EvoBeaker™

Software of the Fittest

Student Workbook for

Tree Thinking

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Credits

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Tree Thinking

Experiments on descent with modification
Tree Thinking

Columbines are a diverse group of wildflowers that live in the mountains of North America, Europe, and Asia. Among their most striking traits are nectar spurs. Nectar spurs are tube-shaped petals that offer a sweet prize at the bottom for the hummingbirds, hawkmoths, and bees that pollinate the flowers.

Another striking thing about columbines is that there are so many different kinds. Some seventy species have been described and given Latin names. The species vary in color, spur length and shape, and flower orientation. Scott Hodges suspected that there is a connection between the nectar spurs of columbines and the number and diversity of species.

Because nectar spurs offer an enticing reward that is nonetheless hard to get, small changes in floral traits can have big effects on pollination success. The graphs at right summarize research by Hodges, in collaboration with Michelle Fulton, on the Sierra columbine (Latin name *Aquilegia pubescens*). This species is pollinated by hawkmoths. It has white or yellow upward-facing flowers with long thin nectar spurs. When Fulton and Hodges tipped Sierra columbine flowers downward, hawkmoths visited the downward-facing flowers only 10% as frequently as upward-facing flowers (graph a). When the researchers shortened the nectar spurs of flowers, by tying them off with thread, the short-spurred flowers were just as attractive to hawkmoths as long-spurred flowers (graph b). But because the moths could sip the nectar without getting as close to the flower, the moths left more of each flower’s pollen behind (graph c).

When small changes in floral traits have big effects on pollination success, populations of plants may rapidly evolve flowers that allow them to specialize on particular species of pollinators. Flowers that are especially attractive to a particular pollinator, but unattractive to others, may get more of their pollen delivered to compatible recipients, and may receive more pollen from compatible donors. They may also diverge dramatically from populations that specialize on different pollinators. Columbines pollinated by hawkmoths tend to resemble the Sierra columbine: They have light coloration, upward-facing flowers, and long spurs. Columbines pollinated by hummingbirds, in contrast, have bright red and yellow downward-facing flowers with short nectar spurs.

To test his hypothesis that the evolution of nectar spurs led to a rapid diversification of columbine species, Hodges needed a tool that would enable him to summarize and collectively analyze the evolutionary history of the columbines and their closest relatives. That tool is the evolutionary tree.

Evolutionary trees have many applications, some of which we explore in other EvoBeaker labs. Evolutionary trees have been used to test the hypothesis that birds evolved from dinosaurs, to determine where the HIV virus came from, and even to prosecute a Louisiana doctor for attempted murder.

This lab will help you learn to read and use evolutionary trees by experimenting with model columbine populations. After you have honed your skills, we will return to Hodges’ analysis of columbines and see whether his hypothesis is correct.
Exercise 1: Columbine Flower Traits

1. Run EvoBeaker.

2. **Under the Labs menu select the Columbine>Designer Flowers experiment.**

The model columbine populations you will experiment with live on a series of peaks in the Rocky Mountains in western North America. These peaks are shown as squares on the left side of the EvoBeaker window. Because our model columbines thrive only at high elevations, the mountain peaks are effectively islands of good habitat floating in an uninhabitable sea of poor habitat.

Columbine seeds typically do not travel far. Instead, they drop to the ground near their parents. The mountain peaks in our model are far apart, so columbine seeds rarely move from one peak to another. A seed can make such a trip only if it gets picked up by an extraordinarily strong wind, or if it gets stuck to the hoof of an elk or the boot of a hiker.

3. There are 8 flower traits that you will look for in the model columbines. To see the traits, **double-click on one of the tiny flowers, such as the one on Peak 1.** You will see a Trait Editor window appear. This window contains an enlarged view of the flower, plus the 8 variable traits listed in pull-down menus.

4. Take the first trait listed, Anthers. Anthers are the male parts of the flower. That is, they are the structures that make pollen. The anthers sit atop hair-like filaments that poke up from the middle of the flower. The columbine you found on Peak 1 has white anthers. To mutate the flower so it has red anthers, **select Red from the pull-down menu next to the word Anthers.**

5. **Go to each of the other traits and mutate them back and forth between their two states.** As you do so, examine the changes in the enlarged cartoon flowers until you are familiar with what each of the traits looks like.

