

Exploring Animal Behavior in Laboratory and Field

Edited by Heather Zimbler-DeLorenzo and Susan W. Margulis



Second Edition



Exploring Animal Behavior in Laboratory and Field

Second Edition

Edited by

Heather Zimbler-DeLorenzo

*Department of Life and Earth Sciences
Perimeter College at Georgia State University
Decatur, GA, United States*

Susan W. Margulis

*Department of Animal Behavior, Ecology, and Conservation
Department of Biology
Canisius College
Buffalo, NY, United States*



ACADEMIC PRESS

An imprint of Elsevier

The evolution of behavior: a phylogenetic approach

7

J. Jordan Price¹, Ken Yasukawa²

¹*Department of Biology, St. Mary's College of Maryland, St. Mary's City, MD, United States;*

²*Department of Biology, Beloit College, Beloit, WI, United States*

Chapter outline

Part I. Student instructions	108
Learning goals, objectives, and key concepts	108
Background	108
Building and interpreting phylogenetic trees	109
Using phylogenies to reconstruct the evolution of behaviors	112
Purpose	113
Methods	113
Activity 1: Whole-class exercise	114
Defining character states	116
Mapping characters onto the tree	117
Results/discussion	117
Questions for in-class discussion	118
Activity 2: Small-group projects	119
References	119
Part II. Instructor notes	121
Classroom management/blocks of analysis	121
Teaching the activity	121
Areas of potential confusion or difficulty for students	123
Another potential modification to the activity	124
Answers to the questions for in-class discussion	124
Appendix: Using Mesquite	126
Creating and editing trees	128
Discrete character state reconstruction using parsimony	129

Part I. Student instructions

Learning goals, objectives, and key concepts

- To understand how phylogenetic trees are constructed and interpreted in evolutionary studies.
- To gain experience scoring behavioral characters and mapping them onto a phylogenetic tree.
- To learn how to infer evolutionary histories of behavior using phylogenetic comparative methods.

Background

Evolution is the underlying principle of biology and, starting with Darwin, animal behavior rested on a firm foundation of evolutionary principles (e.g., [Chapin, 1917](#); [Friedmann, 1929](#); [Wheeler, 1919](#); [Whitman, 1899](#)). For some anatomical traits, evolutionary history can be followed in the fossil record, especially when a relatively complete fossil series of species is available. Unfortunately, behavior does not fossilize, so until time travel becomes a reality or the behavior of extinct animals can be studied using the methods described in the *Jurassic Park* movies, evolutionary studies of behavior must rely on **comparative methods**, by which ancestral states and the pattern of evolutionary changes in behavior can be inferred ([Martins, 1996](#)). Comparative studies of behavior gained considerable momentum in the 1940 and 1950s as influential ethologists such as Konrad Lorenz and Niko Tinbergen championed the view that behavior could be used to infer evolutionary relationships (**phylogeny**) among species and that behavior must evolve in a phylogenetic context. For example, in his study of the evolution of courtship displays of “dabbling” ducks (those that feed from the surface), [Lorenz \(1958\)](#) said, “every time a biologist seeks to know why an organism looks and acts as it does, he must resort to the comparative method.” [Tinbergen \(1963\)](#) included evolution as one of the now famous four areas of animal behavior, and he described the comparative method as follows:

Through comparison [the naturalist] notices both similarities between species and differences between them. Either of these can be due to one of two sources. Similarity can be due to affinity, to common descent; or it can be due to convergent evolution.... The differences between species can be due to lack of affinity, or they can be found in closely related species. The student of survival value concentrates on the latter differences, because they must be due to recent adaptive radiation.

([Tinbergen, 1964](#)).

Tinbergen's characterization of comparative biology foreshadowed the basic concepts of modern phylogenetic comparative methods.

Curiously, from this high point in the evolutionary analyses of behavior, phylogeny was increasingly ignored by students of animal behavior, and by the 1970s, most biologists believed that morphological characteristics were much more valuable for comparative analyses than behavioral characteristics (Brooks & McLennan, 1991; Harvey & Pagel, 1991). With the rise of the “new” comparative biology, behavior has once again been thrust into the evolutionary spotlight (Owens, 2006).

Building and interpreting phylogenetic trees

Comparative studies attempt to identify evolutionary patterns by examining traits of different **extant** (i.e., currently living) organisms. The traits can be molecular, morphological, or behavioral characteristics. All modern comparative methods center on building and interpreting “trees,” or phylogenies, which represent the pattern of shared ancestry among organisms. In other words, phylogenies are like family trees, except that instead of showing the ancestry of individuals in a family, they are “evolutionary genealogies” (Rubenstein & Alcock, 2018). The term *tree* describes the physical appearance of these proposed phylogenetic relationships, which are depicted as branching patterns of evolutionary events. An example of such a tree (or phylogeny) is shown in Fig. 7.1. Fig. 7.1 shows seven hypothetical organisms (A–G) and their phylogenetic relationships. These organisms could represent different species, subspecies, genera, or any other taxonomic group, so in the language of comparative biology, we refer to them as different **taxa**, with each referred

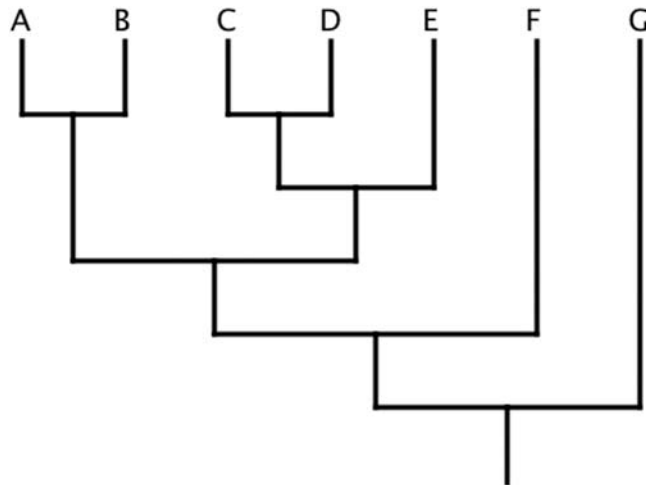


FIGURE 7.1

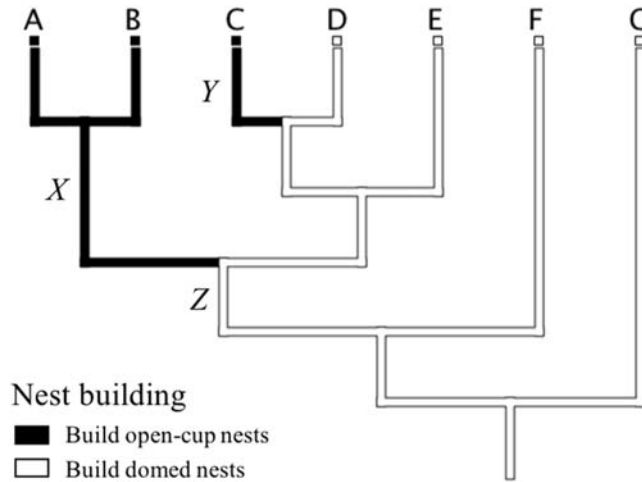
A phylogenetic tree for seven hypothetical taxa, labeled A–G.

to individually as a **taxon**. Extant taxa occur at the **tips** of branches, while all other parts of the tree represent evolutionary ancestors.

