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Origin of Species: Starting the Story with DNA

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This activity starts with a pop bead model of DNA, although the model is presented to students initially not as DNA but as necklaces worn by members of a tribe of humans. Students manipulate the necklaces according to customs of the tribe and discover the simple story of where new species come from: populations split and the resultant daughter populations diverge due to mutation. In our labs we connect the necklace model to a specific example of diversity by analyzing a phylogeny of great apes. The necklaces can also be used to teach about the specificity of restriction enzymes and how these enzymes allow us to distinguish different sources of DNA.

Keywords: origin of species, evolution, speciation, phylogeny, biological diversity, common ancestor, molecular clock, restriction enzyme

Introduction

Where do new species come from? Biologists know, but we don't do a very good job of telling our students what we understand about this subject. A typical textbook discussion dwells on possible mechanisms of geographic isolation and perhaps even raises the problem of sympatric versus allopatric speciation. This kind of discussion, usually near the end of the book, may hold the interest of a graduate student, but it makes a freshman's eyes glaze over. We need to deliver a simple, clear message on this subject. After all, we are in some sense competing with religious beliefs that are stated in extremely simple terms.

In *The Seven Daughters of Eve*, Bryan Sykes reprises an idea put forward by Anthony Edwards in *New Scientist* in 1966:

> "He (Edwards) imagines a tribe that carries with it a pole along which are arrayed 100 discs which are either black or white. Every year, one disc, chosen at random, is changed to the other colour. When the tribe splits into two groups, each group takes with it a copy of the pole with the discs in their current order. The following year they each make one of the random changes to the discs. The next year they make another, the next year another and so on, continuing the custom of one random change every year. Since the changes they make are completely random, the order of the discs on the two poles becomes more and more dissimilar as each year passes." (Sykes 2001:43)

The essence of where new species come from is contained in this imagery. Populations split. Daughter populations diverge because they accumulate different mutations. The story is simple. It is easy to understand. It can be turned into a laboratory activity.

As we worked on developing this lab, the poles with 100 black or white discs became necklaces with four colors of beads, making the reference to DNA more accurate. In our labs (18 student maximum) we divide the class into four groups. The activity can be modified to accommodate more groups in larger-sized classes.

The point of the necklaces is to give students a way to understand biological diversity. The model delivers a clear explanatory message, but it is abstract and students benefit if it is connected to examples. We installed bulletin boards in our lab rooms and each week we hang a poster illustrating diversity in a particular group of organisms. In the weeks leading up to the Origin of Species lab, our instructors point out the posters and raise questions about how students understand where the diversity comes from.

We also go into one example in more depth when we do the necklaces activity. Our method is presented in Appendix A.

The necklaces also offer an interesting way to model the action of restriction enzymes. This is presented in Appendix B.

Student Outline

Learning Objectives

When you have finished this lab, you should be able to do the following things.

1. Discuss the similarities and differences between genealogies and phylogenies.
2. Identify the natural processes that produce new species and explain how the necklace activity modeled those processes.
3. Explain the terms phylogeny, last common ancestor, molecular clock, and multiple sequence alignment.
4. Compare phenotypic and molecular approaches to reconstructing the history of a group of organisms, using the great apes as an example.
5. Interpret patterns in a multiple sequence alignment and a phylogenetic tree.

Introduction

People have always had ideas about why we share the world with so many different kinds of plants and animals. Before the question was addressed scientifically, our explanations came from religious thinking. Sometimes the notion was that divine spirits lived within animals and plants. Other times the notion was that God, or gods, made the various forms of living things.

With the rise of scientific thinking, other explanations became possible. Some new language came into use. Scientists adopted the word **species** to refer to a particular kind of plant or animal. With this new language, our age-old curiosity about the other inhabitants of Earth can be rephrased as "Where do species come from?" It turns out the answer is rather simple. In this lab we look at the origin of species.

What happens to start a new species? [1]

Imagine you (the people in the lab section) are a tribe of humans living by foraging and hunting. Like humans today, you have a fondness for jewelry. Everyone wears a distinctive necklace that shows membership in the tribe. This necklace is 100 beads long and has a precise pattern of four colors. Once every year the tribe holds a special ceremony where they make one change in the necklace pattern. Everyone makes the same change at the same time. Thus each person in the tribe wears an identical necklace but the tribe's pattern changes at one position each year.

There are four necklaces of 100 beads on the lab benches. They are identical now – you can check this. For reasons that will be immediately obvious, we call some of the colors by unusual names. We use alba (Latin for white) and refer to it as A. The other colors are tangerine (T), cherry (C), and green (G). The beads, of course, represent the four different bases found in DNA: Adenine, Thymine, Cytosine, and Guanine. Notice that the starting pattern is a repeated sequence of A, T, C, G.

