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review

PHYLOGENETIC CONSEQUENCES OF CYTOPLASMIC GENE FLOW IN PLANTS

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Abstract

Despite the potential power and extensive use of DNA variation for phylogenetic reconstruction, it has become increasingly apparent that DNA phylogenies are often discordant with organismal phylogenies. Factors responsible for discordance include sampling error, convergence, evolutionary rate heterogeneity, lineage sorting, and reticulation. Of these five factors, reticulation is perhaps the most likely to lead to faulty phylogenetic conclusions in plants due to their high potential for interspecific gene flow. This problem is particularly acute for the clonally inherited cytoplasmic genomes (mitochondrial and chloroplast), where evidence of reticulation would be most easily missed. We found evidence for over 36 examples of chloroplast DNA (cpDNA) capture through hybridization/introgression, many of which were unexpected. In many instances, cytoplasmic exchange appears to have occurred in the absence of significant nuclear gene flow and sometimes the native cytoplasm has been completely displaced by an alien one. Theoretical and experimental evidence suggest that differential cytoplasmic exchange can occur by a variety of processes and with remarkable speed. Unfortunately, it is difficult to demarcate a taxonomic zone of safety because unexpected events of chloroplast capture can occur at a variety of taxonomic levels: among closely related, species, at the sectional level within a genus, and at the generic level. In addition, cytoplasmic transfer between major evolutionary lines during early stages of their divergence has the potential to impact cpDNA phylogenetic reconstruction at all taxonomic levels in angiosperms.

Several approaches are suggested to avoid erroneous phylogenetic conclusions based on cpDNA data, including: (1) comparisons with phylogenetic hypotheses based on nuclear gene sequences, (2) comprehensive sampling methods, and (3) modified data analysis strategies. Suggested strategies for data analysis include separate phylogenetic analysis of character sets from different sources followed by analysis of the combined data sets using algorithms designed to accept reticulations.

Introduction

During the past decade, DNA variation has become increasingly used as the basis for phylogenetic reconstruction in both plants and animals; numerous authors (e.g. Crawford, 1983, 1990; Patterson, 1987; Hillis, 1987; Palmer, 1987; Sytsma, 1990) have argued that molecular data are often preferable to morphological data for phylogenetic inference. Reasons given for this view include the ready availability of large numbers of independent molecular characters, the generally low levels of nonheritable molecular variation, and the apparent selective neutrality of many molecular characters. In contrast, the number of morphological characters suitable for phylogenetic analyses is often very low, morphological characters are often functionally or developmentally correlated, much morphological variation is nonheritable (environmental), and morphological characters often converge when exposed to similar selective pressures. Furthermore, several authors (Gottlieb, 1984; Crawford, 1983; Hilu, 1983) have noted that the genetic basis of many morphological character state differences are poorly understood, sometimes leading to fallacious phylogenetic conclusions (e.g. Sytsma and Gottlieb, 1986; Soltis *et al.*, 1990). Nonetheless, the view that molecular data are generally more 'powerful' than morphological data for phylogenetic inference is not universally held. For example, Donoghue and Sanderson (1991) suggest that many of the perceived advantages of molecular data over morphological evidence may be weakly based at best.

Despite the potential power and extensive use of DNA variation for phylogenetic reconstruction, it has become

increasingly apparent that DNA phylogenies are often discordant with organismal phylogenies. Theoretical and experimental studies (reviewed in Avise, 1989; Doyle, 1987; Sytsma, 1990) have shown that several factors may lead to incongruency between DNA and organismal phylogenies, including sampling error (Saitou and Nei, 1986), convergence (e.g. Yatskievych *et al.*, 1988; Zimmer *et al.*, 1989), evolutionary rate heterogeneity (Doyle, 1987; Avise, 1989), phylogenetic sorting (Doyle *et al.*, 1990; Soltis *et al.*, 1991b), and hybridization/introgression (Doebley, 1989; Rieseberg *et al.*, 1990a; Rieseberg and Brunsfeld, 1991). Although each of these factors has been shown to contribute to phylogenetic discordance, its significance varies greatly depending on the molecule or DNA sequence examined and on the taxonomic group or level chosen for study. For example, phylogenetic sorting has been of special concern to the animal systematist because of the rapid rate of mtDNA sequence evolution (Avise, 1989). Likewise, introgression poses a potentially serious problem for plant systematists because of the high potential for interspecific gene flow in plants (Anderson, 1949). This problem is particularly acute for the clonally inherited cytoplasmic genomes (mitochondrial and chloroplast), where evidence of introgression would be most easily missed (Doebley, 1989; Rieseberg *et al.*, 1990a; Smith and Sytsma, 1990).

Ironically, of the three genomes in plant cells, the chloroplast genome is now the most widely used for phylogenetic inference. Its numerous advantages relative to the nuclear and mitochondrial genomes have been well reviewed (e.g. Birky, 1988; Clegg, 1989a; Crawford, 1990; Palmer, 1985a, 1985b, 1987; Palmer *et al.*, 1988) and include: (1) the relatively small size of the genome; (2) the great abundance of cpDNA within plants; (3) ease of analysis; and (4) the conservative nature of cpDNA evolution, both at the nucleotide sequence level and also in terms of structural rearrangements. Nevertheless, even the earliest of these reviews warned of the potential danger that hybridization, introgression and polyploidy posed for phylogenetic reconstruction based on a clonally inherited molecule. For example, Palmer (1985a) stressed the importance of 'comparing the clonal, generally maternal phylogeny derived from cpDNA with the biparental phylogeny derived from nuclear DNA and other characters under nuclear control' in groups where hybridization appears to be frequent.

It is now evident that chloroplast 'capture' through hybridization and introgression is in fact a serious problem for phylogenetic analysis of cpDNA variation. The reasons for this include the sheer frequency with which unexpected cases of cpDNA capture have already been documented (reviewed in Rieseberg and Brunsfeld, 1991), as well as the fact that based on morphological evidence, hybridization and introgression have been suggested as important evolutionary forces in many plant groups (Grant, 1981; Stebbins, 1950). The purpose of this review is to explore the significance of cytoplasmic gene flow for phylogenetic reconstruction based on cpDNA variation. Although we assume that the evidence presented here for the chloroplast genome holds for both cytoplasmic organelles, this has not been proven. Likewise, our discussion of cytoplasmic gene flow is with reference to flow across species barriers, not within species. Specific questions we wish to address include: (1) What is the extent of cytoplasmic gene flow in plants? (2) Does cytoplasmic gene flow occur more frequently and more readily than nuclear gene flow? (3) Does cytoplasmic gene flow often lead to the complete replacement of a native cytoplasm by an alien one, and how quickly can this process take place? (4) At what taxonomic level(s) is cytoplasmic gene flow a serious problem? (5) What are the phylogenetic consequences of cytoplasmic gene flow? and (6) How can faulty phylogenetic conclusions resulting from cytoplasmic introgression be avoided?

(1) What is the extent of cytoplasmic gene flow in plants?

In plants, unlike animals, hybridization and introgression have long been suggested as important evolutionary forces (Anderson, 1949; Heiser, 1949, 1965, 1973; Stebbins, 1950, 1959, 1969; Grant, 1981). Some plant groups in particular seem especially prone to the occurrence of hybridization and introgression (e.g. *Pinus*, *Geum*, *Quercus*, *Aquilegia*, *Salix* and *Iris*). Perhaps one of the most exciting findings to emerge during the short history of cpDNA systematics is the high frequency with which hybridization and/or introgression have been detected in many plant lineages (Rieseberg and Brunsfeld, 1991). Even at the outset of cpDNA systematics, unexpected examples of chloroplast capture attributed to ancient hybridization or introgression were revealed in *Brassica* (Palmer *et al.*, 1983), *Lycopersicon* (Palmer and Zamir, 1982) and *Pisum* (Palmer *et al.*, 1985). We have attempted to compile a list of all examples of chloroplast capture of which we are aware (Table 1). We found evidence for over 37 examples of cpDNA capture through hybridization/introgression; of these, 28 were deemed to be quite conclusive. This is a remarkably high number of examples given: (1) the short history of cpDNA systematics; (2) the small sample sizes used in many published studies of cpDNA variation, often only one individual per species; (3) the general lack of highly resolved nuclear DNA-based phylogenetic trees for comparative purposes; and (4) the fact that most examples were initially unanticipated by the investigators. Examples of the latter phenomenon include *Brassica napus* (Palmer *et al.*, 1983), *Populus nigra* (Smith and Sytsma,

Table 1. Examples of chloroplast capture due to hybridization/introgression

Example	Evidence for capture[1]	Reference
Argyroxiphium grayanum	C	Baldwin *et al.* (1990)
Brassica napus	C	Palmer *et al.* (1983)
Clarkia sect. *Fibula*	S	Sytsma *et al.* (1990)
Dubautia scabra	C	Baldwin *et al.* (1990)
Fuchsia perscendens	S	Sytsma *et al.* (1991)
F. excorticata	S	Sytsma *et al.* (1991)
Gossypium bickii	C	Wendel *et al.* (1991)
G. aridum	C	Wendel and Albert (1991)
G. cunninghamii	C	Wendel and Albert (1991)
Helianthus annuus ssp. *texanus*	C	Rieseberg *et al.* (1990a)
H. anomalus	C	Rieseberg (1991)
H. debilis ssp. *cucumerifolius*	C	Rieseberg *et al.* (1990a)
H. debilis ssp. *silvestris*	S	Rieseberg *et al.* (1991)
H. deserticola	C	Rieseberg (1991)
H. neglectus	C	Rieseberg *et al.* (1990b)
H. paradoxus	C	Rieseberg *et al.* (1990b)
H. petiolaris ssp. *fallax*	C	Rieseberg *et al.* (1991)
H. petiolaris ssp. *petiolaris*	C	Rieseberg *et al.* (1991)
Heuchera hallii	C	Soltis *et al.* (1991a)
H. micrantha	C	Soltis *et al.* (1991a)
H. nivalis	C	Soltis *et al.* (1991a)
H. parviflora	C	Soltis *et al.* (1991a)
Lycopersicon chilense	S	Palmer and Zamir (1982)
L. chmielewskii	S	Palmer and Zamir (1982)
Persea steyermarkii	C	Furnier *et al.* (1990)
Pisum sativum	C	Palmer *et al.* (1985)
Populus nigra	C	Smith and Sytsma (1991)
Quercus alba	C	Whittemore and Schaal (1991)
Q. macrocarpa	C	Whittemore and Schaal (1991)
Q. michauxii	S	Whittemore and Schaal (1991)
Q. stellata	C	Whittemore and Schaal (1991)
Salix melanopsis	C	Brunsfeld (1990)
S. taxifolia	C	Brunsfeld (1990)
Tellima grandiflora	C	Soltis *et al.* (1991a)
Triticum turgidum	S	Gill and Chen (1987)
Zea perennis	C	Doebley (1989)
Z. mays	S	Doebley and Sisco (1989)

[1]C = convincing evidence for chloroplast capture; S = suggestive evidence for chloroplast capture.