What can we infer from this tree? Well, for one thing, the tree shows us that taxon A and taxon B are each other's closest evolutionary relatives (comparative biologists call them **sister taxa**) because they both diverge from the same **node** (the point at which the **branches** leading to A and B emerge). Taxa C and D are likewise sister taxa, whereas taxon E is more distantly but equally related to both C and D. Together these taxa form a **clade** (a group of taxa sharing a common ancestor, with closer relationships to each other than to members of any other group). In tree terminology, a clade comprises all the branch tips that come from the same node. In Fig. 7.1, A and B are considered a clade, as are C-D, C-D-E, A-B-C-D-E, A-B-C-D-E-F, and A-B-C-D-E-F-G. A clade can also be called a **monophyletic group** (the terms are synonymous), which refers to a group of taxa descended from a single ancestral taxon and including the ancestral species and all descendant species. Note that the common ancestor is included in a clade (or monophyletic group). In tree terminology, taxonomic groups can include ancestors as well as extant taxa. Two other terms that are important in discussing trees are the **root** (base) of the tree and the **internodes** (branches between nodes).

How do we determine relationships among taxa to build a phylogenetic tree? These analyses are based primarily on a careful examination of **characters** across taxa. A phylogenetic character includes a set of possible states or conditions that are thought to evolve from one to the other, with each alternative condition called a **character state** or **trait**. We group organisms together based on **shared derived traits**, which are novel character states that are shared by two or more taxa because they were inherited from a common ancestor in which the trait originated. For example, in songbirds, we can divide the species into those that build open-cup nests and those that build domed nests with roofs (Price & Griffith, 2017). Suppose that open-cup nest building behavior evolved in an ancient species of bird (indicated by X on the tree shown in Fig. 7.2) and that the ancestors of that species built domed nests. Domed nests were thus the **ancestral** state and cup nests the relatively **derived** trait (note that it is also possible for domed nest building behavior to reappear through the loss of the derived trait). This transition from domed to cup nests could have happened anywhere along the internode indicated by X (even though the whole internode is coded as cup nest building in Fig. 7.2). Suppose further that two species, A and B, evolved from that cup nest building ancestor. If species A and B also build cup nests, and if they inherited this behavior from their common ancestor, then cup nest building is a shared derived trait in species A and B and is said to be **homologous** (it was inherited by both species from a common ancestor). The goal of modern taxonomy (called “phylogenetic systematics”) is to organize taxa into clades based on homologous, shared derived traits.

Of course, there are many ways to sort the taxa in Fig. 7.2 into groups that are not monophyletic (not clades) and thus do not reflect evolutionary relationships. For example, a taxonomic group including species A-B-C, based on the presence of cup nests, would be called a **polyphyletic group** because the trait the group is based

**FIGURE 7.2**

A phylogeny showing the same relationships as Fig. 7.1, but with two character states mapped onto the tree: open-cup nest building (indicated by black) and domed nest building (indicated by white). Cup nest building presumably evolved from domed nest building twice on this tree, along branches X and Y. If cup nest building evolved earlier on branch Z, two more transitions back to dome building would have had to occur on the branches leading to taxa D and E, which would be a less parsimonious explanation of evolutionary history.

on (cup nest building) is shared due to the independent evolution of this trait on branches X and Y rather than common ancestry. Cup nest building behavior in this case is said to be a **homoplasy** (it was not inherited from a common ancestor). Polyphyletic groups are based on homoplasies and do not include a common ancestor. Alternatively, a group including just D-E-F-G, based on having domed nests, would be called a **paraphyletic group** because it includes the common ancestor but not all the descendants of that ancestor. Paraphyletic groups are based on homologous, shared ancestral traits rather than on shared derived traits. The easiest way to distinguish polyphyletic and paraphyletic groups is by whether or not they include the group's most recent common ancestor. Both groupings include taxa that are more closely related to nongroup members than to other group members (e.g., D is more closely related to C than to its other group members, and vice versa).

Many well-known animal groups are not monophyletic. Pandas, for example, provide a good example of a polyphyletic group. Despite some striking similarities (including an extra “pseudo thumb” for eating bamboo), giant pandas (*Ailuropoda melanoleuca*) and red pandas (*Ailurus fulgens*) are actually distantly related and did not inherit these traits from a common panda ancestor. Their shared traits are homoplasies that were derived independently through convergent evolution. Reptiles provide a good example of a paraphyletic group because the common ancestor

was a reptile but some descendants of that ancestor, such as birds, are not considered reptiles. Reptiles are grouped together based on shared character states that are relatively ancestral in comparison to those of birds (e.g., scales vs. feathers). As a result, interestingly, some reptiles, such as crocodiles, are actually more closely related to birds than to other reptiles.

Although behavioral characters have been used to construct phylogenies, these days, trees are constructed primarily using molecular data, such as DNA sequences. This is not necessarily because DNA nucleotide characters exhibit less homoplasy than do behavioral characters but because DNA provides so *many* characters—thousands or even billions rather than dozens. Phylogenies are now being constructed based on entire genomes. The phylogenetic trees produced from such molecular analyses have been extremely useful in animal behavior studies in recent decades because they provide an independent and relatively reliable way of estimating phylogenetic relationships. Using them, we can infer the sequence of events through which behavior evolved.

Using phylogenies to reconstruct the evolution of behaviors

Rather than using traits to build a phylogeny, in this exercise, we will use independently generated phylogenetic trees to reconstruct the evolution of behavioral traits. How do we use trees to reconstruct behavioral evolution? **Parsimony** (simple explanations are better than complicated ones) is the most commonly used criterion in both building phylogenetic trees and in using these trees to trace past evolutionary changes in a character. In practice, the parsimony criterion means trying to minimize the number of character-state changes (state transitions) needed to explain the current distribution of character states among extant taxa. For example, let us return to [Fig. 7.2](#). Theoretically, cup nest building behavior could have evolved earlier on in the tree, just once in ancestor *Z* rather than twice in ancestors *X* and *Y*. However, given the current distribution of cup and domed nest building taxa today, this would mean that cup nests were lost at least twice in taxa *D* and *E*. This alternative scenario would therefore involve three evolutionary changes, one gain and two losses of cup nests, rather than just two gains, so we would say that it is a *less parsimonious* explanation.

We can use this same parsimony criterion to determine which character states are relatively ancestral and which are derived; that is, we can determine the order (evolutionary sequence) of character state changes. If cup nests were ancestral to domed nests, for example, our phylogeny suggests that at least four evolutionary changes would have to have occurred, such as domed nests evolving from cup nests four times independently in taxa *D*, *E*, *F*, and *G*. Can you come up with some other possible evolutionary scenarios that would have resulted in the distribution of cup nest building and domed nest building taxa today? Any way you look at it, using these character states on this phylogeny, it is more parsimonious to assume that domed nest building was the ancestral behavior in songbirds.

