

Plant Systematics in the Age of Genomics

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As plant biologists enter a new era in which comparative genomics promises to address fundamental questions in botany, such as unraveling metabolic and regulatory networks, the inestimable value and usefulness of robust systematic studies quickly become clear. In simplest terms, systematic studies can indicate which genomes in the plant kingdom to search, sample, and study for the answers to questions relating to the evolution of chemical and physical structures and their synthesis or ontogeny. After several model and crop species have been sequenced, the next phase of plant genomics will necessarily build on new phylogenies that are greatly assisted by molecular techniques and whose interpretation and applications will be guided by “traditional” botanical knowledge.

Plant systematics was long considered to be an “artful science,” but well before the application of molecular techniques to systematics, semisubjective authority was supplanted by rapidly developing analytical methods and the computers that run them. In the age of genomics, the art of modern plant systematics lies in its applications and its links to other disciplines; conversely, the applications of genomics to an expanding array of plant species will be grounded in plant systematics, itself still based largely on field work and knowing the plants.

Much is new—and much is not—for plant systematics in the age of genomics. Molecular techniques have introduced vast and numerous independent data sets, and there are continual advances in preparing DNA, sequencing genes, aligning sequences, and designing software for interpreting the data. As a consequence of this increased accessibility, mainstream plant systematics has been able to incorporate molecular approaches, which no longer occupy a separate domain but rather constitute part of the normal repertoire of skills for systematic botanists.

The special usefulness of molecular approaches in analyzing phylogenetic relationships at higher ranks has resulted in still unresolved but clearly better and dramatically new classifications, discussed below. These developments, coupled with other advances in phylogenetic analysis, now place systematics in a key position among other disciplines in biology, with increasingly diverse and powerful applications in investigations of biosynthetic and developmental

pathways, natural products, origins and migrations of evolutionary lineages, and conservation. Whether it occupies the hub or spokes, more than ever plant systematics is needed to make the genomics wheel roll.

The biggest non-news is that molecular techniques have in fact not revolutionized methodologies in systematics. Instead, molecular data have rather rapidly been accommodated in existing analytical methods whose revolution—cladistics—had come and for some time had been the new order (Schuh, 2000). Once computers could be harnessed to execute complex pattern analysis and resample data thousands of times for statistical rigor, phylogenetic systematics or cladistics had overruled the authority represented by a few great intellects in favor of greater objectivity and more reproducible results. Systematists were already wrestling with issues about adequate sampling and about merging data sets before DNA sequences began flooding the market.

News perhaps for non-systematists, but not for systematists, is a greater need than ever before for traditional botanical knowledge and activities. Field work, collections, diversity surveys, floras, monographs, and conservation efforts still provide the primary means for working with the physical materials needed for investigations in systematics and genomics (e.g. fresh tissue for RNA extraction and synthesis of cDNA expressed sequence tag libraries), as well as for formulating hypotheses, interpreting the results, and making useful applications of those results, thereby linking the genes to the whole plants and the world outside them.

PHYLOGENETIC ANALYSIS

The field of plant systematics has undergone a renaissance during the last 20 years. This is due primarily to the incorporation of cladistic methodology and subsequently DNA sequence data into phylogenetic reconstruction, which is predicated on the recognition of monophyletic (“natural”) groups. Cladistics represents an approach whereby the data pertaining to terminals (in this discussion, taxonomic groups) are analyzed using the criterion of parsimony to minimize the number of ad hoc hypotheses needed to explain the pattern inherent in the data. The results are represented as “trees” that are termed “unrooted networks.” If the same data set is again analyzed by parsimony, the same answer will be obtained. When used for the purposes of phyloge-