Exercise 2: Watching Evolutionary Trees Grow

1. Imagine that only one of our seven peaks is inhabited by columbines, and that as this population evolves over time seeds occasionally make the long trip from one peak to another to establish a new population. If you could watch this happen over hundreds of generations, what would you see? EvoBeaker can show you, and can summarize the events you would witness in a diagram called an evolutionary tree (also known as a phylogenetic tree or a phylogeny). To see this, **go to the Labs menu and select Columbine>Growing Tree.**

2. The ancestors of the columbines in our mountain range blew in as seeds several years ago and landed on one of the peaks. You can see the population of flowers now living there. **Click on the GO in the Control Panel at the bottom left of the window to let time advance until the original population splits into two populations.** Then **stop the model by clicking on the STOP button** (the one with the red stop sign icon in the Control Panel).

*Population: A population is a group of individuals that live in the same place at the same time. Because travel among peaks is difficult, the columbines on any one of our peaks interbreed with each other, but not with...*
individuals from other peaks. This isolation means that each of our seven populations can evolve independently of the others.

3. Each year as the model runs, all the old flowers set seed and then die. The following spring, the seeds sprout, grow up, and flower. Normally seeds stay on the mountain peak of their parents, but once in a long while, a fierce storm comes through and carries a seed from one peak to another, establishing a new population, which you just watched happen. Look at the evolutionary tree in the Notebook panel. **Describe how the division of one population into two is represented in the tree diagram.**

4. Every so often, a mutation happens in an individual flower in one of the populations. The program indicates this by changing the color of the tiny icon that represents the individual flower. The model is rigged so that new mutations quickly spread through the population in which they arise. **Continue running the model with the GO button** (in the Control Panel) until you see the color of the flowers on one of the peaks change. When the change has spread through all flowers on that peak, **STOP the model.**

5. In addition to the change in color of the little flowers in the Peaks, **describe how the change in a trait is represented on the evolutionary tree diagram.** (Hint: it is shown in two places on the tree diagram – look both the tree itself and at the pictures of the flowers at the branch tips.)

*Traits and Mutations: The model in this lab is rigged to make all changes be advantageous, and therefore quickly spread throughout the populations in which they arise. The model is further rigged so that any given trait changes only once, and so that once changed a trait never reverts to its original state. This is much simpler than real life. In real life, most mutations are disadvantageous, and thus usually disappear before they become fixed in the population. In real life, new traits can arise independently in different populations, and traits can appear and disappear over time. These simplifications we have made are intended to help you see the main points of this lab.*

6. As the changes were happening in Step 4, time was moving forward. **Aside from the time scale on the left, how is the movement of time represented in the evolutionary tree?** (Start the model running again if you want to remind yourself.)
7. Each time a seed blows from one peak to another, we say that the tree diagram *bifurcates*, or splits. The tips of the two resulting branches are the two new populations, drawn at the top of the tree to show that they are currently alive. The base of the two branches come together to show that both new populations came from the same parent population. This parent is the *most recent common ancestor* of the two new populations. **Continue running the model until populations have become established on a couple of other peaks, and then stop the model**

8. **Run the model until 800 years have gone by.** Be patient—evolution takes time. **However, if you have a slow computer, you can stop after 2 or 3 minutes.** Watch the action both on the mountain peaks and on the evolutionary tree. At the end, look at the pictures of the flowers at the tips of the tree branches. Pick a flower picture at the tip of the tree diagram (representing one of the living mountain peak populations). Follow its branch all the way to the base of the tree. **Does the flower picture at the tip reflect all the trait changes that occurred among its ancestors?**

Exercise 3: Building and Reading Trees

1. In the Labs menu, **select the Columbines>Simple Evolving Flowers experiment.**

2. The setup here is the same as last time, except that there are only 4 mountain peaks and 5 traits for each flower. The Notebook on the right will still show the evolutionary tree as it grows, but now no changes will occur unless you make them happen.

3. **Start the model running by clicking on the GO button** in the Control Panel (the arrow icon). Let time advance for 20 - 40 years so there is a little trunk at the bottom of the tree. Then **stop the model from running by clicking on the STOP button** in the Control Panel (the stop sign icon in the lower left of the screen).