1990), *Pisum sativum* (Palmer *et al.*, 1985), species of *Gossypium* (Wendel *et al.*, 1991; Wendel and Albert, 1991), *Salix* (Brunsfeld, 1990), *Dubautia* and *Agroxiphium* (Baldwin *et al.*, 1990), *Helianthus* (Rieseberg *et al.*, 1990a, 1990b, 1991), and *Zea* (Doebley, 1989; Doebley and Sisco, 1989).

The data summarized in Table 1 indicate that cytoplasmic capture does indeed occur in many plant groups. It should be pointed out that this is a minimum frequency of occurrence given the poor sampling schemes typically employed. Furthermore, the numerous documented examples of unexpected cpDNA transfer, some of which occurred in groups not noted for hybridization, indicate that this phenomenon cannot be ruled out in any plant group.

A second question concerns the extent of cytoplasmic gene flow within a lineage. Detailed molecular investigations have now been completed for several plant groups, including *Heuchera* (Soltis *et al.*, 1991a, unpublished), *Helianthus* sect. *Helianthus* (Rieseberg *et al.*, 1988a, 1988b, 1990a, 1990b, 1991; Rieseberg, 1991) and *Salix* sect. *Longifoliae* (Brunsfeld, 1990). These three examples illustrate the extent to which chloroplast capture can occur within a lineage, the complex routes chloroplasts may travel due to hybridization and subsequent chloroplast capture, and the different processes which can lead to chloroplast capture.

One of the best examples of the extent to which hybridization/introgression can occur within a single lineage is demonstrated by a recent study of *Salix* sect.

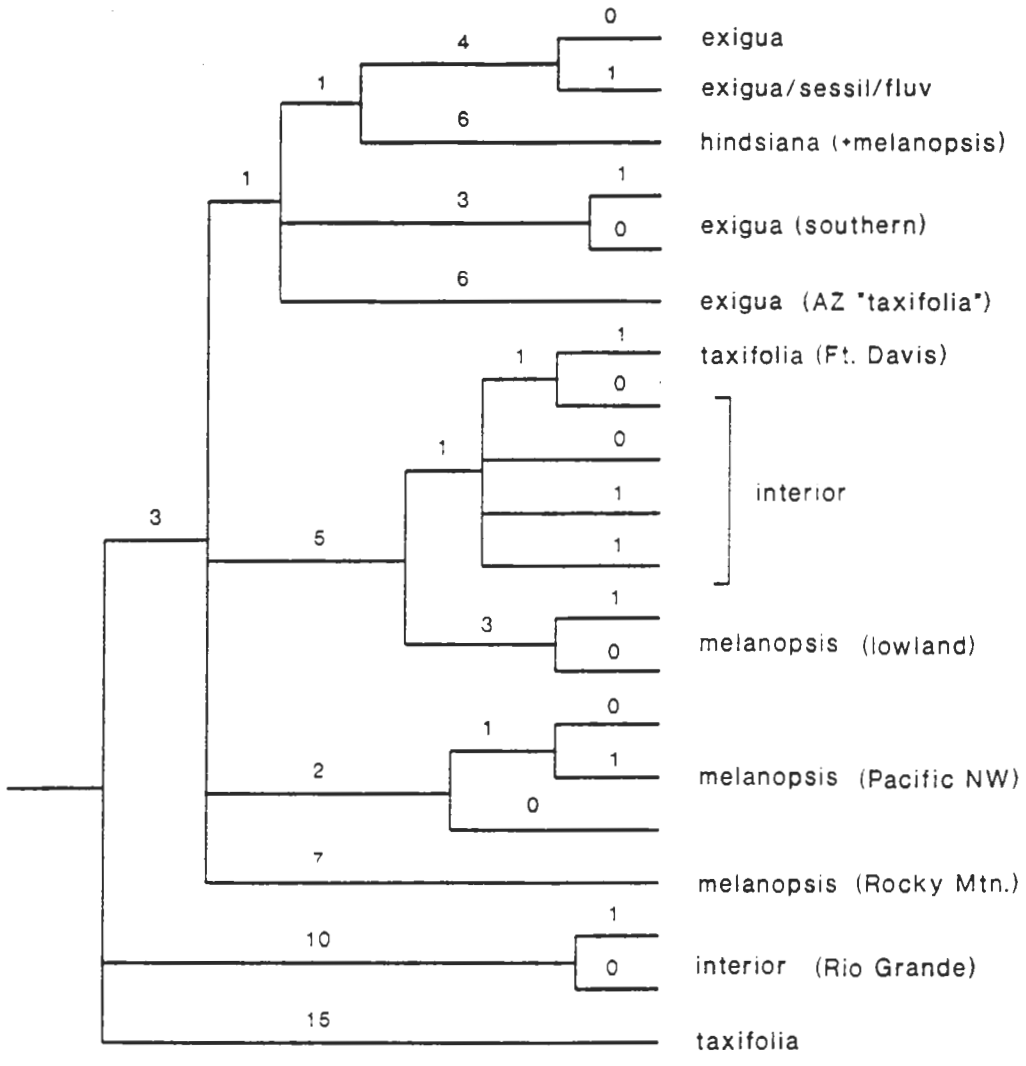

Fig. 1. Shortest Wagner parsimony tree based on cpDNA restriction site data for members of *Salix* section *Longifoliae*. Numbers on branches indicate the number of restriction site mutations supporting each branch. From Brunsfeld (1990).

Longifoliae (Fig. 1). Brunsfeld (1990) documented no fewer than four examples of chloroplast capture in sect. *Longifoliae* using restriction site analysis of cpDNA in conjunction with nuclear markers from enzyme electrophoresis. Perhaps the most intriguing example from this section involves a population of *S. taxifolia* located near Fort Davis, Texas. This species is primarily distributed in central and southern Mexico, but scattered populations occur in isolated, moist habitats as far north as southern Arizona and Texas. The Fort Davis site lies within the range of *S. interior*, a wide-ranging eastern North American member of sect. *Longifoliae*. Common-garden experiments revealed that the Fort Davis material differed morphologically from Mexican *S. taxifolia* and appeared to approach *S. interior* in several characteristics. Molecular analysis revealed that the population had the cpDNA of *S. interior* (Fig. 1) and possessed a mixture of allozymes, including some characteristic of *S. exigua* and *S. melanopsis*, two other members of sect. *Longifoliae*. Lastly, the Fort Davis population currently appears to be hybridizing with *S. goodingii*, a member of sect. *Humboldtianae*, which is also present at the Fort Davis site. Thus, the Fort Davis race appears to be at least the result of gene flow into a relict population of *S. taxifolia* via wind-dispersed seed from a neighbouring population of *S. interior*, and possibly the result of introgression of as many as four or five willow species.

A second apparent example of chloroplast capture resulting from ancient hybridization or introgression in *Salix* involves *S. melanopsis* (Fig. 1). The lowland race of *S. melanopsis*, widespread in western North America, was found to share three restriction sites and two length

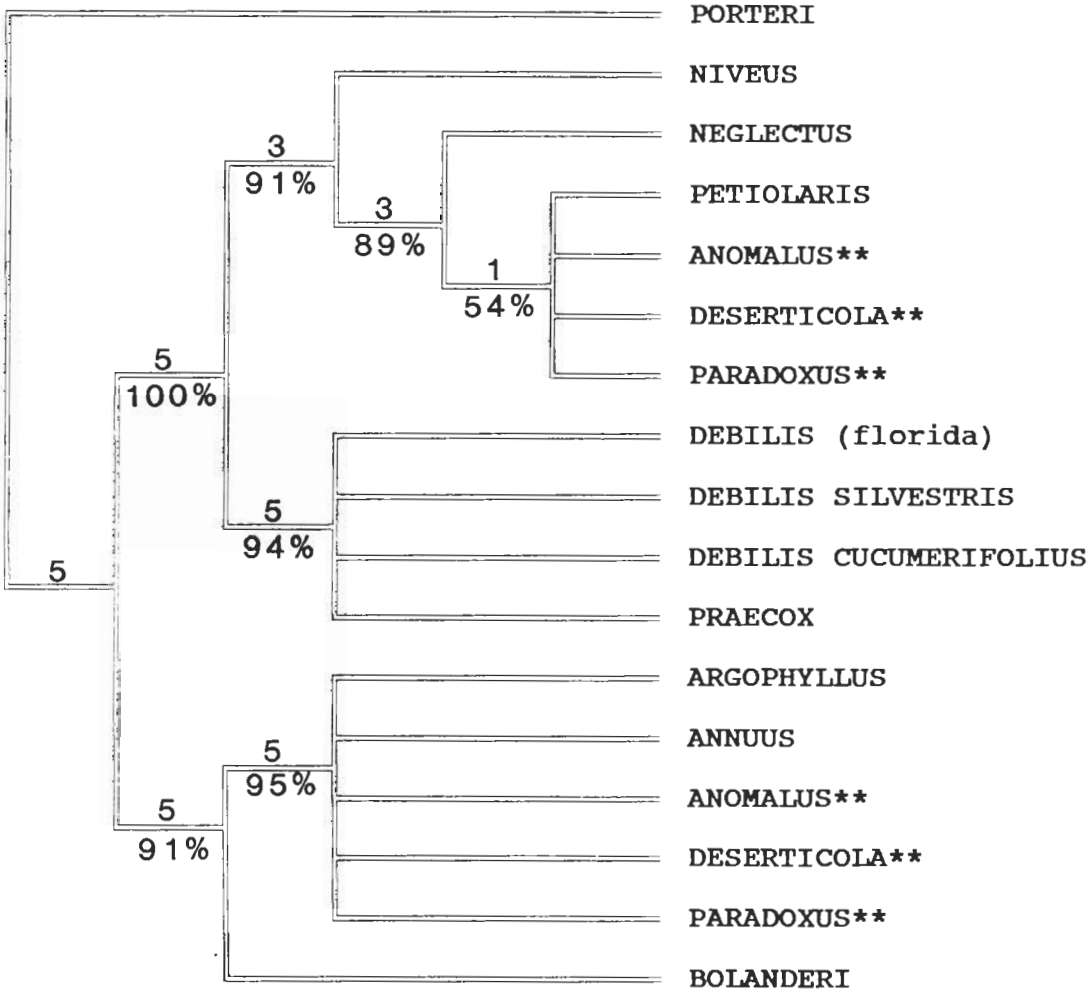

Fig. 2. Single most parsimonious Wagner tree based on rDNA restriction site and length variation in *Helianthus* sect. *Helianthus*. Autapomorphies are not shown. The consistency index was 0.87 (calculated including autapomorphies). Taxon designations are given at the ends of the branches. Percentages indicate the number of times a monophyletic group occurred in 100 bootstrap samples. ** = stabilized hybrid derivative. From Rieseberg (1991).

mutations with *S. interior* of eastern North America. Two other cytoplasmic races of *S. melanopsis* lacked these mutations, but were similar to the lowland race of morphology and allozymes. An early hybridization event may have allowed the lowland race of *S. melanopsis* to 'capture' the cytoplasm of the more distantly related *S. interior*. Hybridization must have occurred early in the evolution of section *Longifoliae* because both lineages possess additional autapomorphic mutations (Fig. 1).

