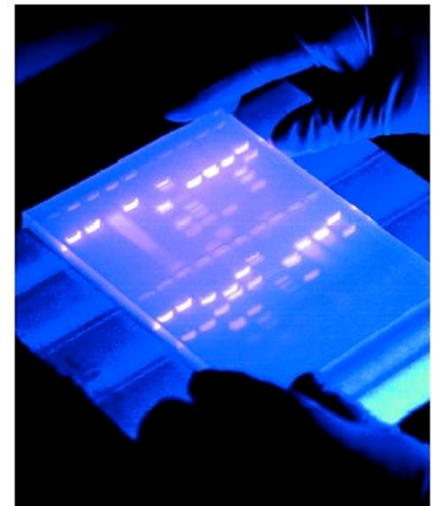
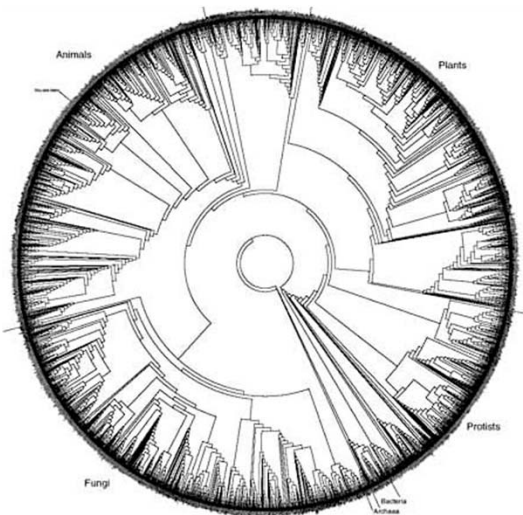




Gels and Trees

Jacob Landis



Recap from Monday

- Pollination syndromes
 - certain flower traits associated with unique kinds of pollinators
- DNA extractions
 - leaf punch for material, Extraction buffer to get DNA into solution
- PCR
 - amplify small targeted fragments of DNA

Tracking Pollinators at home



<http://www.hummingbirdsathome.org/>

Today's goals

- Run PCR product on gel to see bands
- Use morphology data matrix to score 3 characters for all taxa and build phylogeny
- “Obtain” sequence data from PCR product
- Use molecular data to build tree
- Compare differences in morphology trees between groups (if any) and between morphology and molecular trees

Running gels?



Running gels?

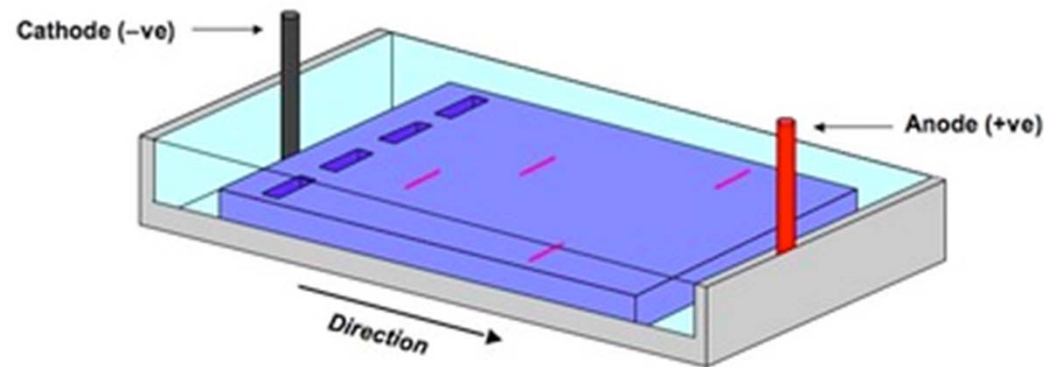


Running gels or gel electrophoresis

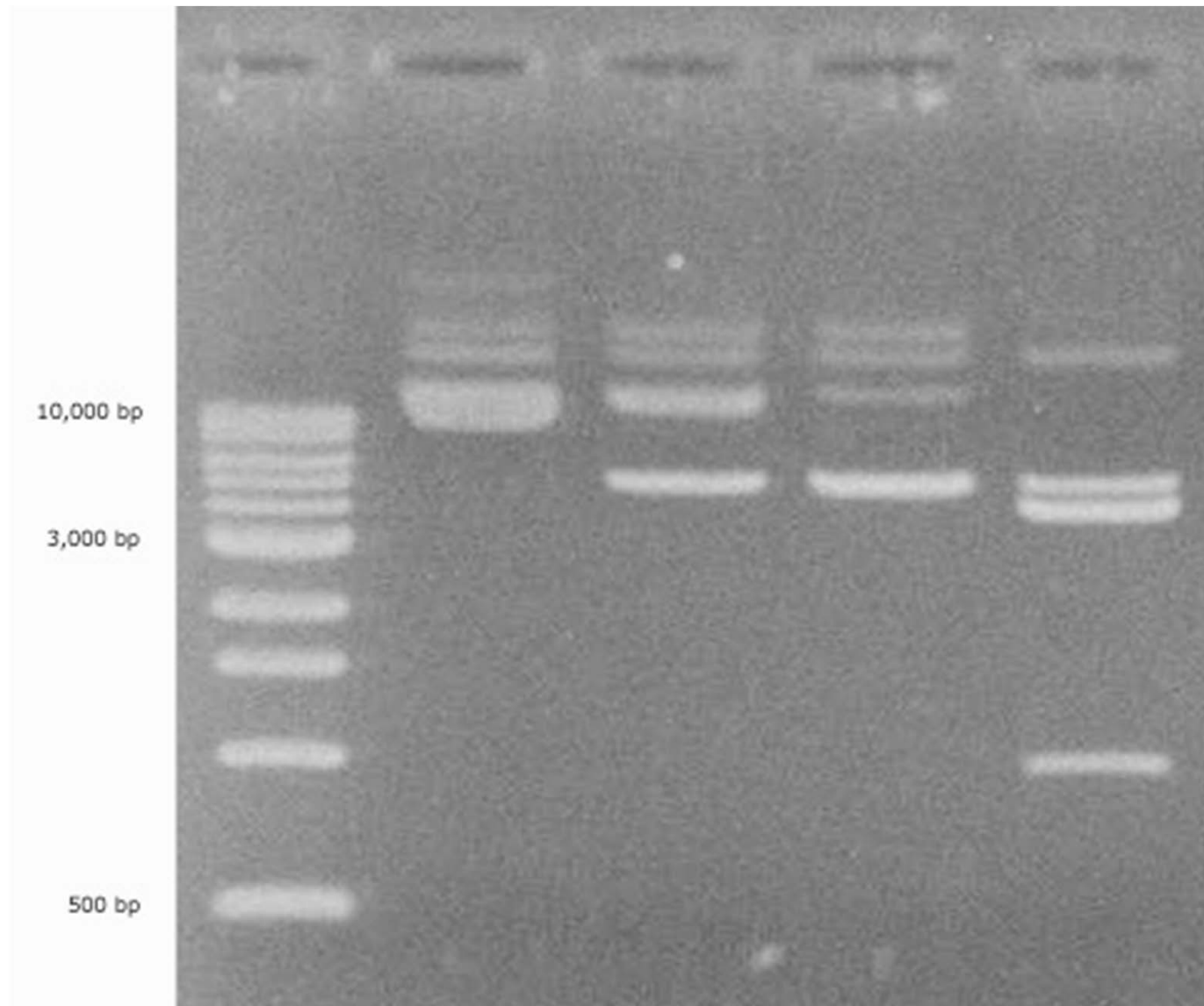


What happens

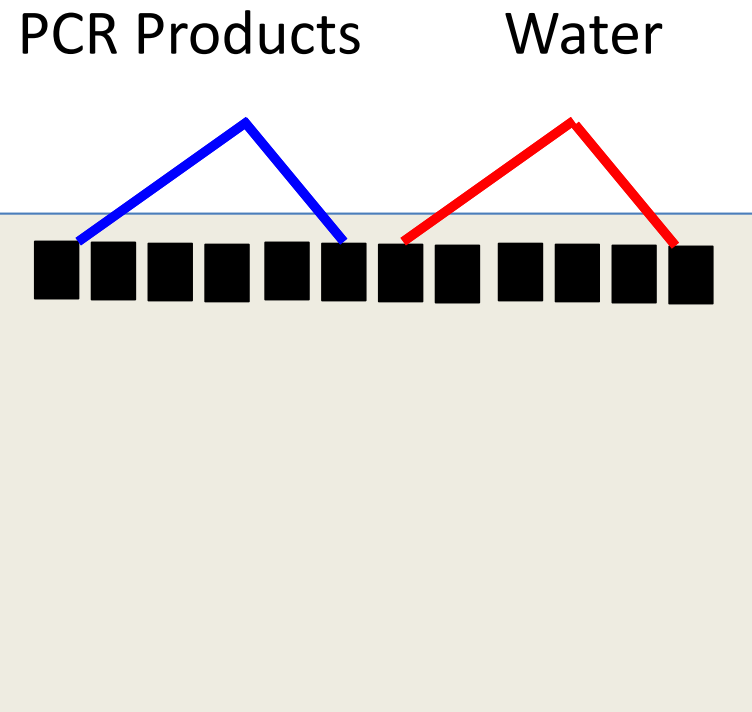
- Method of separating molecules by size
 - DNA negatively charged
 - “Runs to positive” charge
 - Smaller fragments move faster than larger fragments