4. In Exercise 2, you watched as mutations appeared on their own in the columbine populations. In this experiment, you will play mutator, changing the traits of flowers at your whim. Start by changing the anthers of one of your flowers from white to red. To do that, **double-click on one of the flowers in the Peak 1 population.** A Trait Editor window will appear.

5. **Change this flower to have anthers that are red** by selecting Red from the Anthers pull-down menu. **Don’t change any other traits right now.**

6. **Close the Trait Editor window** by clicking on the close icon in the window’s title bar.

7. **Run the model again for 20 – 40 years by clicking on the GO button.** Look at the evolutionary tree. Notice that, as in the last experiment, there is a label showing when the new trait appeared. Note also that the picture at the top shows the current living population with the new trait.
8. Your evolutionary tree should now look like the one shown at right (without the arrows). The evolutionary tree traces the 80 or so previous generations that are ancestors of your current Peak 1 population. The more recent ancestors are indicated by the dark arrow. **What color were their anthers?**

The earlier ancestors are indicated by the white arrow. **What color were their anthers?**

9. There are no storms modeled in this experiment. Instead, you will establish new flower populations on the other peaks yourself, by traveling with seeds stuck to your hiking boots. Before doing this, **draw a diagram of what you think the evolutionary tree will look like when you carry a seed from Peak 1 to establish a new population on Peak 2.** You don’t have to draw pictures of the flowers at the tips, just use the names of the populations.

10. Now go ahead and **carry a seed from Peak 1 to Peak 2 by clicking on a flower in Peak 1, holding your mouse button down, and dragging the flower to Peak 2.**

11. **Start the model running** again and run it for 20 – 40 years.

12. **Stop the model** and look at the tree. **Was your prediction in Question 9 correct?**
What is your evolutionary tree showing?
At right is a picture of the tree that you now have, with some of its parts labeled. Each point along a branch represents a population that lived at a particular point in time. The small black squares mark particularly significant populations. The root is the population you started with. The node marks the population that split to produce two daughter populations. The tips represent the populations that are currently living on Peak 1 and Peak 2.

13. Think about your own family for a moment. Imagine that you have a sister (right). You and your sister are considered closely related. Why?

14. In your answer to Question 13, you probably said something to the effect that you and your sister are considered kin because the two of you share ancestors—namely, your mother and father. You and your sister share lots of other ancestors too, of course. These include your father’s parents, their parents, and so on. Evolutionary biologists refer to the ancestors two individuals share as common ancestors. Note that common, in this context, means “held in common” or “shared.” It does not mean “ordinary.” Who are you and your sister’s most recent common ancestors?

Evolutionary biologists think about the relationships among populations and species in a way that is connected to how we think about our relationships with our kin. Two populations (or species) are related to each other if the individuals living in one of the populations are kin to the individuals living in the other. Likewise, a past population is an ancestor of a present population if individuals that lived in the past population were ancestors of the individuals living in the present population.

15. In the diagram at right, draw an arrow pointing to the most recent common ancestor of the populations currently living at Peak 1 and Peak 2. Label the arrow “most recent common ancestor.”
16. Over time, new traits could arise in the population on either mountain peak that make the flowers on the two peaks look different. **Draw what the tree diagram will look like if the flowers on Peak 1 mutate to have pointy petal tips.**

17. **Change the petal tips of a flower on Peak 1 to “pointy”** (using the Trait Editor).

18. Close the Trait Editor and run the model for another 20 – 40 years.

19. Compare the evolutionary tree on the screen to the one you drew in Question 16 above. **Was your prediction correct?**

20. **Draw what the tree will look like if you transfer a seed from Peak 1 to Peak 3:**

21. **Now click and drag one of the flowers to transplant it from Peak 1 to Peak 3.**

22. Run the model for 20 - 40 years.

23. **Was your prediction in Question 20 correct?**
24. Think about your own family again. Imagine that in addition to a sister you have a cousin (right.) You are related to both your sister and your cousin. To which are you more closely related? Why?

25. Although you might not have used this language, one way to answer Question 24 is to think about your most recent common ancestors with your sister versus your cousin. Who are your most recent common ancestors with your cousin?

26. Who lived more recently (came later), your most recent common ancestors with your sister, or your most recent common ancestors with your cousin?

