0	0
154	0	1	0
155	1	0	0
156	0	1	0
157	1	0	0
158	0	1	0

Table I8. *Q.2.6A Raw Data (continued).*

Response	Correct	Incorrect	No Response
159	1	0	0
160	1	0	0
161	1	0	0
162	1	0	0
163	1	0	0
164	1	0	0
165	0	1	0
166	0	1	0
167	1	0	0
168	1	0	0
169	1	0	0
170	1	0	0
171	1	0	0
172	1	0	0
173	1	0	0
174	1	0	0
175	1	0	0
176	1	0	0
177	1	0	0
178	1	0	0
179	1	0	0
180	1	0	0
181	1	0	0

Table I9. *Q.2.6B Raw Data*

Response	Numbers	Proximity	Branch Length	Node	Not Addressed	No Response
1	0	0	0	2	0	0
2	0	0	0	1	0	0
3	0	0	0	1	0	0
4	0	0	0	2	0	0
5	1	0	0	1	0	0
6	1	0	0	0	0	0
7	1	0	0	0	0	0
8	0	0	0	2	0	0
9	0	0	0	2	0	0
10	1	0	0	1	0	0
11	0	0	1	0	0	0
12	0	0	0	2	0	0
13	0	0	0	2	0	0
14	1	0	0	1	0	0
15	1	0	0	2	0	0
16	0	0	0	0	0	1
17	0	0	0	2	0	0
18	0	0	0	2	0	0
19	0	2	0	1	0	0
20	---	---	---	---	---	---
21	0	0	0	2	0	0
22	0	0	0	0	0	1
23	0	0	0	2	0	0
24	1	0	0	0	0	0
25	1	0	0	0	0	0
26	1	0	0	0	0	0
27	0	0	0	1	0	0
28	0	0	0	0	1	0
29	0	0	0	2	0	0
30	1	0	0	0	0	0
31	0	0	0	2	0	0
32	1	0	0	0	0	0
33	0	0	0	2	0	0
34	0	0	0	0	0	1
35	0	0	0	2	0	0
36	0	0	0	2	0	0
37	1	0	0	0	0	0

Table I9. *Q.2.6B Raw Data (continued).*

Response	Numbers	Proximity	Branch Length	Node	Not Addressed	No Response
38	0	0	0	0	1	0
39	0	0	0	0	0	1
40	1	0	0	0	0	0
41	---	---	---	---	---	---
42	0	0	0	2	0	0
43	0	0	0	1	0	0
44	0	2	0	1	0	0
46	0	0	0	2	0	0
47	1	0	0	2	0	0
48	---	---	---	---	---	---
49	0	0	0	0	0	1
50	0	0	0	2	0	0
51	0	0	0	2	0	0
52	0	0	0	1	0	0
53	1	0	0	0	0	0
54	0	0	0	1	0	0
55	0	0	0	1	0	0
56	1	0	0	0	0	0
57	0	0	0	0	1	0
58	0	0	0	0	0	1
59	0	0	0	0	0	1
60	1	0	0	0	0	0
61	0	0	0	2	0	0
62	0	0	0	0	1	0
63	0	2	0	2	0	0
64	0	0	0	2	0	0
65	0	1	0	0	0	0
66	0	0	0	0	0	1
67	0	2	0	2	0	0
68	1	0	0	0	0	0
69	0	0	0	0	0	1
70	1	0	0	0	0	0
71	0	1	0	0	0	0
72	1	0	0	0	0	0
73	0	0	0	1	0	0
74	0	0	0	1	0	0
75	0	0	0	2	0	0
76	0	0	0	1	0	0
77	---	---	---	---	---	---