Another example in *Salix* shows that cytoplasmic capture has also occurred more recently (Fig. 1). In the upper Sacramento River drainage of California, a population of *S. melanopsis* was found to contain the highly diverged cpDNA of *S. hindsiana*. Although several individuals of *S. hindsiana* were present at the site, there was no indication from morphological or allozyme data that introgression had occurred in the *S. melanopsis* population (Brunsfeld, 1990).

The annual sunflowers of the genus *Helianthus* (Rieseberg *et al.*, 1990a, 1990b, 1991) illustrate both the great extent of cpDNA capture within a lineage as well as two different processes by which chloroplast capture may occur. Members of this group have been subjected to extensive morphological and cytological study and are frequently cited as outstanding examples of the evolutionary significance of hybridization and introgression (Grant, 1981; Stebbins and Daly, 1961). Heiser (1947, 1949, 1951a, 1951b, 1965) has shown that hybridization occurs frequently in the genus, and that hybrids, although highly sterile, can produce some offspring by backcrossing with the parental species. Heiser (1965) suggests that the ecological amplitude and genetic variation in some

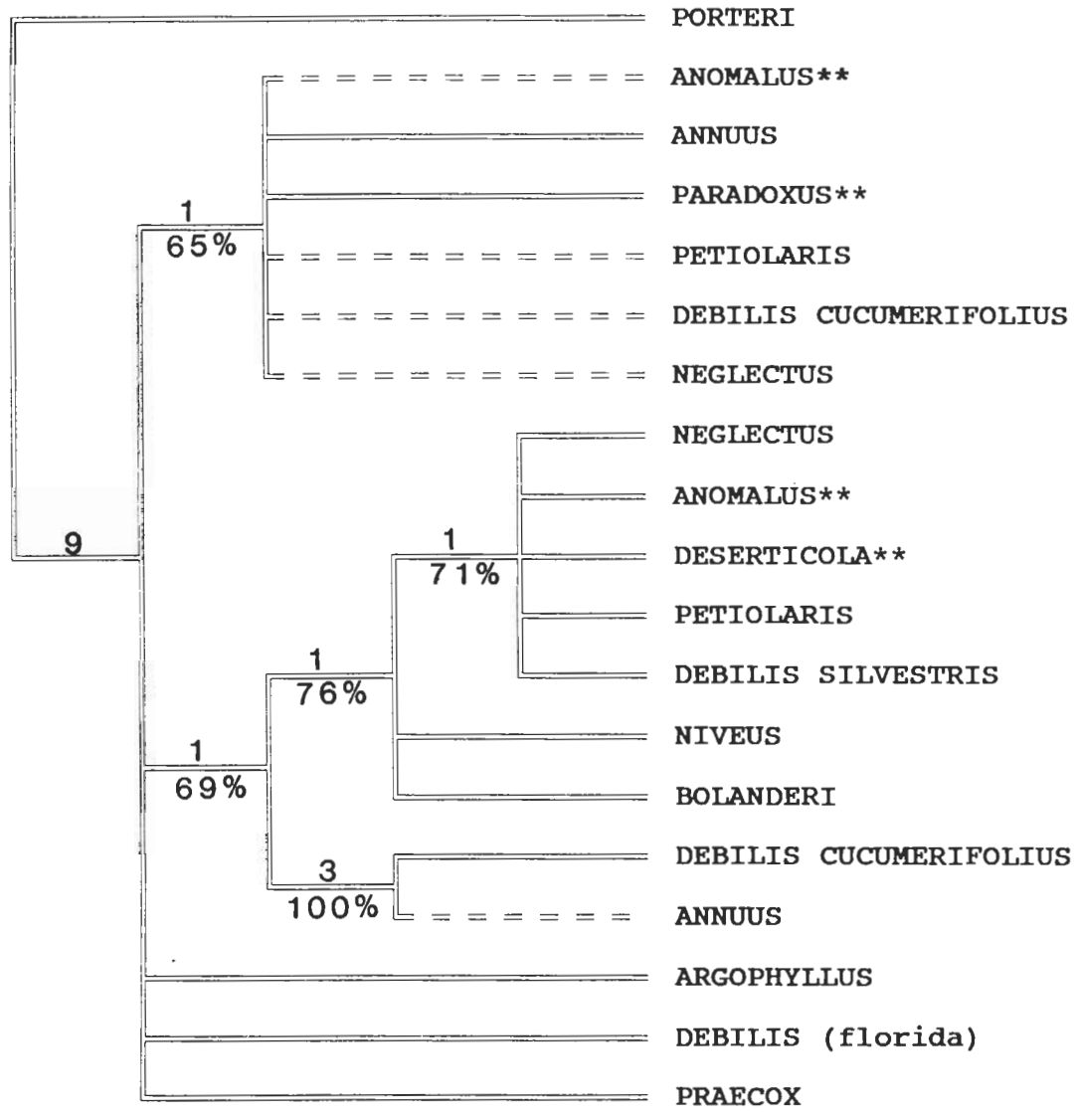

Fig. 3. Single most parsimonious Wagner tree for *Helianthus* sect. *Helianthus* based on cpDNA data. Autapomorphies are not shown. The consistency index was 1.00 (calculated including autapomorphies). Taxon designations are given at the ends of branches, and the number of mutations are given above the branches. Percentages indicate the number of times a monophyletic group occurred in 100 bootstrap samples. Dashed lines indicate discrepancies between morphological classification and cpDNA type which are thought to result from cytoplasmic introgression. ** = stabilized hybrid derivative. From Rieseberg *et al.* (1991).

of these species has been increased by this process. Furthermore, he contends that several geographic races (Heiser, 1947, 1949, 1951a, 1951b, 1965; Heiser *et al.*, 1969) have been derived by introgression.

The evolutionary significance of hybridization and introgression in sect. *Helianthus* has recently been reevaluated in a series of molecular genetic papers (Rieseberg *et al.*, 1988a, 1988b, 1990a, 1990b, 1991; Rieseberg, 1991; Beckstrom-Sternberg *et al.*, 1991). Briefly, nuclear ribosomal RNA genes (rDNA) and cpDNA were used to reconstruct two independent phylogenies for the 23 taxa comprising sect. *Helianthus* (Figs 2 and 3). In addition, over 1000 individuals, representing approximately 220 populations were surveyed for taxonomically informative cpDNA and rDNA mutations, and several thousand plants were assayed for isozyme polymorphisms.

Phylogenetic analysis of the rDNA data (Fig. 2) revealed three principal clades: (1) the '*petiolaris*' clade comprises *H. niveus*, *H. petiolaris*, *H. neglectus*, *H. paradoxus*, *H. deserticola* and *H. anomalus*; (2) the '*debilis*' clade includes all the subspecies of *H. debilis* and *H. praecox*; and (3) the '*annuus*' clade consists of *H. annuus*, *H. argophyllus*, *H. anomalus*, *H. bolanderi*,

H. deserticola. The most significant aspect of the cladogram is the fact that *H. paradoxus*, *H. deserticola* and *H. anomalus* cluster in both the '*annuus*' and '*petiolaris*' clades. These three species combine the rDNA repeat units of *H. petiolaris* and *H. annuus*, species which differ by approximately 20 site or length mutations. The only possible explanation for this pattern is that the three species are stabilized hybrid derivatives of *H. annuus* and *H. petiolaris* [see Rieseberg *et al.* (1990b); Rieseberg (1991) for further details].

The source of the cpDNA(s) captured through this process varies (Fig. 3). All individuals of *H. paradoxus* have the cpDNA genotype of *H. annuus*, whereas all individuals of *H. deserticola* have the cpDNA genotype of *H. petiolaris*. Populations of *H. anomalus*, in contrast, are often polymorphic for the cpDNAs of both parents.

Phylogenetic analysis of the cpDNA data (Fig. 3) suggests a very different set of relationships than that of the rDNA tree. Most notably, four species (in addition to *H. anomalus*) possessed more than one cpDNA genotype, indicative of recent cytoplasmic introgression. Furthermore, several species have actually captured the cytoplasm of another species on more than one occasion. For example, *H. petiolaris* has captured the cytoplasm of *H. annuus* on three separate occasions as evidenced by both geographic location of the introgressed populations and by the *H. annuus* cpDNA genotype captured. It is also noteworthy that *H. annuus* has been either the donor or recipient species in all of the cytoplasmic exchanges detected in *Helianthus*, data which provide some support for Heiser's (1965) concept of *H. annuus* as a compilospecies; that is, a species which is 'genetically aggressive, plundering related species of their heredities' (Harlan and de Wet, 1963).

Removal of the four obvious cases of cytoplasmic introgression from the cpDNA tree (Fig. 3) does not help a great deal in terms of resolving the discrepancies between the cpDNA and rDNA trees. In particular, the placement of *H. bolanderi* and *H. debilis* ssp. *silvestris* is noteworthy. *Helianthus bolanderi* belongs to the '*annuus*' clade based on rDNA data and to the '*petiolaris*' clade based on cpDNA data. Likewise, *H. debilis* ssp. *silvestris* belongs to the '*debilis*' clade based on rDNA and to the '*petiolaris*' clade based on cpDNA. Chloroplast capture through introgression or hybrid speciation may account for the discordant positions of *H. bolanderi* and *H. debilis* ssp. *silvestris* on the phylogenetic trees. Alternatively, the observed discrepancies may result from phylogenetic sorting (Avise, 1989). Although it is difficult to distinguish between these two hypotheses, the former one seems most plausible given the numerous cases of cytoplasmic introgression and hybrid speciation already documented for this group.