27. You are more closely related to your sister than to your cousin because your most recent common ancestors with your sister (your mom and dad) lived more recently than your most recent common ancestors with your cousin (your grandparents). We can, again, use similar reasoning in thinking about the evolutionary relationships among populations and species. In the diagram at right, which arrow points to the most recent common ancestor of the populations currently living on Peak 1 and Peak 3?

28. Which arrow points to the most recent common ancestor of the populations currently living at Peak 1 and Peak 2?

29. Which lived most recently, the most recent common ancestor of the Peak 1 and Peak 3 populations, or the most recent common ancestor of the Peak 1 and Peak 2 populations?

30. Which is more closely related to the current Peak 1 population—the population currently living at Peak 2, or the population currently living at Peak 3?
31. Over the next few years, the following events happen, with 20 – 40 years in between each event:
   - The population on Peak 3 acquires dark petals
   - 20 – 40 years go by.
   - A hiker carries a seed from Peak 1 to Peak 4
   - 20 – 40 years go by.
   - The population on Peak 4 acquires long spur bottoms
   - 20 – 40 years go by.

   Make those changes happen, being sure to run the model for 20 – 40 years between each one.

   Here what the evolutionary tree on your screen should look like now.

32. On the diagram above:
   - Label the most recent common ancestor of the populations on Peak 1 and Peak 3 with the letter B.
   - Label the most recent common ancestor of the populations on Peak 1 and Peak 2 with the letter C.
   - Label the most recent common ancestor of the populations on Peak 3 and Peak 2 with the letter D.

33. Now look at the time scale on the screen. Measure approximately the amounts of time from:

   Peak 1 population to B:

   Peak 1 population to C:

   Peak 3 population to B:

   Peak 3 population to D:
34. Have more years passed since the Peak 3 population split from Peak 1 at B, or since the Peak 3 population split from the Peak 2 population at D?

35. Have more years passed since the Peak 1 population split from Peak 3 at B, or since the Peak 1 population split from Peak 2 at C?

36. Is the population at B an ancestor of C or is the population at C an ancestor of B?

37. Given how much time has passed from each ancestral population to the current populations on the mountain peaks, write down the order of relationships (who is most closely related to whom) among the four currently living populations, and explain why they have that relationship based on the amount of time that has passed from each common ancestor.

When thinking about who is more related to whom, the number of differences in traits between the populations does NOT matter. What counts is how much time (height on the tree diagram) has passed since they shared a common ancestor. Species whose common ancestors were farther in the past are less closely related than species whose ancestors were more recent.
Exercise 4: Flipping Out

1. Right now, the populations are drawn left to right as Peak 1, Peak 4, Peak 3, and Peak 2. Now consider what would happen if you were to flip the Peak 1 / Peak 4 branch of the tree with the Peak 3 branch. The question is, would this make any difference to any of the information given on the tree? To answer this question, drag the Peak 3 picture on the tree to the left, so it switches places with the Peak 1/ Peak 4 branch. Then drag it back to its original position.

2. As you flip the tree back and forth, does the most recent common ancestor between Peak 1 and Peak 3 change?

3. As you flip the tree back and forth, does the branch of the tree on which dark petals arose change? How about the time at which dark petal arose?

4. Does the time when the ancestor of the populations on Peak 1 and Peak 3 split into those separate lineages change?

5. According to the tree diagram, does moving Peak 3 to the left make it older than the Peak 1 population, or do they stay the same age?

6. Did any of the information shown in the evolutionary tree change when you flipped branches around?

7. Based on your answers in 1 through 6, what, if anything, does the left to right order of the populations in the tree tell you?
Exercise 5: Tree Building Challenge

Examine the tree at right. Consider the following questions regarding this tree.

1. Does the tree tell us which peak was occupied by columbines at the start? If so, which peak was it?

2. Does the tree tell us the order in which the remaining peaks were colonized? If so, what is the order?

3. Does the tree tell us which pair of peaks were involved in the most recent colonization event? If so, which peaks were they?

4. In the Labs menu, select the Columbines>Tree Building Challenge experiment.

5. You will see a flower population on one of the peaks. Click on the Reset button (the looping purple arrow in the Control Panel) until the starting population appears on Peak 2, Peak 3, or Peak 4 (NOT PEAK 1).

6. Try to build a tree starting from this population that looks exactly like the tree shown above. Record the steps required below.