Sometimes there are multiple possible evolutionary scenarios that are equally parsimonious. For example, imagine if taxon G were the only domed nest building taxon and all other taxa (A–F) built cup nests. The ancestor, at the root of the tree, could have built cup nests or domed nests and either possibility would be equally parsimonious. How do we decide which scenario is more likely, and thus which character state is ancestral and which derived? In such cases, we can look to additional taxa, called **outgroups**, that are closely related to, but are outside (i.e., not *as* closely related to), the group of interest, which of course is called the **ingroup**. Including outgroup taxa simply increases the size of our tree so that we can determine the directionality of character state transitions. In cases that cannot be resolved using outgroups, sometimes additional information about the characters can help us decide. For example, character state transitions in one direction might be more probable than changes in the other direction. In reconstructing the evolution of flight in birds, for instance, the ability to fly seems far more likely to be lost than gained during evolutionary history simply because it requires so many unique specializations.

When done carefully, comparative studies can help us to infer **adaptation**, or whether the character of interest has evolved into its current state in response to natural selection. For example, building cheap, relatively disposable open-cup nests could have been an adaptation in response to changes in predation, nest parasites, or perhaps even levels of climatic variability. We would need to compare changes in nest building to changes in other characters to see how well they match up. There are a wide variety of methods for statistically comparing such character changes across taxa, ranging from simple to highly complex. Here we will mostly focus on reconstructing the evolution of individual characters.

Purpose

Our general research question in this exercise is: what was the evolutionary sequence of events (i.e., the order of character state changes) that led to the behavioral characteristics we see in a group of species today? To reconstruct these evolutionary changes, you will need to define a set of characters and character states, map the characters onto a phylogenetic tree, and use the principle of parsimony (minimizing the number of character state transitions) to reconstruct when (on which internodes) each character changed from one state to another.

Methods

This exercise can be done with any clade of related taxa. These can be different species, different taxonomic families, or any other level of taxonomic group, as long as you determine their relationships. Here we will use a group of six species from the New World blackbird family (Icteridae). You can do this exercise with paper and pencil or by using a dry-erase whiteboard and colored dry-erase pens, which you

can use to draw the tree, the character states, and evolutionary events with different characters color coded. Alternatively, you can use a free computer program called Mesquite (Maddison & Maddison, 2019; available at <http://mesquiteproject.org>) to visualize evolutionary changes. Directions for using Mesquite are in the Appendix.

This activity consists of two parts. First, the whole class will work on reconstructing the evolution of some behavioral characteristics using the provided phylogeny of blackbirds. Second, working with a partner or in a small group, you will choose another set of taxa and use a phylogeny and the behaviors exhibited by the taxa to reconstruct how those behaviors evolved.

Form working groups of two to four students. If you are going to use a computer to do your analysis, your instructor will take some time to teach you how to use Mesquite. If you are going to do the analyses by hand using paper or a whiteboard, you can proceed directly to the exercises in the following.

Activity 1: Whole-class exercise

You will use the phylogeny in Fig. 7.3, which shows hypothesized evolutionary relationships among six songbird species from the New World blackbird family (Icteridae), including the red-winged blackbird (*Agelaius phoeniceus*) and its evolutionary relatives. This tree is similar to the ones shown in Figs. 7.1 and 7.2 but is turned 90 degrees clockwise. Note that all six species are extant (currently living) and that we are using this hypothesized phylogenetic tree to infer

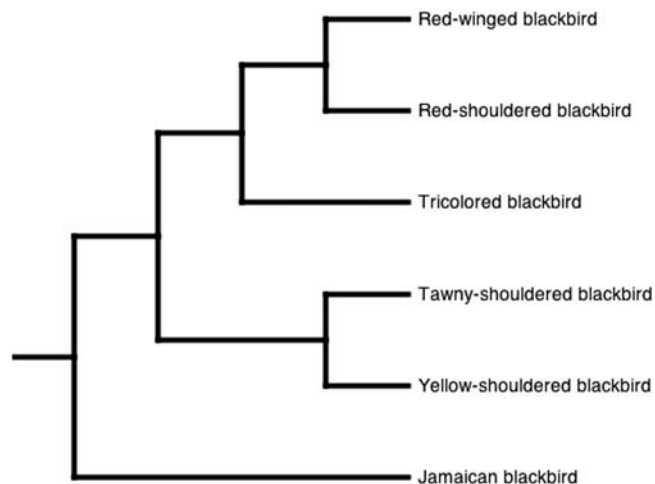


FIGURE 7.3

A phylogenetic tree showing hypothesized evolutionary relationships among six species in the New World blackbird family (Icteridae), based on molecular data (Powell et al., 2014).

evolutionary history from their current character states. This particular phylogeny is based on an extensive comparison of mitochondrial and nuclear DNA sequences (Powell et al., 2014).

A number of studies have been published on the singing and breeding behavior of these species. In fact, the red-winged blackbird is among the most well-studied songbirds in the world (Searcy & Yasukawa, 1995). In some of these species, including red-winged blackbirds, only males produce complex songs, whereas in other species, both males and females produce similar songs at similar rates. In a few species, males and females even coordinate their vocalizations to produce complex duets. In some species, males and females mate as pairs (they are socially monogamous), whereas in others, some males pair with multiple females (they are polygynous). Some breed in the tropics and others breed in the temperate regions of North America. Some migrate annually, whereas others are sedentary and stay in the same areas year-round.

From published studies (Odom et al., 2015; Price, 2009), we can assemble the following descriptions of each species. You will use the following information and Fig. 7.3 to infer the evolutionary history of singing, mating system, breeding latitude, and migratory behavior in this group of animals.

- A.** Red-winged blackbird (*A. phoeniceus*)
Males defend territories using complex vocal displays (song). Females produce a variety of sounds, some of which are as complex as the songs of males, but they do not sing like males. The mating system is polygyny, with some males pairing with multiple females and other males not pairing at all. Most populations breed in the temperate areas of North America and migrate annually.
- B.** Red-shouldered blackbird (*Agelaius assimilis*)
Both males and females defend their shared territory using songs, often by combining their vocalizations into a duet. Males and females are monogamous. They breed in the tropics and do not migrate (they are sedentary).
- C.** Tricolored blackbird (*Agelaius tricolor*)
Males sing and females do not sing. Mating is polygynous. The species breeds in temperate regions and migrates annually.
- D.** Tawny-shouldered blackbird (*Agelaius humeralis*)
Both males and females sing, often by combining their vocalizations into complex duets. Mating is monogamous. They breed in the tropics and are sedentary.
- E.** Yellow-shouldered blackbird (*Agelaius xanthomus*)
Both sexes sing, but males and females do not coordinate their vocalizations as duets. The species is monogamous, tropical, and sedentary.
- F.** Jamaican blackbird (*Nesopsar nigerrimus*)
Both sexes sing, but without producing duets. The species is also monogamous, tropical, and sedentary.

How can we make sense of the diversity of the behavioral and life-history characteristics among these six species? What are the character states, and which ones

are ancestral and which ones are derived? What is the evolutionary sequence of events that produced the current distribution of characteristics we see today?

