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## Onion Plant as an Educational Tool for Phylogenetic Studies: Molecular Analysis and a New Phylogeny?

### Introduction

The laboratory curriculum of the International Biology Olympiad (IBO) involves activities in different areas of botany and zoology. In addition, every year some other areas, such as biochemistry, genetics, ethology and ecology are also included. The IBO curriculum, both at the theory and practical levels, includes topics at the higher secondary level. However, occasionally certain topics at graduate and postgraduate levels may also be included. This is especially true in the case of molecular biology, biochemistry and genetics. Designing challenging laboratories is an exciting experience for teachers and the rest of us involved in the olympiad preparation.

In planning experiments for the 1999 Training Camp, the onion plant was used to bring out several concepts in botany, encompassing morphology, anatomy and biochemistry. Towards this end, a comprehensive view was developed by linking the above aspects. An attempt has also been made to view the onion plant belonging to the family Liliaceae in monocotyledons phylogenetically. A word of caution is needed here. Evolutionary linkages are established by examining commonality or otherwise of several characters and not merely of one or two, as done in this work. But, this approach can give insights into the evolutionary thinking leading to the construction of phylogenetic trees. It can also serve as an useful laboratory exercise. This approach to biology teaching, especially in the more conventional areas of botany and zoology, also helps students to establish structure-function relationships at all levels. Perhaps teachers could also look at other organisms from this perspective.

#### Keywords

Onion plant, evolution of monocots, cladograms, onion phylogenetics, molecular phylogenetics.

**Task 1:** Students were provided with an entire onion plant with roots, bulb and green aerial leaves. In the first part, they were



asked to take longitudinal cuts of the entire plant and draw and label the different parts. This task is carried out at the school/college level, too.

Students had difficulties in identifying the modified part, *bulb*, of the onion plant. They considered the bulb as a stem or a root modification, as revealed by perusal of their answer sheets. However, the longitudinal cuts of the bulb region with attached green leaves reveal that the underground bulb of onion is made up of bases of the aerial leaves, which have become modified – swollen – for storage purposes (*Figure 1*).

What can be the reasons for this confusion in students' understanding of this simple anatomical feature?

**Task 2:** Next, students were required to take a transverse section (TS) of the green and scale-leaves.

TS of green leaves showed the presence of cylindrical, palisade-like cells and round, spongy parenchymatous cells. The presence of palisade-like cells in the green leaves is to be noted since onion belongs to Liliaceae family of monocotyledons. Usually, well-developed palisade tissue is absent in monocots. In contrast, palisade layer is compactly arranged with isodiametric cells in dicots. A layer of palisade-like cells is also observed in some other monocots, such as coconut and grass.

TS of scale-leaves showed only spongy parenchymatous cells (*Figure 2a and 2b*).

*How does one explain to the presence of palisade-like tissue in the green leaves of onion, a monocot?*

**Task 3:** Students were asked to perform biochemical tests on green and scale-leaves. This was done to find out the nature of photosynthetic and storage products. Accor-

