

LABORATORY 7: Clarifying Monet

Introduction

Perhaps when most people take a walk through a garden or a park or the countryside, they see a pleasant blend of greens, along with the colorful blooms of flowers. To plant systematists, however, these scenes are not only aesthetically pleasing, but present a great challenge. There are over 1/4 million species of plants currently recognized, and with many tropical areas of the world poorly documented, there are likely thousands more species yet to be discovered. The challenge plant systematists face is to discover the evolutionary relationships of the world's plants, and subsequently classify the world's species of plants based on how closely they are related to other plants. To do so, a variety of information is used, including morphological data, genetic data, ecological data and even fossil evidence.

In this lab, you will become familiar with the tools most often used by plant systematists in classifying and ordering the world's plants: morphological and genetic data. Although there are a number of groups of non-flowering plants (gymnosperms, ferns, horsetails, mosses, etc.), this lab will focus on the flowering plants, as they constitute more than 80% of the world's plant species.

Exercises

I. Morphological Data

Traditionally, the classification of plants is primarily based on morphological characters (i.e., things that are readily visible or measurable, such as physical size, shape, color). For the flowering plants, the most important morphological characters are found in the flower. Among the important characters are the arrangement and size of the parts of the flower, their number, their color, and even their presence or absence.

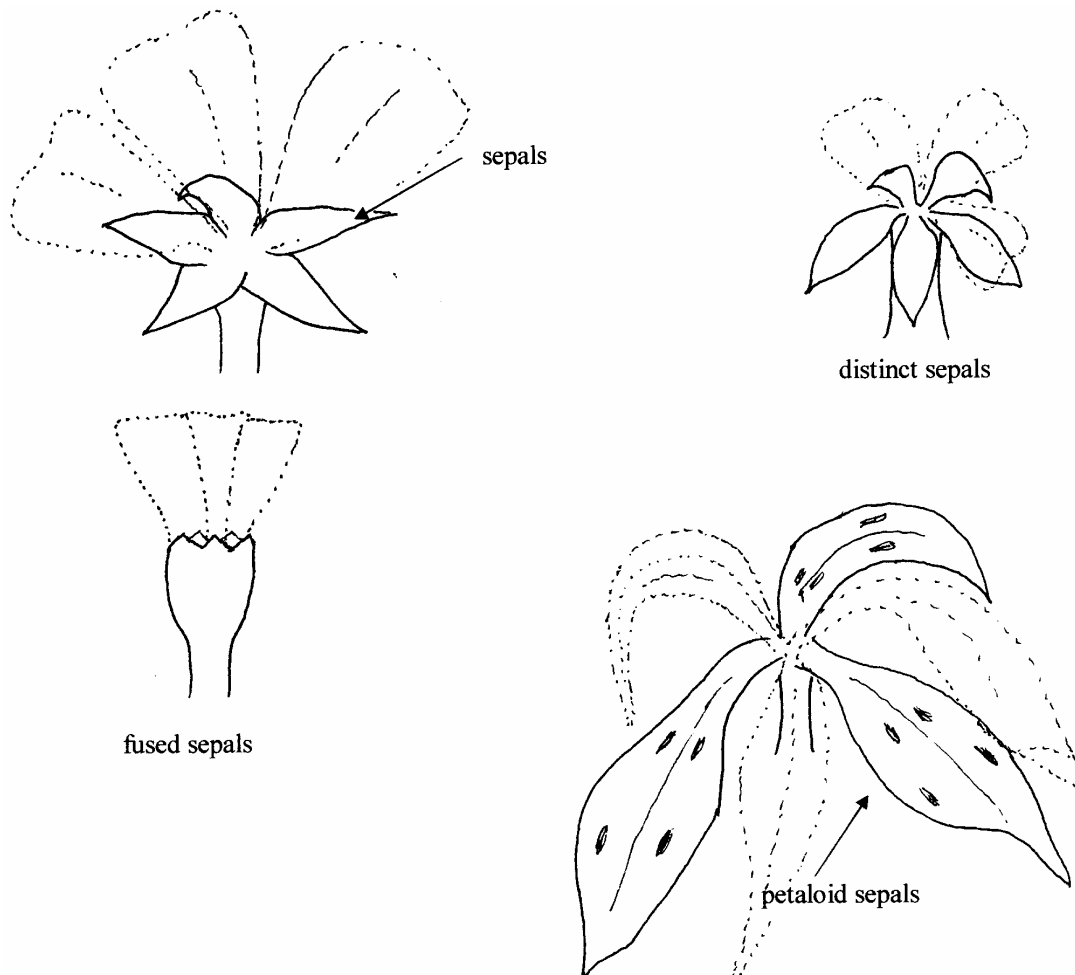
Your group will be given two flowers to work with. Use these flowers to become familiar with the different parts of the flower, and some variations on their characteristics, as outlined in the following sections.

