

PHYLOGENY OF *HELIANTHUS* AND RELATED GENERA

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Summary: Molecular phylogenetic studies have contributed significantly to our understanding of the phylogenetic relationships of *Helianthus*, although several problems remain to be resolved. Molecular data have resolved problems with the circumscription of *Helianthus*. Its sister group is *Phoebanthus*, a narrowly distributed genus of two species from the state of Florida. The sister group to the *Helianthus-Phoebanthus* clade is a diverse set of taxa that occurs in Mexico and South America. These include species now classified as part of the paraphyletic *Viguiera* as well as such distinctive genera as *Tithonia*, *Simsia*, *Pappobolus*, *Scalesia*, *Lagascea*, and *Alvordia*. Incongruence between results based on chloroplast and nuclear-based data sets suggests that hybridization has been involved in the evolutionary history of this group. The nearest outgroup to the clade containing *Helianthus* is composed of members of *Viguiera* sect. *Maculatae*, which are trees and shrubs of Mexico. Other basally diverging groups in the subtribe Helianthinae to which *Helianthus* belongs are primarily woody members now classified in *Viguiera* from Mexico and nearby areas. Within *Helianthus*, divergence appears to be recent, based on an overall lack of divergence between species. The chloroplast-based tree suggests that *Helianthus* includes four phylogenetic lineages, whereas the nuclear ITS sequence data suggests that the perennial species are paraphyletic relative to the rest of the genus, with basally diverging branches consisting of species confined to the southeastern U.S. Because there is a lack of divergence among many groups of species, more variable markers will be required to resolve fully relationships within *Helianthus*.

Introduction

The familiar domesticated sunflower, *Helianthus annuus*, has become a popular plant well known to agronomists and also to home decorators. Less well known is the fact that it is but one of some 50 species in a genus that is native to North America. Together with its close relative, *Phoebanthus*, *Helianthus* forms a distinctive lineage within the subtribe Helianthinae in which it is classified. Subtribe Helianthinae includes a total of about 400 species and has an entirely New World distribution, ranging from eastern North America to Chile and Argentina. The subtribe appears to have its origin in Mexico, and divergence among many of its members is probably recent.

Our view of the systematics of *Helianthus* and its relatives has been heavily influenced by the influx of molecular phylogenetic data (Fig. 1). An initial molecular data set (Schilling and Jansen, 1989; Schilling, 1997) was based on restriction site analysis of chloroplast DNA (cpDNA), which was one of the first approaches to have widespread use in plant phylogenetic studies. The resulting tree thus represents a maternal gene tree, because the chloroplast genome is mostly inherited maternally in flowering plants and is rarely if at all subject to recombination. A data set from sequencing of the nuclear ribosomal internal transcribed spacer region (ITS) has also been obtained (Schilling and Panero, 1996; Schilling et al., 1998), which provides a second, independent gene tree. The results from cpDNA and ITS analyses are mostly but not entirely congruent (Fig. 1).

Circumscription of *Helianthus*

The availability of molecular data has made it possible to refine with confidence the circumscription of *Helianthus*. As a result, several species or groups of species can certainly be excluded from it, and two species of questionable affinities have been included. As now circumscribed, *Helianthus* is geographically coherent, being restricted to North America, but it is not easily diagnosed morphologically. The key feature that classically identifies *Helianthus* within subtribe Helianthinae is the caducous pappus of two awns, with few or no intervening scales. *Helianthus* is also characterized by having pales that are trilobed at the apex. Both of these features are homoplasious in subtribe Helianthinae, and are found in other groups. Furthermore, they are both absent in one species now included in *Helianthus*, *H. porteri*. A third feature that is found throughout *Helianthus* is the well-developed terminal style appendage, but a similar structure is also observed in a few other members of the subtribe.

The relationships of *Helianthus* have also been clarified through molecular phylogenetic analysis. *Phoebanthus*, a small genus of two species with narrowly endemic ranges in the state of Florida, is a near sister group. There is no other single genus that is particularly close to *Helianthus*. The sister group to the *Helianthus*-*Phoebanthus* clade is a large, diverse assemblage that includes several genera, and whose collective range spans areas from Mexico to South America.

A major adjustment in the circumscription of *Helianthus* has involved the transfer of all of the South American species formerly placed in it to the genus *Pappobolus*.

The South American species had been placed in *Helianthus* because they also have a caducous pappus of two awns. *Pappobolus*, which is endemic to the Andes Mountain region, includes species with a wide variety of pappus types (Panero, 1992). The removal of the South American species from *Helianthus* had been suggested prior to molecular studies, and the molecular phylogenetic information adds ample support to this. This makes it clear, for instance, that domestication of the cultivated sunflower, *Helianthus annuus*, did not occur initially in South America.