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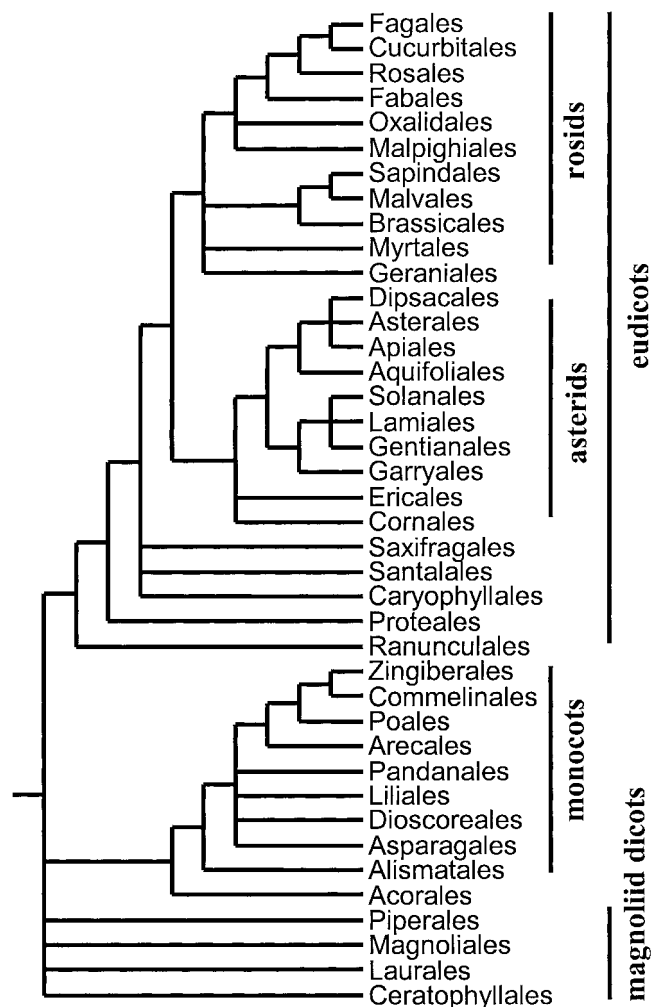


Figure 1. A conservative estimate of phylogenetic relationships among the orders of flowering plants (modified from Angiosperm Phylogeny Group, 1998). Those plants with a single cotyledon (monocots) are a monophyletic clade nested within at least two major lineages of dicots; the eudicots are further divided into rosids and asterids. *Arabidopsis* is a rosid eudicot classified within the order Brassicales; rice is a member of the monocot order Poales.

netic systematics, the tree is rooted between the ingroup (the group under study) and the outgroup, i.e. the group with which the ingroup is compared (compare with Schuh, 2000). This tree becomes a phylogenetic tree that constitutes a hypothesis of the genealogical relationships of the taxa under study and can be subjected to further tests with additional data.

Systematists use cladistics to produce phylogenetic trees that can be used for purposes of phylogenetic classifications. Such trees must remain as hypotheses because there is only one "true" evolutionary tree, which is based upon a long history of life on Earth, and even if we obtained that true tree we would not know it definitively because we cannot directly observe that history. We can assume, however, that such trees from phylogenetic analyses are nearer to the true tree than would be a randomly generated

tree, and these hypotheses of relationships provide our best estimate of genealogy and may be used for starting points for further research in systematics and for understanding the biology of the organisms under study (see section below on useful Web sites for links to phylogenetic studies, nomenclature, herbaria, and specialists).

NEW ALIGNMENTS

These phylogenetic studies and the resulting trees have had a substantial impact on systematists' hypotheses about plant relationships and ultimately on our systems of classification. The new cladograms of higher ranks, based largely on combined gene sequence data from an international team of botanists, differ in a number of fundamental ways from past classification systems.

Perhaps more than any in the previous half century, the comprehensive (down to the rank of family) classification system of flowering plants by Cronquist (1981) has been the most widely followed. That system divided the flowering plants into two major classes that will be familiar to most readers: Magnoliopsida (including all the dicotyledons or "dicots") and Liliopsida (including all the monocotyledons or "monocots"). The long-held notion that the flowering plants are first and foremost separated into monocots and dicots was not supported by cladistic analyses of morphological data, and the need for change was later confirmed by molecular phylogenetic studies. The monocots continue to be recognized as a monophyletic lineage, but that lineage is embedded among separate clades that are characterized by two cotyledons. This led to the realization that two cotyledons are in fact the generalized (i.e. "primitive") condition for seed plants as they occur also in cycads, *Ginkgo biloba*, many conifers, and the Gnetales, while having one cotyledon is uniquely derived (autapomorphic).

