DNA to Darwin Case Study

Elaiosomes and seed dispersal by ants

Version 2.0

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Insect–plant interactions

Insects evolved 400 million years ago and today they are found almost everywhere on Earth. Nearly a million species of insects have been described in the scientific literature, but this is only a fraction of the species that exist. Estimates of the number of insect species range from two to 30 million species. Many groups of insects are poorly known.

The interactions that insects have with plants are a vital part of their ecology. Roughly half of all species of insects eat plants at some stage in their life cycles. Many insect species use plants as shelter and some absorb plant defence chemicals and use them to defend themselves. About 85% of all plant species are pollinated by insects such as bees, flies, butterflies and beetles, while some plants use insects to disperse their seeds and to protect them against other insects and competitors.

Within the insects, there are more than 10,000 species of ants. These have evolved complex social behaviour and numerous and varied adaptations. Ants, together with bees and termites, are one of the most important groups of insects for the ecology of numerous ecosystems. In some tropical areas such as the Amazon basin, their total biomass can be four times that of vertebrate species living there.

In several ant species, some of the workers are used to store honeydew in their abdomen, immobilising them completely.

Weaver ants build nests with leaves and sew them together using the silk from ant larvae.

Leaf-cutter ants collect leaves which they bring back to their nest to use as growing medium for a fungus on which they feed.

Ants have very close relationships with plants. Many of these interactions involve protection of the plant by the ants while the plant provides shelter and nourishment in return. One of the most famous examples of such an interaction is that between acacia trees and ants. The ants protect the trees against herbivores and prevent other plant species from growing in the vicinity of the tree. In return, the tree provides shelter and food to its bodyguards; the ants live in the large hollow thorns on the tree and feed on its sugary sap.
Seed dispersal

The particular interaction in which we are interested here is the dispersal of seeds by ants, the importance of which is increasingly recognised in ecosystems such as in the Cape region of South Africa. Ants collect seeds and bring them to their nests. The advantages to the plant are not completely understood and many hypotheses have been put forward including the protection of the seeds from herbivores and the transfer of the seeds to the richer soil in the ant nest for germination. But what do the ants get out of this?

Seeds dispersed by ants have small structures on their outer surface called elaiosomes, which attract ants and are eaten by them. When ants eat these elaiosomes, the seed is not harmed and studies have shown that in some situations the seeds germinate more easily once the ants have removed the elaiosome.

Whatever are the benefits for each participant, how did this ant–plant interaction evolve? 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 before the ants? Because elaiosomes are used in dispersal by ants, 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. You can build and date an evolutionary tree to estimate when elaiosomes evolved. This will help to explain which ecological and environmental factors could have influenced the appearance of elaiosomes.

Outline of the activity

In this activity, you will create an evolutionary tree (a phylogeny) for members of the milkwort family, Polygalaceae, using the gene *rbcL*, which codes for the large subunit of the ribulose bisphosphate carboxylase (RuBisCo), an enzyme involved in carbon fixation in photosynthesis. The gene *rbcL* is the most widely used DNA region in plant molecular phylogenetics, because all plants have it and it is easy to obtain DNA from the 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, which may cast light on their interaction with ants.
Making a tree

1. Double-click on the document **MilkwortDNAdata.geneious**. This will start up Geneious and load the data into the programme. This file contains 43 DNA sequences that will be used to construct the phylogenetic tree of the Polygalaceae family and some related species. If you examine the sequences by zooming in on them, you will see that they are already aligned to each other so that the tree can be constructed.

2. Create a phylogenetic tree from the data by clicking on the **Tree** button at the top of the window. From the options on the panel that appears, select the **Jukes-Cantor** method, **Neighbour-Joining** and choose **Quillaja saponaria** as the outgroup. Then click **OK**.