A. *The Calyx*

The calyx consists of the sepals, which are found as the outermost whorl or circle of parts of the flower (Figure 1). While the sepals are often relatively small and green, they may range in size greatly, and

can look very similar or identical to the petals (described in the next section). In addition, the sepals may also range from being completely distinct (not attached to one another) to completely fused (forming a tube). Most commonly, sepals are found in numbers of 3, 4, or 5, although they are occasionally altogether absent.

Figure 1.



Questions

Find the sepals on your group's two flowers.

Are the sepals green, or do they look more like the petals of a flower? _____

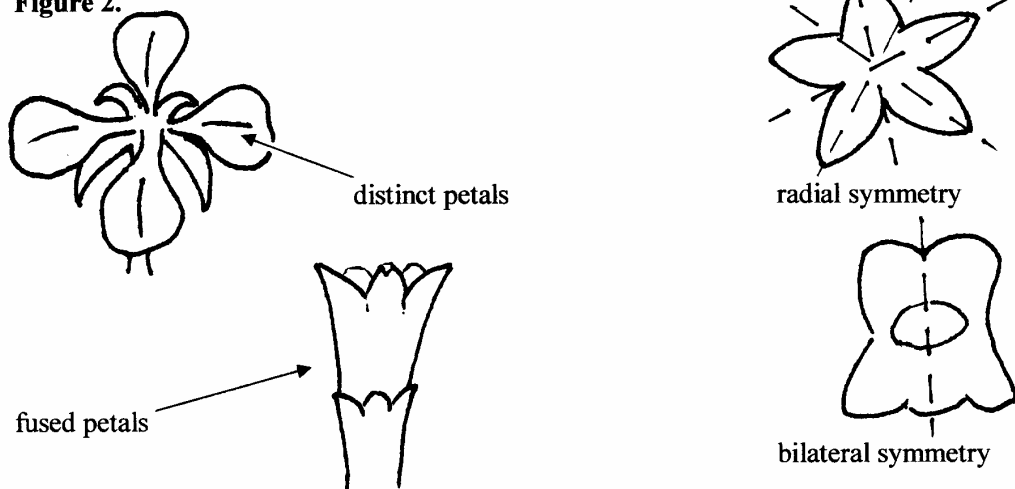
Are they distinct or fused? _____

How many sepals does each of your flowers have? _____

B. The Corolla

The **corolla** consists of the **petals**, which are attached to the interior of the sepals (Figure 2). Like the sepals of the calyx, the petals of the corolla vary greatly. Some are small and inconspicuous, some are large and very showy with bright colors. As most people know, corollas also display a great range of color variation. It may be surprising, though, that color variation is not usually a strong character for determining evolutionary relationships among plants, because petal color changes quite easily. The presence of blue or red petals, however, can in some instances be significant. Like the sepals, the petals can be **distinct** or **fused**. Finally, the corolla may vary in its **symmetry**. For example, look at your flowers' corollas from the top. From this view, draw an imaginary line through the center of the flower (cutting your view into two halves). Do the resulting halves make a mirror image? If you are able to draw lines through the center of your flower in 2 or more directions and obtain mirror images, your flower is said to have **radial symmetry**. If, however, you can only draw a line in one direction through your flower to obtain mirror images, your flower is said to have **bilateral symmetry**. Petals are usually found in numbers of 3, 4, or 5, although they are occasionally absent.

Figure 2.



Questions

Find the petals on your group's flowers.