Defining character states

The first and, at this point, most important step in your analysis is to identify the behavioral characters of interest and their possible states. The simplest characters are those with only two possible states (the so-called **binary characters**). Discuss within your group the characters and their states. For example, singing could be scored as a **multistate character** with three states (only males sing, both sexes sing, and both sexes duet). Note, however, that one of our goals in scoring phylogenetic characters is to define character states that are mutually exclusive, either one thing or another. Are “both sexes sing” and “both sexes duet” mutually exclusive character states, given that females must sing in order to combine their songs with male songs? We therefore recommend that singing be scored as two separate binary characters (presence/absence of female song and presence/absence of duetting). Your group will decide which characters to use, and you will assign two possible states for each of them. Once your group has agreed on characters and states, compare them to those of another group. It is possible that the two groups will not agree. See if you can find a common system of characters and states.

For the purposes of this exercise, the authors have defined five binary characters, but your class could choose to use a different set. Use the information on each of the six species to fill in the appropriate spaces in [Table 7.1](#), which lists the authors’ five binary characters. For some characters the possible states could be “absent” and

Table 7.1 Five behavioral characters for six species of blackbirds, with states not filled in.

Species	Characters				
	Female song	Vocal duets	Mating system	Breeding latitude	Migratory behavior
Red-winged blackbird					
Red-shouldered blackbird					
Tricolored blackbird					
Tawny-shouldered blackbird					
Yellow-shouldered blackbird					
Jamaican blackbird					

“present” (or 0 and 1 in Mesquite), whereas for others the possible states could be more descriptive, such as “monogamous” and “polygynous” (likewise scored as 0 and 1 in Mesquite).

Mapping characters onto the tree

Use the information from [Table 7.1](#) and the hypothesized phylogeny of [Fig. 7.3](#) to “map” the five behavioral characters onto the branches of the phylogeny. For each character in turn, using either paper/whiteboard methods or Mesquite, use the current character states of these species to figure out which state is ancestral and where (on which internode or internodes) the derived state evolved. See if you can find the simplest, most parsimonious evolutionary scenario for each character.

Note that it is possible to find multiple evolutionary histories for a character that involve the same minimum number of changes. In the ancestors of red-winged blackbirds, red-shouldered blackbirds, and tricolored blackbirds, for example, you may find that character state changes could have occurred twice independently in red-winged blackbirds and tricolored blackbirds or once in the common ancestor of these three species and then again (in the opposite direction) in red-shouldered blackbirds. Both possibilities involve the same number of evolutionary transitions and so are equally parsimonious. How do we decide which scenario is more likely? As mentioned earlier, sometimes looking at additional information can be helpful. For instance, biogeographic evidence (based on geographic distributions) and genetic data suggest that red-shouldered blackbirds moved to the tropics relatively recently from a temperate breeding ancestor ([Barker et al., 2008](#)). Thus it seems likely that temperate breeding was gained earlier on in our tree and then lost in red-shouldered blackbirds rather than gained twice independently in red-winged blackbirds and tricolored blackbirds. Does this help us to resolve changes in other characters, such as migration?

Results/discussion

Once your group has completed its phylogeny, discuss the following questions. How did you resolve characters that have more than one possible most parsimonious evolutionary history? Do any character state changes seem correlated with each other (tending to occur together), and do any characters seem likely to influence each other? Once your group has discussed these and any other questions that occur to you, present your phylogeny of blackbird evolution and discuss it with the rest of the class. It is possible that your group’s mapping of behavioral character state transitions will differ from that of another group. Be prepared to defend your proposed evolutionary sequence and to question the sequences of other groups. By what criterion can the class decide which proposed sequence of behavioral state transitions is “best”? It may interest you to know that disagreements in phylogenetics (this kind of analysis) can and do become extremely heated, both in print and in person.

Questions for in-class discussion

If time permits discuss the following questions within your group and then with the whole class. Alternatively, if time is short, each group could be assigned to discuss one question and present its conclusions to the rest of the class.

1. According to some comparative biologists, you should *never use the information you want to study to build your phylogenetic tree*. Why is it important to follow this rule? What happens if you do use the information you want to study to build your phylogenetic tree?
2. Originally, ethologists saw behavior as another set of characters that could be used to construct phylogenies, along with morphological information. These days, however, phylogenies are usually constructed from molecular data. Should we use behavioral data when constructing phylogenies? Why or why not? Which source of information is better? Why?
3. Why is the parsimony criterion used in reconstructing the evolution of character traits? Do you think evolution always necessarily operates according to this principle? Explain.
4. Comparative methods can be used to test whether different characters are correlated with each other across species, such as whether female singing is correlated with monogamy. In making such comparisons, should we always treat each species as an independent “statistical sample” so that, say, five species exhibiting an apparent correlation between female song and monogamy provide more statistical support than just three species sharing these traits? What if these character states are homologous across species, having evolved just once in a common ancestor rather than evolving independently as a result of selection due to similar environments? Does this make a difference?
5. Songbird species that build cup-shaped nests are much more common globally than species that build domed nests, despite the fact that cup nests evolved more recently on the songbird phylogeny (Price & Griffith, 2017). Moreover, most cup-shaped nests are simpler in construction than domed nests. What do these patterns tell us about how the rarity and the complexity of traits relate to the directionality of evolution?
6. In most temperate breeding songbird species, males produce complex songs and females vocalize but do not “sing” like males. Countless studies have investigated why male songbirds sing. Yet, recent phylogenetic studies show that sex differences in singing behavior have generally evolved by females losing song rather than males gaining it (Odom et al., 2014). Likewise, although we often ask why birds fly south in the winter, phylogenetic evidence shows that the ancestors of some migratory songbirds were tropical rather than temperate (e.g., Outlaw et al., 2003; Price, 2009), suggesting that migration evolved as a way to breed in northern regions rather than as a way to winter in southern regions. Do these new findings indicate that previous ideas were wrong?

Activity 2: Small-group projects

Now that you have had some practice, your group can attempt a second analysis with a somewhat more complex example. Our general research question, however, remains the same: what was the evolutionary sequence of events for the behavior or behaviors of interest? You will once again have to define behavioral character states and map character state transitions onto a phylogeny to infer the ancestral states and when each derived state evolved.

Your instructor will provide several data sets for analysis or will tell you how to find appropriate phylogenies and behavioral descriptions. Choose one set and have at it! Devise a table to record the states of the behavioral characters for each taxon. Follow the steps from the whole-class exercise (using either paper/whiteboard methods or Mesquite) to map the behavioral states onto the proposed phylogeny and then to infer the evolutionary history of the behavioral characters of interest.