Table I9. *Q.2.6B Raw Data (continued).*

Response	Numbers	Proximity	Branch Length	Node	Not Addressed	No Response
78	1	0	0	0	0	0
79	0	0	0	0	1	0
80	0	0	0	2	0	0
81	0	0	0	2	0	0
82	1	1	0	0	0	0
83	0	0	0	2	0	0
84	1	0	0	0	0	0
85	0	0	0	0	1	0
86	0	0	0	0	1	0
87	0	0	0	2	0	0
88	0	0	0	0	0	1
89	0	0	0	2	0	0
90	0	0	0	0	1	0
91	0	2	0	2	0	0
92	0	0	0	2	0	0
93	0	0	0	0	0	1
94	0	0	0	1	0	0
95	0	0	0	1	0	0
96	0	1	0	1	0	0
97	0	1	0	0	0	0
98	0	0	0	0	0	1
99	0	0	0	0	0	1
100	0	0	0	1	0	0
101	0	0	0	0	1	0
102	0	0	0	2	0	0
103	0	0	0	1	0	0
104	0	0	0	0	0	1
105	0	0	0	1	0	0
106	0	0	0	1	0	0
107	0	1	0	0	0	0
108	0	0	0	0	0	1
109	---	---	---	---	---	---
110	0	0	0	1	0	0
111	0	1	0	0	0	0
112	0	0	0	0	1	0
113	0	0	0	2	0	0
114	1	0	0	1	0	0
115	---	---	---	---	---	---
116	---	---	---	---	---	---

Table I9. *Q.2.6B Raw Data (continued).*

Response	Numbers	Proximity	Branch Length	Node	Not Addressed	No Response
117	---	---	---	---	---	---
118	---	---	---	---	---	---
119	0	0	0	2	0	0
120	1	0	0	0	0	0
121	0	0	0	2	0	0
122	0	0	0	2	0	0
123	---	---	---	---	---	---
124	---	---	---	---	---	---
125	0	0	0	2	0	0
126	0	0	0	2	0	0
127	0	0	0	2	0	0
128	0	0	0	0	0	1
129	0	0	0	0	0	1
130	---	---	---	---	---	---
131	0	0	0	1	0	0
132	1	0	0	0	0	0
133	1	0	0	0	0	0
134	0	0	0	2	0	0
135	1	0	0	0	0	0
136	0	0	0	1	0	0
137	0	0	0	2	0	0
138	0	0	0	2	0	0
139	0	0	0	1	0	0
140	0	1	0	0	0	0
141	0	0	0	2	0	0
142	0	0	0	2	0	0
143	0	0	0	0	0	1
144	1	0	0	0	0	0
145	1	0	0	0	0	0
146	1	0	0	0	0	0
147	---	---	---	---	---	---
148	0	0	0	0	1	0
149	0	0	0	0	1	0
150	0	0	0	0	1	0
151	0	0	0	0	0	1
152	0	0	0	0	1	0
153	0	0	0	2	0	0
154	1	0	0	0	0	0
155	1	1	0	0	0	0

Table I9. *Q.2.6B Raw Data (continued).*

Response	Numbers	Proximity	Branch Length	Node	Not Addressed	No Response
156	1	0	0	0	0	0
157	0	0	0	0	0	1
158	1	0	0	0	0	0
159	0	0	0	0	1	0
160	0	0	0	1	0	0
161	0	0	0	2	0	0
162	0	0	0	2	0	0
163	0	0	0	2	0	0
164	0	0	0	1	0	0
165	0	0	0	1	0	0
166	1	0	0	0	0	0
167	0	2	0	2	0	0
168	0	0	0	1	0	0
169	0	0	0	2	0	0
170	0	1	0	0	0	0
171	0	0	0	2	0	0
172	0	0	0	2	0	0
173	0	0	0	2	0	0
174	0	0	0	2	0	0
175	0	0	0	2	0	0
176	0	0	0	2	0	0
177	0	0	0	2	0	0
178	0	0	0	1	0	0
179	0	0	0	2	0	0
180	0	0	0	2	0	0
181	0	0	0	2	0	0

Table I10. *Q.2.7A Raw Data*

Response	Correct	Incorrect	No Response
1	0	1	0
2	0	1	0
3	0	1	0
4	1	0	0
5	1	0	0
6	1	0	0
7	1	0	0
8	1	0	0
9	0	1	0
10	1	0	0
11	1	0	0
12	1	0	0
13	0	1	0
14	0	1	0
15	1	0	0
16	1	0	0
17	0	1	0
18	1	0	0
19	1	0	0
20	---	---	---
21	1	0	0
22	0	0	1
23	0	1	0
24	1	0	0
25	1	0	0
26	1	0	0
27	1	0	0
28	0	0	1
29	1	0	0
30	1	0	0
31	0	1	0
32	1	0	0
33	1	0	0
34	1	0	0
35	0	1	0
36	0	1	0
37	0	0	1
38	1	0	0
39	1	0	0