How many petals do your flowers have? _____

Is there the same number of petals as sepals? _____

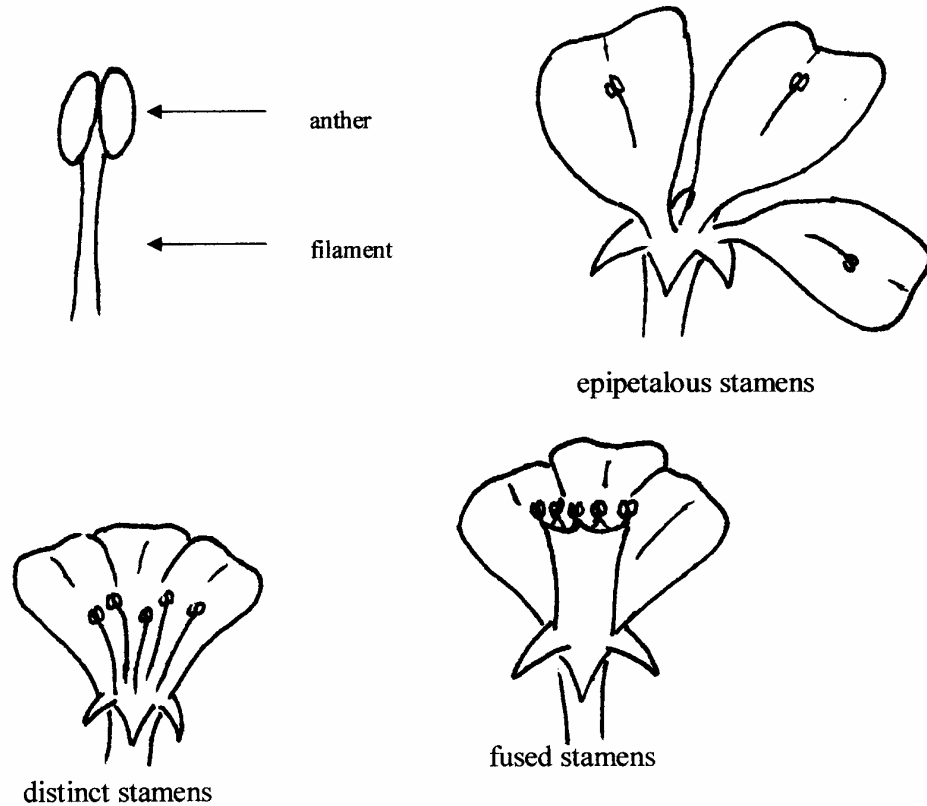
Are the petals distinct or fused? _____

What type of symmetry do your flowers have? _____

C. *The Androecium*

The **androecium** of the flower consists of the **stamens**, which are found interior to the petals (Figure 3). Stamens are made up of two parts: the stalk (called the **filament**) and a head (called the **anther**). The anther is the site of pollen production. Stamens may range in size and color, but generally are not as exceptional in this respect as are sepals and petals. Perhaps more importantly, stamens may be **distinct** from each other or **fused**, or **may actually attach to the petals**. Stamens usually range in number from 2 to more than 10, although they are occasionally absent.

Figure 3.



Questions

Find the stamens on your group's flowers.

How many stamens do your flowers have? _____

Are the stamens the same in number as the sepals and/or the petals? _____

Are the stamens distinct or fused? _____

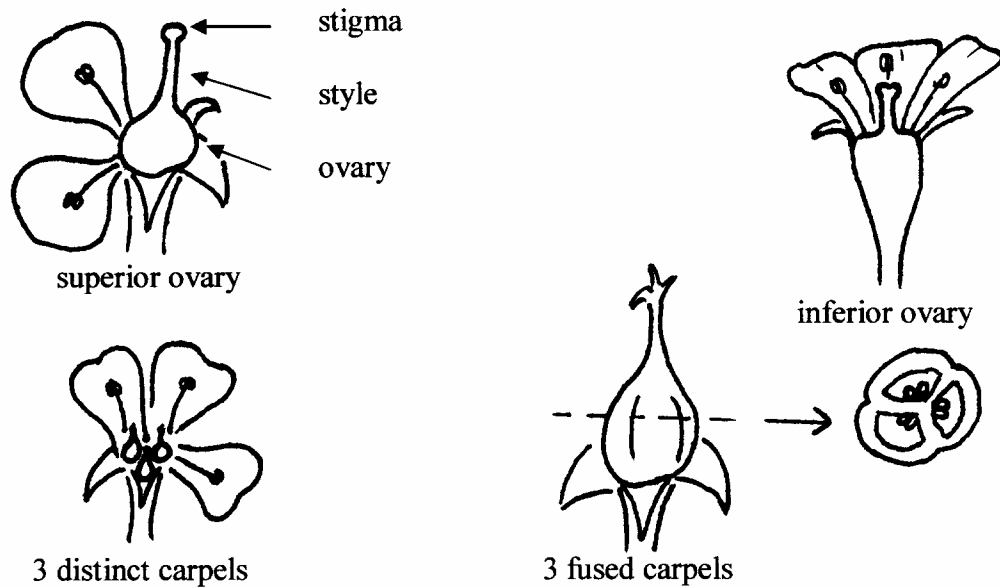
Are the stamens attached to the petals? _____

