A chloroplast DNA inversion marks an ancient evolutionary split in the sunflower family (Asteraceae)

(angiopserm evolution/molecular systematics/Mutisieae/Barnadesinae)

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ABSTRACT We determined the distribution of a chloroplast DNA inversion among 80 species representing 16 tribes of the Asteraceae and 10 putatively related families. Filter hybridizations using cloned chloroplast DNA restriction fragments of lettuce and petunia revealed that this 22-kilobase-pair inversion is shared by 57 genera, representing all tribes of the Asteraceae, but is absent from the subtribe Barnadesinae of the tribe Mutisieae, as well as from all families allied to the Asteraceae. The inversion thus defines an ancient evolutionary split within the family and suggests that the Barnadesinae represents the most primitive lineage in the Asteraceae. These results also indicate that the tribe Mutisieae is not monophyletic, since any common ancestor to its four subtribes is also shared by other tribes in the family. This is the most extensive survey of the systematic distribution of an organelle DNA rearrangement and demonstrates the potential of such mutations for resolving phylogenetic relationships at higher taxonomic levels.

The Asteraceae is one of the largest and economically most important families of flowering plants and consists of 12–17 tribes, approximately 1100 genera, and 20,000 species (1). A combination of several specialized morphological characteristics (e.g., capitula, highly reduced and modified flowers, inferior ovaries, syngenous anthers) strongly supports the naturalness of the family. Cronquist (1) emphasized the distinctness of the Asteraceae by placing it in a monotypic order at the most advanced position within the subclass Asteridae. In addition to its large size, the family has a cosmopolitan distribution and is highly diversified in its habitat preferences and life forms. This diversity includes aquatics, herbs and shrubby trees in temperate, tropical, and arid environments, and trees in tropical rain forests. Species of Asteraceae are of wide economic importance as vegetables (lettuce, artichokes, endive), sources of oil (sunflower, safflower) and insecticides (pyrethrum), and garden ornamentals (chrysanthemum, dahlia, marigold, and many others).

Although there is some controversy concerning its age (2, 3), fossil evidence (4, 5) and biogeographical considerations (6) suggest that the Asteraceae originated in the middle to upper Oligocene (30 million years ago) and subsequently underwent rapid radiation. This rapid diversification has posed special problems for understanding phylogenetic relationships at higher taxonomic levels. Previous attempts (4, 7–10) at constructing phylogenies have relied on comparative anatomical, chromosomal, embryological, micromolecular, morphological, and palynological features. These studies have been largely unsatisfactory because of the repeated parallel and convergent evolution of these characters. For example, three major and highly divergent reformulations of Cronquist’s (1, 4, 7) subfamilial classification for the Asteraceae have been proposed in the last 12 years (8–10).

We are investigating chloroplast DNA (cpDNA) variation in the Asteraceae to resolve phylogenetic relationships at higher taxonomic levels. Our previous study (11) showed that the 151-kilobase (kb) cpDNAs of two species in the family (Lactuca sativa and Barnadesia carophylla) are colinear throughout the genome, with the exception of a single 22-kb inversion. The conservative organization of the chloroplast genome among land plants (12, 13) makes such rearrangements potentially valuable characters for phylogenetic studies. Here we report on the evolutionary direction of the inversion in the Asteraceae by comparing the chloroplast genomes of Lactuca and Barnadesia with that of an outgroup, Petunia hybridra (Solanaceae). We also examine the distribution and phylogenetic significance of this rearrangement.

MATERIALS AND METHODS

cpDNAs were isolated by the sucrose gradient technique (14). Where tissue amounts were limited, total DNA was isolated (15) and further purified by centrifugation in CsCl/ethidium bromide gradients. Restriction endonuclease digests, electrophoresis, transfer of DNA fragments from agarose gels to Zetabind filters (AMF Cuono), and hybridizations were performed as described (11, 14). Recombinant plasmids containing cpDNA fragments from Lactuca and Petunia were described previously (11, 16).