Example



Loading the gel



Run for 30 minutes

Viewing gels

- Usually view under UV light to illuminate DNA
 - Buffer has special dye that lights up



Safe Imager



While gel is running for 30 minutes

- Score all characters for all species as 0 (absence) or 1 (presence) for all species

[illegible]

Running gel and scoring characters



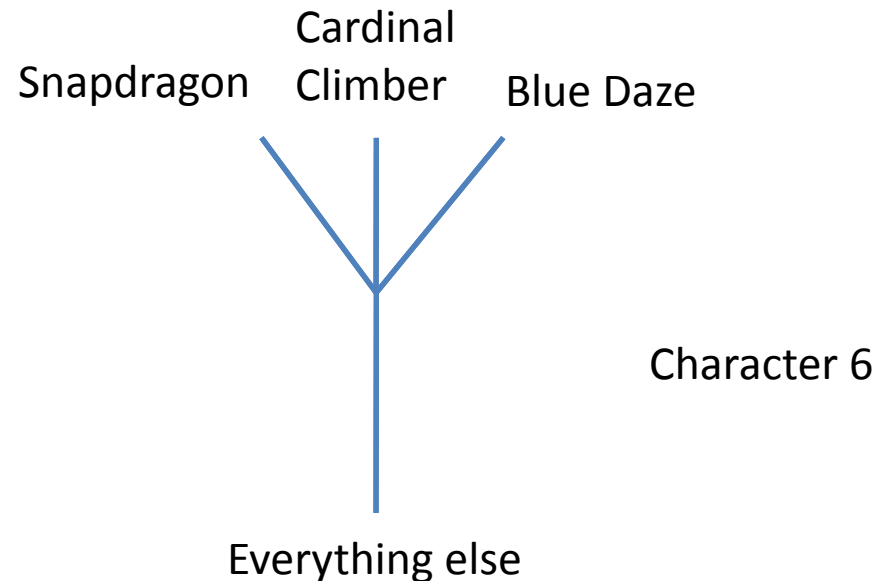
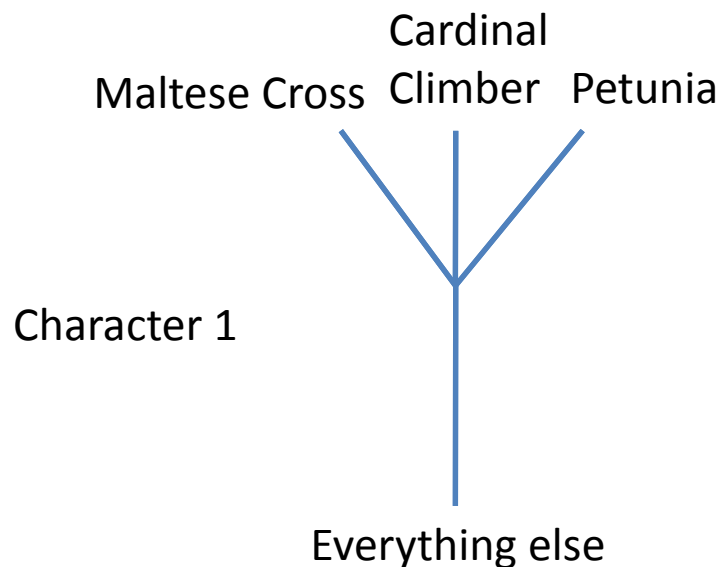
- First 6 wells will be your PCR product from Monday
 - Combine 10 μ L of PCR product and 10 μ L of water
- Last wells will just have 20 μ L of water
- Characters scored as 0 or 1

Choose 3 of the 10 characters on the data matrix that you think will be most informative for grouping plants and score for all 12 species. Don't just pick the first 3

Before we start building phylogenies,
who thinks that the hummingbird
species will be more closely related
to other hummingbird species? And
bees more closely related to other
bee species?

Morphological data

- Before constructing the full tree, pick each character and draw the relationships.
 - Does 1 character give full resolution of the tree?
 - Do the 2 characters differ?



Constructing tree by hand

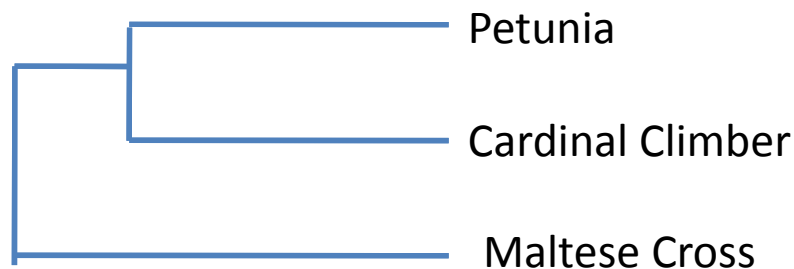
- Build a table with the number of differences between species

species	Color (Red)	Color (Blue)	Flower size	Tubular flower	Symmetry	Petal lobes fused	Stamens exerted	Horizontal Orientation	Individual flowers	Scent
Maltese Cross	1	0	0	0	0	0	0	0	0	1
Cardinal climber	1	0	1	1	0	1	1	1	1	0
Petunia	1	0	1	1	0	1	0	1	1	1

	Maltese Cross	Cardinal Climber	
Cardinal Climber	7		
Petunia	5	2	

Constructing tree continued

- Pick species that have the fewest numbers of differences
 - these are each other's closest relatives
- Work your way out to species with more differences by adding them to your tree



	Maltese Cross	Cardinal Climber	
Cardinal Climber	7		
Petunia	5	2	

Go ahead and construct morphological phylogeny. Use individual characters, and then the combined 3 characters by calculating the distances.

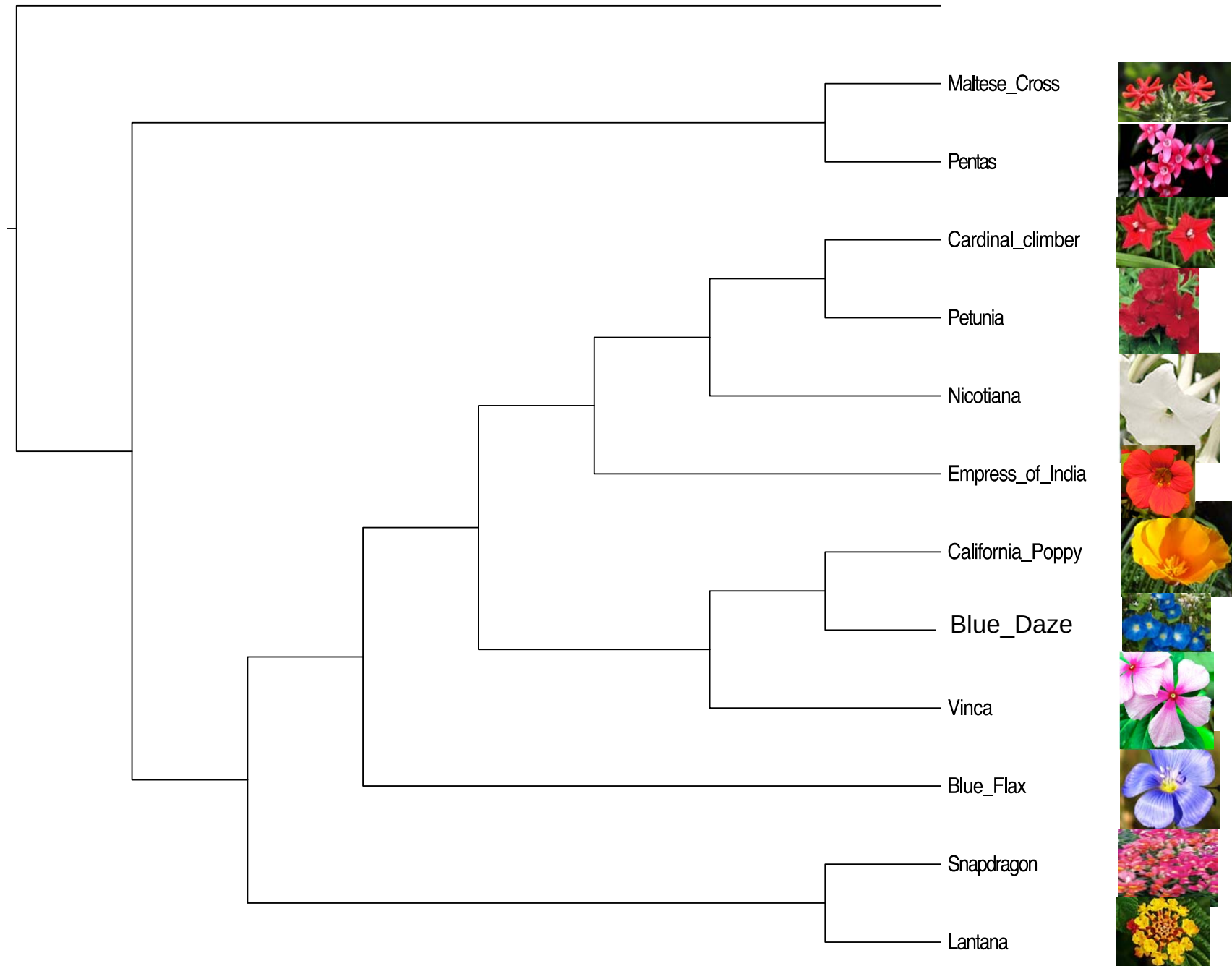
Morphological trees

- How easy was it to score the characters?
 - Did everyone score them the same?
- Compare with a group close to you, are the two phylogenies the same?
 - Why or why not?