Chloroplast capture has also occurred extensively in the genus *Heuchera* (Saxifragaceae), with some examples involving complex routes and processes. Interspecific hybridization, even among species of different sections, has been considered to be of frequent occurrence in the genus (Rosendahl *et al.*, 1936), although few putative examples of hybridization have been examined rigorously (Wells, 1979). Soltis *et al.* (1991a) analysed cpDNA restriction site variation in species representing all five of the sections and 12 of the 13 subsections of *Heuchera* recognized by Rosendahl *et al.* (1936). Two highly divergent cpDNA clades were detected (Figs 4 and 5). One lineage comprises all members of sect. *Rhodoheuchera* analysed (*H. rubescens*, *H. sanguinea*, *H. hemsleyana*, *H. hirsutissima*), as well as *H. hallii* (sect. *Holochloa*), one of two analysed populations of *H. parvifolia* (200) and *H. nivalis* (sect. *Heucherella*). The second lineage comprises the majority of species investigated, representing all sections other than *Rhodoheuchera*; it includes the second population analysed of *H. parvifolia* (166), as well as other species of sect. *Holochloa* (*H. cylindrica*, *H. grossularifolia*, *H. chlorantha*). Morphological and allozyme data, however, indicate that *H. hallii*, *H. nivalis* and population 200 of *H. parvifolia* do not have their closest relatives in sect. *Rhodoheuchera*. The fact that the two populations of *H. parvifolia* analysed appear in the two different major clades within *Heuchera* also indicates that the cpDNA based phylogeny does not accurately reflect phylogenetic relationships. Soltis *et al.* (1991a) suggest that a distinctive chloroplast genotype arose in sect. *Rhodoheuchera* (or the ancestor of that section) and was later captured by populations of *H. nivalis*, *H. parvifolia* and *H. hallii* via hybridization and introgression. These data therefore suggest significant intersectional hybridization in *Heuchera*.

This investigation of cpDNA variation in *Heuchera* also illustrates well the complex routes chloroplasts may travel due to cytoplasmic gene flow. Although populations of *H. nivalis*, *H. parvifolia* and *H. hallii* have apparently obtained their cpDNAs from sect. *Rhodoheuchera*, only *H. parvifolia* has a wide geographic distribution that overlaps considerably with those of some species of *Rhodoheuchera*. Thus, the chloroplast transfer event may have initially involved a species of *Rhodoheuchera* and *H. parvifolia* (Figs 4 and 5, compare phylogenetic positions of populations 166 and 200). A stepping-stone hybridization model can therefore be proposed, initially involving cytoplasmic gene flow between a species of sect. *Rhodoheuchera* and *H. parvifolia*, followed by migration of *H. parvifolia* plants having the captured cytoplasm and subsequent hybridization and transfer of the alien chloroplast genome to *H. nivalis* and *H. hallii*. Adding to the intrigue of this example is the hypothesis that *H. nivalis* is actually a high altitude derivative of

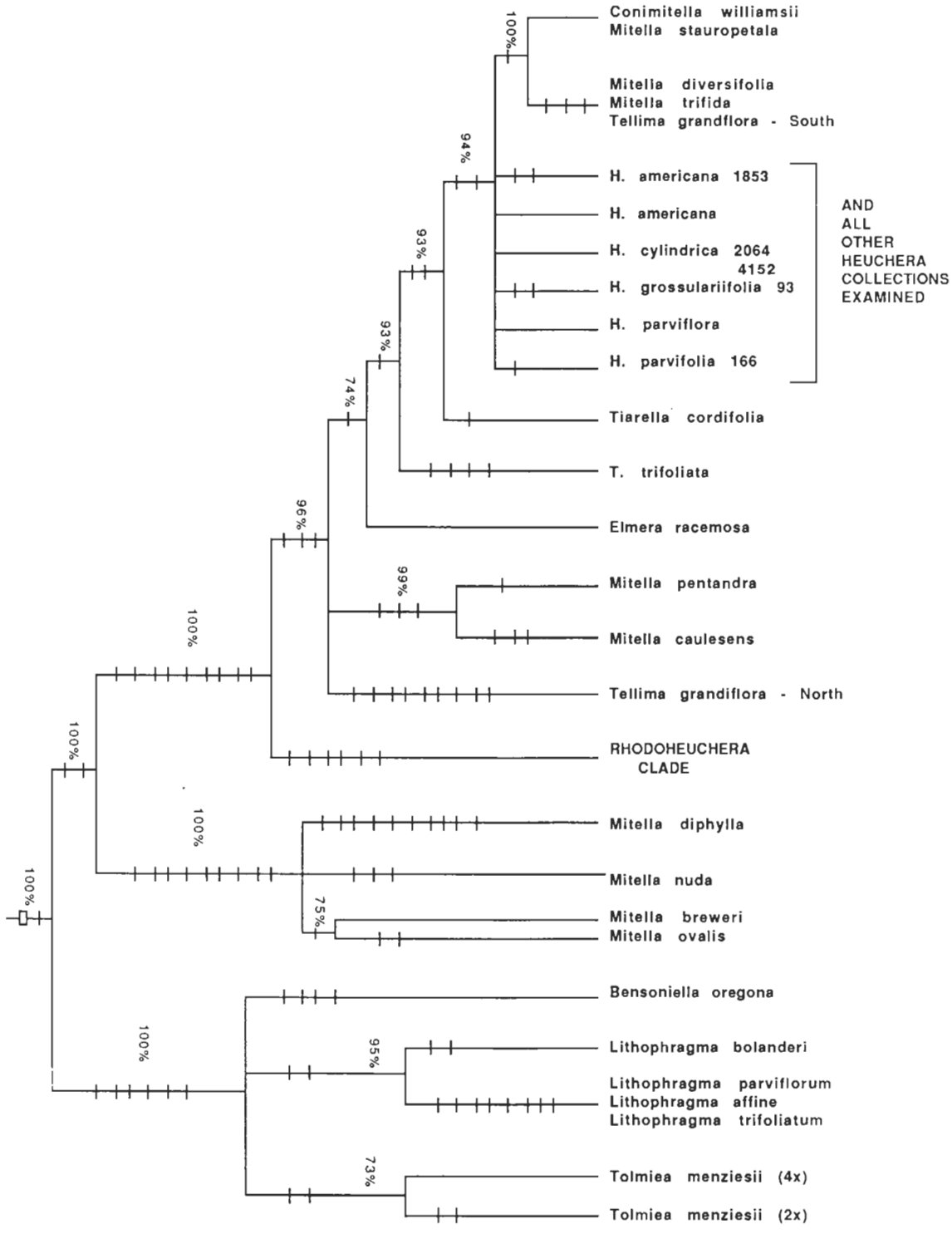

Fig. 4. Most parsimonious phylogenetic tree based on cpDNA restriction site data for members of the *Heuchera* group of genera generated by the MIX program of PHYLIP. Mutations are indicated by slash-marks. On the left of each major branch is the percentage of times that the group defined occurred in 100 bootstrap samples (using the BOOT program of PHYLIP). The consistency index was 0.95 (calculated including autapomorphies). The open box at the base of the tree designates the minimum of 25 restriction site mutations that separate the *Heuchera* group from the outgroup taxa. From Soltis *et al.* (1991a).

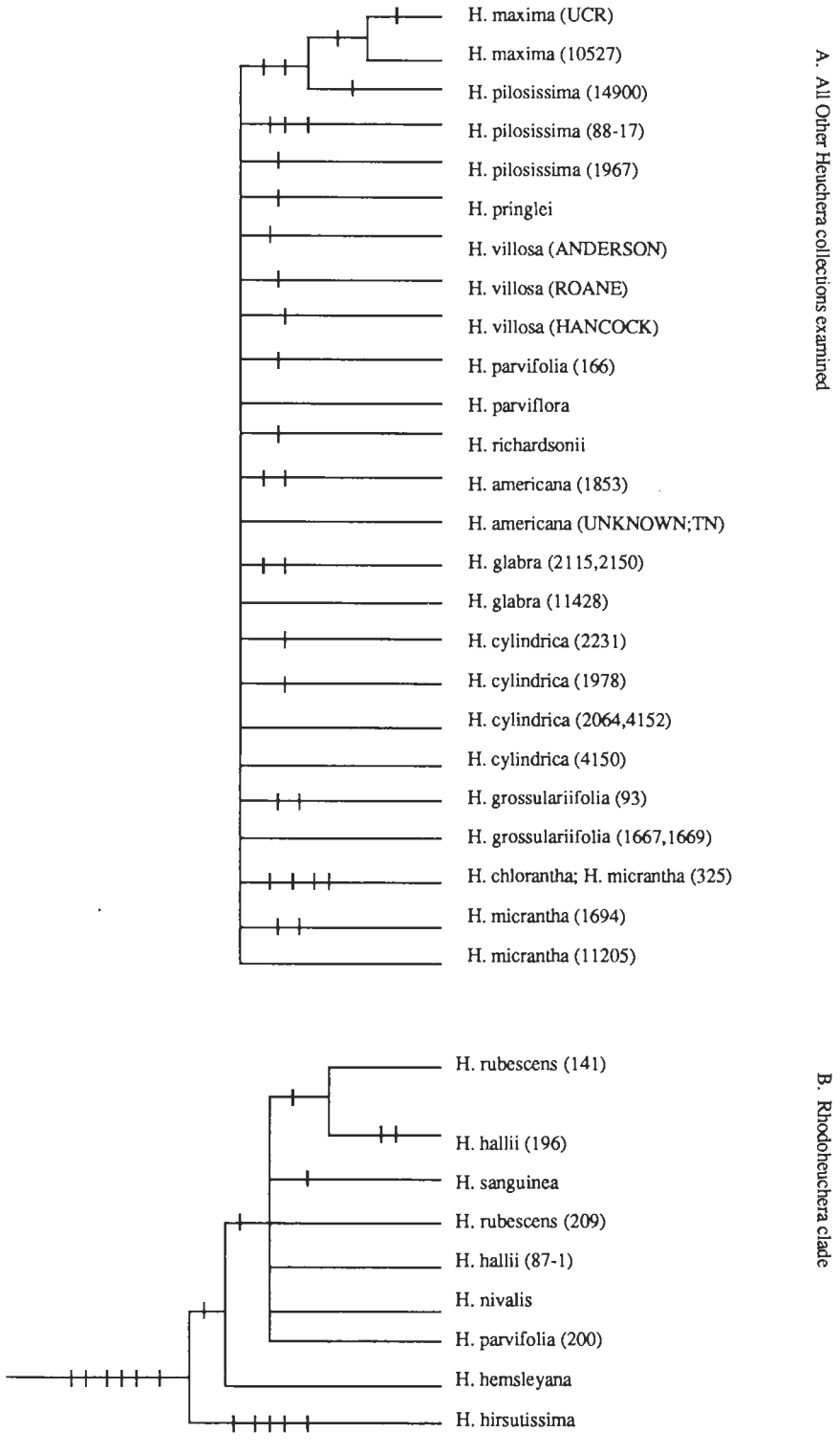

Fig. 5. Most parsimonious phylogenetic tree generated by the MIX program of PHYLIP for species representing the two major clades within *Heuchera*: (A) all other *Heuchera* collections examined other than the *Rhodoheuchera* clade [note: these taxa do not form a monophyletic group (see Fig. 4)]; (B) the *Rhodoheuchera* clade. Mutations indicated by slash-marks. From Soltis *et al.* (1991a).