RESULTS

Filter hybridizations using cloned restriction fragments (16) from petunia (Petunia hybridra, Solanaceae) were performed to assess cpDNA genome arrangement in the Asteraceae. The Petunia genome appears to have the ancestral cpDNA arrangement for angiosperms, since it is colinear with the genomes of a fern, a gymnosperm, and several diverse angiosperms (17–21). Barnadesia cpDNA is colinear with the petunia genome (Fig. 1) and therefore has the same gene order as the ancestral angiosperm type. In contrast, lettuce (Lactuca sativa) cpDNA has a derived inversion in the large single copy region, as evidenced by the hybridization of nonadjacent petunia PrfI fragments of 9.0 and 15.3 kb to the same two regions of the lettuce genome. For example, both of these petunia probes hybridize to 7.5-kb Sac I–Sal I and 6.7-kb Sac I lettuce restriction fragments (Fig. 1). Furthermore, the atpA through rpoB genes have an inverted order and are transcribed in the opposite direction in lettuce relative to Barnadesia (Fig. 1; ref. 11).

Abbreviation: cpDNA, chloroplast DNA.

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Many additional taxa were surveyed for the inversion by performing filter hybridizations using cloned lettuce cpDNA fragments that contain the inversion endpoints. The 7.5-kb Sac I–Sal I and 6.7-kb Sac I lettuce fragments were used as hybridization probes against filter blots containing 12 restriction enzyme digests of DNA from one species of each of 80 genera representing 10 putatively allied families and 16 tribes of Asteraceae (Table 1). The 7.5-kb Sac I–Sal I and 6.7-kb Sac I probes will hybridize to different restriction fragments in those genomes that contain the lettuce inversion. This situation is illustrated in Figs. 2 and 3, in which the two inversion endpoint fragments from lettuce are hybridizing to Sac I fragments of 14.7 and 17.0 kb in Vernonia. Similar hybridization results are evident for Helianthus and Trixis (Fig. 3), which are both members of the Asteraceae. In contrast, in those genomes that are not rearranged, the two lettuce probes will hybridize to two of the same restriction fragments. For example, the 7.5-kb Sac I–Sal I and 6.7-kb Sac I lettuce probes both hybridize to Sac I fragments of 5.8 and 14.9 kb in Barnadesia (Figs. 2 and 3). The autoradiograms (Fig. 3) reveal that representatives of three related families, Cephalaria (Dipsacaceae), Pentas (Rubiaceae), and Scabiosa (Goodeniaceae), also lack the 22-kb inversion.

The results of the inversion survey for all 80 examined taxa are summarized in Table 1. The genome arrangements for 69 of these taxa have been confirmed by constructing complete restriction maps (R.K.J., H. Michaels, and J.D.P., unpublished data). The inversion is absent from all putatively allied families and, within the Asteraceae, from the subtribe Barnadesinae of the tribe Mutisieae. All other examined members of the Asteraceae, including the three other subtribes in the Mutisieae, were found to have the inversion. The 80 genera surveyed represent the major evolutionary lineages within the 16 tribes of Asteraceae and 10 related families. We are confident that the selection of only one species from each genus is an adequate sampling because more extensive studies of 60 species in Carthamus (R. Johnson and J.D.P., unpublished data), Coreopsis (D. Crawford and J.D.P., unpublished data), Hieracium (R.K.J. and J.D.P., unpublished data), and Lactuca (E. Jandourek and J.D.P., unpublished data) have revealed no intragenic variation in chloroplast genome arrangement in the Asteraceae.
We also performed filter hybridizations to a single enzyme digest of DNA from the 80 species, using four restriction fragments (whose sizes and locations are shown in the enlargement at the top of Fig. 1) subcloned from the 6.7-kb Sac I and 7.5-kb Sac I–Sal I fragments. We previously showed (11) that the lettuce inversion endpoints are located very close to the EcoRI sites separating these two pairs of adjacent fragments (Fig. 1, arrows). These smaller, more precise probes hybridize to those genomes containing the inversion in exactly the same manner as to the parental lettuce genome (data not shown). This gives us greater confidence that these taxa have the same inversion as lettuce, rather than a similar but different inversion in the same region of the chloroplast genome.