**Figure 1. Longitudinal section of an onion plant.**

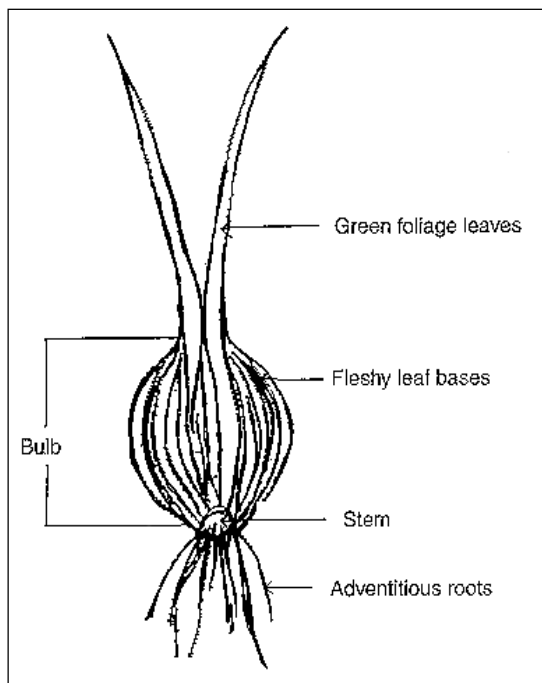
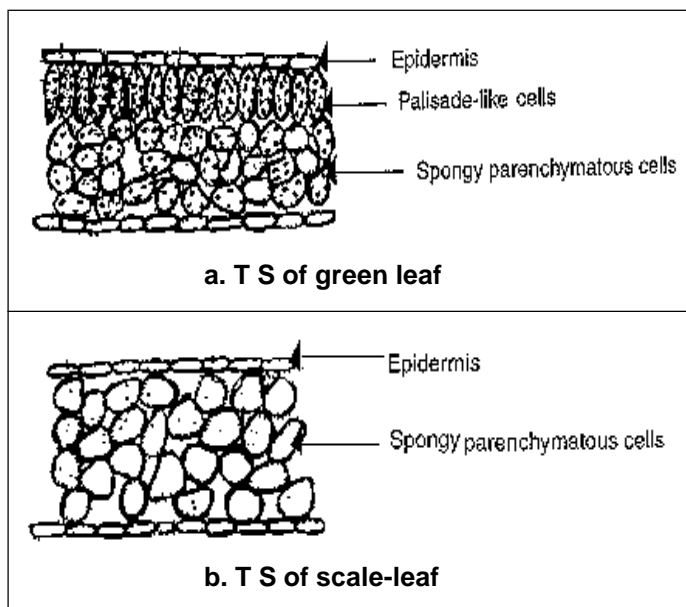


Figure 2.



ding to the given procedure, they had to place TS (or small bits of tissues) of both types of leaves on different slides or in test tubes and test for glucose or starch (*Box 1*).

### Box 1.

**1. Test for starch:** Material + dil.aqueous Lugol's iodine @ blue or blackish colouration indicates the presence of starch.

**2. Test for glucose:** Material + Benedict's solution, warmed over a gentle flame @ orange colour indicates the presence of glucose.

The above-mentioned tests are routine biochemical tests.

- a) Scale leaves are directly used as test material.
- b) For green foliage leaves, pre-treatment of the leaf material is necessary as follows:

Boil the green leaves for 10-15 minutes in water in a small beaker on a hot plate. This preliminary step softens the leaves by breaking down the cell wall. Transfer these softened leaves to a beaker and immerse in ethyl or methyl alcohol. Again warm. This is done to blanch the leaves. Chlorophyll is soluble in alcohol and is extracted from the leaves in about 5 minutes. Thick leaves might take longer. Wash the blanched leaves in water. Spread them in watch glass or slide and then test for starch or glucose as mentioned above.

Both green and scale leaves of onion test *positive* for glucose.



This activity brought out the presence of glucose in both green and scale storage leaves. This confirms that the onion plant photosynthesises and stores its food in the form of glucose. This is also true for monocotyledons, especially the members of Liliaceae. As an extension, one can repeat this task with dicotyledonous green leaves and their storage organs to determine the photosynthetic and storage products. Most dicotyledons convert glucose to starch in the leaves and the storage product is also starch.

*Is it possible to link up the growth pattern of onion plant and, in turn, its energy metabolism, to its biochemistry?*

### Discussion

Let us weave together all the observations of the above tasks. Three main points emerge:

1. Books play an important role in clarifying scientific concepts. Several texts analysed by us describe onion bulb as a modified shoot, as seen in *Box 2*. This description confuses students regarding the exact nature of the modified part, i.e., the lower parts of the green leaves. Out of the seven definitions in the box the one by Campbell, Reece and Mitchell is the least confusing. "...Bulbs are vertical, underground shoots consisting mostly of the swollen bases of leaves that store food".