Procedure

1. Your lab instructor will divide the class into 4 groups so each group has one necklace.
2. Each group has a bag of 100 poker chips. The chips are numbered 1 to 100. Your lab instructor will shake a bag, draw out one chip (without looking), call out its number, and return the chip to the bag.
3. Count to that bead on your necklace and remove it. (Alba (white) beads are numbered to make counting easier.) Put the first bead you take out of the necklace, and all the others you take out later, into the small jar labeled "Beads removed."
4. Next your lab instructor will draw, at random, one poker chip from a second bag. In this bag there are equal numbers of chips labeled A, T, C, or G.
5. Replace the bead you removed with a bead of the color that was just identified by the second random draw. Twenty-five percent of the time this will result in no change, but 75% of the time the color of the bead at that position will change.
6. Fill out the line for year 1 in Table 1 with the information about this change.
7. Repeat this 4 more years, recording the changes in Table 1.
8. At this point each group should have the same pattern in their necklace. Check this and correct any mistakes that were made.

[1] The idea for necklaces is derived from Anthony Edwards, *New Scientist*, 1966, cited in Bryan Sykes, *The Seven Daughters of Eve*, 2001.

9. Your lab instructor will put a figure on the blackboard to illustrate the history of the necklaces. Make a copy for yourself, in Figure 1.

Table 1. Model of how a tribe of humans changes its decorative necklaces over time. The whole lab section makes the same changes each year.

YEAR	POSITION CHANGED	STARTING COLOR	REPLACEMENT COLOR
1			
2			
3			
4			
5			

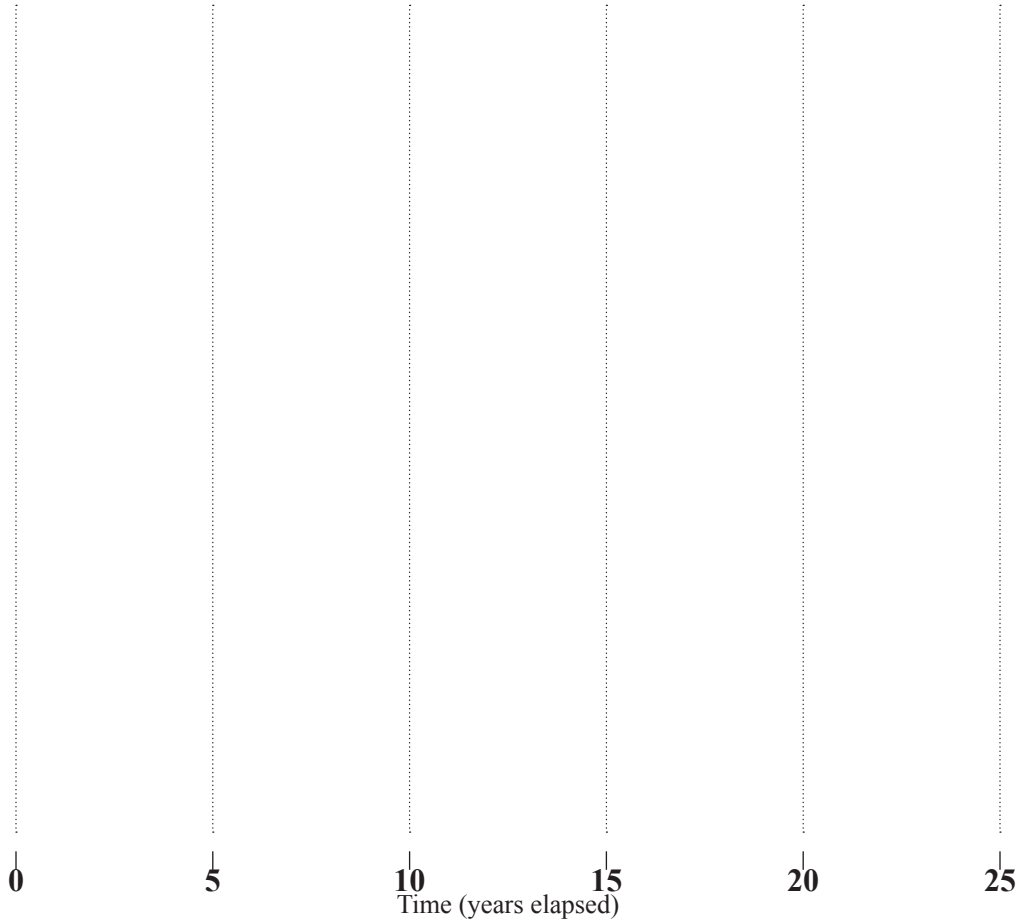

Figure 1. Illustration of branching pattern produced in the tribal necklaces activity.

A branching

Something new happens before year 6. The tribe, for whatever reason, splits in half. From this point onward, there are two populations instead of one.