D. The Gynoecium

The **gynoecium** consists of the **carpels**, which are found interior to the stamens (Figure 4). The carpels contain the ovules which upon pollination (fertilization) will develop into seeds. The carpel consists of three parts: a sticky top (called the **stigma**), a tube (called the **style**), and an enlarged base (called the **ovary**) which holds the ovules. The ovary is also the part of the flower that develops into the fruit.

The most important characters in the gynoecium generally involve the number and fusion of the carpels, and the position of the ovary relative to the petals, sepals, and stamens. Flowers may have **one carpel only**, two to numerous **carpels that are distinct**, or several **fused carpels**. It can be tricky to distinguish between a flower with one carpel and a flower with several fused carpels. To do so, one can check for **lobing** on the top or sides of the carpel, and can check to see if there is more than one chamber inside of the ovary. Usually, the lobing and the number of chambers in the ovary correspond to the number of fused carpels present. The position of the ovary is described as either **superior** or **inferior**. If the petals, sepals, and stamens all attach below the ovary, the ovary is called superior. If the petals, sepals, and stamens all attach at the top of the ovary, it is called inferior. Carpels most often range in number from one to several, although they occasionally are absent altogether.

Figure 4.



Questions

Find the gynoecium on your group's flowers.

How many carpels do your flowers have? (check carefully...)

Are the carpels distinct or fused? _____

Are they the same in number as the petals? Sepals? Stamens?

Are the ovaries of your flowers superior or inferior? _____

II. Constructing a Phylogenetic Tree Using Morphological Data

Now that you are familiar with some of the more common morphological characters used in determining the evolutionary relationships among flowering plants, your group will use this information and the descriptions of several hypothetical species of flowering plants to construct a phylogenetic tree.

A. The Descriptions

Read the descriptions of the flowers of each species below, and try to picture in your mind what the flowers and their parts *might* look like.

- Species A:** calyx: 5 green sepals, all distinct
corolla: 5 white petals, all distinct, radial symmetry
androecium: 10 distinct stamens, not attached to petals
gynoecium: 4 carpels, all distinct, superior ovaries
- Species 1:** calyx: 5 green sepals, all fused
corolla: 5 blue petals, all fused, bilateral symmetry
androecium: 4 stamens, all distinct, all attached to petals
gynoecium: 2 carpels, fused, superior ovary
- Species 2:** calyx: 5 green sepals, all distinct
corolla: 5 red petals, all distinct, radial symmetry
androecium: absent
gynoecium: 4 separate carpels, superior ovaries
- Species 3:** calyx: 5 green sepals, all fused
corolla: 5 red petals, all fused, radial symmetry
androecium: 5 distinct stamens, all attached to petals
gynoecium: 2 fused carpels, superior ovary
- Species 4:** calyx: 5 green sepals, all fused
corolla: 5 red petals, all fused, radial symmetry
androecium: 5 distinct stamens, none attached to petals
gynoecium: 2 fused carpels, superior ovary
- Species 5:** calyx: 5 green sepals, all distinct
corolla: 5 red petals, all distinct, radial symmetry
androecium: 10 distinct stamens, none attached to petals
gynoecium: absent

B. Putting the Characters into a Useful Format

Now that you have an idea of each of the flowers characters, it is important to put the characters into a useful format, so that you may quantify the relationships between the flowers. In order to do this, fill in the rows on the table below according to the descriptions of the different species above, and compare the number of differences

and similarities between each of the flowers. Next, fill in the number of differences each of species 1-5 has with species A below. In this exercise, we will use species A as an **outgroup**. This means that species A represents a lineage of plants that diverged early in time from the lineage that gave rise to all of species 1-5. Hence, species 1-5 can be ordered based on in how many characters each one differs from species A.