(2) The presence of palisade-like tissue in the green leaves of this monocotyledon is unexpected; it gives hints about the phylogenetic position of the onion plant. Phylogeny refers to positioning of any group of organisms with respect to its evolutionary history (*Box 3*). Since the mid-1880's, attempts have been made to work out the phylogenetic position of different families of monocotyledons and dicotyledons. A variety of criteria, the structure and anatomy of the leaf and its venation, the flower (its prominence or its reduction), roots, septal nectaries, cotyledons, etc. have been used for this purpose. But considering the divergence of opinions based on overlaps and exceptions of different morphological characters, the broad pattern of evolution



### Box 2. Descriptions of Onion Bulb in Different Books

Bulb is defined as...

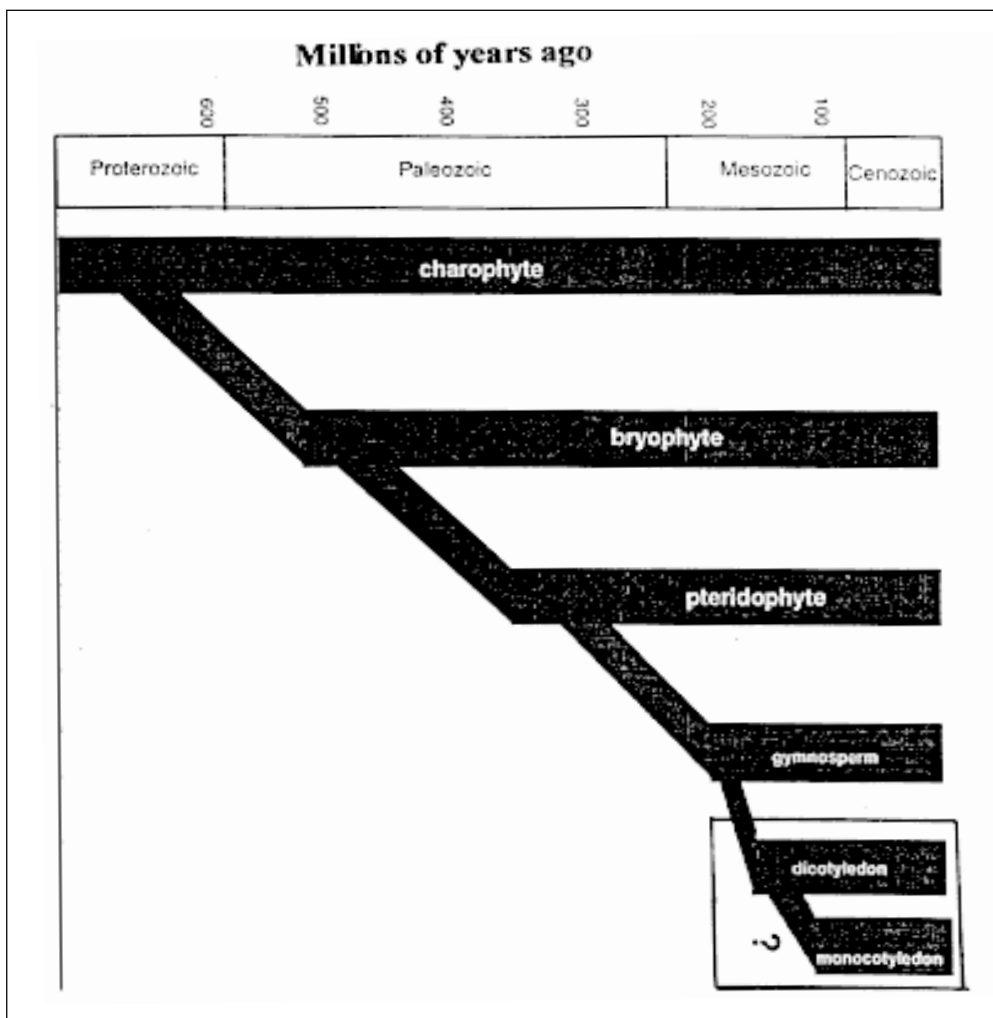
- 1) Oxford Dictionary of Biology; 1999: "...modified shoot with a short flattened stem".
- 2) Dictionary of biology by M S Sood; 1998: "...specialised underground bud with thick, fleshy leaves".
- 3) Biological Science by N P O Green, G W Stout and D J Taylor; 2000: "...is a modified shoot".
- 4) Biology: Part I; Std. XII textbook of NCERT; 1997: "A bulb is characterised by the presence of a highly condensed and discoidal stem and large number of fleshy scale leaves".
- 5) Botany for Degree Students by A C Dutta; 1996: "...is an underground modified shoot (rather a single, often large, terminal bud) consisting of a shortened convex or slightly conical stem, a terminal bud and numerous scale leaves".
- 6) Oxford Secondary Science: *Life Sciences* by Francis Fanthome; 1984: "... is a modified underground shoot"
- 7) Biology by N A Campbell, J B Reece, L G Mitchell; 1999: "Bulbs are vertical, underground shoots consisting mostly of the swollen bases of leaves that store food."