7. On the tree you have made, Peak 1 seems to branch off from the trunk before any of the others. Based on what you did above, does that mean that Peak 1 is more closely related to the original ancestor than the others? Why or why not?
8. How much time has passed between the original ancestor and the current populations on...
   ...Peak 1?
   ...Peak 2?
   ...Peak 3?
   ...Peak 4?

Does this change your answer to Question 7?

9. Think back to the tree you just built. Is the current Peak 1 population shown at the tip of the tree the ancestor of the other three populations? Why or why not?

10. Look back at your answers to Questions 1 - 3 above. Would you change any of them now? Why or why not? (Hint: If your answers weren’t NO, NO, and YES, you should change them.)

As you saw here, no currently living population is an ancestor of another currently living population. They are all equal to each other, just like your cousin is not your ancestor, even if he looks exactly like your grandfather.

In this example, none of the flower traits changed as the populations split, so there are no trait changes marked on any of the branches. But this doesn’t mean that nothing has changed. There may be changes in other parts of these plants, such as the stalk, roots, seeds, and even changes in the genetic sequence that are not shown on this tree, because this tree is only about the flowers.
Exercise 6: Reconstructing Trees

In all of the simulations we have run so far, we have known the true evolutionary history of all our populations. This is because the computer has drawn an evolutionary tree for us as our populations have evolved. In the real world, we virtually never know the true evolutionary history for a set of populations, because we weren’t there to watch the populations evolve. Instead of having the evolutionary tree drawn for us, we have to reconstruct it based on available evidence.

The available evidence is found in the traits of the currently living populations. When populations or species arise by descent with modification from common ancestors, they show a special pattern of similarities and differences. This pattern is often enough to allow us to develop a reasonable hypothesis about their evolutionary history.

1. In the Labs>Columbines menu, select the Growing Tree experiment.

2. Run the model until there are 4 mountain peaks with populations of flowers and at least three novel traits, then click the Stop button.

3. To reconstruct a tree by looking at the traits of currently living populations, you need to look for shared evolutionary innovations. These are novel characters, also known as derived traits that were absent in the earliest common ancestor but are present some of the extant populations. To see how this works, first Click on the Paintbrush tool at the lower right of the Notebook.

4. Select a color from the Color pull-down menu to the right of the Paintbrush tool.

5. Now pick one of the novel traits you see on the tree (for instance red anthers, pointy petal tips, dark petals, yellow spurt tops, etc.). Use your mouse to draw a circle around the living flower populations on the tree that have that trait. (If you make a mistake, you can delete a line as follows: Click on the Select tool (the arrow); Click on the line you want to delete to highlight it; Click on the Trash can button; Finally click again on the Paintbrush to continue drawing lines).

6. Pick a different color from the Color menu and draw another circle around the populations with another of the novel traits.

7. Keep circling populations with shared derived traits until you have a circle for each trait.

8. Note that in each case, you have been circling populations that share evolutionary innovations. The beginning population did not have red anthers, pointy tips, or the rest. The populations that share these evolutionary innovations inherited their shared traits from their common ancestors.

9. Note that your circles do not overlap willy-nilly (haphazardly). Instead, they form nested sets of circles (right). Why is this? (Use the words “common ancestor” in your answer.)
10. Now go to the Labs>Columbines menu and select the Flower Challenge 1 experiment.

11. When you run this model, the program itself will move flowers from island to island and change their traits, without you doing anything. However, the flower populations will be hidden for the first 400 years. You will only be able to see them once all the movement and trait changes have happened. **Start the model running and wait 401 years until the flowers appear on the islands. Then Stop the model.**

12. Your task is to reconstruct a tree representing the evolutionary history of the flower populations on the four peaks. To start, put flower samples into your notebook by **clicking on a flower from each mountain peak, holding down your mouse button and dragging the flower to the Notebook.** Repeat this with a flower from each of the islands so you have four flower samples in your Notebook.

13. **Drag the flowers back and forth** so that flowers that share evolutionary innovations (new traits that weren’t in the earliest ancestral population) are near each other.

14. Based on what you have done so far, **think about where on the screen these flower pictures should be in sitting.** Should they be at the bottom? The top? Lined up in a row or a column? Bunched together? (Remember that you just took these pictures from currently living flower populations on the four mountain peaks).