Each new tribe continues the tradition of changing their necklaces at one position each year, but they do not communicate with each other, so the changes they make are done independently.

10. Your lab instructor will divide your lab section in half.
11. Continue with the procedure for 10 more years. Pay no attention to what the other tribe is doing. Record the changes you make in Table 2.

Table 2. Model of how tribes of humans change their decorative necklaces over time. There are two independent tribes in the lab section; only one is recorded here.

YEAR	POSITION CHANGED	STARTING COLOR	REPLACEMENT COLOR
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			

12. Bring your illustration (Figure 1) up to date.
13. Confirm that the two necklaces managed by your tribe are identical. Correct any mistakes.

How many differences are there between the necklaces of the two tribes at this point?

Another branching

At this point each tribe splits in half again.

14. Your instructor will divide the lab section so there are now four tribes in the room.
15. Continue the same procedure for another 10 years, acting independently of all the other tribes. Record the changes for your tribe in Table 3.

Table 3. Model of how tribes of humans change their decorative necklaces over time. There are four independent tribes in the lab section; only one is recorded here.

YEAR	POSITION CHANGED	STARTING COLOR	REPLACEMENT COLOR
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			

16. Bring your illustration up to date.
17. Your lab instructor will put a data table on the blackboard. This is a place to record how many changes have accumulated in the necklaces between each pair of tribes. Copy these values into the last column of Table 4.
18. Finish off the analysis by completing Table 5, which shows the *average* numbers of changes you observed, compared to the expected values.

Table 4. The number of differences between pairs of necklaces, compared to the maximum number of differences possible, and to the expected number of differences.

Necklace pair	Max changes In 100 bases	Expected:75% of max changes In 100 bases	Observed differences between pairs of necklaces
1 and 2	20	15	
1 and 3	40	30	
1 and 4	40	30	
2 and 3	40	30	
2 and 4	40	30	
3 and 4	20	15	

Table 5. Comparison of expected and actual numbers of changes between pairs of tribes with branching points at different times in history.

Pairs	Expected	Actual (average)
Set A (pairs 1&2, 3&4)	15	
Set B (pairs 1&3, 1&4, 2&3, 2&4)	30	

You know the actual history of these four necklaces. Do the data you collected in Table 4 reflect that history? Comment on how they do.

How well do your average values match the expected values in Table 5?

What explanations can you offer for any discrepancies between expected and actual values in Table 5?

Suppose you did not know the history. Discuss how you could use the necklaces, as they appear at the end of 25 years, to construct a reasonable approximation of the actual history.

What does all this mean?

There are two elements to this story:

1. There is a source of change;
2. Populations split, or branch, once in a while.

These simple elements have interesting consequences.

Imagine two individuals who belong to different sub-tribes encountering each other. They know immediately how long it has been since their ancestors were in the same tribe. All they have to do is compare their necklaces, which act as a kind of clock, keeping a record of how long it has been since their sub-tribes branched from each other.

Necklaces as DNA

Where do new species come from? They come from earlier species. All it takes is for one population to split into two populations. Actually, there is one more necessary element, and that is a source of change. In biology, that source of change is mutation.

The kind of mutation we modeled with the necklaces, when one of the letters A, T, C, G replaces one of the other letters, is called a single-base substitution.

How often do single-base substitutions happen in real DNA? Making new copies of DNA is a very, very accurate process. One current estimate suggests that a mistake occurs about 1 time for every half billion letters copied. That is an extremely low error rate, but it is a source of change and it means this:

- When a population splits in two, the two populations start to accumulate different mutations and they become more and more different from each other over time, *just because of mutation.* This continuous accumulation of mutations has been called a **molecular clock** and it has proven to be very useful in estimating how long ago the ancestors of currently living species split into different lineages.

To give our model a sense of biological realism, we need to adjust the time factor of Figure 1. Although guesswork is involved, a reasonable conversion might be to make one year of necklace time equal 5 million years of actual time.

Using this conversion and looking at the necklaces as sequences of DNA,

- How many years back in time would you have to go to find the place where necklaces 1 and 2 were identical?

- When did the **last common ancestor** of necklaces 3 and 4 exist?

- How long ago did the ancestral population of necklaces 1 and 3 split into two populations?

Materials

Our lab sections are divided into four groups. Each group gets the following supplies.

- A necklace of 100 pop beads in a large Petri dish
- The necklace is a repeating pattern of white, orange, red, green
- Each white bead is numbered
- A bag of 100 poker chips, numbered from 1 to 100
- A bag of 20 poker chips, labeled A, T, C, G, five chips of each
- A small jar labeled "Beads removed"
- An extra jar of each color of bead

In making the necklaces we discarded about 10% of the beads because the hole was too large, causing the necklace to fall apart. We found that numbering the white beads was essential; it is too difficult to count out to the correct place without this aid. Sharpie marker numbers will wipe off the beads. We used paint pens to get a permanent number.