of monocotyledons and dicotyledons remains unclear (*Figure 3*). One school of taxonomists has considered monocots to be a distinct lineage of angiosperms, the other being dicotyledons as

### Box 3. Phylogenetic Trees

The evolutionary relationships among a group of taxa are conveniently illustrated by means of a phylogenetic tree, also called a *dendrogram*. The branching pattern of the tree is referred to as its *topology*. In a *rooted tree* the tree is connected to a *root* from which a unique evolutionary path leads to all other nodes. An *unrooted tree* does not have such a root and it only specifies the relationship between all the units in it. Thus an unrooted tree cannot really be considered a phylogenetic tree as it lacks the arrow of time. There are many methods of constructing trees, and some researchers claim that trees made on the basis of DNA sequence data may better reflect ancestor-descendent relationships among taxa than those based on morphological traits. In the literature you will find two vehemently opposed camps – that of the '*cladists*' and '*pheneticists*'. Cladists construct *cladograms* which are only concerned about the number of branches, which branch connects to which branch, and the branching order. Pheneticists construct *phenograms* which are concerned with the degree of similarity between organisms. Cladograms and phenograms will be identical only when there is a linear relationship between evolutionary time and the degree of dissimilarity between taxa. The *maximum parsimony* method is an example of the method used to construct cladograms and UPGMA (*unweighted pair-group method with arithmetic mean*) is similarly an example of the method used to construct phenograms. Cladograms can also be constructed using *maximum likelihood* methods.





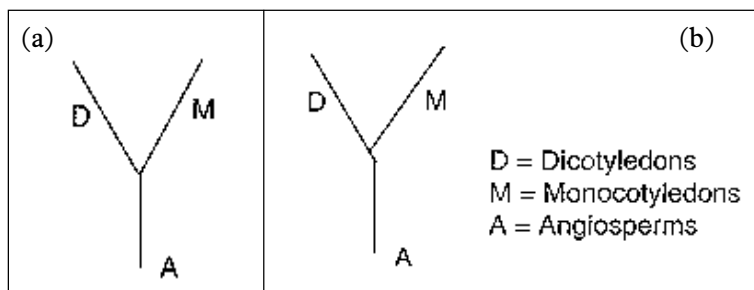
**Figure 3. Probable cladogram of plant kingdom.**

seen in *Figure 4a*. According to another viewpoint, monocots are considered to have evolved from primitive dicots. Hence the monocots must follow rather than precede the dicots in any proper linear sequence. This diversification of monocots from dicots is supposed to have taken place early in the evolution of angiosperms as in *Figure 4b*.