H. parvifolia (Rosendahl *et al.*, 1936; Soltis *et al.*, unpublished). Thus, during the speciation process *H. nivalis* may have obtained a chloroplast genome that originated in sect. *Rhodoheuchera* from ancestral populations of *H. parvifolia*. Alternatively, hybridization between *H. parvifolia* and *H. nivalis* could have transferred the *Rhodoheuchera* cytoplasm to *H. nivalis*. Naturally occurring hybrids have been reported between *H. parvifolia* and *H. nivalis* and also between *H. nivalis* and *H. hallii*.

Still another example of 'stepping stone' chloroplast transfer in *Heuchera* involves *H. micrantha* (sect. *Euheuchera*) and *H. chlorantha* (section *Holochloa*) (Figs 4 and 5). Although morphologically and isozymically distinct, some populations of these two species share five cpDNA site gains. All available evidence suggests that several populations of *H. micrantha* captured the cytoplasm of *H. chlorantha*, even though no hybrids have been reported between these species in nature. Allozyme and cpDNA data suggest an initial chloroplast transfer from *H. chlorantha* (which is strictly diploid) to diploid populations of *H. micrantha*; an autopolyploid event later followed, resulting in some autotetraploid populations of *H. micrantha* that possess the chloroplast genome of *H. chlorantha*.

(2) Does chloroplast gene flow occur more frequently and more readily than nuclear gene flow?

The above data demonstrate the high frequency of cytoplasmic gene flow in angiosperms, as well as its extent within certain lineages. These data do not necessarily prove, however, that cytoplasmic genomes are any more susceptible to hybridization/introgression phenomena than nuclear genes. It might be argued, for example, that the frequency of chloroplast capture observed is simply a reflection of the evolutionary significance of hybridization and introgression in plants and that detailed phylogenetic studies using nuclear genes would be equally vulnerable to these phenomena. There are several reasons, however, why this does not appear to be the case. First, the maternal inheritance and vegetative segregation of organelles results in organelle genes having an effective population size approximately one-quarter that of nuclear genes (Birky *et al.*, 1983). This leads to a corresponding increase in the rate of gene fixation by drift and a decrease in expected gene diversity. Thus, the likelihood of maintaining two divergent cpDNAs in a population (e.g. native and alien) over long periods of time is much less than for nuclear genes, and the chances of detecting and correctly diagnosing cases of cytoplasmic introgression are therefore greatly reduced.

A second reason for the greater susceptibility of organellar-based phylogenies to introgression is the empirical evidence suggesting that nuclear genes may be exchanged less freely between species than chloroplast genotypes. In *Quercus*, for example, Whittemore and Schaal (1991) examined chloroplast DNA and nuclear ribosomal DNA variation in five sympatric species of white oak native to the eastern United States. A cladogram based on cpDNA data did not reflect morphologic species boundaries, but was strongly correlated with geographic distribution. Furthermore, in cases where a mixed stand of two species was examined, the common cpDNA genotype in both species was the same. In contrast, distribution of rDNA markers consistently followed species boundaries, suggesting nuclear genes may be exchanged less freely between species.

Detailed comparisons of cytoplasmic gene flow versus nuclear gene flow have also been made in the genus *Helianthus* (Rieseberg *et al.*, 1990a, 1991, unpublished; Dorado *et al.*, 1991). Rieseberg *et al.* (1990a, unpublished) used cpDNA and rDNA markers to assess levels of nuclear and cytoplasmic introgression between *H. debilis* ssp. *cucumerifolius* and *H. annuus* ssp. *texanus*, two sunflowers from eastern Texas. Analysis of 14 populations (154 individuals) of *H. annuus* ssp. *texanus* revealed that 10 plants from seven populations had cpDNAs like that of *H. debilis* ssp. *cucumerifolius*, and 16 individuals from 10 populations combined the rDNA repeat units of *H. annuus* ssp. *texanus* and *H. debilis* ssp. *cucumerifolius* in varying proportions (Table 2). Thus, levels of nuclear and cytoplasmic gene flow from

Table 2. Distribution and relative proportion of *H. debilis* ssp. *cucumerifolius* cpDNA and rDNA markers in populations and individuals of *H. annuus* ssp. *texanus*

	cpDNA		rDNA (*debilis*)[1]			
Population	*annuus*	*debilis*	0	0–0.1	<0.1–0.9	0.9–1.0
AT1	19	0	18	1	0	0
AT2	4	0	3	1	0	0
AT3	10	0	10	0	0	0
AT4	4	1	4	1	0	0
AT5	10	1	9	2	0	0
AT6	23	2	25	0	0	0
AT7	11	0	9	1	1	0
AT8	25	0	22	3	0	0
AT9	3	3	4	2	0	0
AT10	10	0	9	1	0	0
AT11	8	1	9	0	0	0
AT12	2	1	1	1	0	1
AT13	10	1	11	0	0	0
AT14	5	0	4	0	1	0

[1]Numerical headings refer to the proportion of *H. debilis* ssp. *cucumerifolius* rDNA marker in individuals of *H. annuus* ssp. *texanus*.

H. debilis ssp. *cucumerifolius* into *H. annuus* ssp. *texanus* are roughly equivalent. In contrast, the interspecific flow of chloroplast genotypes is much higher (10 times) than that for rDNA markers in the opposing direction (Table 3). Analysis of 11 populations (270 individuals) of *H. debilis* ssp. *cucumerifolius* revealed that 193 individuals from nine populations had the cpDNA genotype of *H. annuus* ssp. *texanus*, whereas only 20 individuals from four populations had detectable rDNA markers of *H. annuus* ssp. *texanus* (Table 3).

Likewise, analysis of cpDNA versus rDNA introgression between races of *H. annuus* and *H. petiolaris* from southern California revealed much higher levels of cytoplasmic gene flow than nuclear gene flow (Dorado *et al.*, 1991). *Helianthus annuus* and *H. petiolaris* are widespread polytypic species with similar ranges, occurring commonly in the western United States and less frequently eastwards. Both species appear to be recent introductions into southern California, but are now quite common. Of the 137 individuals (six populations) of *H. petiolaris* surveyed, all but four have the cpDNA genotype of *H. annuus* (Table 4). In contrast, only two plants had rDNA markers of *H. annuus*. No introgression was observed from *H. petiolaris* into *H. annuus*.

Phylogenetic evidence also suggests a predominance of cytoplasmic gene flow over nuclear gene flow in *Helianthus* (Rieseberg *et al.*, 1991; Rieseberg, 1991). As observed in *Quercus*, the cpDNA phylogeny for sect. *Helianthus* is strongly correlated with geographic distribution, not taxonomic categories. That is, geographically proximal taxa are generally most closely related in terms of cpDNA. In contrast, rDNA variation is generally concordant with taxonomic boundaries; in fact, the rDNA-based phylogenetic tree is much more similar to a morphologically-based phylogenetic tree for this group (Schilling and Heiser, 1981) than to the tree based on cpDNA data.

Phylogenetic evidence from a number of other plant groups also suggests that interspecific exchange of chloroplast genotypes occurs more readily and more frequently than does exchange of nuclear genes. For example, in two of the four cases of chloroplast capture in *Salix* (Brunsfeld, 1990), there was no evidence of nuclear gene introgression, although both isozyme and morphological characters were examined. Likewise, the several instances of cytoplasmic capture detected in *Heuchera* do not appear to have been accompanied by nuclear gene flow as assessed using isozyme markers.

Three of the most clear-cut cases of cytoplasmic capture apparently in the absence of significant nuclear introgression are in *Gossypium* (Wendel *et al.*, 1991; Wendel and Albert, 1991), *Populus* (Smith and Sytsma, 1990) and *Zea* (Doebley, 1989). The best example from *Gossypium* involves the Australian (C-genome or G-genome) species, *Gossypium bickii*. This species, along with *G. nelsoni* and *G. australe*, are morphologically similar arid zone species included in sect. *Hibiscoidea* of subgenus *Sturtia*. These three species are quite distinct morphologically from the rest of the genus, a relationship confirmed based on isozyme and rDNA evidence. Yet, the cpDNA of *G. bickii* is most similar to that of *G. sturtianum* of sect. *Sturtia*. Significantly, Wendel *et al.* (1991) examined numerous accessions of *G. bickii* with reference to isozyme and rDNA markers, but no *G. sturtia* nuclear markers were detected.

Smith and Sytsma (1991) analysed restriction site variation in cpDNA and rDNA in nine species of *Populus*. The most parsimonious trees based on cpDNA data indicate that *P. nigra* (one of the black poplars, sect. *Aegeros*) has a chloroplast genome divergent from the American cottonwoods of its own section. The chloroplast

Table 3. Distribution and relative proportion of *H. annuus* ssp. *texanus* cpDNA and rDNA markers in populations and individuals of *H. debilis* ssp. *cucumerifolius*

	cpDNA		rDNA (*annuus*)[1]			
Population	*annuus*	*debilis*	0	0–0.1	<0.1–0.9	0.9–1.0
DC1009	2	2	4	1	0	0
DC1023	18	0	14	1	2	1
DC1028	0	30	30	0	0	0
DC1018	0	34	33	1	0	0
DC1066	51	0	47	2	1	1
DC1025	21	8	28	0	1	0
DC1017	17	0	16	1	0	0
DC1004	19	2	16	3	2	0
DC1027	6	0	6	2	0	0
DC1011	30	0	29	1	0	0
DC1068	29	1	30	0	0	0

[1]Numerical headings refer to the proportion of *H. annuus* ssp. *texanus* rDNA marker in individuals of *H. debilis* ssp. *cucumerifolius*.