Table I10. *Q.2.7A Raw Data (continued).*

Response	Correct	Incorrect	No Response
40	1	0	0
41	---	---	---
42	0	1	0
43	0	1	0
44	0	1	0
46	0	1	0
47	0	1	0
48	---	---	---
49	0	0	1
50	0	1	0
51	0	1	0
52	0	1	0
53	0	1	0
54	0	1	0
55	0	1	0
56	1	0	0
57	1	0	0
58	1	0	0
59	0	1	0
60	1	0	0
61	0	1	0
62	0	0	1
63	1	0	0
64	0	1	0
65	0	1	0
66	1	0	0
67	0	1	0
68	0	1	0
69	0	0	1
70	1	0	0
71	1	0	0
72	1	0	0
73	0	1	0
74	1	0	0
75	1	0	0
76	0	1	0
77	---	---	---
78	1	0	0
79	0	1	0

Table I10. *Q.2.7A Raw Data (continued).*

Response	Correct	Incorrect	No Response
80	0	1	0
81	1	0	0
82	1	0	0
83	1	0	0
84	1	0	0
85	1	0	0
86	1	0	0
87	0	1	0
88	0	0	1
89	0	1	0
90	1	0	0
91	0	1	0
92	0	1	0
93	0	0	1
94	1	0	0
95	0	1	0
96	0	1	0
97	0	1	0
98	1	0	0
99	0	1	0
100	1	0	0
101	0	1	0
102	0	1	0
103	0	1	0
104	1	0	0
105	1	0	0
106	1	0	0
107	0	1	0
108	0	1	0
109	---	---	---
110	0	1	0
111	1	0	0
112	0	0	1
113	0	1	0
114	0	1	0
115	---	---	---
116	---	---	---
117	---	---	---
118	---	---	---
119	0	0	1

Table I10. *Q.2.7A Raw Data (continued).*

Response	Correct	Incorrect	No Response
120	1	0	0
121	1	0	0
122	1	0	0
123	---	---	---
124	---	---	---
125	0	1	0
126	0	1	0
127	0	1	0
128	1	0	0
129	0	1	0
130	---	---	---
131	0	1	0
132	1	0	0
133	1	0	0
134	1	0	0
135	1	0	0
136	0	1	0
137	1	0	0
138	0	1	0
139	0	1	0
140	1	0	0
141	1	0	0
142	0	1	0
143	1	0	0
144	1	0	0
145	1	0	0
146	1	0	0
147	---	---	---
148	0	1	0
149	1	0	0
150	0	1	0
151	1	0	0
152	0	0	1
153	1	0	0
154	1	0	0
155	1	0	0
156	0	1	0
157	1	0	0
158	1	0	0

Table I10. *Q.2.7A Raw Data (continued).*

Response	Correct	Incorrect	No Response
159	1	0	0
160	0	1	0
161	0	1	0
162	0	1	0
163	0	1	0
164	0	1	0
165	1	0	0
166	1	0	0
167	0	1	0
168	1	0	0
169	0	1	0
170	0	1	0
171	0	1	0
172	0	1	0
173	1	0	0
174	1	0	0
175	1	0	0
176	0	1	0
177	0	1	0
178	1	0	0
179	0	1	0
180	0	1	0
181	0	1	0

Table I11. *Q.2.7B Raw Data*

Response	Numbers	Proximity	Branch Length	Node	Not Addressed	No Response
1	0	0	0	2	0	0
2	0	0	0	1	0	0
3	0	0	0	0	0	0
4	1	0	0	0	0	0
5	2	0	2	0	0	0
6	1	0	0	0	0	0
7	1	0	0	0	0	0
8	2	0	0	0	0	0
9	0	0	0	2	0	0
10	1	0	0	0	0	0
11	1	0	0	0	0	0
12	1	0	0	0	0	0
13	0	0	0	2	0	0
14	0	1	0	0	0	0
15	1	0	0	0	0	0
16	1	0	0	0	0	0
17	0	0	0	2	0	0
18	1	0	0	0	0	0
19	1	0	0	0	0	0
20	---	---	---	---	---	---
21	1	0	0	0	0	0
22	0	0	0	0	0	1
23	0	0	0	2	0	0
24	1	0	0	0	0	0
25	1	0	0	2	0	0
26	1	0	0	0	0	0
27	0	1	0	0	0	0
28	0	0	0	0	1	0
29	1	0	0	0	0	0
30	1	0	0	0	0	0
31	0	0	0	2	0	0
32	1	0	0	0	0	0
33	2	0	0	0	0	0
34	0	0	2	0	0	0
35	0	0	0	2	0	0
36	0	0	0	2	0	0
37	0	0	0	0	0	1
38	1	0	0	0	0	0