Two other species that had been placed in *Helianthus* because of their pappus type have been returned to *Viguiera*. Erroneous information regarding its habit had led to the placement of one of these, *V. similis*, with the annual members of *Helianthus* (Heiser et al., 1969). This reflects in part the location of *V. similis* in a remote area of Baja California, Mexico, and the fact for many years the only herbarium material that was available for it was fragmentary. More recent information shows that it is clearly a shrub, like other members of *V.* subgen. *Bahiopsis* with which it is clearly allied. The relationships of the other species removed from *Helianthus*, *V. phenax*, are less well resolved. This species was placed in *Helianthus* under a synonym, *H. ludens*, and confusion regarding its affinities again reflects in part the scanty information available for it. It is an annual that is abundant locally in an area of Durango, Mexico, and apparently occurred as a waif in Texas, from where a collection was described as a *Helianthus*. It is still not completely clear what its exact relationships are, but it is clearly not a *Helianthus*, despite its caducous pappus.

Molecular data allow confirmation of the placement in *Helianthus* of two species whose affinities were not certain. The placement of one, *H. agrestis*, had been questioned because it has several distinctive traits for *Helianthus*, including tuberculate cypselas and self-compatibility. The other species, *H. porteri*, has been placed in *Viguiera* (or in *Heliomeris* by those who recognize it as distinct) by most botanists. As recently as 1985 a thorough review of morphological and chemical data done as part of a Ph.D. dissertation concluded that it was best placed in *Viguiera*. The molecular data, however, agree with chromosome number, crossing behavior, and biogeographic evidence in indicating that *H. porteri* is accurately placed in *Helianthus*. The inclusion of *H. porteri* in *Helianthus* creates a problem in the morphological circumscription of the genus, because it lacks two of the three apparent synapomorphies of the genus. The pales of *H. porteri* are not trilobed at the apex, and it entirely lacks a pappus. It does, however, have the prominent terminal style appendage that characterizes *Helianthus*. There are several other members of subtribe Helianthinae that exhibit a similar terminal style appendage, so there is not a single absolute synapomorphy to allow morphological diagnosis of *Helianthus*.

Relationships of *Helianthus* to other members of subtribe Helianthinae

The nearest relative to *Helianthus* is clearly *Phoebanthus*, a small genus of two species restricted to areas in the state of Florida in the southeastern U.S. In fact, the cpDNA tree would suggest inclusion of *Phoebanthus* within *Helianthus*, although the ITS tree suggests that the two are sister genera (Fig. 3).

The *Helianthus-Phoebanthus* clade is distinctive within subtribe Helianthinae. This clade is supported by 11 restriction site changes in the cpDNA tree and five base pair substitutions in the ITS tree. There have not yet been any morphologically synapomorphies discovered that diagnose unambiguously this clade.

Beyond *Phoebanthus*, there is no single genus that can be identified as being nearest to *Helianthus*. The sister group to the *Helianthus-Phoebanthus* clade is a large and diverse group that includes species now classified in *Viguiera* as well as the distinctive genera *Tithonia*, *Simsia*, *Pappobolus*, *Scalesia*, *Lagascea*, and *Alvordia* (Fig. 1, 2). There is no morphological synapomorphy that characterizes this group, and they occur over a wide area of Mexico, Central and South America. In general, plants of this lineage are herbaceous perennials, although there are also annuals and a few woody species (notably *Pappobolus* and *Scalesia*).

An aspect of the sister clade to *Helianthus* is that there is significant incongruence between trees obtained from cpDNA and ITS results, respectively (Fig. 2). In some cases, the incongruence appears to be most logically explained by chloroplast transfer through past hybridization. For example, *Simsia dombeyana*, which is one of only two South American species of a mostly Mexican genus, is placed by cpDNA within the clade of South American species of *Viguiera*, whereas the ITS tree places it in a derived position within *Simsia*. The latter position is consistent with its morphology, because it barely differs from *S. foetida* (Spooner, 1990). Similarly, the cpDNA tree would suggest that *Tithonia* is diphyletic, with some species part of the *Simsia* clade and others close to *Viguiera* subgen. *Amphilepis* (Fig. 2.) In contrast the ITS tree shows *Tithonia* to be entirely monophyletic and sister to *V.* subgen. *Amphilepis*. Again, the result from ITS is most consistent with morphological observations. A problem with an explanation that involves hybridization between genera is that such hybrids have never been documented, either in nature or from artificial crosses. It suggests that wide hybridization should be possible, however.