References

- Barker, F. K., Vandergon, A. J., & Lanyon, S. M. (2008). Species status of the red-shouldered blackbird (*Agelaius assimilis*): Implications for ecological, morphological, and behavioral evolution in *Agelaius*. *The Auk: Ornithological Advances*, *125*, 87–94.
- Brooks, D. R., & McLennan, D. A. (1991). *Phylogeny, ecology, and behavior: A research program in comparative biology*. Chicago: University of Chicago Press.
- Chapin, J. P. (1917). The classification of the weaver birds. *Bulletin of the American Museum of Natural History*, *37*, 243–280.
- Friedmann, H. (1929). *The cowbirds*. Springfield, Illinois: Charles C. Thomas.
- Harvey, P. H., & Pagel, M. D. (1991). *The comparative method in evolutionary biology*. Oxford: Oxford University Press.
- Lorenz, K. (1958). The evolution of behavior. *Scientific American*, *199*, 67–78.
- Maddison, W. P., & Maddison, D. R. (2019). *Mesquite: A modular system for evolutionary analysis* (v. 3.61). See <http://mesquiteproject.org>.
- Martins, E. P. (Ed.). (1996). *Phylogenies and the comparative method in animal behavior*. Oxford: Oxford University Press.
- Odom, K. J., Hall, M. L., Riebel, K., Omland, K. E., & Langmore, N. E. (2014). Female song is widespread and ancestral in songbirds. *Nature Communications*, *5*, 3379.
- Odom, K. J., Omland, K. E., & Price, J. J. (2015). Differentiating the evolution of female song and male–female duets in the new world blackbirds: Can tropical natural history traits explain duet evolution? *Evolution*, *69*, 839–847.
- Outlaw, D. C., Voelker, G., Mila, B., & Girman, D. J. (2003). Evolution of long-distance migration in and historical biogeography of *Catharus thrushes*: A molecular phylogenetic approach. *The Auk: Ornithological Advances*, *120*, 299–310.
- Owens, I. P. F. (2006). Where is behavioral ecology going? *Trends in Ecology & Evolution*, *21*, 356–361.
- Powell, A. F. L. A., Barker, F. K., Lanyon, S. M., Burns, K. J., Klicka, J., & Lovette, I. J. (2014). A comprehensive species-level molecular phylogeny of the new world blackbirds (Icteridae). *Molecular Phylogenetics and Evolution*, *71*, 94–112.

- Price, J. J. (2009). Evolution and life history correlates of female song in the New World blackbirds. *Behavioral Ecology*, 20, 967–977.
- Price, J. J., & Griffith, S. C. (2017). Open cup nests evolved from roofed nests in the early passerines. *Proceedings of the Royal Society B: Biological Sciences*, 284, 20162708.
- Rubenstein, D. R., & Alcock, J. (2018). *Animal behavior: An evolutionary approach* (11th ed.). Oxford: Oxford University Press.
- Searcy, W. A., & Yasukawa, K. (1995). *Polygyny and sexual selection in red-winged blackbirds*. Princeton: Princeton University Press.
- Tinbergen, N. (1963). *Social behaviour in animals: With special reference to vertebrates*. London: Methuen & Co.
- Tinbergen, N. (1964). On aims and methods of ethology. *Zeitschrift für Tierpsychologie*, 20, 410–433.
- Wheeler, W. M. (1919). The parasitic *Aculeata*, a study in evolution. *Proceedings of the American Philosophical Society*, 58, 1–40.
- Whitman, C. O. (1899). Animal behavior. In C. O. Whitman (Ed.), *Biological lectures, woods hole* (pp. 285–338). Boston: Ginn & Co.

Part II. Instructor notes

Classroom management/blocks of analysis

This exercise can be done in two sections. The whole-class exercise can be completed in a single lab period of 2–3 h, with the small-group projects being done next, outside the lab time. For the whole-class exercise, allow about 30 min for students to define the character states and discuss them. If you plan to use Mesquite (see [Appendix](#)), then plan to spend about 30 min showing the program to students by using an example data file to walk them through the various functions and to give them some time to “play” with the program on their own. Once they understand how Mesquite works give them 30–60 min to build their character matrix, construct the tree, trace character histories, and discuss their results. Make sure you leave enough time for each group to explain their character state reconstructions to the rest of the class or lab.

Students can do their small-group projects outside the lab or classtime, but they should plan to leave some time at the end of the lab period to answer questions they have about the project. Most students are not familiar with “tree thinking,” so the time spent introducing and reinforcing concepts and methods to them is extremely important.

Groups of three to four students work best, although pairs of students can also produce very good work. In some cases, four students may be too many, especially if one student is reticent. In other cases, four students are too few because none of them take to “tree thinking.” Careful monitoring during the whole-class exercise and asking for progress reports during the small-group projects will help ensure that each group is making progress.

Teaching the activity

Preclass preparation and potential variations

This exercise could be done by hand with paper and pencils or better yet by giving each group a dry-erase whiteboard and several colors of dry-erase pens. Cheap white laminate wall covering can be purchased in 4 × 8-foot sheets and cut into 2 × 4-foot boards to be given to each group.

Hand methods will be sufficient for simple trees and characters, such as in the whole-class exercise, but may prove to be more difficult for more complex trees and sets of characters, as would be encountered in the small-group projects. Some students may feel more comfortable using a computer, whereas others might be

Table 7.2 States of five behavioral characters for six species of blackbirds.

Species	Characters				
	Female song	Vocal duets	Mating system	Breeding latitude	Migratory behavior
Red-winged blackbird	0	0	1	1	1
Red-shouldered blackbird	1	1	0	0	0
Tricolored blackbird	0	0	1	1	1
Tawny-shouldered blackbird	1	1	0	0	0
Yellow-shouldered blackbird	1	0	0	0	0
Jamaican blackbird	1	0	0	0	0

bewildered by all of Mesquite's various functions (in the [Appendix](#), we describe only a very small fraction of the program's features). Decide in advance whether you want to do the exercise by hand, on computers, or as some combination of the two. You may also choose to map other characters onto the blackbird phylogeny, such as plumage colors. If you decide to have the students use Mesquite, you should plan to spend a few hours beforehand learning its ins and outs so you can answer their questions (they will have many).

The small-group projects can be based on trees that are provided by the instructor, or students can be asked to find trees in the published literature or other sources. Some excellent websites for generating evolutionary phylogenies are *Time-Tree* (<http://timetree.org>) and the *Tree of Life Web Project* (<http://tolweb.org/tree/>). Student groups could build their phylogenies based on those relationships for a wide variety of taxonomic groups, although birds may be your best bet given the many phylogenetic and behavioral studies that have been published.

In-class preparation

Once students define their character states (as in [Table 7.2](#)), they can map their behavioral characters onto the tree shown in [Fig. 7.3](#) by hand using paper/whiteboards or on a computer using Mesquite. If they do this by hand, first have the students record the character states of each of the five behavioral characters near the tips of the tree. It is easiest to have them record each character on a separate line, and perhaps using a different color, so they will need five lines for each species (one for female song, one for duetting, etc.). Once the character states are recorded at the tips, the students can focus on each character in turn to try to figure out which

is the ancestral state and where (on what branch or internode) each derived state evolved. Typically, these evolutionary events are depicted by drawing a short line across the branch or internode and writing the name of the derived state next to it. Good examples are presented in [Brooks and McLennan \(1991\)](#) and [Rubenstein and Alcock \(2018\)](#).