With this background information based on morphological and anatomical characters, teachers and students can dwell on the phylogenetic position of onion belonging to Liliaceae. Where would they place this plant, having some characters of monocots



**Figure 4. Probable branching patterns of angiosperms.**



and some of dicots? The presence of palisade-like tissue in the green leaf suggests proximity of the onion plant to dicots. The onion plant could also be considered to have split early from dicots in the evolution of seed plants. To support this argument, the many characteristics of monocotyledons, such as parallel-venation, absence of cambium, dissected stele, and adventitious roots are all considered as *apomorphic* characters, that is phenotypic characters that have evolved after a subsidiary branch diverged from a major branch of a phylogenetic tree. Morphological characters of some other Liliaceae members, like *Smilax* and *Dioscorea*, also suggest the proximity of this family to dicots. (On the other hand, viewed from the clade<sup>1</sup> occupied by dicots, several families of dicots, such as Ranunculaceae, e.g. *Clematis* share characters with monocots, again giving hints about their phylogenetic position.) Thus the onion plant, and several Liliaceae members, could be considered as a connecting link between dicotyledons and monocotyledons.

<sup>1</sup> A group of organisms evolved from common ancestors.

(3) The presence of glucose (as an energy reservoir) in both green and swollen storage tissues of onion is a characteristic of monocotyledons. In contrast, starch is the end storage product in dicots, with a few exceptions, e.g., sucrose is the storage product in sugar beet.

Starch biosynthesis has been studied in great detail. It is known that the enzyme AGPase, i.e., ADP Glucosyl Pyrophosphorylase facilitates the first step in the conversion of glucose to starch. This and other starch synthesizing enzymes are absent or inhibited in monocots. This leads to accumulation of glucose as the storage product. Lack of enzyme machinery for starch



biosynthesis is responsible for the difference of storage products in dicots and monocots. This biochemistry supports the bi/annual growth pattern of most monocotyledons, which necessitates a quick supply of energy easily provided by glucose (in contrast to starch, which needs a complex converting mechanism).

### Conclusion

The origin, evolution and nature of flowering plants intrigued botanists even before Darwin. In attempts to answer these questions, in the early 19th century, people studied the gross morphology and anatomy of plants, and generated several phylogenetic schemes (trees). However, they soon realised the incongruence of these trees with several overlaps and gaps. Now molecular biology seems to be changing the entire systematics scene, not only in botany but also in the entire living domain, including microorganisms. In the 1960's, Carl Woese of the University of Illinois, Urbana, USA, pioneered the use of ribosomal RNAs to classify bacteria. In the mid-1990's, plant molecular taxonomists relied on comparison of single gene sequences, such as the chloroplast-based *rbcL* or a nuclear gene encoding the 18S RNA of cytoplasmic ribosome (*18S rDNA*), among different plants to construct evolutionary trees. However, they soon realised that more reliable evolutionary trees could be obtained by comparing large number of genes in hundreds of plant types. Hence data from multiple genes were put together and statistically analysed using sophisticated computer software.