Species	# sepals	distinct or fused	color	# petals	distinct or fused	color	symmetry	# stamens	distinct or fused	stamens attached to petals?	# carpels	distinct or fused	superior or inferior ovary (ies)
A													
1													
2													
3													
4													
5													

Differences from Species A.

Species 1 differs in _____ characters from species A.

Species 2 differs in _____ characters from species A.

Species 3 differs in _____ characters from species A.

Species 4 differs in _____ characters from species A.

Species 5 differs in _____ characters from species A.

Questions

1. Based on the morphological data, which species do you think is most closely related to species A? _____

Which species seems most distantly related to species A?

Are there more than one species that differ in the same number of characters from species A? _____

2. If species 1-5 are put in order from those which numerically differ the least from species A to those which differ most, what are the possible orders?

A _____

A _____

3. Do you think that some characters listed on the table are more important than others? _____

If so, how might you take this into account? _____

C. Constructing a Phylogenetic Tree

A phylogenetic tree is a diagram that attempts to show the evolutionary relationships among organisms. In general, organisms that are similar in more of the characters under study are more closely related in an evolutionary sense. When constructing or interpreting phylogenetic trees, there are several important concepts to keep in mind:

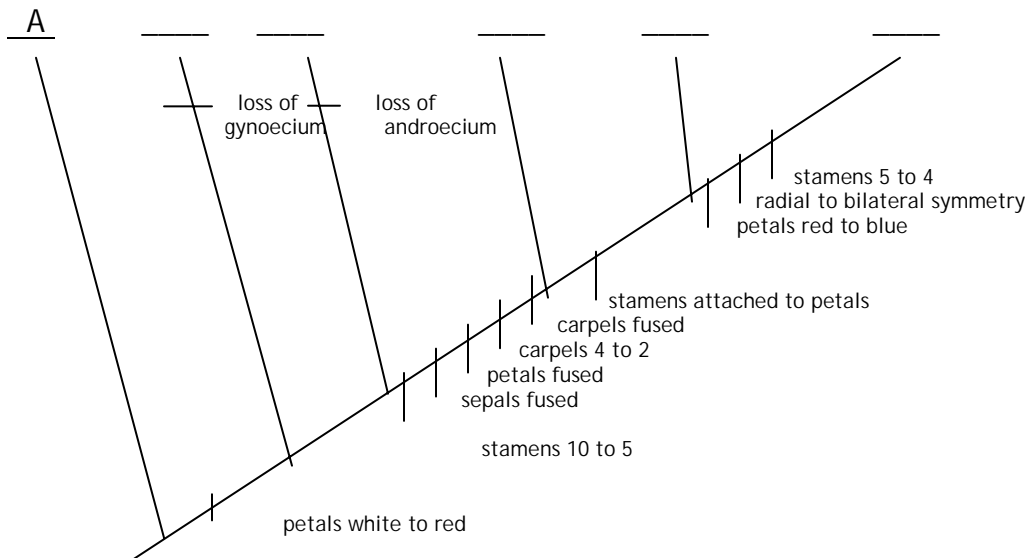
- species under consideration only go at the end of lines, not at the nodes (intersections of lines)
- the nodes represent instances of divergence, where a hypothetical single ancestral species split into exactly two species, each of which gave rise to a different lineage of organisms
- changes in characters are noted on phylogenetic trees between successive nodes, or between a node and the end of a line
- the distance between species or groups on a phylogenetic tree does not directly indicate time, but species or groups placed more closely

together (i.e., more recently sharing a common ancestor), are more closely related

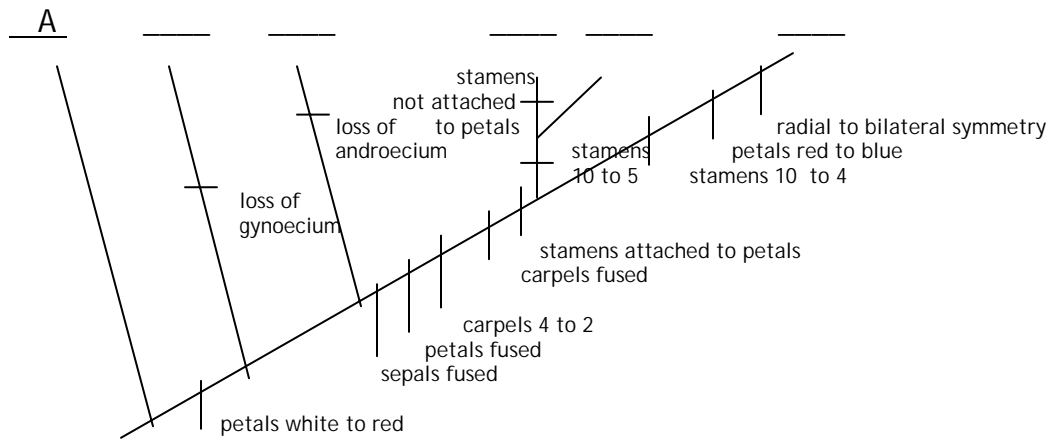
- the most probable trees are usually those with the fewest differences between adjacent species (i.e., the least amount of changes in characters. For example, instead of creating a phylogenetic tree in which flower petal color changes from red to white to red to white and back to red again, a more probable phylogenetic tree might have (if possible) only one or two color changes.

