

## Exercise 4: Phylogenetics of Insects

### Introduction

One of the most profound implications of Darwin's Theory of Evolution by Natural Selection is that all life can be traced back to a single ancestor. The tree of life may never be fully resolved, but there have been advances in analytical techniques that have enhanced our view of evolutionary history. The reconstruction of invertebrate evolutionary history, and in particular, that of the arthropods, has a controversial history, and debate still surrounds hypotheses of arthropod phylogeny. Within the arthropods, phylogeneticists agree that the major lineages are monophyletic. We have examined similarities and differences among orders of Hexapoda, to both examine evolutionary relationships and to develop a classification system of hexapods. **Phylogenetic analysis** is the examination of such relationships, and the development of a classification system arises from the best-supported hypothetical cladograms.

To gain a better appreciation of insect classification, phylogeny, and biodiversity you will perform a phylogenetic analysis of Class Insecta. You will also use this opportunity to increase your familiarity with insects. Using parsimony, we will assume that if two taxa, at whatever level, share a certain characteristic, that characteristic was inherited from a common ancestor in the past. The two taxa subsequently speciated and evolved other new characteristics. The greater the number of recently **derived** (or evolved) characters that are shared by two taxa, the more closely they are related.

This exercise will provide you with insights into the problems encountered when attempting to place a species into its most appropriate groups. From the knowledge we gain, either as neophyte taxonomists or as experienced evolutionary biologists, the process of assigning relationships is much the same. Keep in mind that in this exercise we will initially use external morphological features, but once identified to the order, we will use other information from our textbook to construct our phylogenies. Modern biologists use information from a whole range of biological disciplines, such as paleontology, comparative embryology, biochemistry, molecular biology, and genomics to reconstruct evolutionary history. The data from all of these disciplines can be compared and evaluated to form robust associations.

### Objectives:

1. To learn how phylogenetic analysis is used to explore the evolutionary history of organisms.
2. To explore the evolutionary history of insects, and understand the shared, derived characteristics of the major taxonomic groupings of insects above the order level.
3. To become more familiar with the 30 orders of insects.
4. To work as part of a team to select the morphological features important in determining evolutionary history of insects.
5. To construct a hypothesis for the evolution of the major groups of insects using phylogenetic analysis of morphological and other features, and to compare that hypothesis with published hypotheses.

### Materials:

- Representative adult insect specimens, descriptions, and figures from all orders of Class Insecta
- Representative non-insect arthropods (Chilopoda, Diplopoda, Crustacea, or Chelicerata)
- Textbook Appendix

### Procedure

Recall that all of the organisms within a phylum will possess the characters that define the phylum, but a class within that phylum will also have specific defining class characteristics. Further, if two species within an order are closely related, they have evolved from a common

ancestor more recently, and hence, share more newly derived characters than organisms from two different orders. Organisms from two different orders may only share unmodified, or ancestral characters that define the class. Insects, in addition to possessing jointed appendages common to all arthropods, have more recently evolved other identifying characteristics that define the class.

The characters to use for an analysis of shared relationships are an important consideration for phylogenetic analysis. Not all potential characters are homologous (= similarity due to inheritance from a common ancestor), easy to characterize, or unambiguous to determine. One of your goals is to learn the **process** of phylogenetic analysis. Your performance will not be based on whether you correctly identify the evolutionary relationships; however, you will be evaluated according to how you think about the process of hierarchical classification. Remember that we can't directly observe real evolutionary relationships, so consequently the tree you construct is a **hypothesis** about the evolution of these organisms and can be tested by including more characters. If the new characters do not fit the tree, then the original hypothesis is rejected and a new one is constructed. Sound familiar?

- a. Begin by choosing morphological characters based on your knowledge and examination of insect external morphology. Consider carefully the characters you select. Consider the defining characteristics of Class Insecta – will they be helpful in separating orders within the Class? Characters that you might consider include tagmosis, number of wings, shape and pattern of forewings and hindwings, presence, shape, and number of segments of antennae, presence and number of tail structures (cerci, pincers, or filaments), type of mouthparts, structure of accessory mouthparts, type of development, shape and similarity of forelegs, midlegs, and hindlegs.
- b. Examine the representative species and note differences and similarities between them in a variety of characters that either I've identified or that you choose. For each specimen, record the state of each character (state is the actual form, or type, of a character).
- c. Include an **outgroup** in your analysis. An outgroup is an organism or taxa known to be closely related to the organisms in question, but not categorized with those organisms. The outgroup allows us to determine which states are shared and ancestral, and which are newly derived by assuming that all the shared states of the **ingroup** and outgroup are ancestral. In this way, the outgroup polarizes the ingroup; we know by comparison with the outgroup that the organisms comprising the ingroup are all more closely related to each other than they are to other organisms. Choice of outgroup is very important; choosing a group that is too distantly related or that is actually part of the ingroup could bias our results. I will help you choose an outgroup and ancestral characters that will help to unite the hexapods into one monophyletic group.
- d. Make a table and list the state of each character for each order (specimen). A certain character, such as the possession of wings, may be found in different numbers, or not at all in particular orders. You may choose to record the presence of absence of wings, or the actual number of wings, 0, 2, or 4.
- e. Identify the specimen to order and determine states of other characters that define the order. These may be developmental or physiological characters.
- f. While looking at your table, recognize states shared by more than one group. Shared states indicate a common ancestry, and sharing the same state of a character with the outgroup indicates a more primitive state. That particular state is thus old and organisms possessing the state belong to the same lineage. Shared, ancestral states will not help us to elucidate phylogenetic trees if the ingroup and outgroup possess it. I will suggest such a character for you to examine in the representative organisms