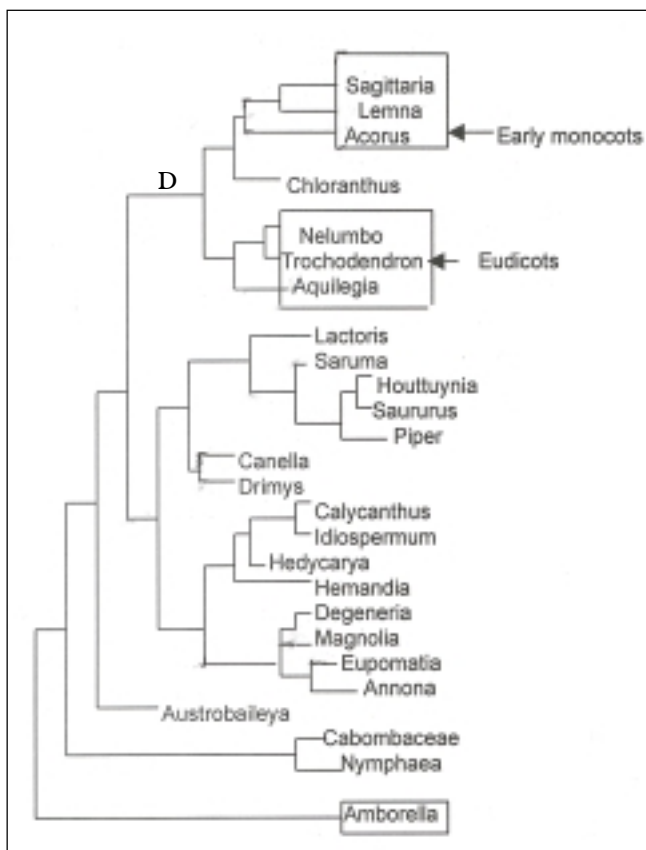
In the context of phylogenetic trees involving the origins of angiosperms, including the monocots, in recent years two groups working separately have come up with important insights leading to a consensus regarding the root of the angiosperms. Sarah Mathews and Michael Donoghue from the Department of Organismic and Evolutionary Biology of the Harvard University, USA, have used the phytochrome genes, *PHYA* and *PHYC*, which are duplicate genes (*Figure 5*). On the other hand, data from two plastid genes (*rbcL* and *atpB*) and one nuclear gene



**Figure 5. Consensus tree from analysis of *PHYA* and *PHYB* sequences.**

**Note the positions of *Amborella*, early monocots and eudicots.**

(after S Mathews and M Donoghue, 1999. Statistical values at different levels of the tree have been omitted.)



(*18S rDNA*) were pooled together by Pamela and Douglas Soltis from Washington State University, Pullman, USA, and Mark Chase from the Jodrell Laboratory attached to the Royal Botanical Gardens, Kew, UK. Both these groups have used different genes, compared their sequences in several hundred plant species, and arrived at certain conclusions, with special focus on the basal region of their respective trees. Their results show that *Amborella* is at the root of the angiosperm tree, with *Nymphaea* and *Austrobaileya* occupying the first branches of this tree. This is partially indicated in the phylogenetic tree of Mathews and Donoghue using *PHYA* and *PHYC* genes (Figure 5).

Both these groups of scientists have also positioned the early monocots and eudicots, which are the more recent and dominant dicots, on their respective phylogenetic trees. (To be precise,



### Box 4. How to Make a Cladogram?

A large number of phylogenetic trees of angiosperms, based on morphological characters, especially the structure of the flower, have been put forward at varied times by different workers, such as Adolf Engler, A P de Candolle, G Bentham and J D Hooker, C E Bessey, J Hutchinson, A L Takhtanjan and A Cronquist. Soon the weaknesses of these trees became apparent with overlaps and gaps in characters across branches. In 1950, Willi Hennig, an entomologist, came up with the cladistic method of classification of different organisms based on their evolutionary history. Cladistics is based on determining the branching points in a phylogenetic tree and delineates groups of individuals/organisms on the basis of characters with common origins. The resulting phylogenetic tree is called a cladogram, with branches known as clades, emerging from branch-points called nodes. This method acquired importance in 1980s with extensive use of computers and statistical tools.

#### Method of Constructing a Cladogram:

a) Choose taxa or groups of organisms, which interest you. They should have a monophyletic origin (origin in a single ancestral species), with taxa lying on different clades.

(For example, consider maize, *Pinus*, fern and *Riccia*.)

(b) Zero down on some striking heritable characters. Check if these characters are present or absent in the chosen taxa. Take care to see that all taxa are unique.

In this example, the heritable characters are:

1. Multicellular embryos in maize, *Pinus*, fern and *Riccia*.
2. Seeds in maize and *Pinus*.
3. Conducting tissue in maize, *Pinus* and fern.
4. Double fertilization in maize.
5. True root system in maize, *Pinus* and fern.
6. Secondary growth in maize and *Pinus*.