It appears that a more natural (monophyletic) grouping of the angiosperms would consist of the "eudicots" (see Fig. 1), with typically three pollen apertures; the monocots; and the magnoliid dicots, comprising several diverging lineages. This is harmonious with the formal higher rank classification proposed recently by an international team of plant systematists calling themselves the Angiosperm Phylogeny Group (1998). Theirs was the first attempt to realign the orders and families of flowering plants into a phylogenetic system based on an analysis of molecular data. A summary of that system is presented in the form of a phylogenetic tree or cladogram (Fig. 1 is modified from that); a number of helpful Web sites concerned with plant phylogenies, plant names, and botanical specialists and institutions are presented at the end of this article.

A SPECTRUM OF APPLICATIONS

Phylogenetic studies already have direct applications in agriculture, natural products chemistry, biomedical sciences, and other disciplines. In the age of genomics, the breadth and power of these applications will increase as interpretation of phylogenies helps to identify those taxa for which genomic treatments will answer fundamental and in some cases long-standing questions about metabolic and regulatory networks in all the evolutionary corners of the plant kingdom (e.g. Ohlrogge and Benning, 2000).

The evolutionary origin of symbiosis between plants and nitrogen-fixing bacteria living in root nodules represents an example of applications in agriculture. Known to occur in only 10 different flowering plant families, it had traditionally been thought that these families were only distantly related to one another. In the new system of angiosperm classification, however, all nitrogen-fixing angiosperms are members of only four ordinal clades (Fabales, Cucurbitales, Rosales, and Fagales) that are linked as a monophyletic group. This information suggests that the predisposition for root-nodule symbiosis and nitrogen fixation in angiosperms evolved only once (Soltis et al., 1995). From a phylogenetic perspective, therefore, the investigation of genes that contribute to nodule formation would be best studied within other genomes from this clade, rather than randomly throughout the angiosperms.

Phylogenetic trees are useful also in directing research in natural products chemistry. Taxol is a compound known only from the yew family (Taxaceae). If we wish to look for additional sources of taxol and other taxanes, rather than randomly sample the plant kingdom, we can focus on the nearest relatives as understood from a phylogenetic tree of the seed plants. This in turn leads us to investigate the Podocarpaceae because current analyses indicate that this is the sister group to the yew family, i.e. these two conifer families share a more recent common ancestor with each other than either does to any other plant family. That is indeed the case, and taxanes have recently been reported in the Podocarpaceae (Stahlhut et al., 1999).

Chemical information is often used in data sets for phylogenetic analysis (e.g. Stevenson, 1990a). The compound β -N-methylamino-L-Ala (BMAA, a non-protein alpha amino acid) was first discovered in the cycad genus *Cycas*; it binds to Glu receptors and is implicated in the ailment Guam Dementia. Subsequently, it has been demonstrated to occur in all other cycads examined and in no other plants (Norstog and Nicholls, 1997), so the shared derived compound BMAA is termed a "synapomorphy" for the cycads. It is also highly toxic to most herbivores. After Glu receptors were discovered in plants (Lam et al., 1998; Chiu et al., 1999), treatment of *Arabidopsis* with BMAA led to the discovery that a plant-derived neurotoxin may act as a signaling molecule

in plants, involved in the control of morphogenesis (Brenner et al., 2000). New collaborative studies on cycad genomics initiated by The New York Botanical Garden, Cold Spring Harbor Laboratories, and New York University are being used to identify cycad genes involved in the synthesis of this neurotoxin and to identify its potential target genes in plants, among other applications. In addition, because of the group's basal phylogenetic position, sequences from a cycad genome will be valuable for evolutionary studies.

Mustard oil glucosinolates are known to be produced in at least 15 different families of plants. Depending on the system of classification, the biochemical pathway for these secondary metabolites was thought to have arisen and then to have been lost on multiple occasions. Molecular phylogenetic information, however, indicates that all mustard oil-producing families are part of the same ordinal clade, Brassicales, with the exception of the genus *Drypetes* (a member of Malpighiales), indicating only two evolutionary origins for mustard glucosinolates (Rodman et al., 1993). Moreover, these mustard oils are derived from two different biosynthetic pathways, thus showing that even though the final product may be the same, the ways they were synthesized are not; hence they are not the same compounds in an evolutionary, i.e. historical, sense. This new information clearly has profound implications for the plant biologist interested in the genetic basis for mustard oil glucoside synthesis in the plant kingdom.