The pop beads are from Ward's Natural Science.

Green	361536
Orange	361535
Red	361530
White	361534

To number the white beads we used a Brite-Mark® valve action paint marker, obtained from McMaster-Carr, New Brunswick, New Jersey.

Notes for the Instructor

The lab instructor is actively involved in managing this activity. For the first five years, the instructor draws the poker chips and announces the outcome of each draw to the class. At year 5, the instructor begins to draw the history, in a copy of Figure 1 on the blackboard, as a horizontal line from year 0 to year 5. S/he adds the number of actual changes of bead color that occurred in the first five years above the first horizontal line. S/he also has each group compare their necklaces to at least one other necklace to be sure no mistakes have been made. It is a good idea to put a differently colored piece of tape, or an identifying extension of beads, on each necklace at this time, so each group will be able to keep track of their own necklace after further side-by-side comparisons are made. The instructor then divides the class into two "tribes" and records this branching on the blackboard as a vertical line at year 5. Each new tribe begins to draw their own poker chips starting with year 6.

At year 15 the instructor again steps in to 1) update the figure with two new horizontal lines, 2) label each of these lines with the number of actual changes that occurred in each lineage, 3) have each tribe compare its two necklaces and correct any mistakes, 4) divide each tribe in half again, and 5) record these new divisions as two vertical lines added to the figure at year 15.

At year 25 the instructor updates the figure on the blackboard once more, including the number of actual changes that occurred in each lineage in the years 16 to 25. S/he directs each group to do a side-by-side comparison with each of the other three necklaces. This is an opportunity to tell the students that this is how biologists compare DNA sequences from two different organisms; for example, this is how the well-circulated piece of information about the high degree of similarity between chimpanzees and humans is derived. The values from these side-by-side comparisons are put into the last column of a copy of Table 4 that the instructor has put on the blackboard.

The instructor leads a class analysis of how the observed values in the fourth column differ from the expected values in the third column, and also differ (probably) from the values on Figure 1. Students need the instructor's help in learning how to sum the values between any two pairs of final necklaces by adding the number on each segment, starting at one final necklace, going back to the last common ancestor, and then out again to the second final necklace. These values can be added as a fifth column. Students are able to propose good explanations for the sources of these differences, but the instructor needs to lead this discussion.

The necklace activity can be adapted for larger classes. It is desirable to have a small number of people per necklace so the group interacts well, but more necklaces can be added to a class. We have found that it pays to plan ahead by drawing out a desirable final phylogenetic tree. From this, the way to number the final tribes, and to subdivide early larger tribes at chosen times, can be worked out. Tables 2 and 3 do not have to be changed; when a tribe is split off, the instructor only has to say something like "continue to year 17 and stop there." Table 4 enlarges rapidly as the number of necklaces increases, but it is very manageable with six in a class.

Acknowledgement

Stephanie Oliet was a great collaborator in developing the necklaces activity.

Literature Cited

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About the Author

Robert Ketcham works at the University of Delaware. Since 1988 he has coordinated large-enrollment labs for both science majors and non-science majors. He is currently working with the non-science majors' class, which is titled Principles of Biology with Laboratory. His goal is to have students in this class get rich lab experience in three "principle" areas: Cell Theory, Chromosomal Theory of Inheritance, and Evolutionary Theory. Cell Theory and Chromosomal Theory of Inheritance are in place now. He is working on Evolutionary Theory.

Appendix A: Great Apes

Introduction

The necklaces activity can be done by itself, but students gain a lot from having a biological example to go along with it. We use the great apes and their cytochrome b sequences. This is inherently interesting since modern humans and Neanderthals are included. Another advantage is that it is a small and nicely balanced data set that we can analyze without using computers. We know from past experience that our students do not do well when we ask them to use a computer program during lab, so we found ways around this problem. For instructors who are not confronted with this limitation, the basic version of Geneious (**http://www.geneious.com/default,387,home.sm)**, available as a free download, is a nice program to use.

In our situation we give students a multiple sequence alignment (MSA) that has been printed out in color. Students work in groups of 4 or 5 (the groups from the necklaces) which means the printing has to be large enough to be viewed simultaneously by several people. The protein sequence is shorter than the DNA sequence, of course, so we use that. Still, cytochrome b is 380 amino acids, and the MSA is 34 feet long at the font size we use, so it has the physical form of a scroll that students unroll down the length of a lab bench and then adjust to expose different sections.

We give students a chance to become familiar with the nature of a MSA by asking them to interpret the patterns found at specific positions. This amounts to proposing when, in the history of great apes, a particular mutation took place, a direct utilization of their experience with the necklaces. We also ask each group to go down the full length of the MSA to count the differences between one pair of great apes. This is tedious work, of course, and we provide the data for all the other possible pairs.