Full data set

		Characters										
Taxa		1	2	3	4	5	6	7	8	9	10	11
1	Maltese Cross	1	0	0	1	0	0	0	0	1	0	
2	Cardinal climber	1	0	1	1	1	1	1	0	0	1	
3	Petunia	1	0	1	1	1	0	1	0	1	1	
4	Snapdragon	0	0	0	0	1	0	0	1	0	1	
5	California Poppy	0	0	1	0	0	0	0	0	1	0	
6	Empress of India	0	0	1	0	1	0	1	1	0	0	
7	Morning Glory	0	1	1	0	0	1	0	1	1	0	
8	Pentas	0	0	0	1	0	0	0	0	0	0	
9	Lantana	0	0	0	0	0	0	0	0	0	1	
10	Vinca	0	0	1	0	1	0	0	0	1	0	
11	Blue Flax	0	1	0	0	1	0	0	0	0	0	
12	Nicotiana	0	0	1	1	1	0	1	0	0	0	

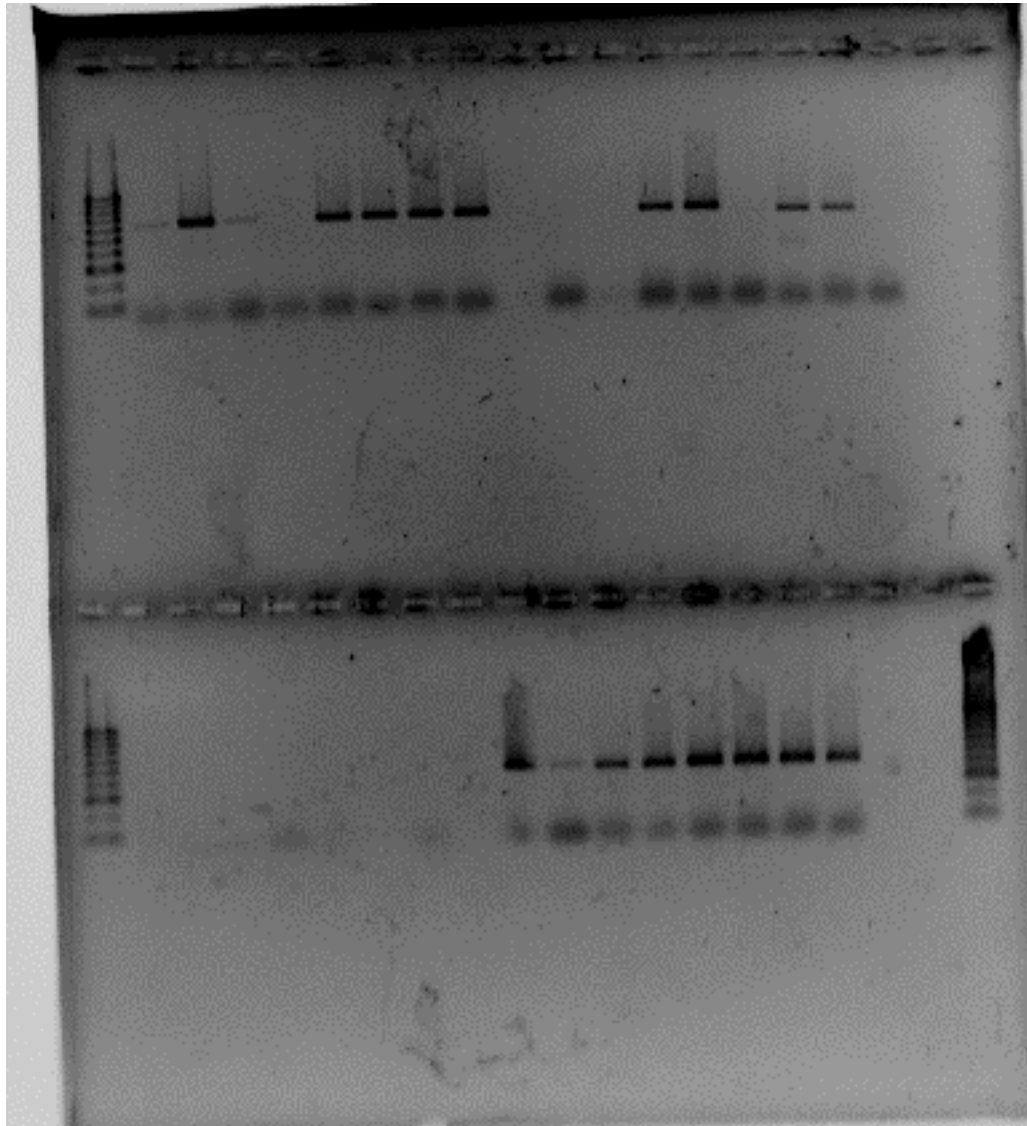
Morphology tree



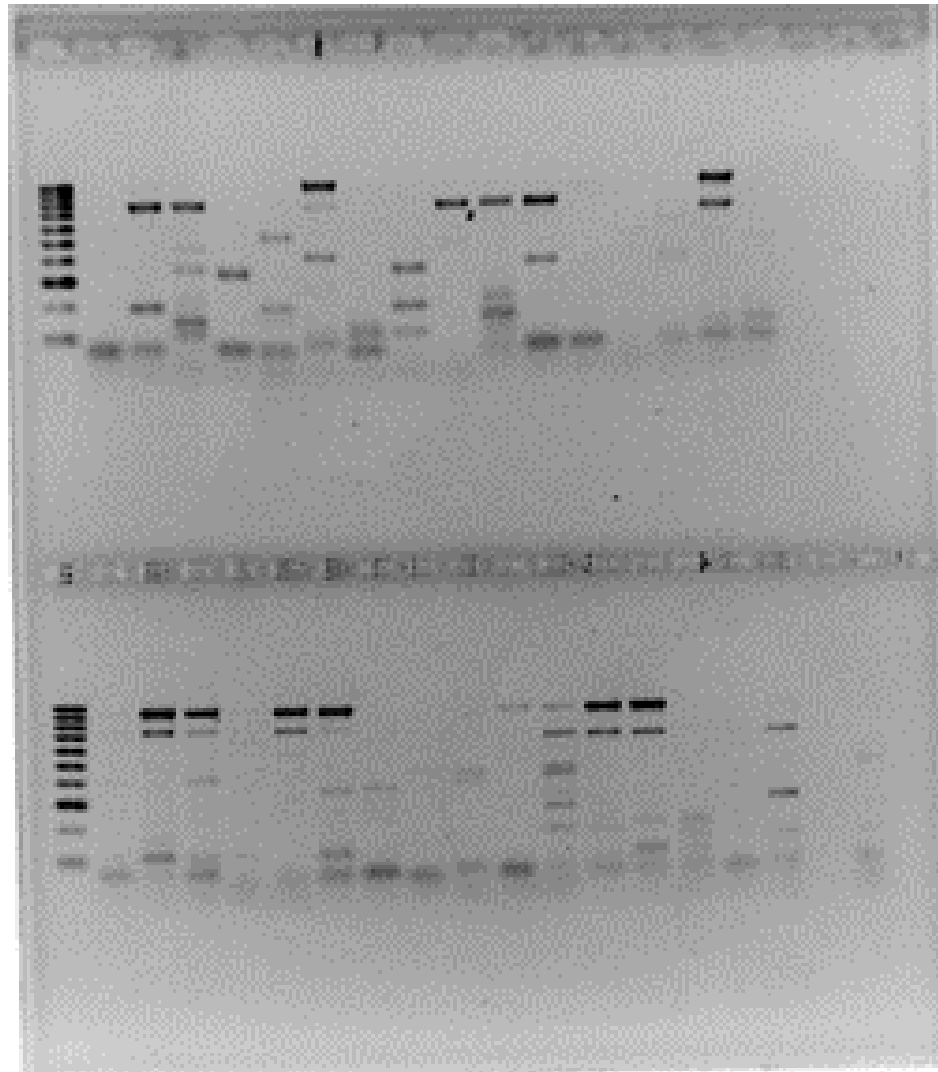
Back to Molecular



Good Gel Results

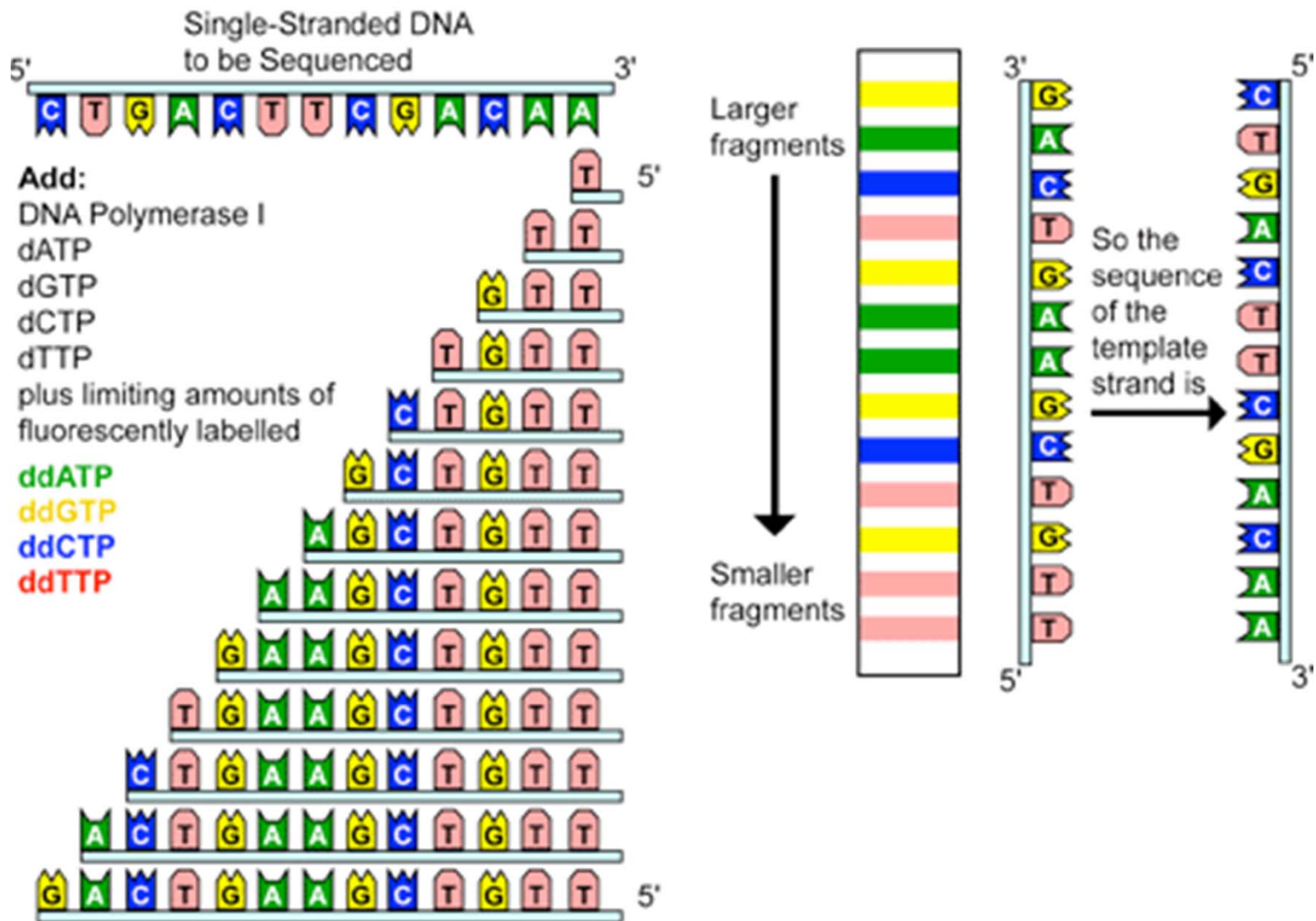


Bad Results



Good results move on to the next
step, but before we get there,
we'll wrap up the morphology
aspect

Sequencing



Results

AACTTTCAAATTCAGAGAAACCCCGGAATTAAGAAAAATGGGCAATCCTGAGCCAAATCCTGTCTTCTCA
AAACAAAGGTTTCAGAAAGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGA
GTTGACTGTGTTGGTAGAGGAATCTTTCCATCAAACTTCAGAAAAGATGAAGGATAAATCTATCTATTG
AATACTATATCAAATTATTAATGATGGCCCGAATCTGTATCTGTATTTTTCTATATTAAAAATGGAAGAA
TTGGTGTGAATTGATTCCACATTGAATAAAGAATGGAATATTCATTCATTAAATCATTCACTCCATAGTC
TGGTAGATCTTTTAAAGAACTGATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATAC
CGGCAACAATGAAATTTATAGTAAGAGGAAAATCCGTGCGACTTTAAAAATCGTGAGGGTTCAAGTCCCTC
TATCCCCAAAAAGGGCCTTTATCCTATCCCTTTTTTAGTGGTTCTCCTTTATCTTTCTCATTCTTTTAGA
AACGTATTGGGGCGTAAATGACTTTATCTTATCACATGTGATATAGAATGCACATACAAATTAAGCAAGG