RECIPROCAL ILLUMINATION AND THE APPLICATIONS OF PHYLOGENETIC STUDIES

When morphological characters are used in a data matrix, they are coded as states of a character based upon presumed evolutionary homology, and phylogenetic analysis can be used to examine their developmental equivalence. The example of the two origins of the mustard oils demonstrates the problems of character coding based upon descriptors and of divorcing descriptors from process (Sattler, 1993; Weston, 2000). A descriptor may be accurate at a structural level but not at the developmental level, and this can be detected through phylogenetic analysis and subsequent "reciprocal illumination", the process of using the phylogenetic tree to test the notion that a character reappearing independently is not really the same (i.e. evolutionarily homologous) despite its apparent similarity.

An example of reciprocal illumination concerns the origin of storage products in the seeds of some monocotyledonous plants. There is a storage tissue derived from cells of the nucellus occurring in some monocots that is termed "perisperm" and is formed as storage tissue instead of the more typical endosperm. Phylogenetic analyses of the monocots using both morphological and molecular sequence data have

shown that perisperm has evolved three times within the monocots, thus indicating that not all perisperm is the same or minimally had the same history. Perisperm has been described for *Acorus*, the putative sister taxon to the rest of the monocots, for *Hydatella*, nested within the monocots, and for *Cyanastrum*, also nested within the monocots in a different clade from *Hydatella*. Recent work has demonstrated that the perisperm of *Acorus* is derived from the epidermis of the nucellus and that of *Hydatella* is derived from subepidermal layers of the nucellus, while *Cyanastrum* in fact does not even have perisperm but rather its seed storage tissue is derived from the chalazal tissue of the ovule (Rudall, 2000). Thus, as the tree indicated, these are not homologous but different in origin, despite having a similar function and appearance. The implication is that perhaps different genes are controlling storage tissue development in these three taxa.

Clearly, the construction of phylogenetic trees, tempered by testing with reciprocal analysis, is a powerful tool for understanding the biology of plants. This is taking on increasing importance for our understanding of development and processes; in turn, this knowledge can be applied to various purposes that include improving agriculture, exploring natural products chemistry, and contributing to biomedical science. In the current age of genomics, the applications of phylogenetic studies constitute one of several major criteria for deciding which plant genomes to tackle next.

CHOOSING CLADES

For future comparative genomics studies, the importance of classification systems based on solid phylogenetic information cannot be overemphasized. Zoologists have already recognized this fact and have recently begun to debate which mammalian species should be targeted for sequencing now that the human genome has been nearly completed. Candidates for new model taxa include the mouse (*Mus musculus*), rat (*Rattus norvegicus*), chimpanzee (*Pan troglodytes*), and rhesus monkey (*Macaca mulatta*). As O'Brien et al. (2001) pointed out, however, these candidate species may not necessarily be the best from a phylogenetic perspective, as they are all members of the same evolutionary lineage. Instead, the authors proposed a phylogenetically more even sampling that includes organisms rather distant from as well as quite close to human beings, while factoring in other criteria such as economic value, biomedical relevance, and genome size.

The same approach should be applied to plants. With the Arabidopsis genome sequenced (Arabidopsis Genome Initiative, 2000), and that of rice (*Oryza sativa*) nearing completion, we will soon be able to make direct comparisons between these two species, which are phylogenetically distant. Subsequent ge-