With the great apes, it was appropriate to create a data set that contained four genera, with each genus having two species or subspecies. This is simple enough to allow the lab instructors to talk through how the phylogeny is derived from the matrix table of pair-wise differences and we provide the final phylogenetic figure in the lab manual.

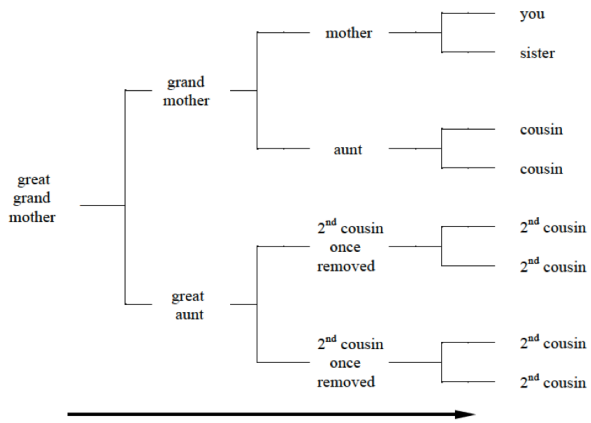
We find that it helps to introduce students to the idea of phylogeny and to the great apes in a lab that precedes the Origin of Species lab. At this time we give each group of students a pack of 8 photographs of adult males. We also give them an example of a genealogy to illustrate a branching pattern like the one we ask them to create for the great apes. Each group proposes a phylogeny for the apes after studying the photographs, and the proposals from different groups are put on exhibit for comparison. It seems to work well to separate the phenotypic analysis of the photographs from the genotypic analysis of protein sequences by this extra week. Thinking about evolution is a new experience for so many of our students that extra time is helpful. In the intervening week we give them a homework assignment that deals again with the great apes. They must choose between two possible branching patterns and defend their choice by reference to the phenotypic traits in the photographs. The materials we use in these preliminary activities are provided here.

Preliminary In-class Assignment for Great Apes

Useful information

Next week you will do the lab called Origin of Species. It will introduce the logic of how living species are related to each other. Here are a few points to consider now.

1. When we find several species that are very similar to each other, we conclude that they share a common ancestor. This means if we go back far enough in time, we will find that only one species existed, the common ancestor.
2. The idea is really the same as what we find in genealogy:



3. The big difference between genealogy diagrams and diagrams of phylogeny is the much longer time scale for phylogenies, which are often measured in millions of years. Both kinds of diagrams show history as a series of branching events.

Grading rubric for Great Apes Phylogeny assignment

Excellent (3 pts)	Good (2 pts)	Fair (1 pt)	Poor (0 pts)
The choice of scheme A or B is clearly stated. Reasons for making that choice are clearly stated. Evidence obtained from the photographs is used purposefully in arguing for the appropriateness of the choice of scheme. Difficulties encountered in making the choice of scheme are discussed. Presentation is easy to read and understand. Good paragraph structure, sentence structure, and punctuation are used.	The choice of scheme A or B is clearly stated. Reasons for making that choice are stated. Evidence obtained from the photographs is used in arguing for the appropriateness of the choice. Difficulties encountered in making the choice of scheme may be discussed. Presentation can be understood for the most part, but the writing could use editing to improve its readability. Good paragraph structure, sentence structure, and punctuation are used for the most part.	The choice of scheme A or B may be stated. Reasons for making that choice may be presented using reference to evidence obtained from the photographs. Difficulties encountered in making the choice of scheme may be mentioned. Thinking behind the choice is not easy to understand because presentation needs substantial editing. Paragraph structure, sentence structure, and punctuation could be improved	The choice of scheme A or B may be stated and reasons for making that choice may be presented. Evidence from photographs may be mentioned. Argument is very difficult to follow because writing is not clear. Rewriting would be needed to make it easy to read and understand.

The Great Apes

Your lab instructor will give your group a pack of 8 photographs. Each photo shows an adult male of one species of great ape. These are species that are alive today.[2],[3]

How are these great apes related to each other?

Your assignment is to study the photographs and then propose an answer to this question.

Your lab instructor will give you a large sheet of newsprint to work on.

Are there easy conclusions you can reach – ones that every person in your group agrees with?

When you have gotten beyond the obvious, try this approach for making more progress:

- Consider just one trait at a time. Some possibilities: head shape, hand shape, length of trunk compared to length of limb, kind of locomotion, etc.
- Describe this trait for each specimen.
- Repeat the procedure for other specific traits.

Now use this information to make a diagram of how you imagine the history of this group of animals. It should be a branching pattern, like the genealogy diagram (on the back of this sheet).