because it is an important trait in the evolution of both groups and you should recognize it as such. It will not assist you in constructing your tree.

- g. States not shared among all groups are derived, indicating that they are more recently evolved or the organisms possessing them belong to different lineages. Many character states will be derived and unique to a particular species. These will not help us very much to construct a phylogeny of Class Insecta when considering the order level. What we need are derived states that are shared by an entire order, which we can then use to separate that order from others in our phylogenetic tree.
- h. Compare the selected character states of the insects with those of the outgroup. Ancestral states shared by all specimens are also possessed by the outgroup, since the ingroup derived new states after splitting (evolving) from the outgroup. We know by comparison with the outgroup that we can polarize the ingroup. That is, our ingroup organisms are all more closely related to each other than they are to the outgroup. Character states thus found in many taxa of the ingroup and not at all in the outgroup should be considered shared and possibly ancestral within the ingroup.
- i. Choose a character shared by all members of the ingroup, but not by the outgroup to initially separate the outgroup from the ingroup. You now have two distinct groups; insects have been separated from other arthropods.
- j. You now have the basic method used to build your tree. The characters that have only two states, that is, shared (ancestral) and one derived state can be used to divide any group of taxa in two.
- k. Let's say we have an insect taxon that shares the character state of having no wings with the outgroup. We conclude, according to our protocol, that lacking wings is the ancestral state of that trait. For other characters, different states are possessed by other taxa that we cannot designate as ancestral or derived within the insect ingroup. Designate the first taxa, which lacks wings like the outgroup, as the new functional outgroup. Then examine the other characters and compare the states of each for the functional outgroup and the rest of the ingroup. Consider the states possessed by the functional outgroup ancestral within the original ingroup, and determine if any orders within the ingroup possess those states. If they do, you can readily subdivide the ingroup into two smaller groups based on a certain state shared by at least one taxon in the ingroup and the functional outgroup.
- l. This method helps you to consistently separate the ingroup into smaller and smaller groups by branching off certain taxa on your tree along the way. Keep repeating this process until all specimens (orders) are separated and are on a unique branch.

### Worked Example

- a. Use the example below in Table 4.1, which uses differences in molecular sequences, to practice the method. Data in the table represent states for six characters among six hypothetical species. There is at least one potential homoplasy in this table, and it is there to remind you to be aware of convergent evolution and evolutionary reversals while constructing your tree.
- b. Identify the most different, or ancestral, species, relative to the other groups, which will be our outgroup. In Table 4.1, this is Species A.
- c. Draw a tree with a short, main trunk to indicate shared ancestry of all six species. Near the bottom, draw a bifurcation, such that two branches separate Species A from the rest of the group. Species A is at the tip of one of these two branches; all the other species will be on branches of the other trunk. You have effectively polarized the ingroup (Species B through F).
- d. From within the remaining species to be judged, find a new "functional outgroup"

using the same process used in step “b.” Repeat the process of separating one species or a small group of species from the rest of the group, until all species are at the tip of their own branch.

<b>Table 4.1: States of six characters for six hypothetical species.</b>						
	Species A	Species B	Species C	Species D	Species E	Species F
Character 1	Absent	Absent	Absent	Absent	Present	Present
Character 2	0	0	2	2	4	4
Character 3	Single	Double	Double	Double, Bifurcated	Double, Bifurcated	Double, Bifurcated
Character 4	Absent	Absent	Present	Present	Present	Present
Character 5	6	8	8	8	8	8
Character 6	Single	Double	Triple	Triple	Double	Triple

**Present your results and interpretations as follows for Write-up #1**

- a. Turn in the table and the phylogenetic tree you and your partners constructed.
- b. Include a short paragraph that outlines the logic you employed to construct your tree and the decisions made along the way.
- c. Perform a self-analysis of your tree with the published phylogeny in your textbook (Fig. 7.2).