(c) Next, the nature of characters, original or derived, technically called polarity of characters, is determined. The absence or presence of these characters in the outgroup branch (terminal taxon(s) used to root the cladogram) helps in determining the polarity of characters.

Consider Charophyte as an outgroup taxa (O) showing the presence of chlorophyll. This character is also present in the taxa, maize, *Pinus*, fern and *Riccia*. Next; polarity analysis is done as in Table 1.

(d) Taxa are grouped according to their shared, *derived* characters, i.e., synapomorphic characters. Care has to be taken NOT to include primitive or ancestral characters in these taxa.

Box 4. continued...

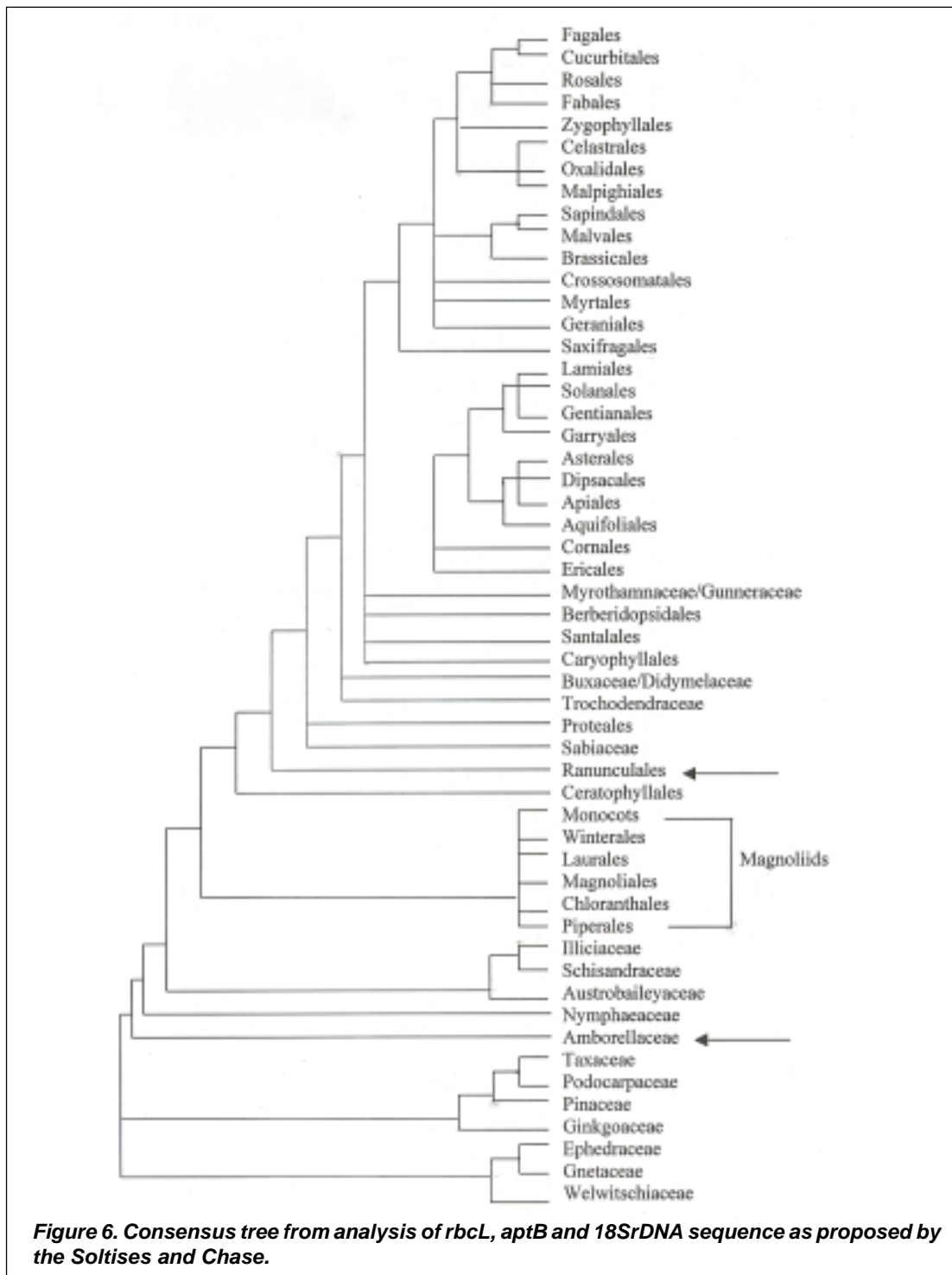




these are networks or unrooted trees where the ancestor (=root) has not yet been established. An unrooted tree can also be rooted on any of its branches.) For instance, Mathews and Donoghue have positioned the monocots somewhere at the top of the network of *PHYA* and *PHYC*. These monocots – *Sagittaria*, *Lemna* and *Acorus* – have diverged from the same branch (D in *Figure 5*), which has given rise to eudicots (*Nelumbo*, *Trochodendron* and *Aquilegia*). Soltis and Chase have refined the position of different monocots, including Liliales containing the progenitor of the present-day onion, though not with a very high degree of confidence. Pooling the databases of two plastids and a nuclear gene, they have assigned “... *Acorus* as a sister branch that has given rise to the rest of the monocots. Further, Alistamales is the next branching monocot, sister to a large clade containing six main lineages: Petrosaviaceae, Dioscoreales, Pandanales, Liliales, Asparagales and Commelinoids” (not shown in *Figure 6*). These monocot clades and their positioning, though largely related are weakly supported (<80%) in terms of overall confidence levels. Further, based on this work, some scientists have even questioned the division of angiosperms into monocots and dicots. According to them, monocots are derived from a group of dicots with monosulcate pollen as seen in Magnoliidae. Eudicots with triaperturate pollen are seen to emerge from this monosulcate clade. Among dicots, Ranunculales and Proteales are seen to form the base of eudicots and not Magnoliids as considered till recently based on morphological data (*Figure 