Start at the left edge and write "common ancestor" where the genealogy has "great grand mother." Then draw whatever branching pattern you think is appropriate, and label the end points on the right with the names of the great apes (chimp 1 and chimp 2 is good enough). These go in the positions where the genealogy has "you", "sister", "cousin", "2nd cousin".

If your group does not agree on one phylogeny, draw two or more. These are hypotheses, not certainties.

What does the history of the great apes look like? We have a major problem in answering this question: no one was around to record all the changes that took place along the way, as you did with the necklaces.

What we have to work with are the currently living species. We can make phylogenies based on phenotypes, as you did last week with the great ape photos. We can also make phylogenies based on genotypes. To use genotypes, comparable sequences of DNA from two or more organisms are lined up side-by-side and the places where A's, T's, C's, and G's do not match are counted. This can also be done using proteins instead of DNA; places where amino acids differ are counted.

Your lab instructor will give you a **multiple sequence alignment** for the cytochrome b protein in the 8 great apes. Cytochrome b is part of a complex of proteins that uses the oxygen you breathe to release energy from the food you eat; it is present in virtually all organisms. Each letter represents one amino acid. Each sequence is 380 amino acids long.[4], [5]

[2] We are giving Neanderthals honorary status as alive today. They actually went extinct 20,000 to 30,000 years ago.

[3] The two gorillas are generally considered to be subspecies rather than completely separate species. Whether Neanderthals and modern humans are species or subspecies is a hotly debated topic. The two orangutans and the two chimpanzees are definitely different species.

[4] The Neanderthal sequence is missing its last two amino acids. Presumably those could not be extracted from the fossil bone. We will not count those two positions when we compare the Neanderthal sequence to the sequences of other apes.

[5] These protein sequences were obtained by extracting DNA from a tissue sample from each ape, sequencing the part of the total DNA that included the cytochrome b gene, and then converting the DNA information into its protein equivalent. When biologists determine sequences like these, they deposit the information in a public database operated by the National Center for Biotechnology Information. We downloaded these sequences from that database.

Table 1. Number of amino acid differences in cytochrome b between two pairs of great apes.

		Bonobo	Robust Chimpan-zee	Western Gorilla	Western Lowland Gorilla	Modern Human	Neander-thal	Bornean Orangutan	Sumatran Orangutan
		Pan paniscus	*Pan troglo-dytes*	*Gorilla gorilla*	*Gorilla gorilla gorilla*	*Homo sapiens sapiens*	*Homo sapiens neander-thalensis*	*Pongo pygmeaus*	*Pongo abelii*
Bonobo	*Pan paniscus*	---	16	27	27		24	42	41
Robust Chimpan-zee	*Pan troglo-dytes*		---	27	26	25	23	42	39
Western Gorilla	*Gorilla gorilla*			---	5		28	40	35
Western Lowland Gorilla	*Gorilla gorilla gorilla*				---	28	30	41	38
Modern human	*Homo sapiens sapiens*					---		46	
Neander-thal	*Homo sapiens neander-thalensis*						---	43	44
Bornean Orangutan	*Pongo pygmeaus*							---	14
Sumatran Orangutan	*Pongo abelii*								---

The whole multiple sequence alignment is too long to fit on a lab bench. You will have to look at just part of it at a time and scroll back-and-forth to see other parts.

Questions

Find position 164. If you assume that all the great apes had a common ancestor, what is the "best guess" for which amino acid that ancestor had at position 164?

When, in the history of the great apes, did the mutation occur that changed the amino acid at position 164?

Now move to position 171. What was the probable amino acid in this position in the common ancestor?

When did the mutation at this position take place?

Positions 193 and 194 are similar but not identical. Describe two different ways the pattern at position 194 might have come to be.

What is the simplest explanation for the pattern at position 212?

The multiple sequence alignment is the first step in preparing a **phylogenetic tree** based on cytochrome b data. The next step is to count all the differences between each pair of apes. This has been done for most pairs and the results are given in Table 1 on the next page. Your assignment is to complete Table 1 by counting the difference between modern humans and 1) the bonobo, 2) the western gorilla, 3) the Neanderthal, and 4) the Sumatran orangutan.

The next step in making a phylogenetic tree is usually done by computer. It involves a lot of calculations so it is not much fun to do by hand. Fortunately, this particular set of data is pretty easy to analyze "by eye."

Notice that the smallest values in the Table 1 are between the two apes that are in the same genus. This fits with what you saw in the photographs last week – they easily fell into 4 pairs: two chimpanzees, two gorillas, two humans, and two orangutans. We can be confident that the last branch points in the phylogeny are going to be the ones that split these pairs into two separate species or subspecies. We can ignore those splits temporarily and concentrate on the earlier branch points, the ones that split the different genera from each other.

To help sort out the earlier splits, the data have been simplified and put into Table 2. These new data values show the average differences between genera (e.g., between the two chimpanzees and the two gorillas).