Quillaja is the only genus of the family Quillajaceae and is thought to be the closest relative of the other three families in the order Fabales (Polygalaceae, Surianaceae and Leguminosae). The selection of an outgroup helps to show which events happened first, giving a ‘direction’ to the tree.
3. Adjust the presentation of the tree so that *Quillaja* sits at the bottom of the window, like this:

You can change the appearance of the tree by selecting different options in the menu panel to the right of the tree diagram.

If *Quillaja* is not at the foot of the tree, under *Formatting* in the menu panel, ensure that the **Ordering** is **decreasing**.

Also ensure that the tree has a root, by selecting **Show Root Branch** and moving the **Root Length** slider to the right, if necessary.
4. The next task is to work out where on the evolutionary tree elaiosomes first appeared. To do this, you must mark on the tree which type of fruit or seed is characteristic of each species. The information needed to do this (for the milkwort family only) is given in the table below:

<table>
<thead>
<tr>
<th>Seeds with elaiosomes</th>
<th>Fleshy fruits or seeds with a juicy appendage</th>
<th>Fruit or seed with a wing-like appendage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comesperma elusifolium</td>
<td>Atroxima afzeliana</td>
<td>Monnina xalapensis</td>
</tr>
<tr>
<td>Comesperma hispidulum</td>
<td>Atroxima liberica</td>
<td>Securidaca diversifolia</td>
</tr>
<tr>
<td>Heterosamara tatarinowii</td>
<td>Barnhartia floribunda</td>
<td>Securidaca virgata</td>
</tr>
<tr>
<td>Muralta angulosa</td>
<td>Carpolobia alba</td>
<td></td>
</tr>
<tr>
<td>Muralta heisteria</td>
<td>Carpolobia goetzii</td>
<td></td>
</tr>
<tr>
<td>Polygala alpicola</td>
<td>Eriandra fragrans</td>
<td></td>
</tr>
<tr>
<td>Polygala arillata</td>
<td>Moutabea acueta</td>
<td></td>
</tr>
<tr>
<td>Polygala bracteolata</td>
<td>Nylandta spinosa</td>
<td></td>
</tr>
<tr>
<td>Polygala chamaebuxus</td>
<td>Xanthophyllum affine</td>
<td></td>
</tr>
<tr>
<td>Polygala erioptera</td>
<td>Xanthophyllum octandrum</td>
<td></td>
</tr>
<tr>
<td>Polygala guyaquilensis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polygala ligustrioides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polygala lindheimeri</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polygala rehmannii</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polygala teretifolia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polygala vulgaris</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. On a paper print-out of the tree diagram (on the next page), draw a coloured dot or letter next to each living (extant) species, showing the type of seed or fruit that that species has, like this:

- Elaiosome
- Juicy fruit or seed
- Winged fruit or seed

6. Once each species in the tree has been assigned a fruit or seed type, you need to determine which type was probably found on the internal nodes of the tree, that is, on the ancestors of the species used to make the tree.

If two closely-related species share the same type of seed, then their most recent common ancestor (the first node shared by these two species) will have the same type (for example, node 1 in the figure below). If two closely-related species have different types of seed,
elaiosomes and seed dispersal by ants

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

Polygalaceae

Polygala alpicola
- Polygala vulgaris
- Polygala bracteolata
- Polygala teretifolia
- Polygala eriptera
- Polygala rehmannii
- Muraltia angulosa
- Nylandtia spinosa
- Muraltia heisteria
- Polygala chamaebuxus
- Polygala arillata
- Heterosamara tatarinowii
- Comesperma hispidulum
- Comesperma esulifolium
- Monnina xalapensis
- Polygala gewyquilensis
- Polygala lindheimeri
- Securidaca diversifolia
- Securidaca virgata
- Polygala ligustrioides
- Atroxima azeliana
- Atroxima liberica
- Carpolobia alba
- Carpolobia goetzii
- Eriandra fragrans
- Moutabea aceueta
- Barnhartia floribunda
- Xanthophyllum affine
- Xanthophyllum octandrum
- Bauhinia gilvum
- Bauhinia syringifolia
- Brenierea insignis
- Griffonia physocarpa
- Bauhinia galpinii
- Adenolobus pechuelii
- Cercis canadensis
- Cercis siliquastrum
- Bobgunnia fistulosa
- Swartzia cardiospermum
- Gleditsia trianthes
- Cadellia pentastylos
- Suriana maritima
- Quillaja saponaria

Leguminosae

Surianaceae

Quillajaceae (Outgroup)
however, then their most recent common ancestor should be marked with coloured dots of both types because it is not possible to say with certainty which one it had (for example, nodes 2 and 4, below).