Table 4. Distribution and relative proportion of *H. annuus* cpDNA and rDNA markers in populations and individuals of *H. petiolaris* from Southern California

	cpDNA		rDNA (*annuus*)[1]			
Population	*annuus*	*petiolaris*	0	0–0.1	<0.1–0.9	0.9–1.0
OD2060	13	1	17	0	2	0
OD2059	27	0	28	0	0	0
OD2062	39	0	46	0	0	0
OD2064	15	0	15	0	0	0
OD2070	28	3	30	0	0	0
OD2071	15	0	16	0	0	0

[1]Numerical headings refer to the proportion of *H. annuus* rDNA markers in individuals of *H. petiolaris*.

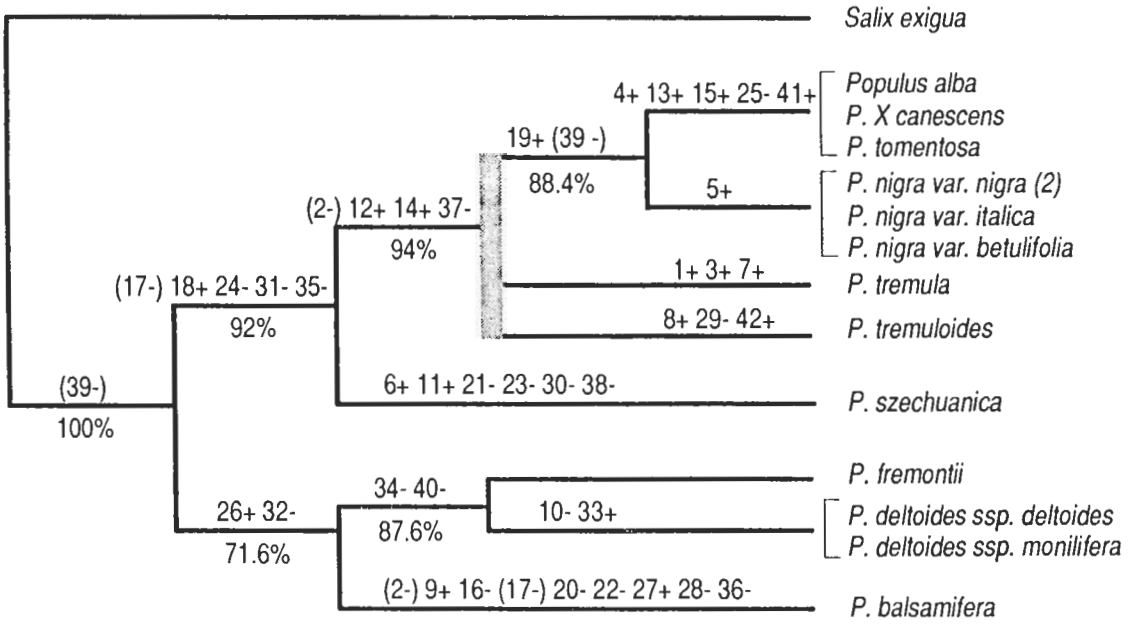

Fig. 6. Consensus tree of the three shortest Wagner parsimony tree using cpDNA restriction site mutations for species of *Populus*. Grey bar represents the unresolved trichotomy. Identification numbers for specific mutations appear along the branches. Gains and losses of sites are designated by '+' and '−', respectively; parentheses indicate convergences. From Smith and Sytsma (1990).

genome of *P. nigra* apparently was derived from *P. alba* (one of the white poplars, sect. *Populus*) (Fig. 6). In contrast, the rDNA genotype of *P. nigra* was similar to that possessed by other black poplars; no rDNA markers of the *P. alba* lineage were detected. Smith and Sytsma suggest that *P. alba* or its immediate ancestor acted as the maternal parent in a hybridization event with *P. nigra*. Subsequent backcrosses to the paternal parent (*P. nigra*) gave rise to present day *P. nigra* with a chloroplast genome of *P. alba* and the nuclear genome of the paternal species.

Doebely (1989) detected eight individuals from one population of *Zea perennis* that possessed a markedly different chloroplast genome from other collections of the same species (Fig. 7). Furthermore, these same eight individuals were morphologically typical of *Z. perennis* and were also tetraploid as is *Z. perennis*, rather than diploid as are other taxa of *Zea*. The cpDNA data, in conjunction with allozyme, cytological and morphological evidence, clearly argue for the incorporation of a foregin chloroplast genome into *Z. perennis*, in the absence of detectable nuclear gene flow. The plants having the typical plastid genome are positioned with *Z. mays*, whereas the remaining collections show a close relationship to *Z. diploperennis*.

At least one case of nuclear gene flow in the absence of cytoplasmic gene flow has been reported in plants. Introgression of allozymes, morphological and terpene characters has been detected between populations of lodgepole (*Pinus contorta*) and jack pine (*Pinus banksiana*) (Critchfield, 1985; Forrest, 1980; Wheeler and Guries, 1987). In contrast, no evidence of significant cpDNA introgression was observed in these same species (Wagner *et al.*, 1987), despite extensive sampling of both sympatric and allopatric populations (including some of the same populations on which previous reports of introgression were based). Thus, although the ease and frequency of organellar transfer across species barriers appears to be much greater than that of nuclear genes, this tendency is by no means absolute. It is noteworthy that inheritance of cpDNA is paternal in *Pinus*, but generally maternal in most of the other examples cited.

Study of hybrid zones in both plants and animals has revealed that the permeability of species barriers is largely dependent on selection, linkage and recombination (Hewitt, 1989). Advantageous or neutral alleles will be slowed significantly only if they are tightly linked to a locus or loci with considerable heterozygous disadvantage. Thus, one explanation for the differential between cytoplasmic and nuclear gene flow is selection against nuclear but not cytoplasmic genes (Barton and Jones, 1983; Powell, 1983). Strong concerted selection acting on loci scattered throughout the nuclear genome could greatly reduce overall nuclear gene flow due to linkage (Barton and Bengtsson, 1986; Whittemore and Schaal, 1991). The explanation may be particularly

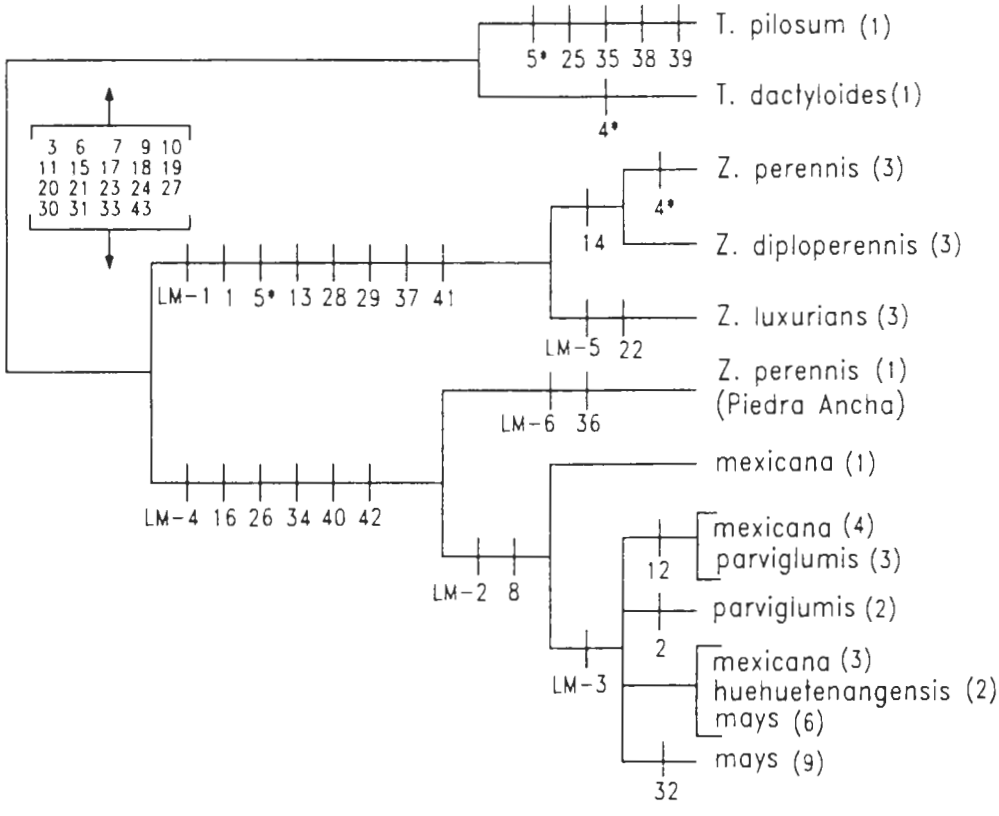

Fig. 7. Wagner parsimony phylogenetic analysis for the genus *Zea* based on cpDNA data. Two species of *Tripsacum* served as outgroups. Identification numbers for specific mutations appear along the branches. The number in parentheses at the ends of the branches are the number of populations possessing that particular chloroplast genome. From Doebley (1989).

applicable to groups such as *Quercus*, where species are thought to be maintained by strong selection for groups of coadapted alleles (Whittemore and Schaal, 1991).

A second possibility is differential relative fitness among cytoplasm-nucleus combinations (Wendel *et al.*, 1991). In this scenario, natural selection would favour one of the alien/native cytoplasm-nuclear combinations. It is noteworthy that in allopolyploids, the nuclear contribution of the paternal parent seems to be more easily lost or altered than that of the maternal parent (Song *et al.*, 1988). Perhaps a similar process occurs in diploid hybrids or introgressants. Alternatively, the alien cytoplasm might be adaptively superior to the native one (Rieseberg *et al.*, 1981). Frank (1989) has shown that a native cytoplasm could be largely replaced by an alien one if the alien cytoplasm has a slight fitness advantage through relative ovule success. This process would be promoted by cytoplasmic male sterility (CMS) (Frank, 1989). It is noteworthy that CMS has been observed for some crosses in both *Helianthus* and *Gossypium*.

Another possible explanation for the differential between cytoplasmic and nuclear gene flow involves the input of a hybrid propagule into a population of one parent or the other (Gyllensten and Wilson, 1987). Male sterility in first generation hybrids and the very first generations of backcrosses could quickly lead to cytoplasmic capture in the absence of significant nuclear exchange. Alternatively, in a dioecious species, input of one or two females from one species into populations of another, combined with hybrid male sterility, would be an even more efficient mechanism for cytoplasmic transfer in the absence of nuclear gene flow (Aubert and Solignac, 1990). This may provide a mechanism for differential cytoplasmic gene flow among dioecious species of *Salix* sect. *Longifoliae*.