AATCCCTATGTGAATGATTCACAATCAATAGCATTACTCACACTGAACTTACAAAGACGTCTTTTTTGAA
AATCCAAGAAATTACATACAGGACTTGAGCAAACTTTCCAATTCCCCCCTTGACCTTTAATTGACATA
GACCCCAGTGCTCTCATAAAATGAGCATGGGATGNTAGATTGG

Constructing a molecular tree

- Get sequence data
 - Data from a common chloroplast marker used in plant systematics studies
- Align sequences for similarity
 - Automatic aligners such as muscle or clustal
- Construct phylogeny
 - many options, but we'll do a parsimony analysis

Alignments

C	T	T	A	G	A	T	C	G	T	A	C	C	A	A	-	-	-	A	A	T	A	T	T	A	C
C	T	T	A	G	A	T	C	G	T	A	C	C	A	C	A	-	T	A	C	-	T	T	T	A	C
A	T	T	A	G	A	T	C	G	T	A	C	C	A	C	T	A	T	A	A	G	T	T	T	A	C
C	T	T	A	G	A	T	C	G	T	T	C	C	A	C	-	-	-	A	C	A	T	A	T	A	C
A	T	T	A	G	A	T	C	G	T	A	C	C	A	C	-	-	-	A	T	A	T	A	T	T	C
A	T	T	A	G	A	T	C	G	T	A	C	C	A	C	-	-	-	A	T	A	T	A	T	A	C
C	T	T	A	G	A	T	C	G	T	A	C	C	A	C	-	-	-	A	C	A	A	T	T	A	C
C	T	T	A	G	A	T	C	G	T	A	C	C	-	-	-	-	-	A	C	A	A	A	T	A	C

Parsimony

- Construct tree with the fewest number of steps necessary
 - species with the fewest number of changes will be more closely related to each other

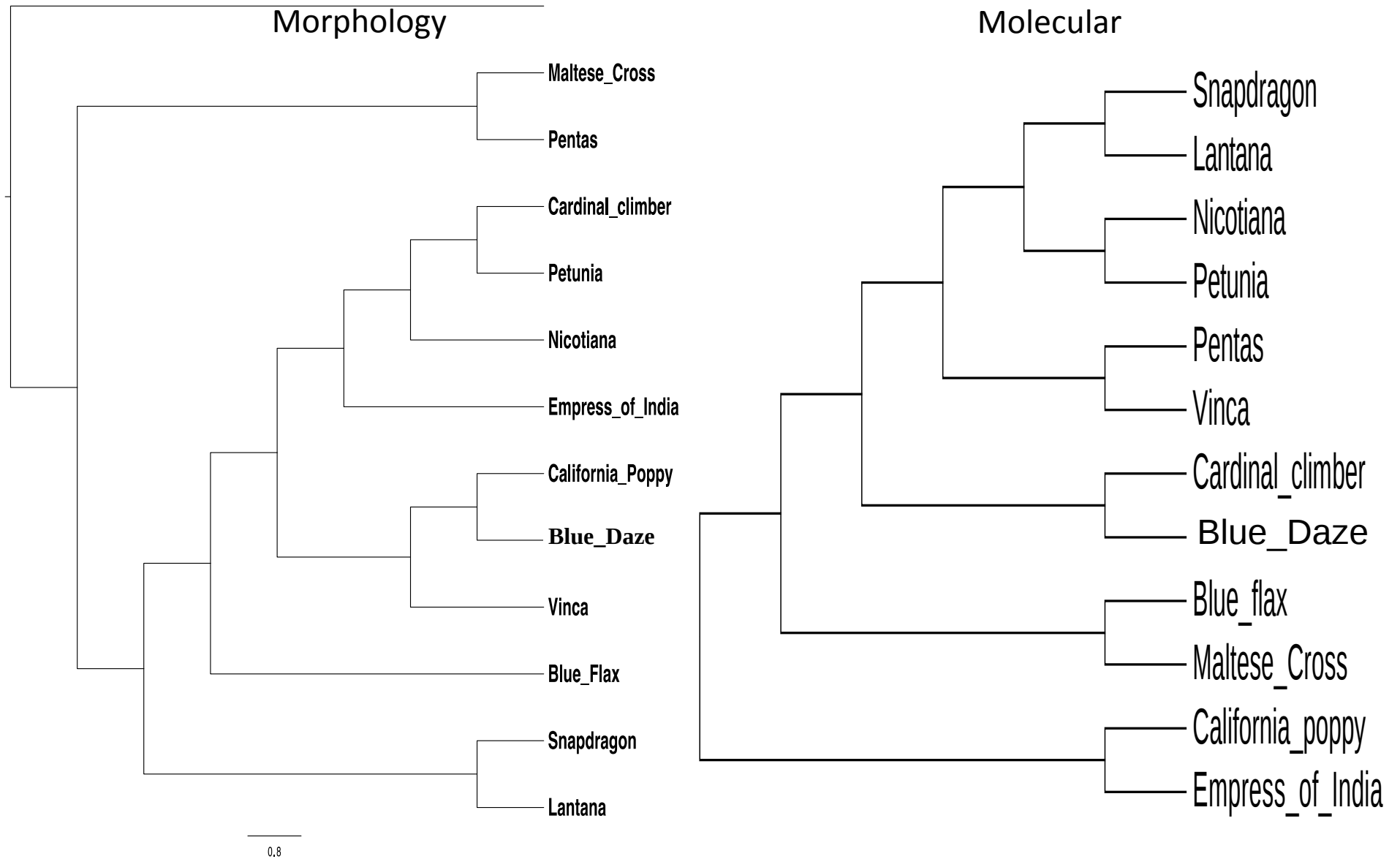
C	T	T	A	G	A	T	C	G	T	A	C	C	A	A	-	-	-	A	A	T	A	T	T	A	C
C	T	T	A	G	A	T	C	G	T	A	C	C	A	C	A	-	T	A	C	-	T	T	T	A	C
A	T	T	A	G	A	T	C	G	T	A	C	C	A	C	T	A	T	A	A	G	T	T	T	A	C
C	T	T	A	G	A	T	C	G	T	T	C	C	A	C	-	-	-	A	C	A	T	A	T	A	C
A	T	T	A	G	A	T	C	G	T	A	C	C	A	C	-	-	-	A	T	A	T	A	T	T	C
A	T	T	A	G	A	T	C	G	T	A	C	C	A	C	-	-	-	A	T	A	T	A	T	A	C
C	T	T	A	G	A	T	C	G	T	A	C	C	A	C	-	-	-	A	C	A	A	T	T	A	C
C	T	T	A	G	A	T	C	G	T	A	C	C	-	-	-	-	-	A	C	A	A	A	T	A	C