Areas of potential confusion or difficulty for students

Whether they map behavior by hand or with Mesquite, your students should begin to think about the evolution of behavior in a very different way. Tree thinking will be new for most students, so at each step, it would be a good idea to spend some time talking about what they did, what they found, and what it means.

For both the whole-class exercise and small-group projects, students may have trouble scoring characters into clearly defined character states. In the whole-class exercise, for example, some may want to lump female song and duets into a single multistate character. Emphasize that character states should be mutually exclusive and that using lots of simple binary character states is usually much easier to interpret than using fewer, more complicated characters.

Students might also have trouble when there is more than one possible most parsimonious evolutionary history for a character (as in the ancestors of red-winged blackbirds, red-shouldered blackbirds, and tricolored blackbirds). A trait may have been gained twice independently or gained once and lost once; both possibilities involve the same number of evolutionary transitions. Such issues are common in evolutionary reconstructions. Talk about how we might try to resolve such uncertainties.

In the small-group projects, you may want to discourage students from choosing very complex trees (in excess of 30 taxa) or poorly resolved trees with many **polytomies** (unresolved relationships in which more than two branches come from a single node). For example, you could suggest that they use trees with fewer than 15 taxa or you could provide trees for them to use. If they use Mesquite, the most difficult step your students will face is converting a default random tree into the tree that they have chosen to analyze (see [Appendix](#)). Moving branches around in Mesquite is easy, but if students do it without thinking about what they are doing, the result will be a very messy tree and a lot of wasted time. Also, students may not realize at first that trees showing the same relationships can look different. For example, selecting sister taxa and rotating their branches at the node will change the positions of the species but will not change their relationships at all. A quick demonstration on a chalkboard/whiteboard would help show this to your students.

Whatever your students choose to do for their small-group projects, once they find a system to analyze, they will need to do the same three steps as in the whole-class exercise: (1) obtain a tree showing relationships among the taxa of interest, (2) define behavioral character states and enter these data into a table or into Mesquite, and (3) map (trace) the behavioral character transitions on the tree.

Another potential modification to the activity

Early comparative studies of behavior often used behavioral characters to construct trees. There is really no reason why we could not do that as part of this exercise, especially given the tools at our disposal. Students could use Mesquite to construct one tree using a set of behavioral characters (i.e., they could use shared derived character states to define relationships, using only behavioral characters) and then compare that tree to another one constructed from other characters (e.g., molecular sequences or morphological character states). In some cases the behavioral trees could be used to resolve polytomies, or vice versa.

Answers to the questions for in-class discussion

1. The idea that one should *never use the information you want to study to build your phylogenetic tree* is an attempt to avoid circular reasoning. If, for example, you constructed a phylogeny using behavioral characters and then used that phylogeny to reconstruct the evolutionary sequence of events for those same characters, it is pretty obvious that you would not have an independent assessment of evolutionary history. “Phylogenetic systematics” (the field of biology focused on determining evolutionary relationships among organisms) seeks to construct phylogenies that are independent of assumptions about phylogenetic history, so systematists usually construct trees based on molecular data such as DNA sequences. A molecular phylogeny provides an independent hypothesis for phylogenetic relationships among taxa, which can then be used to provide information about the evolution of the behavioral characters.
2. Are behavioral characters useful in constructing phylogenies? For any character, the critical distinction made by comparative biologists is between homologies, which can indicate phylogenetic relationships, and homoplasies, which can be misleading about phylogeny (Brooks & McLennan, 1991). Two species can share a derived behavioral state by homology just as they could share a morphological state or a DNA nucleotide sequence by homology. Although it is perhaps less common these days to use behavioral characters to construct trees, there is no a priori reason to assume that they are less valid than other characters. Some biologists assume that behavioral characters tend to be highly variable and are subject to modification by experience. Yet morphological features are also subject to environmental influences, and DNA sequences can be extremely variable across taxa with high levels of homoplasy. Molecular sequences are so frequently used in building trees because they provide so *many* characters, not necessarily because each character (i.e., each nucleotide) is necessarily more informative than other types of characters.
3. Parsimony is a working assumption used by systematists. Theoretically, when building a phylogenetic tree, if we did not limit the number of times each character transitioned between states, we could generate huge numbers of potential trees from the same set of data. How would we decide which tree reflects the actual pattern of relationships among taxa? Clearly, we need some kind of

rule by which we can choose a tree (remembering that any tree is a hypothesis for the true phylogeny). Choosing the most parsimonious tree, the one with the fewest possible character changes, is simply a rule we can follow to select among all the possibilities. However, the most parsimonious tree may not necessarily be the correct tree. Evolution can proceed randomly or in response to complex and changing environmental conditions, so evolution may not necessarily operate according to the principle of parsimony.

4. When testing whether different characters are correlated with each other across taxa, should each species be considered as an independent sample, even if shared character states among these species are due to shared ancestry (homology) and thus are not really independent? Modern comparative methods use phylogenies to overcome this inherent difficulty, and they have provided lots of valuable information for biologists interested in the evolutionary history of behavior. Taking phylogenetic relationships into account allows us to make comparisons among taxa that are statistically independent. In other words, if three blackbird species exhibit both female song and monogamy because both traits evolved in a common ancestor, we should not consider them as three independent examples showing a statistical correlation between these traits. Each of these traits may have evolved just once in the past, perhaps together but perhaps not. In contrast, if our analysis showed that female song was lost or gained every time the species transitioned between monogamy and other mating systems, then that would provide good evidence for an evolutionary correlation. Understanding phylogeny allows us to see if traits truly have evolved together.
5. Interestingly, although cup-shaped nests evolved more recently than domed nests, most of the world's songbirds (approximately 75%) build cup-shaped nests (Price & Griffith, 2017). Cup nests are also generally simpler to construct. These observations illustrate two important points. First, although biologists have often assumed that the ancestral bird nest was cup shaped, mostly because this design is so widespread, there is no reason to assume that the current prevalence of a trait indicates the order of events in the evolutionary past. Second, evolution does not always proceed from simple to complex. Indeed, there are many examples (including the evolution of female song) in which complex behavioral patterns have become lost or simplified during evolutionary history.
6. Many biologists, including Charles Darwin himself, have explained the complex singing behaviors of male songbirds as having evolved through the influence of sexual selection for elaborate male traits. Recent findings, however, show that singing by both sexes was the ancestral state in songbirds and that female song has been lost repeatedly across the songbird phylogeny (Odom et al., 2014). Thus it might be more appropriate to ask why so many females *do not* produce complex songs, rather than just focusing on why males do. In a similar way, phylogenetic evidence that temperate migratory species evolved from tropical, nonmigratory ancestors suggests that we should be asking why birds migrate north to breed, in addition to asking why they migrate south in the winter. These new findings from phylogenetic studies do not necessarily mean that old evidence is wrong. Rather, they bring up new questions and suggest new ways of thinking about the evolution of animal behavior.

Appendix: Using Mesquite

Mesquite (available at <http://www.mesquiteproject.org>) is a free computer program that can help students to visualize evolutionary sequences by mapping evolutionary events (changes in character state) onto phylogenetic trees. The following directions are for Mesquite version 3.61 (Maddison & Maddison, 2019).