A fourth explanation involves some type of founder event. In this scenario, which is not wholly separable from several of the above explanations, founders or colonists would be introgressive individuals carrying alien cytoplasms in a predominantly native nuclear background (Gyllensten and Wilson, 1987). For example, Dorado *et al.* (1991) suggested that the presence of an alien cytoplasm in Southern California *H. petiolaris* may be best accounted for by two of the above factors, founder effect and hybrid male sterility through CMS. They further suggest that the spread of an alien cytoplasm

throughout a species area can be accomplished more easily and rapidly by population growth than by cpDNA migration.

A final explanation for differential cytoplasmic exchange has been suggested by Wendel *et al.* (1991) for *Gossypium*. Briefly, they argue that the process of semigamy (Turcotte and Feaster, 1967), where gamete fusion occurs without nuclear fusion, could lead to fixation of the nuclear genome of a male donor into a foreign cytoplasm in a single generation.

(3) Does cytoplasmic gene flow often lead to the complete replacement of native cytoplasm by an alien one, and how quickly can this process take place?

The high frequency with which unexpected examples of chloroplast capture have occurred should in itself suggest caution to the systematist employing cpDNA data for phylogenetic inference. The ultimate concern, however, is how often does this cytoplasmic introgression lead to complete replacement of native cytoplasm? It is self-evident that if introgression often leads to complete cytoplasmic replacement within a species, no amount of sampling will allow detection.

There is little doubt that the fixation of an alien cytoplasm has occurred within individual populations of a species. For example, all individuals of four populations of *H. petiolaris* had a *H. annuus* cytoplasm as did all plants from five populations of *H. debilis* ssp. *cucumerifolius*. In some of these instances, all plants comprising a natural population were sampled.

There are few studies, however, that have employed sufficiently detailed sampling strategies to adequately demonstrate complete cytoplasmic replacement within a species. The two best examples are probably from *Gossypium* and *Populus*. Wendel *et al.* (1991) showed that all seven accessions of *G. bickii* sampled had an alien cytoplasm. Likewise, Smith and Sytsma (1990) found that all four accessions of *P. nigra* analysed had a *P. alba* cytoplasm. All sampled populations (usually only one) of several other taxa, including *G. aridum* and *G. cunninghamii* (Wendel and Albert, 1991), *Heuchera hallii* and *H. nivalis (Soltis et al.*, 1991a) and *H. debilis* ssp. *silvestris* (Rieseberg *et al.*, 1991) also appear to be fixed for a foreign cytoplasm. It is quite possible if not likely, however, that in most of these cases the implementation of comprehensive sampling strategies will lead to the discovery of the native cytoplasm in at least a portion of the species range. This would seem particularly likely for widespread species.

It can be predicted that the greater the time since the cytoplasmic transfer event, the greater the chance of complete replacement within a lineage. Likewise, the more widespread the taxon, the less likely complete replacement will occur. Nonetheless, some estimate of the rapidity of this process would be useful. For example, is complete replacement unlikely in recently founded species? If so, should discrepancies between organelle trees and nuclear trees for closely related taxa be attributed to lineage sorting rather than cytoplasmic replacement?

Perhaps the most striking evidence for the rapid replacement of a native cytoplasm by an alien one involves the race of *H. petiolaris* from Southern California described above (Dorado *et al.*, 1991). Herbarium records suggest that *H. petiolaris* was introduced into Southern California during the mid-1940s. The first collections were made in 1947 and the species is now rather common. Yet, all but four of the 137 individuals (six populations) sampled had the cytoplasm of *H. annuus*, a more common sunflower in the Southern California basin (Table 4). Thus, the *H. petiolaris* cytoplasm was almost completely replaced by a *H. annuus* cytoplasm in less than 50 years. To account for the rapidity of this process, Dorado *et al.* (1991) suggest that a single *H. annuus* propagule was introduced into a founder population of *H. petiolaris* followed by hybridization and CMS. Male sterility in hybrids and introgressants would quickly lead to a preponderance of individuals carrying exclusively *H. annuus* cpDNAs and predominantly *H. petiolaris* nuclear genes. Continuing this scenario, individuals from the founder population subsequently colonized the remainder of Southern California, resulting in the present pattern of cpDNA distribution. Alternatively, it is possible that the plants of *H. petiolaris* which invaded Southern California had captured an *H. annuus* cytoplasm prior to their introduction. This hypothesis seems unlikely, however, given the presence of both '*annuus*' and '*petiolaris*' cpDNAs in some populations of Southern California *H. petiolaris* (Table 4).

Likewise, Whittemore and Schaal (1991) note that oaks were absent from most of east North America during the Pleistocene and only reinvaded the central and northern part of their study area during the past 10,000 to 15,000 years (i.e. ca. 100 generations). Nonetheless, mixed populations had the same chloroplast genotype and cpDNA variation was correlated with geographic distribution rather than species boundaries. These data not only suggest extremely high levels of cytoplasmic gene flow, but also the potential for rapid cytoplasmic replacement.

Experimental evidence for the potential rapidity of cytoplasmic exchange in the absence of nuclear exchange has been provided by a study of mtDNA introgression in *Drosophila* (Aubert and Solignac, 1990). They introduced a single virgin female of *D. simulans* (initial frequency 0.03) into a population of *D. mauritiana*. In three generations the *D. mauritiana* mtDNA was almost

entirely displaced. Parallel introgression of nuclear genes was constrained due to hybrid male sterility.

The results from these studies suggest that cytoplasmic introgression leading to replacement can occur extremely rapidly. Thus, chloroplast capture and subsequent displacement can lead to phylogenetically misleading results in recently diverged species as well as more ancient lineages.

(4) At what taxonomic level(s) is cytoplasmic gene flow a serious problem?

Unexpected examples of cpDNA capture due to hybridization/introgression have now been documented at a variety of taxonomic levels: (1) among closely related species [e.g. *Zea* (Doebley, 1989), *Helianthus* (Rieseberg *et al.*, 1990a, 1990b, 1991, unpublished), *Salix* (Brunsfeld, 1990) and *Pisum* (Palmer *et al.*, 1985)]; (2) among more distantly related congeneric species [e.g. the intersectional level in *Gossypium* (Wendel *et al.*, 1981; Wendel and Albert, 1991), *Heuchera* (Soltis *et al.*, 1991a) and in *Populus* (Smith and Sytsma, 1990)]; and (3) at the intergeneric level between genera of the *Heuchera* group (Soltis *et al.*, 1991a). Examples of cpDNA capture among closely related species and among sections have been discussed above. An example of cpDNA capture among genera is presented in Figure 4 and discussed below.

The *Heuchera* group comprises nine genera (*Bensoniella*, *Conimitella*, *Elmera*, *Heuchera*, *Lithophragma*, *Mitella*, *Tellima* and *Tolmiea*) that are united by similar chemistry, karyotypes, morphology and the host preferences of parasitic rusts, as well as cpDNA restriction site data (Spongberg, 1972; Wells, 1979; Soltis, 1988; Soltis *et al.*, 1991a, unpublished). Analysis of populations from throughout the range of the monotypic *Tellima* revealed that populations from northern (TELLNO) and southern (TELLSO) portions of the range of this species appear on well-separated branches of the cpDNA-based phylogeny (Fig. 4). TELLSO populations have a chloroplast genome identical to that observed in two species of *Mitella*, *M. trifida* and *M. diversifolia*, raising the possibility of intergeneric hybridization and chloroplast capture. In support of this possibility is the fact that intergeneric hybridization has been documented relatively frequently in the *Heuchera* group (although no hybrids have been reported between *Tellima* and *Mitella*). In contrast, TELLNO populations have a unique chloroplast genome characterized by numerous autapomorphic mutations, suggesting that the chloroplast genome of this *Tellima* lineage was not obtained via hybridization. Electrophoresis of allozymes showed high genetic similarity (I = 0.99) among all populations of *Tellima grandiflora* analysed, which included both TELLNO and TELLSO populations (Rieseberg and Soltis, 1987). Thus, based on allozyme data, there is no evidence of a foreign nuclear genome in any *Tellima* populations. Furthermore, allozyme data also indicate the distinctiveness of all *Tellima* populations compared to *M. diversifolia*, *M. stauropetala* and *Conimitella* (*M. trifida* was not available for electrophoretic analysis).

The many examples of chloroplast capture discussed here (Table 1) provide ample evidence of the potential significance of cytoplasmic gene flow for phylogenetic inference among congeneric species or even closely related genera of many plant groups. Nevertheless, these examples do not address the potential of chloroplast capture for obscuring phylogenetic relationships at still higher taxonomic levels. One argument is that reticulation will be phylogenetically significant only within the limits of crossability (e.g. Clegg, 1989b). This view would exclude consideration of hybridization for phylogenetic interpretations at higher taxonomic levels (tribes, families and orders) in most plant groups. Other workers (e.g. Stebbins, 1950; Grant, 1953) suggest that the lack of clear discontinuities between major evolutionary lines in plants results from hybridization between these lineages during the early stages of their divergence. As a result, in their view, several generations of plant taxonomists have been unable to devise a generally satisfactory and truly phylogenetic classification system for angiosperms.

Although it is not now possible to estimate the significance of hybridization/introgression for phylogenetic inference at higher taxonomic levels, the high levels of sequence divergence observed between lineages which have exchanged chloroplasts suggests that this phenomenon may pose potential difficulties for higher-level phylogenetic reconstruction. For example, cpDNA sequence divergence values between species of *Gossypium* which have recently exchanged cpDNAs range from 0.88 to 1.02% (Wendel *et al.*, 1991). These estimates are nearly equivalent to the amount of sequence divergence between the cpDNAs of the A-genome and D-genome *Gossypium* species, whose parental lineages are thought to have diverged between 6 and 11 million years ago (Wendel, 1989). Even more astounding is the high level of sequence divergence between *Brassica nigra* and *B. oleraceae* (2.4%), two species which recently hybridized to form the allotetraploid *B. campestris* (Palmer *et al.*, 1983; Palmer, 1985). Thus, hybridization and potential chloroplast transfer can occur between species that have been isolated for 15–20 million years. Obviously, if cytoplasmic exchanges commonly occur between lineages approximating these divergence times, the possibility of hybridization affecting phylogenetic reconstruction at any level in angiosperms cannot be ignored—although the results of reticulation may no

longer be distinguishable from those of convergence, lineage sorting, and sample error.