If you have a node which is assigned two fruit or seed types and a species or node with only one (such as species D and G), then their most recent common ancestor will probably have had the type they have in common (as in nodes 3 and 5). Try to apply these principles to the members of the Polygalaceae family in the tree using information from the table.

7. Look at the tree and try to determine which branch the evolution of elaiosomes and occurred on (it is not possible to tell exactly).

8. Now you have an idea of where on the tree dispersal by ants first appeared, we need to estimate when this happened. Molecular ‘clock’ dating can be used to estimate this.

Here are the molecular clock estimates for the main nodes on the tree:

<table>
<thead>
<tr>
<th>Species 1</th>
<th>Species 2</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenolobus pechuelii</td>
<td>Gleditsia tricanthos</td>
<td>59.0</td>
</tr>
<tr>
<td>Xanthophyllum affine</td>
<td>Securidaca virgata</td>
<td>62.4</td>
</tr>
<tr>
<td>Barnhartia floibunda</td>
<td>Securidaca virgata</td>
<td>60.0</td>
</tr>
<tr>
<td>Atromixia afzeliana</td>
<td>Securidaca virgata</td>
<td>52.3</td>
</tr>
<tr>
<td>Heterosamara tatarinowii</td>
<td>Atroxima liberica</td>
<td>40.7</td>
</tr>
<tr>
<td>Heterosamara tatarinowii</td>
<td>Polygala rehmannii</td>
<td>35.3</td>
</tr>
<tr>
<td>Muralitia angulosa</td>
<td>Polygala rehmannii</td>
<td>34.1</td>
</tr>
<tr>
<td>Polygala alpicola</td>
<td>Polygala rehmannii</td>
<td>16.6</td>
</tr>
</tbody>
</table>

This table gives an estimate of when the first species (Species 1) shared a common ancestor with the second species (Species 2). The ‘time’ is in millions of years ago.

Write the times at the appropriate places on the branches of the tree. The first one (59 million years ago) has been done for you.
Questions

a. Where on the tree did elaiosomes evolve? (Hint: most species above this point/node will have elaiosomes; those below it will not).

b. Between which dates do elaiosomes appear to have evolved?

c. From the fossil record, we know that ants evolved about 100 million years ago but they were not as important in these ancient ecosystems as they are today. Amber deposits collected around the world show a marked increase in ant fossils towards the end of the Eocene epoch—about 40–45 million years ago. Was this before or after the appearance of elaiosomes?

d. Does this support the suggestion that elaiosomes evolved to provide ants with a ‘reward’?

e. About 50 million years ago, in the late Paleocene and early Eocene period, global temperatures on Earth reached high levels that have not been surpassed since. The sea surface temperature increased by 5–10 °C in 10,000 years, and one recent study suggests that at least at one site, rainfall declined by as much as 40%. As mentioned in the introduction to this Case Study, some research has suggested that elaiosomes may help in the absorption of water and seed germination. What does this suggest about the possible reasons for the evolution of elaiosomes?

Further information


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.

Antweb
Antweb is a unique source of information about ants, including photographs of nearly 6,000 species: www.antweb.org

Life in the undergrowth (2005) BBC Worldwide Ltd. Region 2, PAL: two DVDs. 2 Entertain Video. ASIN: B000ASALQA. The complete series presented by David Attenborough. 245 minutes. Episode 5, ‘Supersocieties’, deals with social insects, including ants.