Inspecting Table 2 shows that all the largest values include orangutans. Larger values, of course, mean that the branch point was further back in time, so we conclude that the first split eventually produced modern orangutans on one side and all the other apes on the other side. In other words, the great ape phylogeny fits scheme A of the homework, the unbalanced one.

All the other data values in Table 2 are pretty similar, though the ones that include gorillas are slightly larger. This means that two splits happened in a fairly short period of time (geologically speaking) and that the first split separated off the lineage that eventually produced the living gorilla species. The second split then separated the chimpanzee and human lineages.

Figure 1 shows a tree phylogeny of these great ape species that was produced (by a computer program) from the cytochrome b data. The length of lines is based on the number of differences accumulated in the lineages. Molecular data lets us draw lines whose *relative* lengths make sense. In order to put an actual time scale on a molecular phylogeny requires calibration with fossils that have been dated.

Figure 1 agrees very well with phylogenies for the great apes that biologists had produced before molecular data became available. Combining information from phenotypic analyses, molecular sequence analyses, and fossils suggests the key events in the history of the great apes were:

- Orangutans split off about 14 million years ago;
- Gorillas split off about 7 million years ago;
- Humans and chimpanzees separated about 6 million years ago.

Table 2. Average number of amino acid substitutions between genera of great apes.

	Chimpanzees	Gorillas	Humans	Orangutans
Chimpanzees	---	26.75	24.25	41
Gorillas	26.75	---	28.5	38.5
Humans	24.25	28.5	---	43.75
Orangutans	41	38.5	43.75	---

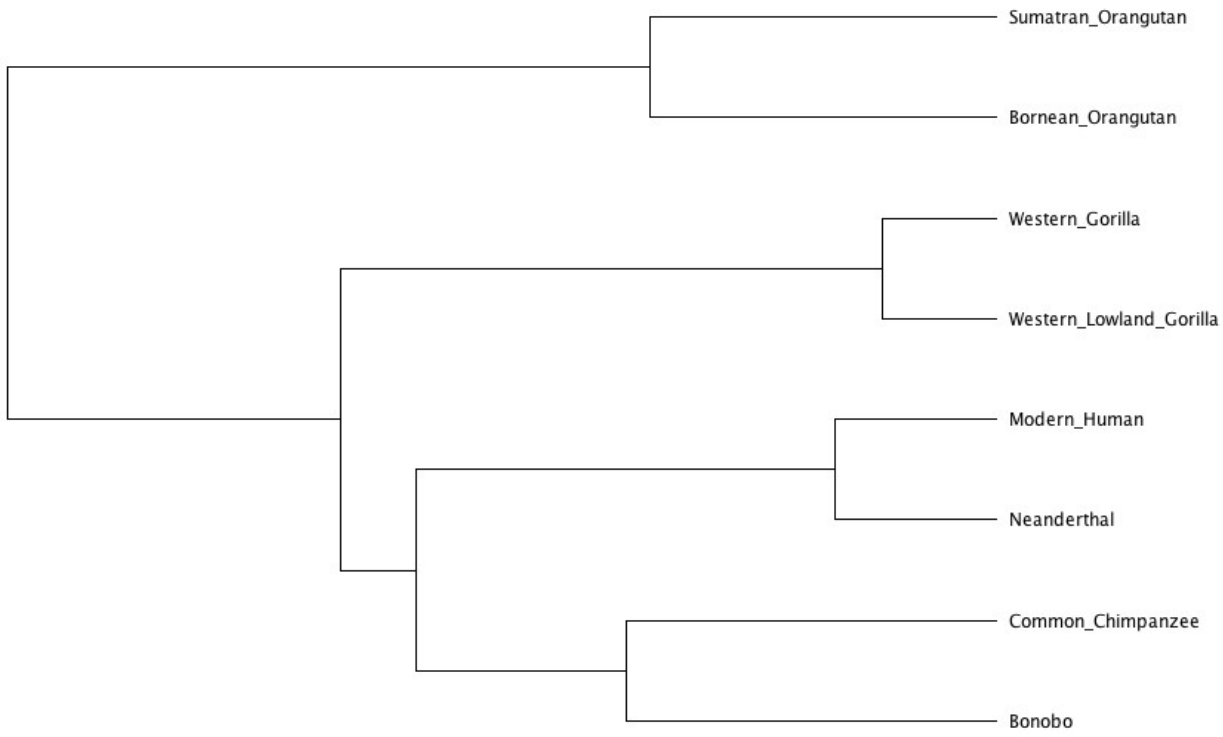


Figure 1. Phylogeny of great apes according to cytochrome b data.

Questions

In what ways is this phylogeny similar to the one you prepared as homework?

In what ways is it different?

Describe how a biologist would go about fitting an extinct lineage of apes into this phylogeny, if no molecular data were available. What tools would s/he have available?