The fact that the basal-most diverging clades consist of groups that are primarily or exclusively found in Mexico suggests that subtribe Helianthinae originated in this region (Fig. 1). Subsequent divergence of the subtribe led to various distinctive groups both in Mexico and also in other areas. Most of the movement out of Mexico appears to have been to Central and South America. The *Helianthus-Phoebanthus* clade is the only group to be present in eastern North America. The molecular phylogenetic trees are consistent in suggesting that *Viguiera* sect. *Maculatae* is the next most basal diverging group in subtribe Helianthinae to *Helianthus*. *Viguiera* sect. *Maculatae* is endemic to Mexico and is woody, including large shrubs and small trees.

Divergence within *Helianthus*

The most striking feature of molecular phylogenetic studies of *Helianthus* to date is that there is strikingly little variation within the genus for either ITS or cpDNA (Table 2). In addition, there is incongruence between results from the two molecular markers (Fig. 3). The lack of divergence in particular has limited the conclusions that can be drawn regarding relationships within *Helianthus*. This observation was extended by our recent survey of variation for the trnT-trnF spacer/intron region. Comparison of *H. porteri*, *H. angustifolius*, and *H. giganteus* revealed only 2 base pair changes and 6 insertion/deletion changes from a total of 1,417 bases that were sequenced for this region of the cpDNA genome (Table 2). By contrast, studies of this gene region in other plant families have revealed extensive variation, sometimes even within a single species.

The incongruence between results from cpDNA and ITS data sets for relationships within *Helianthus* is not readily explainable, although it may reflect past hybridization between different lineages. The cpDNA results would suggest a relatively straightforward division of the genus into four main lineages (Fig. 3), with *Phoebanthus* as part of the perennial group. The ITS data suggest a more complex scenario in which the perennial species are paraphyletic to the rest of the genus, based on placement of *H. heterophyllus* and *H. carnosus* as basally branching clades. In the ITS tree, *Phoebanthus* is placed as the sister group to *Helianthus*, rather than as part of the perennials.

Neither the tree from cpDNA nor the one based on ITS is in accord with the current infrageneric classification of *Helianthus* (Schilling and Heiser, 1981). Section *Helianthus*, which contains the bulk of the annual species including *H. annuus*, is shown to be monophyletic in both trees. Both trees also place the peculiar annual species, *H. agrestis* and *H. porteri*, as distinctive lineages. In neither tree is *H. sect. Ciliares* shown to be monophyletic. The three species of *H. ser. Ciliares* are a monophyletic ingroup to the perennial species in both trees. For the most part, however, there are few differences among the perennial species from either data set.

The lack of variation leaves unresolved the relationships of the polyploid species of *Helianthus*, all of which are perennial. It has not been possible even to identify the maternal genome of any of the polyploids. The only exception to this involves the sterile triploid, *H. x multiflorus*, in which it has been possible to show that the cpDNA genome is from a polyploid species and that it contains rDNA genomes from both *H. annuus* and a perennial species. Thus, further work is needed to identify molecular markers that show the appropriate, high level of variation that will be needed to resolve relationships of the polyploid species of *Helianthus*.

It is potentially notable that the basally diverging clades of *Helianthus* in the ITS tree all are species of the extreme southeastern U.S. with relatively narrow geographic distributions. This suggests the hypothesis that after migration to North America from a probably Mexican origin, the ancestral lineage to *Helianthus* may have been limited during glaciation events to areas near the southeastern coastline. Thus, *Phoebanthus*, *H. carnosus*, *H. heterophyllus*, *H. agrestis*, and *H. porteri* may represent relictual species

from one or more early rounds of divergence in *Helianthus*. The subsequent migration from the southeastern U.S. across much of North America and differentiation to form the current suite of species may have been relatively recent.

References

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Table 1. *Helianthus* – examples of molecular divergence for chloroplast DNA restriction sites (cpDNA), nuclear ribosomal internal transcribed spacer (ITS) and chloroplast-encoded trnT-trnL-trnF spacer/intron regions. b.p, base pairs; n.a., not available.

	cpDNA	ITS	trnL intron*
within <i>Helianthus</i>	0-0.25%	0-4%	0%
<i>Helianthus-Phoebanthus</i>	0-0.12%	2.4-5.5%	n.a.
<i>Helianthus-Eupatorieae</i>	n.a.	6-30%	0.6-1.0%

*cpDNA spacer/intron sequence data for *Helianthus angustifolius*, *H. giganteus*, *H. porteri*:

	length b.p.	indels	b.p. changes
trnT-trnL spacer	562	4	1
trnL intron	467	0	0
trnL-trnF spacer	388	2	1
TOTAL	1,417	6	2