Open Mesquite and select File > New. Provide a name for the file (e.g., “Blackbird evolution”) and a location where it will be saved. When you click OK, a New File Options window will appear. The Make Taxa Block should be checked (check it if it is not checked), enter the number of taxa (6 in our example), check Make Character Matrix, and hit OK. In the New Character Matrix window enter the number of characters (5 in our example). There are several options in the Type of Data menu; for this exercise select Standard Categorical Data. Click OK.

A Project Window will open showing an empty Character Matrix (Fig. 7.4). Here you can enter the names of taxa, characters, and character states. There are several tools along the left side that allow you to manipulate the matrix. When you hold the cursor over each of the buttons, a description of what it does appears at the bottom of the window.

Click on the Edit tool and use it to enter each taxon name along the left column and character name along the top as shown earlier in Table 7.1. Then enter your

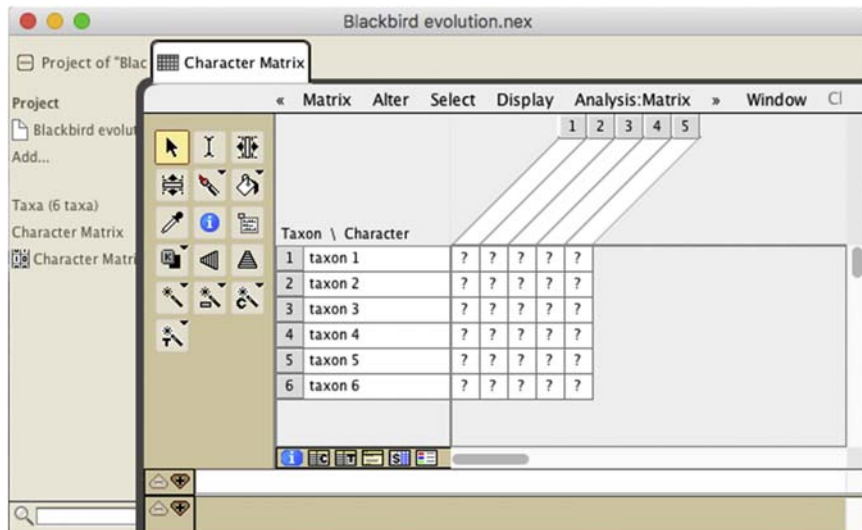


FIGURE 7.4

Project window with empty character matrix.

Taxon \ Character	1	2	3	4	5
1 Red-winged blackbird	0	0	1	1	1
2 Red-shouldered blackbird	1	1	0	0	0
3 Tricolored blackbird	0	0	1	1	1
4 Tawny-shouldered blackbird	1	1	0	0	0
5 Yellow-shouldered blackbird	1	0	0	0	0
6 Jamaican blackbird	1	0	0	0	0

FIGURE 7.5

Character matrix with taxa, characters, and states entered.

previously defined binary character states in each cell as either a 0 or 1, as in Fig. 7.5. For example, you could score species without female song as 0 and with female song as 1, and you could score monogamous species as 0 and polygynous species as 1. In this simple example, we have only five characters with just two states each, but in other cases, there could be more characters, which could have two or more discrete states. Alternatively, our characters could be continuous rather than discrete or could be nucleotides rather than behavioral categories. Each has a specific option in the Type of Data menu (see earlier discussion).

A matrix filled with 0s and 1s is often not the easiest thing to interpret; we would be better served if the character states were more descriptive. You can name the character states as follows. At the bottom left are five small buttons next to a small blue “i”; each looks like a little window. Select the one that is second from the right, the Show State Names Editor Window (this is also available in a drop-down menu as Matrix > Edit State Names). Select the Edit tool and use it to give names to our characters and their states (Fig. 7.6). Now go back to the Character Matrix by clicking the tab at the top of the window. As you can see, all the 0s and 1s have been replaced with character state names. One thing to note is that when adding or editing character states in the matrix you still need to type a 0 or 1, even if the states now have more descriptive names.

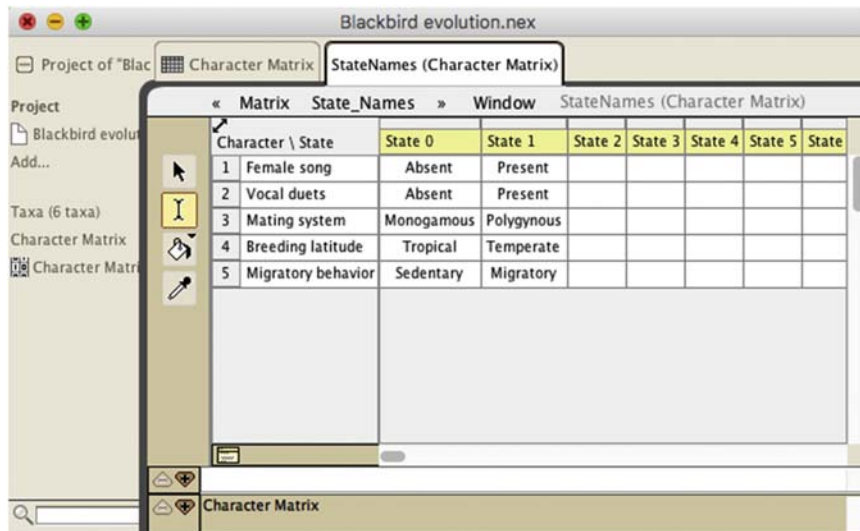


FIGURE 7.6

Entering names for each character state.

Creating and editing trees

There are ways to import previously constructed phylogenies into Mesquite, but this is a little beyond the scope of our exercise. Here we will manually recreate the tree shown in Fig. 7.3. Select Taxa & Trees > New Tree Window > With Tree To Edit By Hand. A window tab will appear with a randomly organized tree of our six taxa (Fig. 7.7), which we will need to modify to match the relationships in Fig. 7.3. Click on the Select tool (the arrow) and use it to click on the various branches and drag them over to connect with others. We suggest starting with pairs of sister species (e.g., red-winged blackbird and red-shouldered blackbird). Note that for sister taxa, it does not matter which one is to the right and which one is to the left—they just have to branch from the same node. The order in which you click and drag determines left and right positioning, so if you want a topology that is identical to that shown in Fig. 7.3, you may need to use Edit > Undo a few times (or the Interchange branches tool) to get the particular branching pattern you want. By default, Mesquite will draw a Square Tree, but you can change it to a different form using Display > Tree Form. You can rotate the tree using Display > Orientation. You can also change other aspects of the tree, such as the thickness of the lines (Display > Line Width) and the angle of the taxon names (Display > Names > Taxon Name Angle). When you are happy with how your tree looks save it using Tree > Store Tree in Tree Block As