(5) What are the consequences of chloroplast capture for phylogenetic reconstruction?

Of particular interest to the focus of this review is the fact that the many examples discussed above or listed in Table 1 also demonstrate the ease with which cytoplasmic gene flow can lead to faulty phylogenetic hypotheses. In the *Heuchera* group for example, it is easy to imagine how the erroneous conclusion could have been reached that the monotypic *Tellima* is closely allied with *Mitella diversifolia*, *M. trifida*, *M. stauropetala* and *Conimitella williamsii* (Fig. 4). Even if Soltis *et al.* (1991a) had sampled extensively, but only in the southern portion of range of *Tellima*, cpDNA data would still have led to this erroneous phylogenetic conclusion. Nuclear markers from enzyme electrophoresis reinforce the phylogenetic conclusions based on detailed sampling of cpDNA variation; that is, *Tellima* is not most closely related to *Conimitella* and the three *Mitellas*, with which it appears in the cpDNA tree. In *Gossypium* (Wendel *et al.*, 1991) and *Populus* (Smith and Sytsma, 1990), however, even relatively detailed population sampling was not sufficient to demonstrate the misleading nature of the cpDNA phylogeny. Only with evidence from nuclear genes (isozymes and nuclear rDNA in *Gossypium* and rDNA in *Populus*) could phylogenetic hypotheses compatible with previous taxonomic treatments for these groups be obtained.

The two most extreme examples, however, may be in *Quercus* (Whittemore and Schaal, 1991) and *Helianthus* (Rieseberg *et al.*, 1991). The great extent and rapidity of cytoplasmic gene flow in these two groups suggests that the cpDNA-based phylogenies for these taxa may be misleading and inadequate, regardless of population sampling regimes employed.

It is noteworthy that the majority of examples of cpDNA capture (Table 1) were revealed due to either detailed population sampling and/or by comparison with other biosystematic data sets—evidence from the nuclear genome being of particular importance. In contrast, imagine a group of congeneric plant species subjected to cpDNA analysis for which nuclear markers are not available and for which a sampling strategy is employed where only one, or perhaps several collections are used per species. It is easy to visualize how cpDNA transfer could lead to an inaccurate phylogeny. What is particularly worrying is that in such an instance, cytoplasmic gene flow might not even be suspected, and the faulty phylogeny would be viewed as a dependable phylogenetic hypothesis. Furthermore, the numerous documented examples of unexpected cpDNA transfer (see Table 1), some of which occurred in groups not noted for hybridization, indicate that this phenomenon cannot be ignored in any plant group. In addition, it is difficult to demarcate a taxonomic zone of safety because as noted above unexpected chloroplast transfer might occur at the specific, sectional or even generic levels, and cpDNA exchange between major lines early in their evolution may serve to obscure phylogenetic relationships even at higher levels. One can safely assume that the likelihood that chloroplast capture will go undetected and result in erroneous phylogenetic hypotheses increases the smaller the sample sizes employed. The probability of error also is greater in poorly understood groups for which little biosystematic data are available.

(6) How can faulty phylogenetic conclusions resulting from cytoplasmic introgression be avoided?

The obvious question that arises from this review is how best to avoid faulty phylogenetic conclusions resulting from cpDNA transfer. Perhaps the most effective solution is comparison of the cpDNA-based phylogenetic tree with one based on a nuclear gene sequence or sequences. Nuclear genes differ from organelle genes in their patterns of evolutionary divergence, transmission and linkages, thus revealing different aspects of the evolutionary history of the populations or species being studied. Furthermore, theoretical and empirical evidence (above) indicate that nuclear genes may be less vulnerable than organelle genes to hybridization/introgression phenomena.

Although nuclear genes are not the focus of this proposal, it is important that nuclear genome variation also be treated with caution when used for phylogenetic reconstruction. It has been evident for more than a decade that nuclear components are subject to an extraordinary degree of flux and turnover, which can also lead to numerous incongruencies between nuclear gene trees and organismal phylogenies (Dover, 1987; Hancock and Dover, 1990; Drouin and Dover, 1990). Many nuclear genes occur as duplicate copies or are members of multigene families, either present in tandem arrays or dispersed throughout the genome. Duplicate genes pose difficulties for the assessment of homology because the duplication event may predate the origin of the species being compared. In addition, it has been observed that multigene families often display a higher degree of sequence homogeneity within species than among species. This phenomenon has been termed 'concerted evolution', and has been shown to result from sequences within a gene family being corrected against each other by processes of unequal crossing over, gene conversion, transposition, slippage replication or RNA-mediated exchanges (Drouin and Dover, 1990). Furthermore, reciprocal or non-reciprocal exchanges can occur between genes through several of these mechanisms, resulting in recombinant genes, or apparent reticulation. All of these

phenomena can distort the evolutionary history of nuclear genes relative to that of the organisms under investigation and thus confound phylogenetic interpretations. It is noteworthy that ribosomal RNA genes (rDNA), which are often used for phylogenetic reconstruction, are known to be susceptible to many of these phenomena (e.g. Hancock and Dover, 1990; Hillis *et al.*, 1991). There are now several examples where discordant phylogenies are derived from different rDNA families (e.g. 5S rRNA versus 18S-25s rRNA) and also from different regions of the same family [e.g. Herzog and Maroteaux (1986) versus Lenaers *et al.* (1989)]. Thus, for nuclear genes as well as cytoplasmic genes, comparisons with phylogenetic hypotheses based on other nuclear gene sequences or cytoplasmic genes are essential to avoid faulty phylogenetic conclusions.

A second way to avoid erroneous phylogenetic hypotheses due to cytoplasmic introgression is by employing adequate sampling strategies. Unfortunately, analysis of many population samples in species-level phylogenetic studies is often deterred because it is expensive and time-consuming. Likewise, the number of species or genera included in higher-level phylogenetic studies is constrained due to expense and time. We suggest, therefore, that additional population samples simply be surveyed with regard to phylogenetically informative mutations, rather than the entire battery of restriction endonucleases or the entire sequence surveyed in the original phylogenetic study. Although it might be argued that introgression with unknown taxa might be missed using this approach, this seems unlikely if autapomorphic mutations were included in the study. It should also be noted that even the most extensive sampling strategies may not be sufficient to detect chloroplast capture, since the native cytoplasm appears to have been completely displaced by an alien one in some taxa.

Although extensive population sampling may be one of the easiest methods for avoiding erroneous phylogenetic conclusions based on cpDNA data, this approach has had little support in the systematic community. Rather, many investigators argue that the conservative evolution of cpDNA and generally low levels of intraspecific variation make extensive population sampling redundant. Although we agree that levels of cpDNA variation within most species are indeed low (several notable exceptions are reviewed in Soltis *et al.*, 1991b), we argue that this fact has little to do with choice of sampling strategy. The question that must be addressed instead is the likelihood that a character set from any given individual or population within a species will reflect accurately the phylogenetic history of that species. For the conservative chloroplast genome, ironically, the likelihood may be lower than expected due to high levels of cytoplasmic gene flow, possibly even lower than other data sets such as allozyme, flavonoids and morphology, whose phylogenetic utility has sometimes been criticized due to 'high levels of intraspecific variation'. Perhaps it would be more accurate to view levels of intraspecific variation as an indicator of the utility of a specific character set for intraspecific phylogenetic reconstruction rather than as a justification for sampling strategies employed in species-level phylogenetic studies.

Other approaches for reducing the vulnerability of phylogenetic systematics to cytoplasmic gene flow concern methods of data analysis. First, as emphasized by Smith and Sytsma (1990), phylogenetically informative characters from different sources (e.g. morphology, cpDNA and nuclear DNA) should not be combined prior to cladistic analyses. Rather, each data set should be analysed separately, with subsequent examinations and explanations of congruence or discordance. Second, if data from different sources are ultimately combined (this may be desirable since it would allow the greatest phylogenetic resolution and confidence), then algorithms must be developed which allow for reticulation. These algorithms would determine whether significant reductions in length or homoplasy could be achieved in a phylogenetic tree by postulating one or more reticulations. Presently, these calculations must be performed manually (e.g. Nelson, 1983; Funk, 1985; Rieseberg, 1991).

A potential alternative to developing wholly new algorithms for the phylogenetic analysis of reticulation would be the use of compatibility analysis (Estabrook, 1978) rather than maximum parsimony. This approach chooses a tree topology that maximizes the number of informative compatible sets among different DNA sequences of interest. Visual inspection of incompatible sites might allow identification of introgressed sequences. This approach has been successfully employed to detect reticulate producing processes such as gene conversion among members of the actin gene family in potato (Drouin and Dover, 1990). Drouin and Dover (1990) also employed a G-test to detect long regions of gene conversion between relatively divergent genes, a procedure which might be useful for detecting reticulation among species as well.

Conclusions

Recent papers have provided excellent reviews of the strengths of cpDNA variation for phylogenetic reconstruction (e.g. Birky, 1988; Clegg, 1989a; Crawford, 1989; Palmer, 1985a, 1985b, 1987; Palmer *et al.*, 1988; Sytsma, 1990). As a result, plant systematists increasingly rely on cpDNA evidence for phylogenetic inference. Nevertheless, despite the advantages of cpDNA data for phylogenetic reconstruction, the numerous unexpected examples of chloroplast capture through hybridization and introgression already revealed in the short history of

cpDNA systematics suggest a need for caution in the application of organellar variation to phylogenetic reconstruction in plants. Cytoplasmic gene flow in the absence of significant nuclear gene flow can occur through a variety of processes and often with remarkable speed. Furthermore, empirical evidence suggests that this process may even lead to the complete replacement of a native cytoplasm by an alien one. It is also clear that unexpected events of chloroplast capture can occur at a variety of taxonomic levels: among closely related species, at the sectional level within a genus, and at the generic level. In addition, cytoplasmic transfer between major evolutionary lines during early stages of their divergence has the potential to impact cpDNA phylogenetic reconstruction at all taxonomic levels in angiosperms, although it is recognized that the effects of introgression may be difficult to distinguish from convergence or lineage sorting in these instances.

Several approaches are suggested to avoid erroneous phylogenetic conclusions based on cpDNA data. These include comparisons with phylogenetic hypotheses based on nuclear gene sequences, comprehensive sampling methods and modified data analysis strategies. Suggested strategies for data analysis include separate phylogenetic analysis of character sets from different sources followed by analysis of the combined data sets using algorithms designed to accept reticulations.

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