**DISCUSSION**

The 22-kb inversion must be derived within the Asteraceae since all putative outgroup families lack this rearrangement. Furthermore, more inclusive outgroups, including 30 additional families of angiosperms, a gymnosperm, and a fern, also lack the inversion (12, 13, 17–20). There are two alternative explanations for the phylogenetic distribution of the inversion (Fig. 4). The most parsimonious interpretation is that the three genera in the Barnadesiinae primitively lack the inversion and that this derived mutation groups all other Asteraceae together. Alternatively, the inversion occurred in the common ancestor of the entire family and subsequently reverted in the Barnadesiinae. The former explanation seems more likely, both on a parsimony basis (23) and, more compellingly, because cpDNA inversions are rare among land plants (12, 13). This particular inversion appears to have occurred only once in some 400 million years of land plant evolution. Furthermore, independent cladistic studies using data from restriction site mapping (unpublished) and morphology (24) support the phylogeny shown in Fig. 4 and place the Barnadesiinae as an ancestral lineage within the Asteraceae.

The distribution of the cpDNA inversion within the Asteraceae (Table 1, Fig. 4) defines the primary evolutionary split within this large and important family of flowering plants, and thus it has significant phylogenetic implications. Indeed, one of the most controversial systematic issues within the family has been the identification of the most primitive lineage. A
Data rooted difficult, convergent best the the are (7-9, tive have also been suggested Vernonieae, other for the Heliantheae have characters of putatively primitive morphological number Ver, Vernon. Numbers of the filters refer to hybridization probes. Numbers alongside the filters indicate fragment sizes in kb. 

FIG. 3. Hybridization of cloned (11) lettuce restriction fragments to Sac I digests of DNA from eight representative species from the Asteraceae and related families. Bar, Barnadesia; Cep, Cephalaria; Hel, Helianthus; Lac, Lactuca; Pen, Pentas; Sca, Scabiosa; Tri, Trisia; and Ver, Vernonia. Numbers above the filters refer to hybridization probes. Numbers alongside the filters indicate fragment sizes in kb.

number of putatively primitive morphological and anatomical characters have been used to hypothesize an ancestral position for the Heliantheae (2, 4, 7, 9, 25). However, four other tribes, the Cardueae, Mutisieae, Senecioineae, and Vernonieae, have also been suggested as being most primitive (7-9, 26). The primary reasons for this lack of agreement are the uncertainty about which family or families constitute the best outgroup and the high incidence of parallel and convergent evolution in the characters that have been used. Identification of primitive character states has thus been difficult, whereas the cpDNA inversion is unambiguously rooted and appears free of parallelism and convergence. The data presented here, together with the two recent morphological and restriction site studies cited above, clearly indicate that the Barnadesiinae is the primitive group within the Asteraceae. This conclusion agrees with recent suggestions (8, 9, 26) that the Mutisieae contains the most primitive taxa in the family.

Our identification of the Barnadesiinae as the most primitive lineage in the Asteraceae provides support for suggestions that the Asteraceae originated in montane South America (2, 6, 27, 28), as the eight genera of this subtribe are centered in the northern Andes (22). Our results are consistent with suggestions (8, 26, 29) that bilabiate (two-lipped) flowers and woody habit, which are common features in the Barnadesiinae, are primitive within the Asteraceae. This suggests affinities between the Asteraceae and the families with some bilabiate or woody members, including the Campanulaceae, Lobeliaceae, Goodeniaceae, and Stylidiaceae.

The distribution of the cpDNA inversion also provides insights into phylogenetic relationships within the Mutisieae. Our data confirm previous suggestions (22, 30, 31) that the Mutisieae is not a monophyletic group (i.e., one derived from a common ancestor not shared by any other tribes in the Asteraceae), since three of its four subtribes are more closely related cladistically to 15 other tribes than they are to the Barnadesiinae (Table 1, Fig. 4). The uniqueness of the subtribe Barnadesiinae is evident in its lack of the 22-kb cpDNA inversion and its distinctive pollen (31-33) and floral (30) morphology.

This is the most extensive survey of the systematic distribution of a structural mutation in an organelle genome and clearly demonstrates the potential of rearrangements for resolving phylogenetic relationships at higher taxonomic levels. Detailed studies of cpDNA inversions (12, 13) in other flowering plant families, including the economically important grasses and legumes, should be equally valuable in making major phylogenetic groupings among and within these families.

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