Below are several possible phylogenetic trees that represent the morphological data from the previous section. With your group, fill in the blanks on the phylogenetic trees with the appropriate species number. It will help to refer to the data table from the previous section.

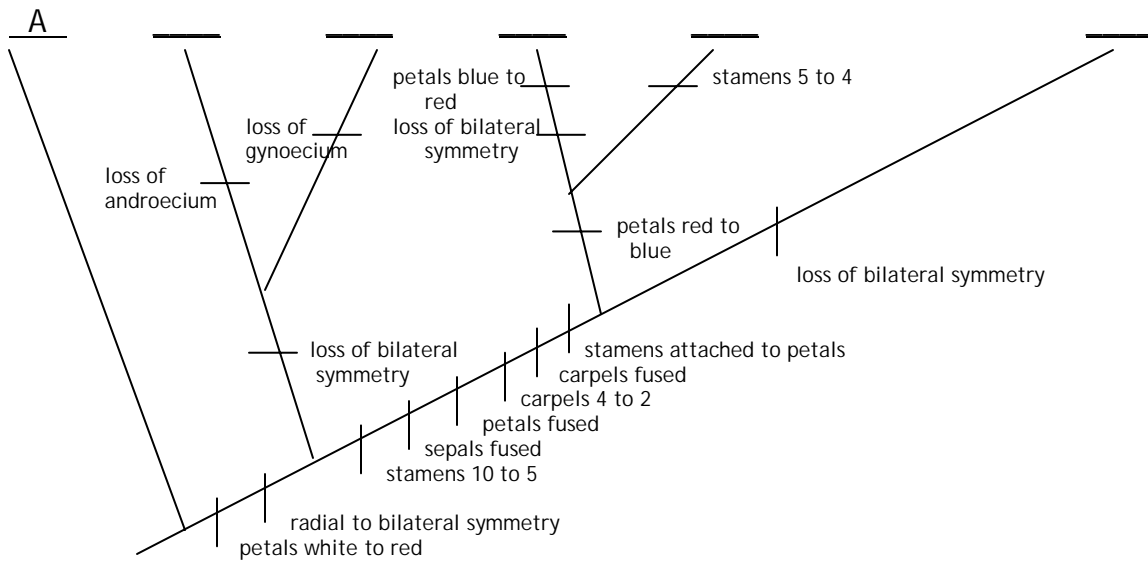
Tree 1.



Tree 2.



Tree 3.



Questions

4. Does there seem to be more than one plausible answer?

If so, how do you account for this? _____

Do you think that there are more than these three possible solutions? _____

Can you think of any way that might be used to narrow down the number of possible solutions (make the relationships clearer)?

5. From the morphological data, is there any way to tell in which order species 2 and 5 should go on the phylogenetic trees?

III. Genetic Data

In the last 15-20 years, genetic data has become an increasingly powerful and important tool for discerning evolutionary relationships. The utility of genetic data has been greatly aided by the development of relatively inexpensive and efficient equipment (including DNA sequencers and polymerase chain reaction (PCR) systems) that allow researchers to investigate the DNA of organisms much more easily than in the past.