Appendix B: Restriction Enzymes

Introduction

Instructors who teach about restriction enzymes may find this extension of the necklaces activity useful. It certainly is fun, and it is always exciting to see how the random mutations applied to the necklaces produce different patterns of fragments. The way we set it up is to cut the necklaces three separate times with three different recognition sequences, reconstructing the necklace between each enzyme. We also keep it simple by cutting in only one direction.

The models of restriction enzyme recognition sequences have to be very similar to the initial ATCG sequence of the necklaces. We use ATCGATCC for enzyme A, ATCGATCGAT for enzyme B, and ATCGATCGATCG for enzyme C. Students tend to overlook the instruction to reassemble their necklace after each restriction enzyme assay and they have been known to put the pieces back together in the wrong order. Instructors need to watch for these mistakes.

On its own, the model leads students to think of restriction enzymes as strings of DNA bases; this needs to be contradicted with an explanation that the short strands are only models of the recognition sites of the enzymes.

The role of gel electrophoresis can be included by having students transfer the pattern they collectively put on the blackboard to a sheet of 2-cycle semilog graph paper. We do this as an "activity sheet" the students submit at the end of lab. We give them photocopies of the semilog graph paper with the axes prepared for the specific situation they are graphing.

Restriction Enzymes

In the Origin of Species lab you saw how mutations cause two populations to become different after a branch point. This week you will see one of the ways we can measure these differences at a DNA level.

The technique makes use of restriction enzymes. These are enzymes produced by bacteria, presumably as protection against viruses. Viruses do attack bacteria and since a virus is little more than a piece of DNA, it makes sense that cutting DNA would be protective for the bacteria.

To see how a restriction enzyme works, get out your bead necklace, which you saved at the end of the Origin of Species lab. Remember that at the end of that lab there were four different necklace patterns. We are going to see if we can tell them apart by using restriction enzymes.

Procedure

1. You will find three short strands of beads in a plastic Petri dish. The strands are labeled A, B, or C with a small tag on a string.
2. Stretch your necklace out straight on the lab bench. Take the short strand labeled A out of the Petri dish and line it up alongside your necklace, starting at position 1 of your necklace, and with the free prong of your necklace and the free prong of the short strand facing in the same direction.
3. Does the short strand match perfectly with your necklace? If it does, go to step 4. If it does not, slowly slide the short strand along your necklace until you find a perfect match.
4. When you find a perfect match, break your necklace apart at the position that matches with the place the string is attached to the short strand.
5. Continue to slide the short strand along your necklace, stopping wherever you find a perfect match and breaking your necklace at the site of the string.
6. When you have finished, you will have one piece for each time you found a perfect match, plus one more piece from before the first match. (You will have n + 1 pieces, where n is the number of times you found a perfect match.) If you found no perfect matches, you will have just one piece that is 100 beads long.
7. Go to Table 1 and record how many pieces you end up with.
8. Now count how many beads are in each piece. Record these values in Table 1 under "Sizes of pieces".
9. Put your necklace back together, as you found it when you started today.
10. Repeat the procedure from step 1 through step 8 with short strand B.
11. Put your necklace back together, as you found it when you started today.
12. Repeat the procedure from step 1 through step 8 with short strand C.

Table 3. Results of cutting a bead necklace with three different restriction enzymes.

Restriction sequence	Number of pieces	Sizes of pieces
A		
B		
C		

Now we need to find out if we can tell the different necklaces apart by the number of pieces produced by the restriction enzymes and the sizes of those pieces. Your lab instructor will show you how to put your results on the blackboard. Each of the four "tribes" will put their results up. *Please be careful – you need to put them where your instructor tells you.*

There will be three sets of lines on the board, representing the three different short strands. The pattern shows you what you get after **gel electrophoresis** of DNA cut with restriction enzymes. (It is *almost* what you get – see the activity sheet.)

NAME: SECTION:

Gel electrophoresis allows you to separate pieces of DNA according to how big they are. Small pieces travel faster through the gel than larger pieces. However, the relationship between size and speed is not linear, it's logarithmic. In other words, small pieces of DNA travel much faster than large pieces of DNA.

The illustration on the blackboard shows a linear relationship between size and speed. What you are going to do now is to make a logarithmic illustration of the same data. It is easy, really, if you use semi-log graph paper.

Get one sheet of 2-cycle semi-log paper from your instructor. Look at how it is labeled; notice that it is organized like the pattern on the blackboard.

Now transfer the data from the blackboard to your graph paper.

When you are done you have a picture of a gel for this model experiment. Staple your illustration to this activity sheet before you turn it in.

Assume you had no way of knowing the sequence of beads in the necklaces. Suppose all you could do is cut them with A, B, and C. Discuss the evidence you'd have that the four necklaces are not all identical.

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