15. As you did above, **use different colors to circle all the flowers that have red anthers, all the flowers that have pointy petal tips, all with long spur bottoms, dark petals, and red spur tubes.**

16. All of your columbines share an evolutionary innovation that makes them columbines. This novel trait is nectar spurs. **Draw one big circle around all of your flowers to indicate that they share this key derived trait, which they have all inherited from their common ancestor.**

17. **From your circles, find two flowers that must represent sister populations (that is, they share a more recent common ancestor with each other than either does with any other population). Explain why you think these two are sister populations, based on your multicolored circles.**

18. **To connect the two sister populations together with a tree branch, click on the Tree Builder tool (the U-shaped icon) in the lower right of the Notebook.**

19. **Click on one of the flower populations you want to connect and then, holding the mouse button down, drag the mouse to the other one and let go.** You will see a tree branch connect the two. The most recent common ancestor of the two populations you have connected will be shown as a small square at the base of the new branch.

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20. Now look for the next largest circle you have. The populations within that circle must share a most recent common ancestor just a little older than the ones you’ve already identified. **Connect the populations within this circle together using the Tree Builder tool.** This may involve connecting a common ancestor to an extant population, or it might involve connecting two common ancestors to each other.

21. If you make a mistake, you can delete a branch as follows: Click on the Select button (the arrow icon) in the lower right of the Notebook panel. Click on the square at the base of the branch you want to delete. You will see a red outline drawn around that square to show that it is selected. Then click on the Trash button (the trash can icon) at the lower right of the Notebook panel. The branch will disappear.

22. Continue looking for larger circles and connecting populations together until you have all the extant populations connected to the tree.

23. There are labels for each of the possible flower traits at the bottom of the Notebook. **Click on the Select tool** (the arrow in the lower right of the Notebook). **Then drag the trait labels to the appropriate branch of the tree to show where each trait changed.**

24. Now check whether your reconstruction matches the true evolutionary history of your populations. **Select Tree View from the Views menu at the top of the screen.** When you check Tree View, a new view will appear below the Notebook that shows the real tree. Compare your reconstruction with the real tree. (You may want to drag branches around in the real tree so the flower populations are in the same order in both). Did you get your reconstruction correct? Are your flowers connected correctly? Are they positioned at the proper time? If not, explain the mistake you made.

25. If you did not reconstruct the tree correctly, **load the Flower Challenge 1 experiment again and repeat steps 11 – 24.** See if this time you can reconstruct the tree accurately.

26. As a final challenge to yourself, **load the Flower Challenge 2 experiment** from the Labs>Columbine menu. In this experiment, there are 7 mountain peaks and the full set of traits that you saw at the beginning of this lab. **Run this model for 701 years until the flowers all appear. Then see if you can reconstruct the evolutionary history of all 7 populations.**
Why are there so many species of columbines?

Now that you have honed your tree thinking skills, we can return to Scott Hodges’ hypothesis about why there are so many species of columbines. Hodges suspected that the evolution of nectar spurs created an opportunity for rapid evolutionary diversification. He tested this hypothesis with the evolutionary tree shown here.

The tree shows the evolutionary history of 15 species of columbines (Latin name *Aquilegia*, abbreviated as *A*), plus several of their kin, as reconstructed by Scott Hodges and Michael Arnold (1995) based on genetic data. (Other EvoBeaker labs explore how this kind of reconstruction works). The currently living species don’t all line up horizontally at the top because on this tree branch length indicates not time, per *se*, but accumulating genetic differences in regions of the genome not subject to natural selection. The shorter the travel distance from one branch tip to another via their most recent common ancestor, the more closely related are the species at the tips.

Find the most recent common ancestor of all 15 columbines. Find, also, the point on the tree at which nectar spurs appeared. The two match up. As we have said before, nectar spurs are the shared evolutionary innovation that defines the columbines.

Note that branches on the tree are more densely packed, and the branch lengths are shorter, among the columbines than elsewhere on the tree. This is not because Hodges and Arnold went looking for columbines that were closely related. Instead, it appears that *all* columbines are closely related. As Hodges had hypothesized, the evolution of nectar spurs apparently resulted in rapid evolution and diversification among columbine populations. Quite likely, it is because they have nectar spurs that there are so many species of columbines.
Bibliography