Genetic research in plants differs slightly from that in other organisms. Animal systematists, for example, investigate mitochondrial or nuclear (from the nucleus) DNA. Plant systematists, on the other hand, often focus on the DNA of the chloroplast genome (**cpDNA**). cpDNA has several advantages over other sources of DNA:

1. the chloroplast genome is small (120 - 200 kilobases long), allowing the examination of the entire genome in many cases
2. there is only one copy of most genes
3. the chloroplast genome evolves fairly slowly, but different parts may evolve at different rates
4. chloroplasts are maternally inherited, so all of the chloroplasts in a given plant will have the same DNA

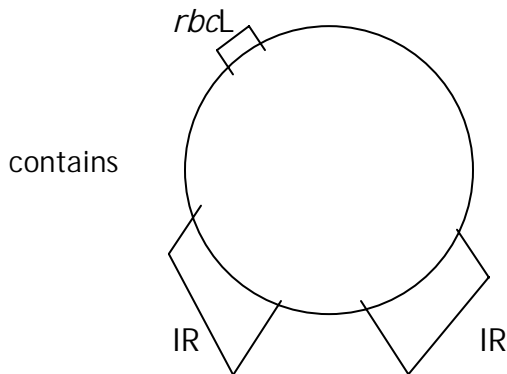
Currently, the most common approaches to utilizing cpDNA involve **nucleotide sequencing** and the identification of **structural changes** in the cpDNA.

A. cpDNA Sequencing

The sequencing of cpDNA involves obtaining the exact order or nucleotides (e.g. adenine, thymine, cytosine, guanine) in a chosen segment of the chloroplast genome. Currently, the most common segment of cpDNA used in phylogenetic studies is the *rbcL* gene (Figure 5). This gene is a sequence of about 1430 nucleotides that codes for the large subunit of the enzyme (protein) RuBisCo. As you may already be aware, RuBisCo is the enzyme that captures carbon dioxide during photosynthesis, initiating the biological process that is ultimately responsible for virtually all of the carbohydrates on the planet.

The general concept of using sequence data is the same as for morphological data, i.e., organisms with more similarities are more closely related. In this case, however, it is the similarities in the nucleotide sequence of the DNA or organisms that is compared.

Figure 5. The chloroplast genome.



The chloroplast genome is circular, 120 - 200 kilobases in length, and usually two approximately 22 kilobase inverted repeats (IR).

Below are sequences of *rbcL* cpDNA for the same hypothetical plants you worked with in the previous section. The sequences below, of course, are shorter than real *rbcL* sequences. It would be too labor-intensive to work with a full-length gene sequence without the aid of computers. With your group, determine which species appear to be most closely related according to the

sequence data, by comparing them to species A and to each other. It may help to use the following steps:

1. Block or square out any differences that species 1-5 have with species A

ex.

Species	Sequence
A	A A G T A
1	A A G A T
2	A A G T T
3	A A G T A

2. Place species 1-5 in order based on their similarities/differences to species A
3. Refine this order by placing species 1-5 based on their genetic similarities to each other. For example, if two species have the same number of differences with species A, determine which of the other species they share more similarities with, and place them in the resulting order.

rbcL Sequences for Species A, 1-5

Species	Sequence Data	# Differences from Species A
A	A A T C G A T A G G G C T G G G G A A C G	-----
1	A C C G A G T A T G A T T G G G G A A G A	_____
2	A A T G G A T A G G A A T G G G G A A C C	_____
3	A A C G G G T A T G A A T G G G G A A G C	_____
4	A A C G G G T A G G A A T G G G G A A G T	_____
5	A A T G G A T A G G A C T G G G G A A C C	_____

Now, refer again to the phylogenetic trees constructed from the morphological data, and place species 1-5 on each of the trees according to their genetic relatedness.

Questions

6. Are there now any trees which seem more probable than the others? _____

Does the same tree fit best with both morphological and genetic data? _____

7. Do you think you can make a better interpretation of the phylogenetic relationships with two sources of data rather than one? Why? _____

B. Structural Changes

Structural changes in DNA generally involve large scale substitutions, deletions, or inversions. **Substitutions** occur when pieces of DNA are added to the genome or replace existing parts of the genome.