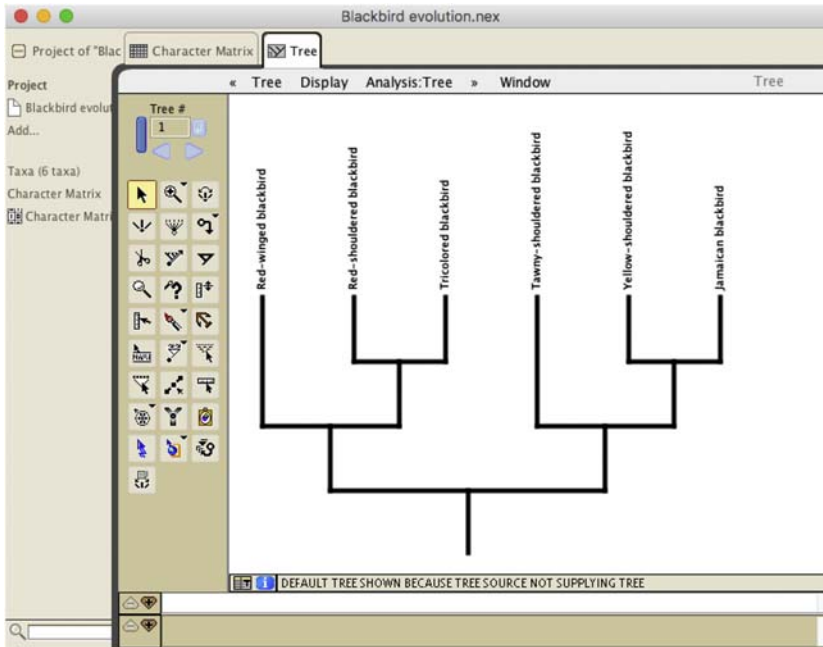


FIGURE 7.7

Default tree before moving branches.

Discrete character state reconstruction using parsimony

With the Tree Window open select Analysis:Tree > Trace Character History. Select Parsimony Ancestral States and hit OK. Ancestral states for our characters will appear on the branches (Fig. 7.8), similar to how they were shown in Fig. 7.2. You can look at the reconstructions of each character by clicking on the arrows in the Trace Character box (which is usually at the bottom left of your Tree Window). In Mesquite, branches are shown with the colors of all the possible states that they could have under parsimony. Thus if there are two most parsimonious scenarios on a branch, it will be shown as unresolved with both character state colors (e.g., white and black). When you move the cursor over a branch, you will see the character state or states at the bottom of the Trace Character box. You may have to move this box up to see its bottom, or so it does not obscure parts of your tree.

Now we are ready to interpret the evolution of our characters on the blackbird phylogeny. For each character, which state is ancestral and which is more recently derived? Do any character state changes seem correlated with each other, and do any characters seem likely to influence each other? Do any characters exhibit multiple possible evolutionary scenarios that are equally parsimonious? How will we

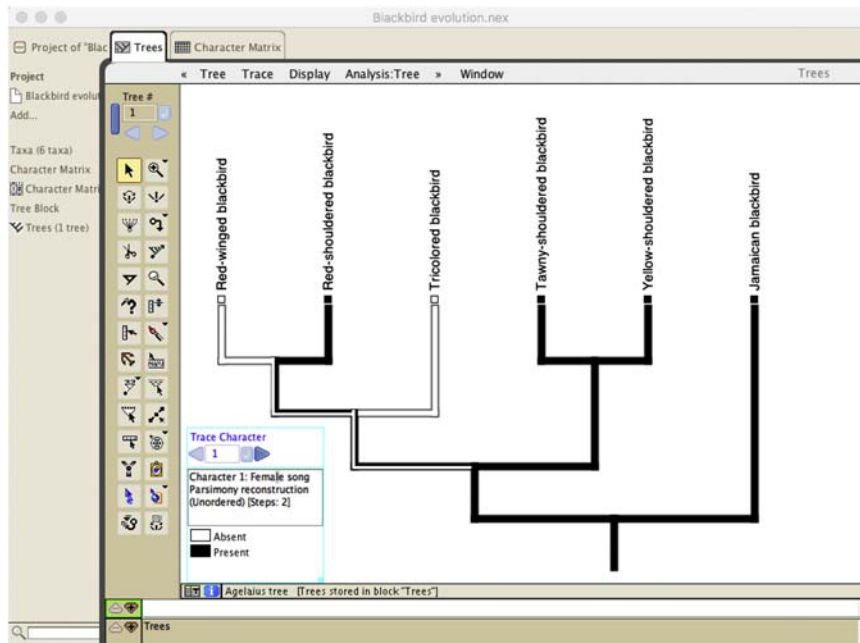


FIGURE 7.8

Ancestral states for female song reconstructed on the blackbird phylogeny.

resolve these? Note that the tree itself is a hypothesized phylogeny, so these evolutionary changes are also hypotheses.

If you change a character state in your matrix, the results will automatically appear in your tree (i.e., the tree is “hot linked” to the character matrix). Go to the Character Matrix window and change the character state of the character you are currently tracing in your tree window for one of the taxa. The character state will automatically change in the tree window. This is a general feature of Mesquite; whenever you change something in one window, it will affect what is happening in all the windows that are related to it.

The abovementioned description of Mesquite covers only a small fraction of the functions it offers for evolutionary studies. The best way to learn Mesquite is to play with it. Students may also want to spend some time going through the Mesquite manual at <http://www.mesquiteproject.org/>.

Second Edition

Exploring Animal Behavior in Laboratory and Field

Edited by Heather Zimbler-DeLorenzo and Susan W. Margulis

Exploring Animal Behavior in Laboratory and Field, Second Edition provides a comprehensive manual on animal behavior laboratory activities. This new edition brings together basic research and methods, presenting applications and problem-solving techniques. It provides all the details to successfully run designed activities while also offering flexibility and ease in setup. The exercises in this volume address animal behavior at all levels, describing behavior, theory, application, and communication. This is an important resource for student educators, researchers, and practitioners who want to explore and study animal behavior.

The field of animal behavior has changed dramatically in the past 15 - 20 years, including a greater use and availability of technology and statistical tools. In addition, animal behavior has taken on a more applied role in the last decade, with a greater emphasis on conservation and applied behavior, hence the necessity for new resources on the topic.

Key Features

- Offers an up-to-date representation of animal behavior
- Examines ethics and approvals for the study of vertebrate animals
- Includes contributions from a large field of expertise in the Animal Behavior Society
- Provides a flexible resource that can be used as a laboratory manual or in a flipped classroom setting

About the Editors

Heather Zimbler-DeLorenzo is currently an Associate Professor of Biology at Perimeter College at Georgia State University. She received her PhD in Biological Sciences from Auburn University. Dr. Zimbler-DeLorenzo has been a member of the Animal Behavior Society since 2004 and held the position as Chair of the Education Committee for Animal Behavior Society from 2018 to 2021.

Susan W. Margulis is currently a Professor and Department Chair of Animal Behavior, Ecology, and Conservation and a Professor of Biology at Canisius College. Dr. Margulis received her PhD from the University of Chicago in Evolutionary Biology. She has been a member of the Animal Behavior Society since 1980 and has served on the Society's Education Committee and Executive Committee.



ACADEMIC PRESS

An imprint of Elsevier
elsevier.com/books-and-journals

ISBN 978-0-12-821410-7



9 780128 214107