Open MEGA5

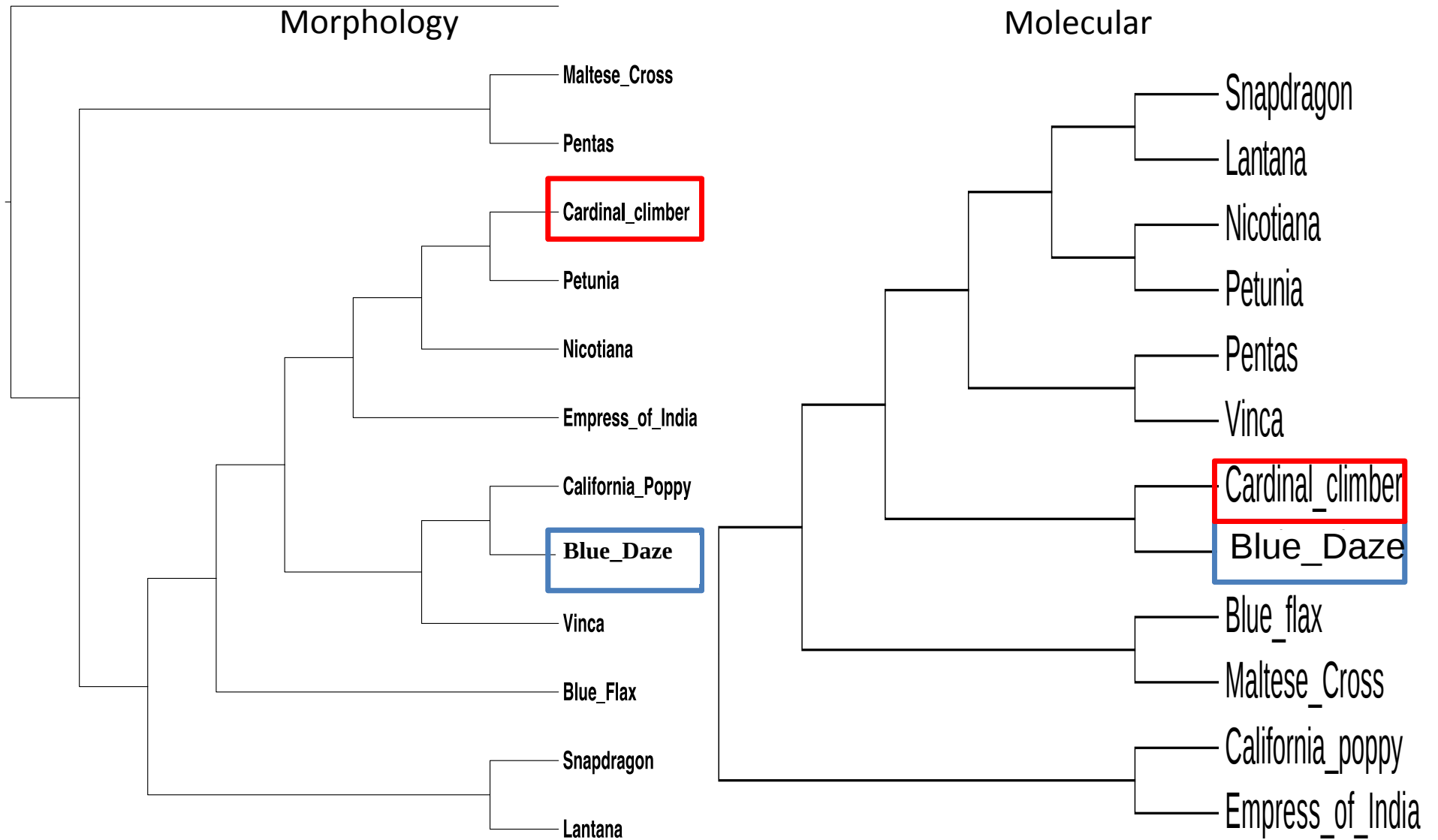
Exploration

- Try different alignment options, Muscle instead of Clustal
- Build tree using Maximum-likelihood, neighbor joining, or other options
- Use data set 2
 - Chloroplast (data 1) versus nuclear (data 2)