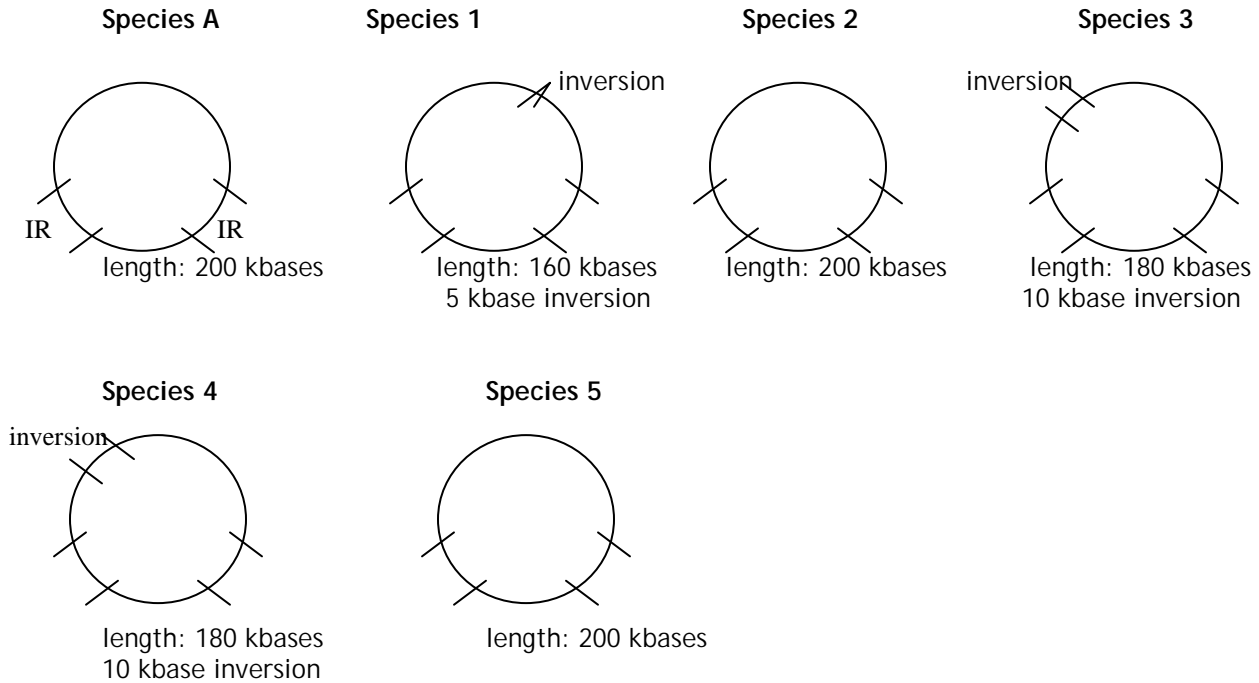
Deletions occur when pieces of DNA are removed from the genome.

Inversions occur when a piece of DNA is taken out of the genome, "turned around," and re-inserted backwards. Structural changes in cpDNA may range in size from several hundred bases long to over 80 kilobases long (remember, the chloroplast genome is only 120 - 200 kilobases long total!). One example of a structural change occurs in certain members of the legume family - several groups in this family are missing one of the two inverted repeats normally present in the cpDNA.

As one might surmise, large scale changes in a genome could prove disastrous. Because of this, structural changes are relatively infrequent. In turn, if certain plants or groups of plants have identical structural changes, there is a good chance that they are relatively closely related, as the chance of the same structural change happening more than once (in separate lineages) is usually not great.

Below are diagrams of the chloroplast genomes for our hypothetical plant species. Take a moment to compare them with respect to any substitutions, deletions (pay attentions to the genome length), or inversions.

Chloroplast Genomes for Species A, 1-5



Now, make a final determination of which of the three original phylogenetic trees is most plausible, incorporating the data on structural changes.

Questions

8. If you now take into account all three sources of data (morphological, sequence, and structural changes), which tree seems best supported? _____

Would it be correct to say that the tree you have chosen represents the true evolutionary relationships among our hypothetical plant species? Why or why not? _____

9. Does the structural genetic data support the *rbcL* sequence data? Does it support the morphological data?

10. If you had to choose only one source of data, morphological or genetic, which would you choose? Why? _____

Extra Credit (2 points)

If the DNA data (both sequence and structural) turned out to be identical for species 2 and 5, can you think of another possible explanation concerning their relatedness? _____

IV. Finishing Up

For your lab report, please answer all ten of the numbered problems throughout the lab. For some of the questions, you will already have the answers from working through the lab; other questions will require more thought. **All** questions must be answered in **complete sentences**, and your report must be **typed**. Some answers will require only one sentence while others will require several sentences to answer the question well. If you would like to write more than a short paragraph for any answer, please feel free to do so. Remember, though, that you should strive for clarity in your answers, however long or short they may be. This lab report is due one week from today.