Table I11. *Q.2.7B Raw Data (continued).*

Response	Numbers	Proximity	Branch Length	Node	Not Addressed	No Response
39	0	0	0	0	0	1
40	1	0	0	0	0	0
41	---	---	---	---	---	---
42	0	1	0	0	0	0
43	0	0	0	1	0	0
44	1	0	0	0	0	0
46	0	0	0	2	0	0
47	0	0	0	2	0	0
48	---	---	---	---	---	---
49	0	0	0	0	0	1
50	0	0	0	2	0	0
51	0	0	0	2	0	0
52	0	0	0	1	0	0
53	0	1	0	0	0	0
54	0	0	0	1	0	0
55	0	0	0	1	0	0
56	1	0	0	0	0	0
57	0	0	0	0	0	1
58	0	0	0	0	0	1
59	0	1	0	0	0	0
60	1	0	0	0	0	0
61	0	0	0	2	0	0
62	0	0	0	0	1	0
63	1	0	0	0	0	0
64	0	0	0	2	0	0
65	0	1	0	0	0	0
66	0	0	0	0	0	1
67	0	2	0	2	0	0
68	1	0	0	0	0	0
69	0	1	0	0	0	0
70	1	0	0	0	0	0
71	0	0	0	1	0	0
72	1	0	0	0	0	0
73	0	2	0	2	0	0
74	1	0	0	0	0	0
75	1	0	0	0	0	0
76	0	0	0	1	0	0
77	---	---	---	---	---	---
78	1	0	0	0	0	0

Table I11. *Q.2.7B Raw Data (continued).*

Response	Numbers	Proximity	Branch Length	Node	Not Addressed	No Response
79	0	0	0	0	1	0
80	0	0	0	2	0	0
81	0	0	2	0	0	0
82	1	0	0	0	0	0
83	1	0	0	0	0	0
84	1	0	0	0	0	0
85	1	0	0	0	0	0
86	1	0	0	0	0	0
87	0	0	0	2	0	0
88	0	0	0	0	0	1
89	0	0	0	1	0	0
90	0	0	0	0	0	1
91	0	0	0	2	0	0
92	0	0	0	2	0	0
93	0	0	0	0	0	1
94	1	0	0	0	0	0
95	0	0	0	1	0	0
96	0	0	0	1	0	0
97	0	0	0	0	1	0
98	0	0	1	0	0	0
99	0	0	0	2	0	0
100	1	0	0	0	0	0
101	0	1	0	0	0	0
102	0	1	0	0	0	0
103	0	0	0	2	0	0
104	1	0	0	0	0	0
105	1	0	0	0	0	0
106	1	0	0	0	0	0
107	0	1	0	0	0	0
108	0	0	0	1	0	0
109	---	---	---	---	---	---
110	0	0	0	2	0	0
111	1	0	0	0	0	0
112	0	0	0	0	1	0
113	0	0	0	2	0	0
114	0	1	0	0	0	0
115	---	---	---	---	---	---
116	---	---	---	---	---	---
117	---	---	---	---	---	---