Differences between trees



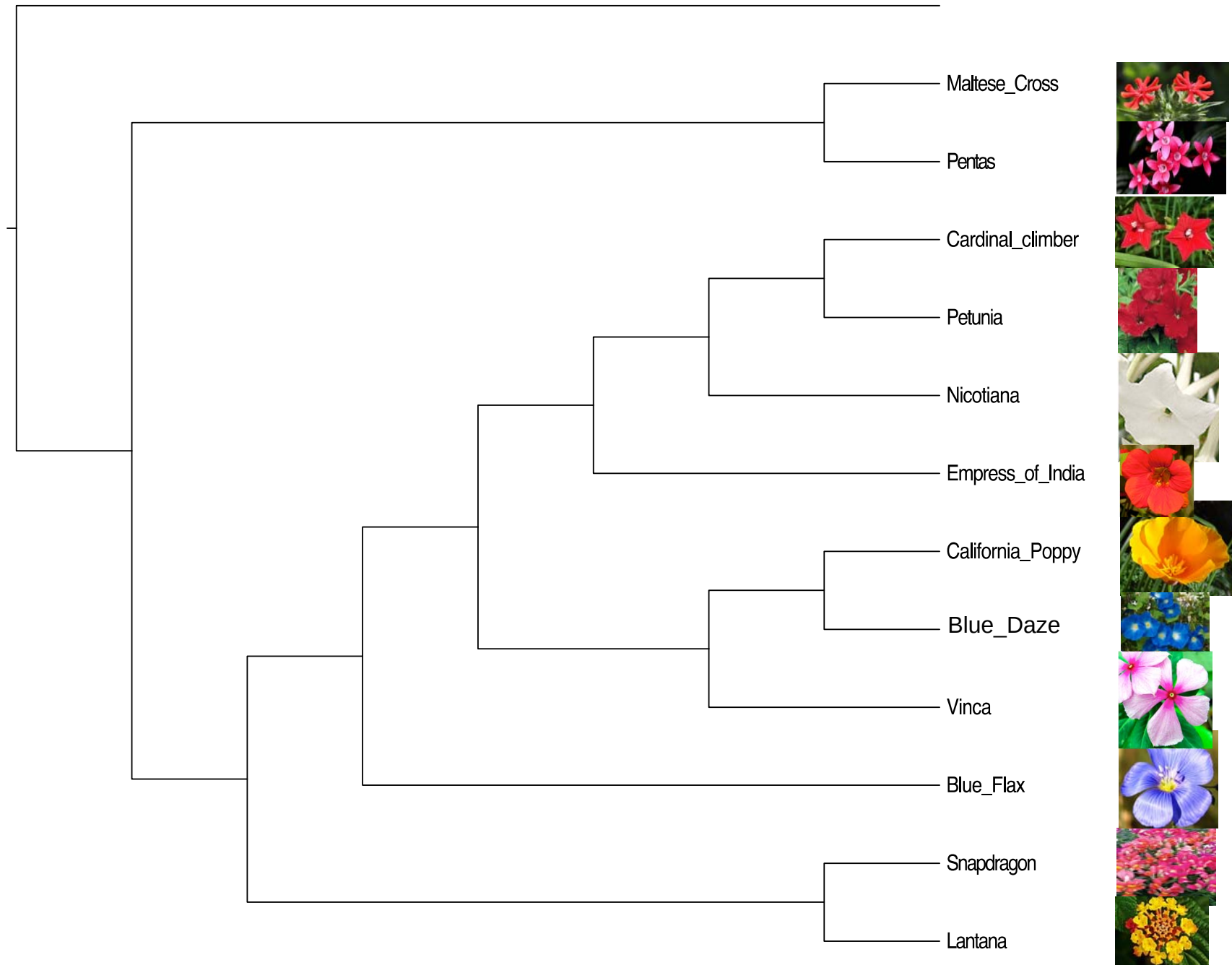
Differences between trees



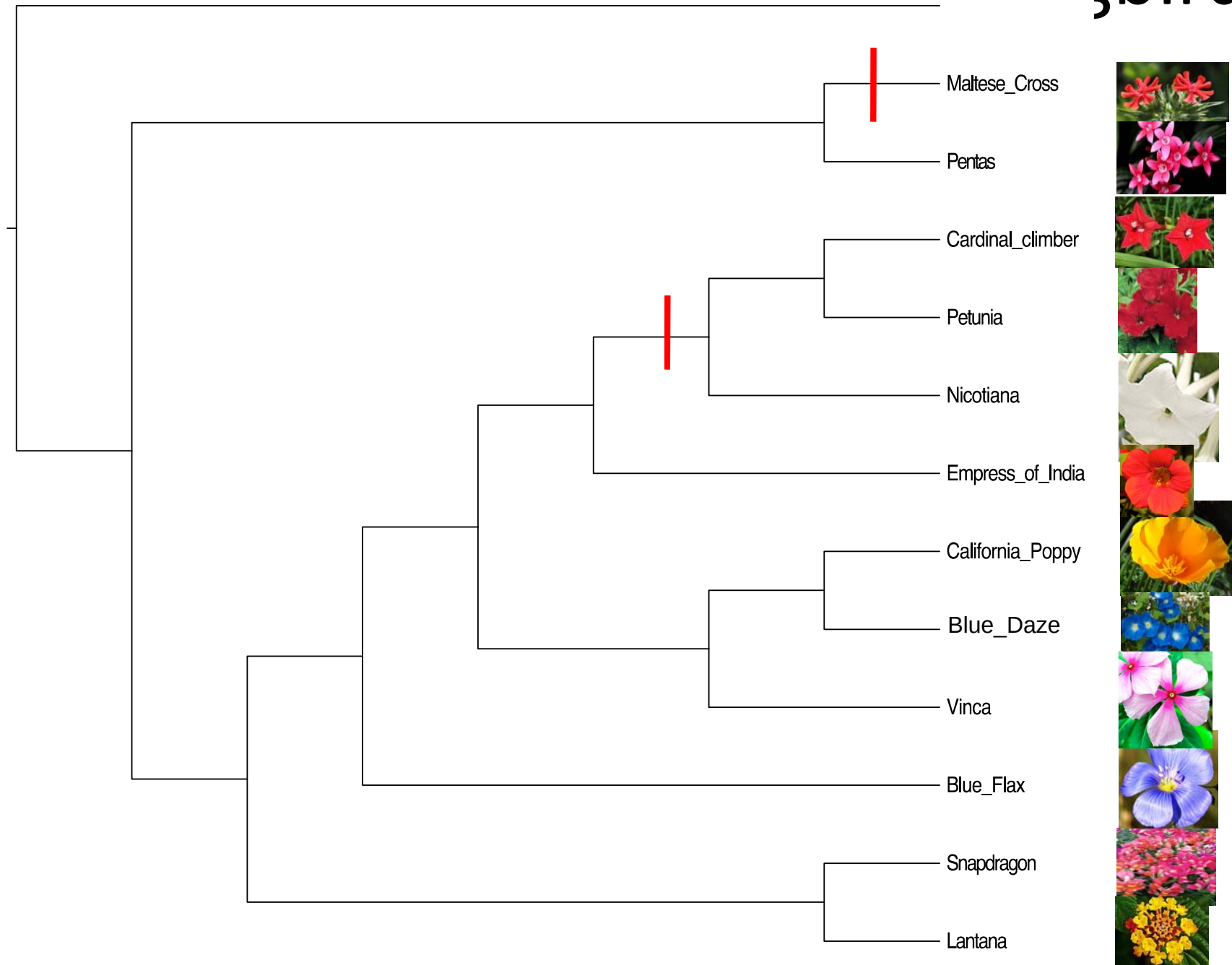
Wrap up

- Do we have a “correct tree”? If so which do you think it would be?
- Which method, morphological or molecular, would you want to use on a data set with 12 species?
 - How about 100 species?
- How many times have the different pollinator types arisen?