nome sequencing of other model plant species, such as tobacco (*Nicotiana tabacum*), snapdragon (*Antirrhinum majus*), corn (*Zea mays*), tomato (*Lycopersicon esculentum*), or *Gerbera jamesonii* is warranted, but it should be noted that these are all derived from the same common ancestor as Arabidopsis and rice. Selection of species for genomic studies must consider phylogenetic position, such that the sampling allows comparisons among closely related genomes as well as among distantly related ones throughout the plant kingdom. We propose that the next wave of model plants should include species from these three categories: (a) species not too distantly related to currently used model taxa (e.g. other members of the order Brassicales close to Arabidopsis); (b) representatives of species several lineages derived from the same common ancestor as Arabidopsis and rice but close to neither (e.g. Lamiales and Caryophyllales); and (c) angiosperms that are not descendants of the last common ancestor of rice and Arabidopsis, i.e. the point of divergence between the monocots (which include rice) and the eudicots (which include Arabidopsis). Examples of the latter are the Piperales and Laurales (see Fig. 1).

BACK TO BASICS

In the genomics age, molecular techniques represent an immensely powerful resource that, surprising to some, greatly increases rather than obviates the need for non-molecular data sets, the botanists who can interpret them, and the means for generating them, i.e. field work, floristic studies, herbarium collections, and the building blocks of basic taxonomy, morphology and anatomy. Sequences and cladograms are merely tools, and one has to know the plants in order to pose the questions, structure the sampling, select the characters (including which genes), and interpret the results. As the examples of applications discussed above show, cladograms often pose as many questions as they answer; there is no reciprocal illumination if there is no light on the other end.

Plant systematics provides molecular and phylogenetic investigations with the physical and informational *materia prima* for the data sets that make them tick, and this can be achieved only through greatly increased activity in basic taxonomic and floristic research, and vastly accelerated and better-coordinated programs of field work and plant collections. A useful relationship between systematics and those molecular and phylogenetic investigations depends heavily on accurately identified specimens, well-prepared ancillary collections (silica-dried material and others), and carefully planned sampling of taxa and/or populations representing the fullest possible range of geography, variation, and phylogeny in the group. Monographs and floras are the most efficient way of generating the needed information

and specimens, as well as putting into practice the extensive phylogenetic realignments now under way. Taxonomists are needed to execute the numerous nitty-gritty nomenclatural changes involved (Hamamel, 2001).

Returning to the example of the cycads, participation in long-term floristic projects (e.g. Stevenson, 1991) and years of field work ultimately powered what has followed. Field work has documented the diversity of the group, including the discovery of a new genus in this conspicuous group in recent years (Stevenson, 1990b). It produces the physical material that has formed the basis not only for monographic work in the family (e.g. Stevenson, 1992) but also for chemical and subsequently for molecular investigations. Work on the labyrinthine nomenclature of this group (Stevenson and Sabato, 1986), which contains a number of ornamentals, established which legitimate genus and species names are linked to which historic reference specimens. In turn, morphology- and anatomy-based taxonomic work, culminating in monographs and revisions (e.g. Hill, 1996), has provided the means for identifying the specimens being used for anything from floras to genomics. The data sets produced by the taxonomic work are the raw material for generating the cladograms that are making it possible to thoroughly sample the group (i.e. one taxon from each clade) for any subsequent investigation, including natural products (Norstog and Nicholls, 1997) and genomic and other molecular investigations (e.g. Brenner et al., 2000). Thorough sampling of the cycads and related groups for the presence of BMAA subsequent to its discovery in *Cycas circinalis* (Vega and Bell, 1967) tested and confirmed the hypothesis that this compound is unique to the cycads, resulting in the focus on that group by the current genomic investigations of cycads mentioned above.

CONSERVATION

Floristic work, built on taxonomy and phylogenetic analyses that often incorporate geographic data, drives plant conservation. Data on the distributions, habitat preferences, and population structures of organisms identify centers of endemism and of diversity, as well as rare and/or threatened species; this constitutes the first layer of information for setting conservation priorities.

