Elaiosomes and seed dispersal by ants

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Elaiosomes are fleshy appendages that are found on many sorts of seeds. No one is quite sure why they evolved. Because ants often carry such seeds back to their nests and eat the elaiosomes, leaving the seed apparently unharmed, one suggestion is that they evolved as a ‘reward’ for ants, encouraging them to disperse the seeds. An alternative proposal is that elaiosomes may help the seed to absorb and retain moisture for germination.

In this activity, students build a phylogenetic tree of plants from the Polygalaceae. Many of the species in this family produce seeds with elaiosomes. From the dated tree, students infer when elaiosomes might have evolved and compare this with when ants are known to have evolved and become abundant in ecosystems. This casts light on the possible reasons for the evolution of elaiosomes.

Ants or climate?

There are numerous examples of plant–insect associations, often with both parties benefitting from complex relationships that have co-evolved over millions of years. Whatever the benefits for each participant in the current Case Study, what were the selection pressures that contributed to the evolution of this ant–plant interaction? Because elaiosomes attract ants and cause them to disperse seeds that have them, one might assume that plants evolved these structures in response to the presence of ants and their potential as dispersers. However, some studies suggest that elaiosomes could also be involved in seed germination and water intake. Did the evolution of ants lead to the appearance of elaiosomes on seeds which then led to the dispersal of seeds by ants or did the elaiosomes evolve for some other reason before ants became common in ecosystems?

Chloroplast DNA

A dated evolutionary tree will provide an estimate when elaiosomes evolved and help students speculate about the ecological or environmental factors could have influenced the appearance of elaiosomes.

In the computer-based activity, students produce an evolutionary tree (a phylogeny) for members of the milkwort family, Polygalaceae, using 43 DNA sequences from the gene rbCL, which codes for the large subunit of the ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCo). The gene rbCL is the most widely used DNA region in plant molecular phylogenetics, because all plants have it and it is relatively easy to obtain DNA from the numerous chloroplasts where it is located.
The Polygalaceae is a relatively large family of about a thousand species of trees, shrubs, lianas and herbs distributed almost all over the world. This group also has a variety of seed and fruit dispersal systems, including dispersal by wind, birds, mammals and of course, ants. The Polygalaceae are closely related to three other families: the small groups Surianaceae and Quillajaceae and the very large pea family, Leguminosae, which is the third-largest family of flowering plants after orchids and daisies. Together these four families form the order Fabales.

Because the age of the Leguminosae is known from the fossil record, dates can be placed on the tree, providing a rough estimate of when elaiosomes evolved.

Molecular dating

A technique called molecular dating has been used to provide an estimate of the dates of nodes on the evolutionary tree.

In the current exercise, one fixed date which is known from the fossil record has been used as a starting point from which the other dates on the evolutionary tree have been estimated. The software used for this is called 'PATHd8' and although it is freely-available (see 'Scientific papers'), its UNIX-based command line interface is probably too complex for many school students to use. Several key dates have therefore been generated in advance and are supplied to students.

This exercise is in fact a greatly simplified version of work described in a research paper (Forest et al, 2007). In that work, DNA sequences from many more species were available, more sophisticated software was used and at least four known dates were used to help calculate the dates on the phylogenies. The current exercise is therefore a demonstration of the principle of molecular dating.

The molecular dating method assumes that the amount of change in DNA sequences is constant through time — in other words that DNA changes act as a ‘molecular clock’. You should be aware, however, that the basis of the molecular clock hypothesis is hotly-debated in the scientific community because many studies have shown that changes in the rate of mutation in DNA sequences vary greatly through time — and thus invalidate the very basis of the molecular clock hypothesis.

Outline of the activity

There are three parts to the activity:

- Building a phylogenetic tree of family Polygalaceae with closely-related families from order Fabales used as outgroup taxa (Surianaceae, Quillajaceae and Leguminosae) using the Neighbor-Joining algorithm in Geneious;
• Mapping seed/fruit types onto the resulting phylogenetic tree to determine where on the tree seeds with elaiosomes first appeared. This is done manually using coloured pens;
• Dating the phylogenetic tree using some key dates that have been generated using other software and are provided.

After the practical exercise, a discussion of how climatic changes can lead to evolutionary innovations would be appropriate, but it is important to emphasise that the changes induced by human activities that we witness today are too sudden to allow adaptation of most species to new environmental conditions; they will probably lead to mass extinctions.

**General reading**


*Reading the story in DNA: A beginner’s guide to molecular evolution* by Lindell Bromham (2008) Oxford University Press (Paperback) ISBN: 978 0199290918. An engaging textbook on molecular evolution, which assumes no specialist mathematical knowledge and takes the reader from first principles. Although it’s aimed at undergraduates, this superb book contains sufficient detail for PhD students, yet parts will appeal equally to 16–19 year-olds.

*Evolution of the insects* by D. Grimaldi and M.S. Engel (2005) Cambridge University Press, Cambridge. ISBN: 978 0521821490. One of the most beautiful and complete books on the evolution of insects. With the help of the most up-to-date molecular phylogenetic trees and more than 900 illustrations and photographs, the authors cover the breadth of insect diversity.

**Scientific publications**

Most of these publications require paid access.


Rutschmann, F. (2006) Molecular dating of phylogenetic trees: A brief review of current methods that estimate divergence times. *Diversity and Distributions* 12 (1) 35–48. doi: 10.1111/j.1366-9516.2006.00210.x. Note that PATHd8 is not mentioned in this review because it predates that software, but the concept behind this programme and an earlier version of it are discussed.
Requirements

Software
The software required, Geneious, can be downloaded free-of-charge from: www.geneious.com. The software is available for Windows, Macintosh and Linux operating systems. Only the free, ‘basic’ version of the software is required for this activity.

DNA sequence data
Students will need the Geneious document containing 43 aligned DNA sequences from Polygalaceae and some related plant families: MilkwortDNAdata.geneious

Students’ worksheets
Students will require copies of Student’s Guide, pages 2–9. Each student or working group will need three different-coloured pencils or pens.

Presentations
Teachers may find the QuickTime animation and PowerPoint or Keynote presentations helpful for introducing this exercise. QuickTime may be downloaded free-of-charge from the Apple web site: www.apple.com/quicktime

Educational aims
The activity reinforces students’ understanding of DNA structure. It introduces the principle of alignment, generating an evolutionary tree from molecular data and using Molecular clock to date such trees.

It encourages students to speculate about the interactions between species and the ecological and environmental factors that shape their evolution.

Prerequisite knowledge
Students will need to understand the basic structure of DNA. They will need to know that chloroplasts have their own DNA and to be taught the principle of sequence alignment (the Introductory Activities, which are in a separate document, will be useful here).

Answers to the questions on the worksheets

a. This is shown in the side presentations and on the next page.
b. Between 52.3 and 60.0 million years ago.
c. Elaiosomes evolved before ants became abundant in ecosystems.
d. This suggests that elaiosomes initially evolved for some other purpose than providing ants with a ‘reward’.
e. Climate change may have been one of the factors that led to the evolution of elaiosomes in the Polygalaceae.
Results

Numbers represent millions of years ago

- **Fleshy seeds**
- **Elaiosomes**
- **Winged seeds**

The date of this split is assumed to be 59 million years ago.