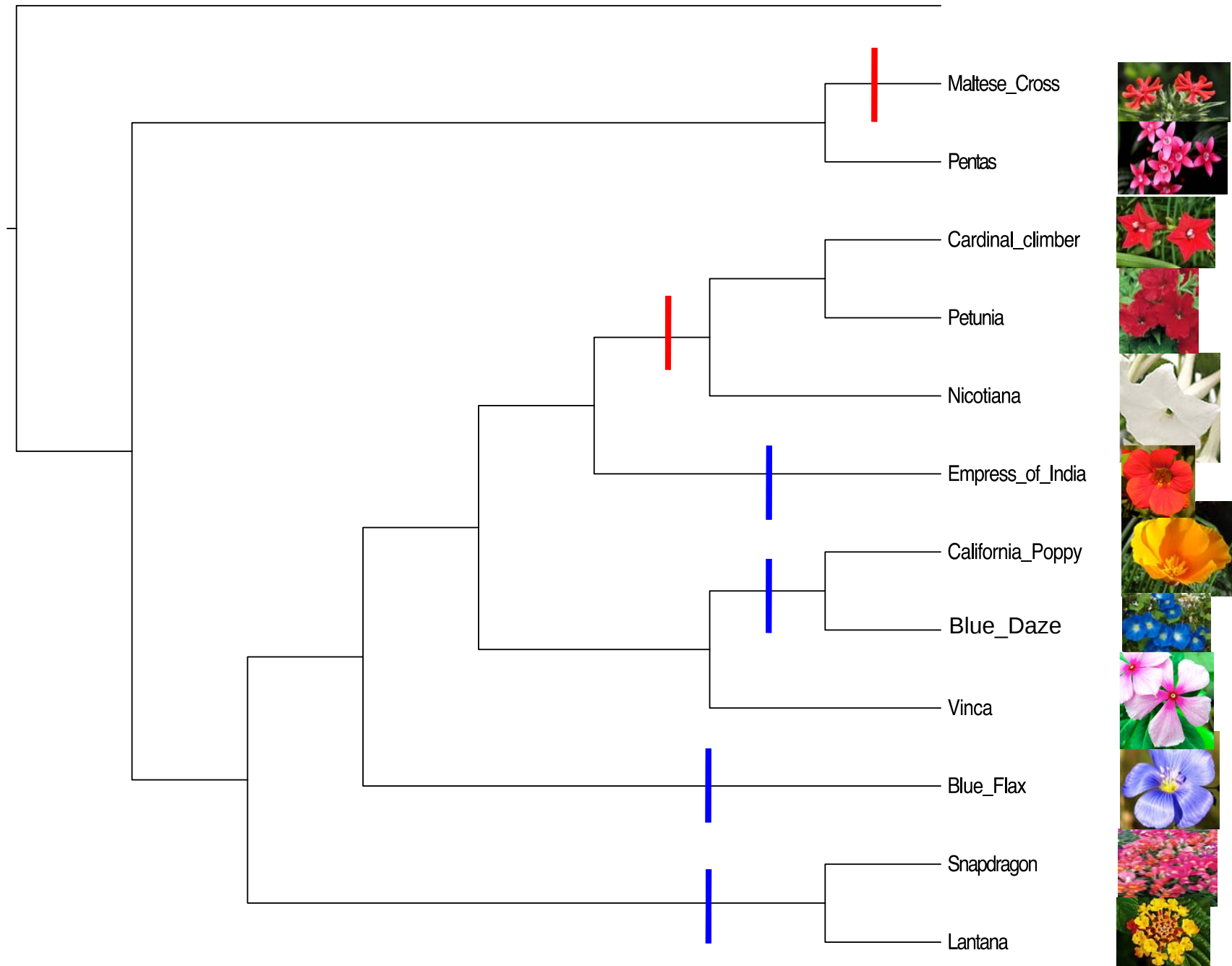
Morphology tree



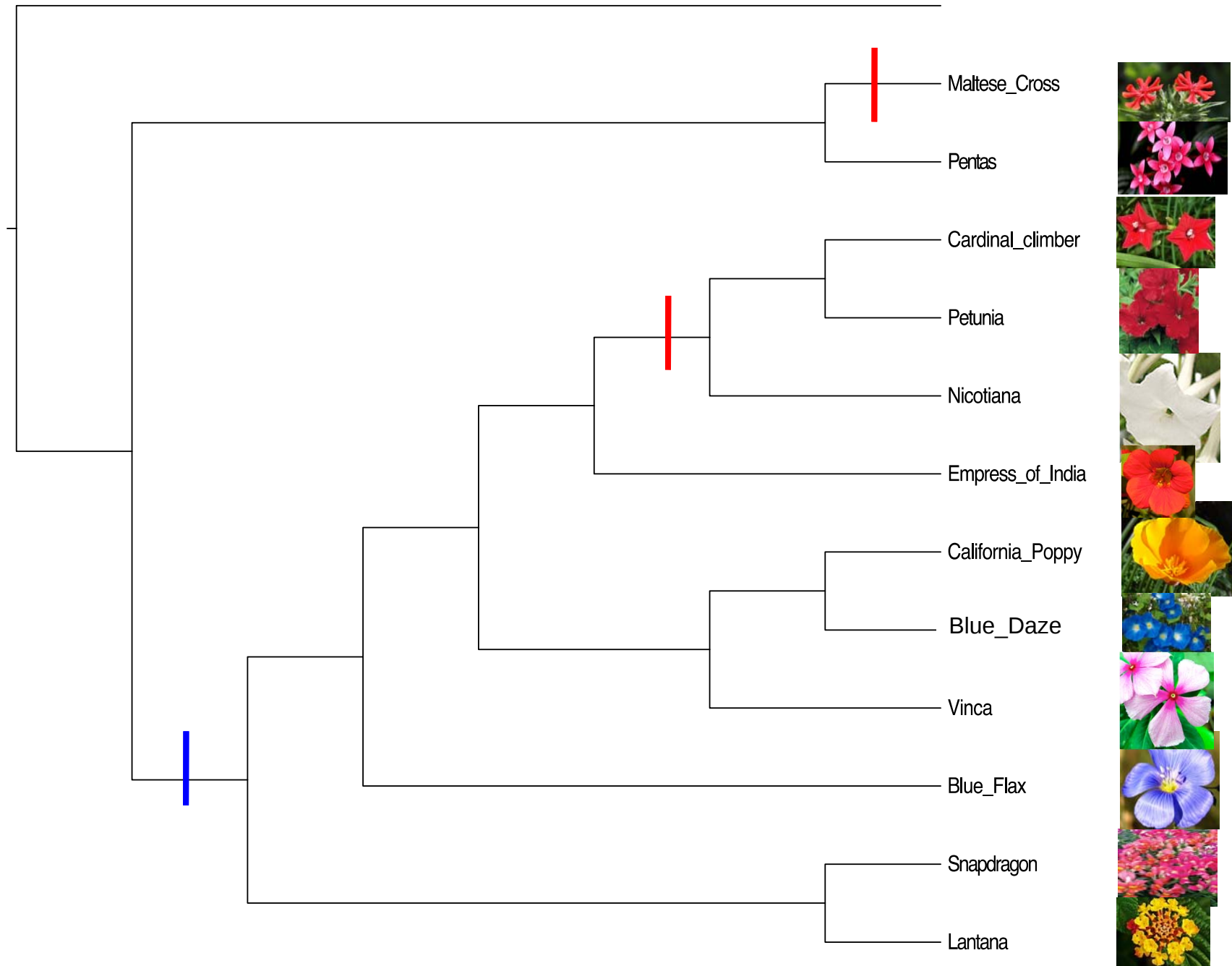
Morphology tree - hummingbird



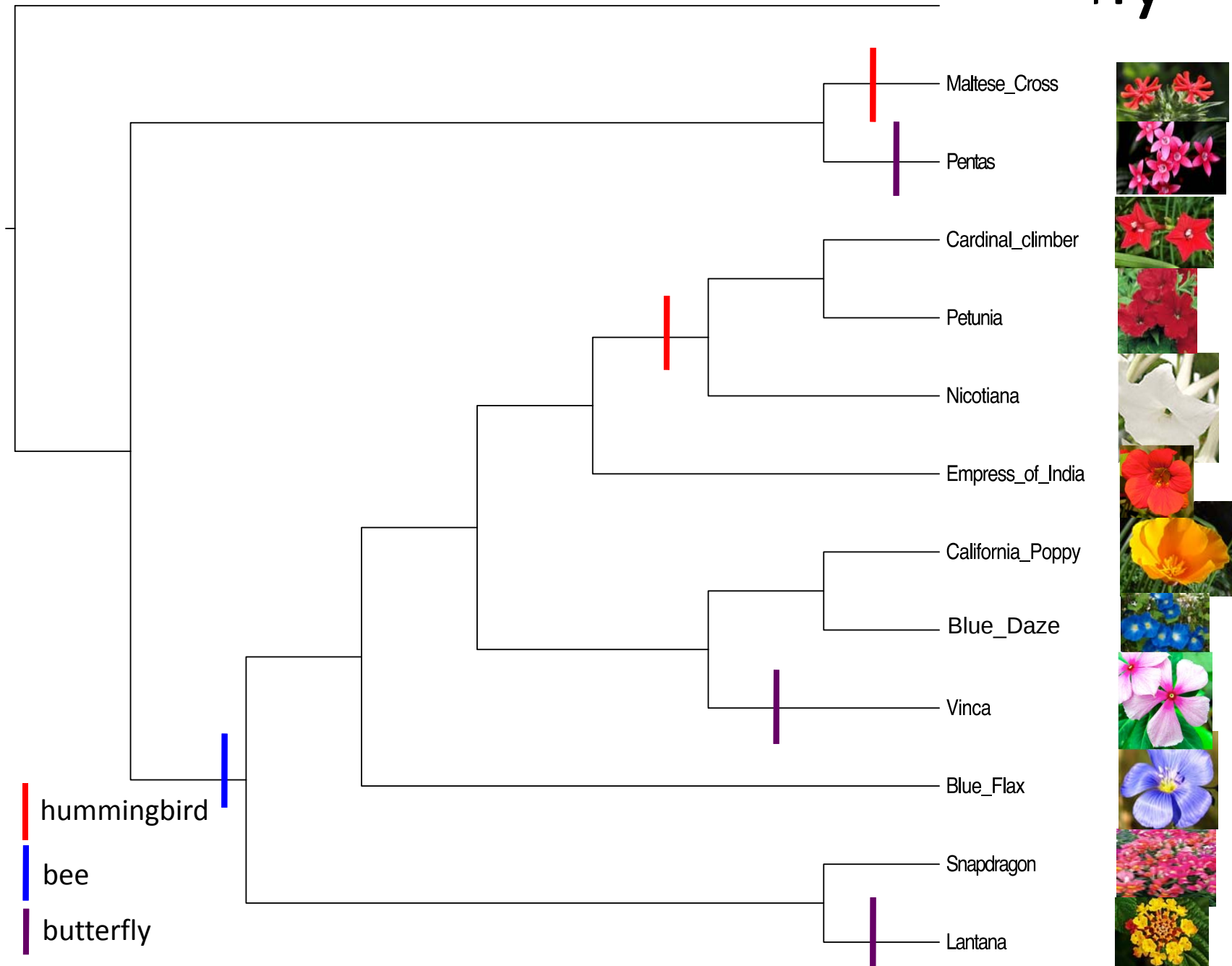
Morphology tree - bee



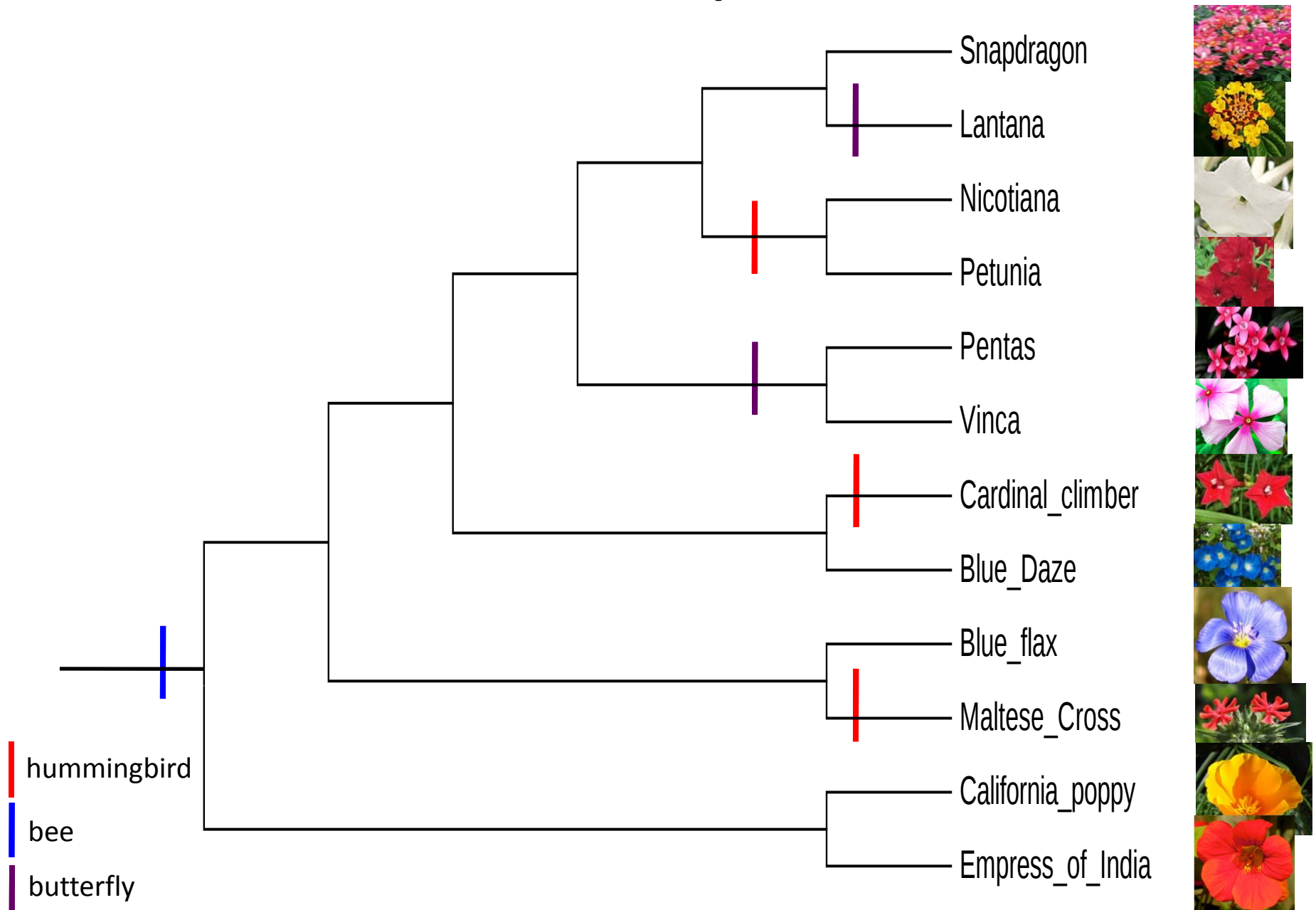
Morphology tree - hee



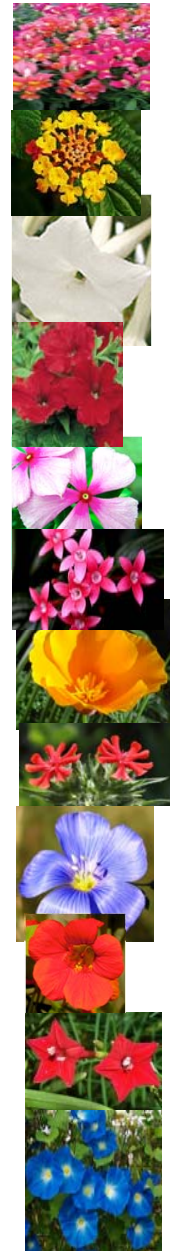
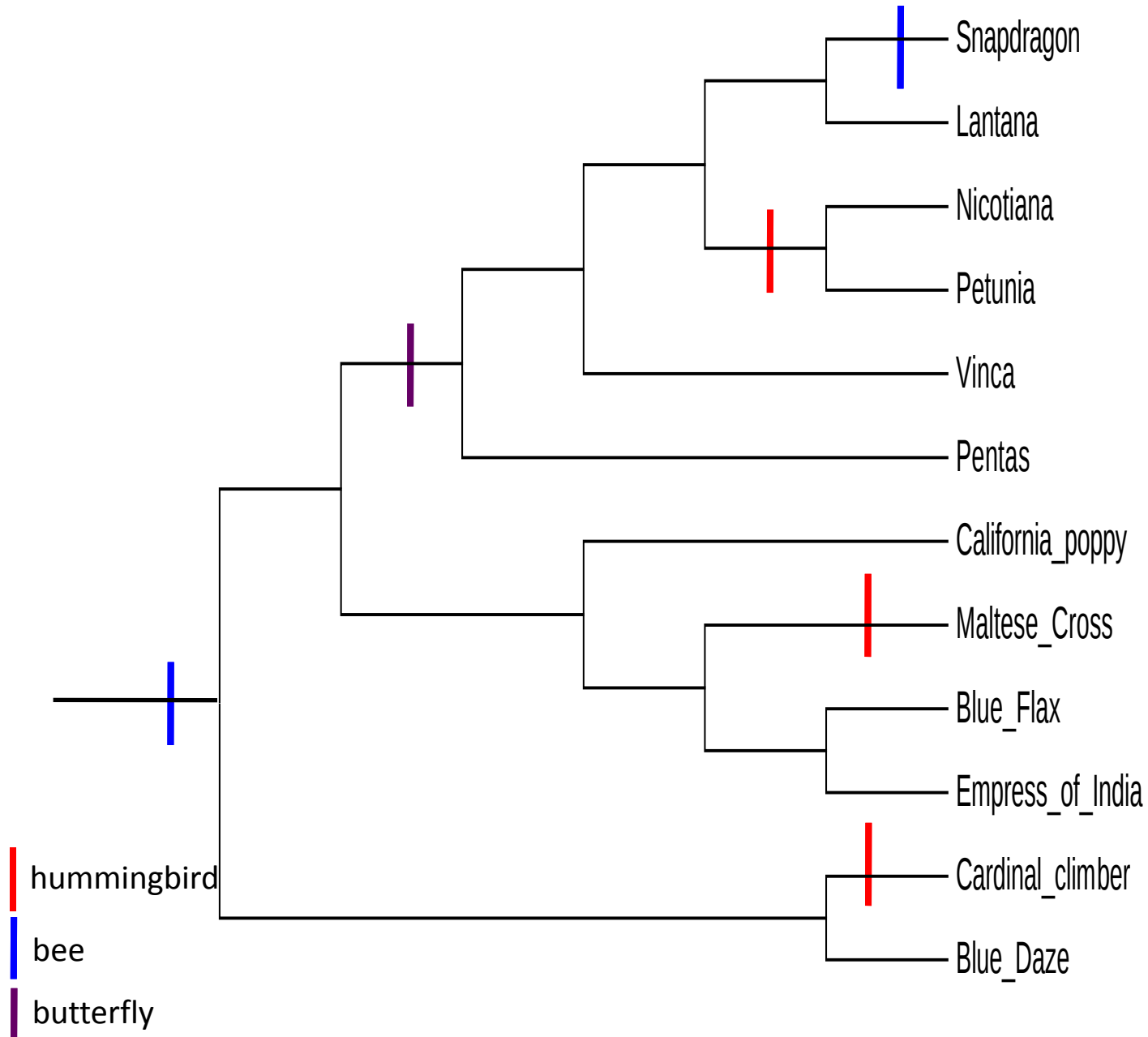
Morphology tree - butterfly



Chloroplast



Nuclear

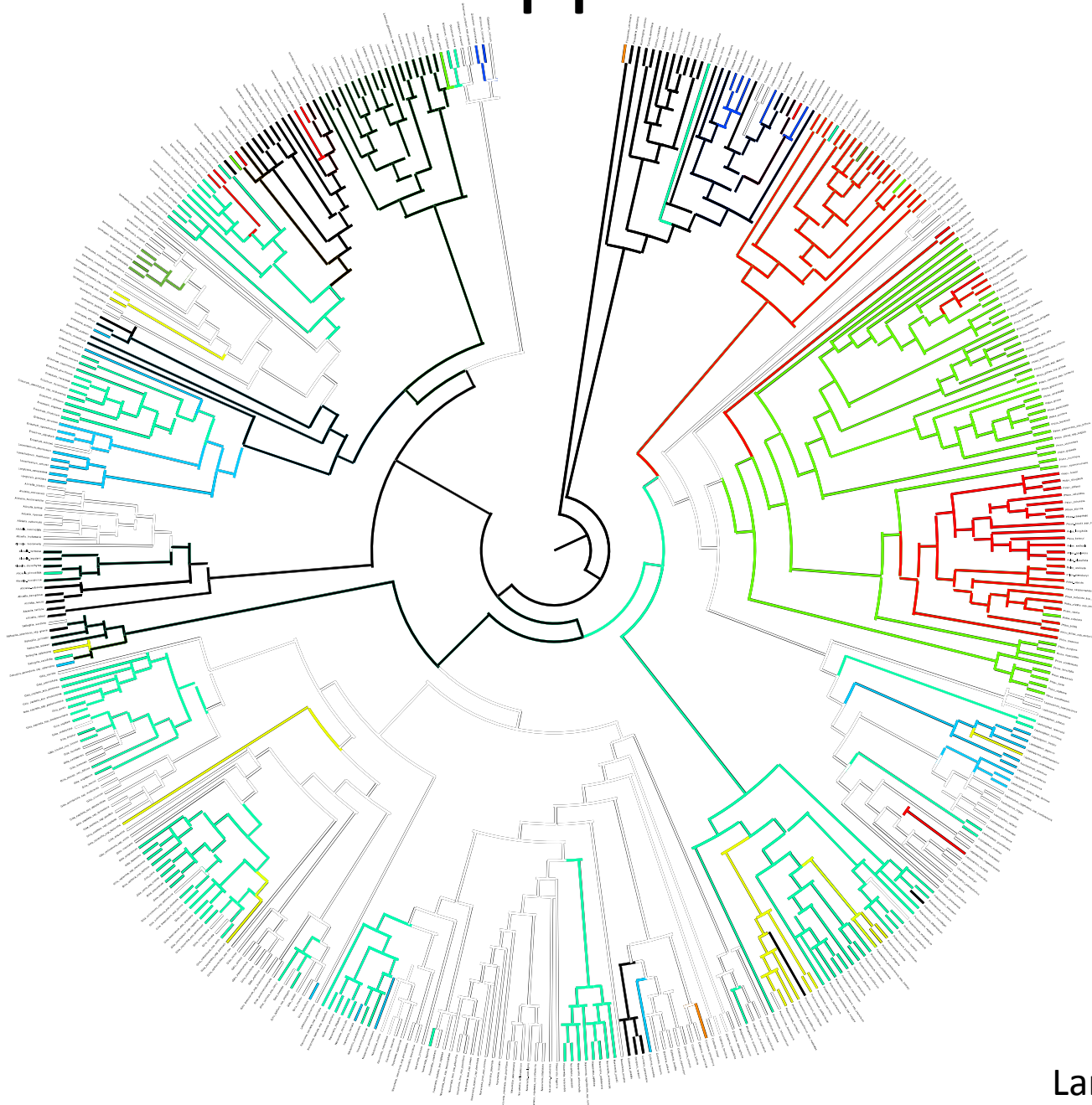


Evolution of Pollinators

- Based on your morphological and molecular trees, how many times have each of the following pollinator syndromes originated?

	Morphological	Chloroplast	Nuclear
Hummingbird	2	3	3
Bee	1	1	2
Butterfly	3	2	1

Actual Application



Landis et al., 2013

Summary

- Number of times pollinators have changed greatly relies on which phylogeny you are using
- Difficulties with using morphological data, especially when multiple people are collecting data

Any Questions