Sound phylogenetic trees can establish another layer of information and analysis for maximizing the conservation of genetic diversity, the basis of biodiversity. Habitat destruction and threats of extinction continue while the major realignments of taxa highlight how much of the plant kingdom is still a biological black hole in many ways: as pointed out above, to date most plant taxa used in some way as model taxa fall in one clade, so entire sectors of plant diversity (e.g. families or genera endemic to a small

region) could go extinct before we have any notion of significant differences in chemistry, developmental processes, or even gene regulation. Because we cannot realistically save all areas from habitat destruction, phylogenetic studies can help us make intelligent choices that will allow us to conserve areas with the highest genetic and hence taxic diversity. It should also allow us to identify evolutionarily significant species whose DNA should be stored in banks; this will complement existing efforts to create DNA banks for rare and threatened species (e.g. Randell and Morden, 1999). It is also necessary to conserve the engines of evolution: phylogenetic analyses incorporating molecular data sets can help guide the protection of evolutionary processes by illuminating historical migrations and geographic and ecological origins.

CONCLUSIONS

During the 1980s, plant systematics underwent a decade-long revolution in methodology that has enabled the discipline to adapt itself to absorb and analyze the subsequent avalanche of molecular data sets, and now molecular techniques are part of the mainstream of plant systematics. In the age of genomics, the horizons of plant systematics have vastly expanded. New phylogenetic realignments and diversifying links to other disciplines give systematic studies an increasingly important role in targeting species for genomic treatments and other research that will help elucidate—and conserve—the evolution of metabolic pathways and developmental processes. Execution and interpretation of the entire effort will continue to be grounded in botanical field work and traditional botanical knowledge.

Useful Web Sites

A number of Web sites help link plant systematics more effectively to other disciplines; until relatively recently, even the “correct” name of a plant was the domain of a rather limited group of specialists who can navigate taxonomic nomenclature. Members of the scientific community as a whole now have far better access to accepted names and taxonomic synonyms. They can examine the most recent substantiated phylogenetic realignments and the literature that produces them. They can search more easily for the closest relatives of a given taxon and/or representatives of a given plant group in a geographic region. They can locate specialists in a given taxonomic group and botanists working in a given geographic area. The following are some of the more important Web sites for these purposes:

- *Index Herbariorum* (<http://www.nybg.org/bsci/ih/ih.html>), formerly available in only occasionally updated hard-copy editions but now continually updated on-line, is a database of information about all the world's herbaria. It can be searched by a

number of fields including country, city, and botanist; significantly for our purposes here, it can be searched by taxonomic group to locate specialists in that group.

- TROPICOS (<http://mobot.mobot.org/W3T/Search/vast.html>) is one of several sites (none of them completely flawless) that provide accepted names, authors, and publications of plant taxa. It also provides literature in which the names appear, some taxonomic synonyms, and lists of specimens and maps based on the holdings of the Missouri Botanical Garden herbarium.
 - International Plant Names Index (IPNI; <http://www.ipni.org/index.html>). IPNI, a collaborative effort of the Royal Botanic Gardens, Kew, The Harvard University Herbaria, and the Australian National Herbarium, has integrated the indexes of those three institutions to create a database (still being refined) of the names and associated basic bibliographic details of all seed plants. It can be searched by plant name, authors of names, publications, and collectors of the type collections.
 - Gray Index (<http://django.harvard.edu/grayindex/grayindex.htm>). The Gray Index of the Harvard University Herbaria is one of the three index resources that make up IPNI (see previous entry); it contains the records only of New World taxa published after 1886.
 - Diversity of Life Dot Org (<http://www.plantsystematics.org/javatree.htm>), a site under construction that treats plants and animals. For plants it starts with the three-gene, 567-taxon tree of Soltis et al. (2000) and is providing detailed cladograms of various plant groups and in-depth, heavily illustrated family treatments.
 - Angiosperm Phylogeny Website (<http://www.mobot.org/MOBOT/Research/APweb/>; Stevens, PF. Version 2 August 2001), launched in July 2001 and undergoing further construction, is the newest of a number of sites that provide the most recent available alignments in plant phylogeny (now following the Angiosperm Phylogeny Group, 1998) and the literature behind them. At this site, one can click on a terminal taxon (orders at this point) or internal node to obtain a listing and description of group members, key literature citations, and photos. One can also go directly to a description/discussion of the characters themselves, lists of accepted and synonymized families and orders, or related links.
 - TAXACOM (<http://usobi.org/archives/cgi-bin/wa.exe?SUBED1=taxacom&A=1>) is an electronic bulletin board/discussion group for news and issues related to systematics.
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