6*). With this background information, scientists are hopeful of soon resolving major systematic challenges, such as monocot placement and gymnosperm ancestry. Of course, all this will be achieved with more gene databases and still powerful computer programs. To speed up this important task, a global consortium, called the Green Plant Phylogeny Research Coordination Group, has been set up.

The need is to create large-scale molecular data of monocots and all seed plants, to be followed by their synthesis into a congruent phylogenetic tree. This tree would bring out the extent of





congruence (agreement), if any, with the different phylogenetic trees of angiosperms obtained with morphological data. In the conflict between molecules and morphology, one should not be surprised if there are not many major shifts in the details of angiosperm classification. For example, in the case of classification of microbes, the entire 'tree of life' has been shaken up by the use of genome data. Biology Olympiad laboratory work has the potential of stirring students and teachers to think about these issues.

In a recent article in *Current Science*, P Pushpangadan from the National Botanical Research Institute, Lucknow and K Narayanan Nair from the Tropical Botanic Garden and Research Institute, Thiruvananthapuram, have recommended several strategies and challenges to enhance the future of systematics and biodiversity research in India. Among the many steps (p. 636), two are relevant to our discussion: Point 6: conducting integrated taxonomic research for multi-disciplinary characterisation of biogenetic resources at morphological, cytological, molecular and genetic levels. Point 8: capacity building and infrastructure development in classical and modern systematics. These two points are major long-time efforts requiring inputs of hundreds of scientists, teachers and students. Perhaps the current article could be used at college levels to initiate these efforts.

### Suggested Reading

- [1] A C Dutta, *Botany for degree students*, Oxford University Press, 2000.
- [2] N A Campbell, J B Reece and L G Mitchell, *Biology*, Addison Wesley Longman Inc., California, 1999.
- [3] Soltis S Pamela, Soltis E Douglas and M W Chase, Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology, *Nature*, 402, 402-404, 1999.
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