Table I11. *Q.2.7B Raw Data (continued).*

Response	Numbers	Proximity	Branch Length	Node	Not Addressed	No Response
118	---	---	---	---	---	---
119	0	0	0	0	1	0
120	1	0	0	0	0	0
121	1	0	0	0	0	0
122	1	0	0	0	0	0
123	---	---	---	---	---	---
124	---	---	---	---	---	---
125	0	1	0	0	0	0
126	1	0	0	2	0	0
127	0	0	0	2	0	0
128	0	0	0	0	0	1
129	0	0	0	1	0	0
130	---	---	---	---	---	---
131	0	0	0	1	0	0
132	1	0	0	0	0	0
133	1	0	0	0	0	0
134	1	0	0	0	0	0
135	1	0	0	0	0	0
136	0	0	0	1	0	0
137	2	0	0	0	0	0
138	0	0	0	0	1	0
139	0	0	0	1	0	0
140	1	0	0	0	0	0
141	0	0	0	0	0	1
142	0	0	0	2	0	0
143	2	0	0	0	0	0
144	0	0	0	0	0	1
145	1	0	0	0	0	0
146	1	0	0	0	0	0
147	---	---	---	---	---	---
148	0	1	0	0	0	0
149	0	0	0	0	1	0
150	0	0	0	1	0	0
151	0	0	0	0	0	1
152	0	0	0	0	1	0
153	1	0	0	0	0	0
154	1	0	0	0	0	0
155	1	0	2	0	0	0

Table I11. *Q.2.7B Raw Data (continued).*

Response	Numbers	Proximity	Branch Length	Node	Not Addressed	No Response
156	1	0	0	0	0	0
157	1	0	0	0	0	0
158	1	0	0	0	0	0
159	2	0	0	0	0	0
160	0	0	0	1	0	0
161	0	0	0	2	0	0
162	0	0	0	2	0	0
163	0	0	0	2	0	0
164	0	0	0	1	0	0
165	1	0	0	0	0	0
166	1	0	0	0	0	0
167	0	0	0	2	0	0
168	2	0	0	0	0	0
169	0	0	0	2	0	0
170	0	0	0	1	0	0
171	0	0	0	2	0	0
172	0	0	0	2	0	0
173	1	0	0	1	0	0
174	1	0	0	0	0	0
175	1	0	0	0	0	0
176	1	0	0	0	0	0
177	0	0	0	2	0	0
178	1	0	0	0	0	0
179	0	0	0	2	0	0
180	0	0	0	2	0	0
181	0	0	0	2	0	0

Table I12. *Q.2.7B Alternative Idea Raw Data*

Response	Equally Related
1	1
2	1
3	1
4	0
5	0
6	0
7	0
8	0
9	1
10	0
11	0
12	0
13	1
14	0
15	0
16	0
17	1
18	0
19	0
20	---
21	0
22	0
23	1
24	0
25	0
26	0
27	0
28	0
29	0
30	0
31	1
32	0
33	0
34	0
35	0
36	1
37	0
38	0
39	0

Table I12. *Q.2.7B Alternative Idea Raw Data (continued).*

Response	Equally Related
40	0
41	---
42	0
43	0
44	0
46	1
47	1
48	---
49	0
50	1
51	1
52	1
53	0
54	1
55	1
56	0
57	0
58	0
59	0
60	0
61	1
62	0
63	0
64	1
65	0
66	0
67	1
68	0
69	0
70	0
71	0
72	0
73	1
74	0
75	0
76	1
77	---
78	0
79	0
80	1

Table I12. *Q.2.7B Alternative Idea Raw Data (continued).*

Response	Equally Related
81	0
82	0
83	0
84	0
85	0
86	0
87	1
88	0
89	1
90	0
91	1
92	1
93	0
94	0
95	0
96	0
97	0
98	0
99	0
100	0
101	0
102	0
103	1
104	0
105	0
106	0
107	0
108	0
109	---
110	1
111	0
112	0
113	0
114	0
115	---
116	---
117	---
118	---
119	0
120	0

Table I12. *Q.2.7B Alternative Idea Raw Data (continued).*

Response	Equally Related
121	0
122	0
123	---
124	---
125	0
126	1
127	1
128	0
129	0
130	---
131	1
132	0
133	0
134	0
135	0
136	0
137	0
138	0
139	1
140	0
141	0
142	1
143	0
144	0
145	0
146	0
147	---
148	0
149	0
150	1
151	0
152	0
153	0
154	0
155	0
156	0
157	0
158	0
159	0
160	1

Table I12. *Q.2.7B Alternative Idea Raw Data (continued).*

Response	Equally Related
161	1
162	1
163	1
164	1
165	0
166	0
167	1
168	0
169	1
170	1
171	1
172	1
173	0
174	0
175	0
176	0
177	1
178	0
179	1
180	1
181	1