Acta Bot. Neerl. 45(3), September 1996, p. 243–262

REVIEW

Molecular marker incongruence in plant hybrid zones and phylogenetic trees

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CONTENTS

Key-words: molecular markers, hybridization, introgression, cytoplasmic gene flow, molecular phylogeny.

INTRODUCTION

One of the striking observations from the young field of molecular evolutionary biology has been the high frequency of cases where taxon-specific markers are found to differ widely in both phylogenetic and geographic distribution. This pattern has been observed most frequently when cytoplasmic markers are compared with those of nuclear origin (Rieseberg & Soltis 1991), but similar observations are often made for comparisons among markers of nuclear origin only (Rieseberg & Ellstrand 1993). These observations are important to phylogenists because they provide 'footprints' of past evolutionary events, information that may be critical to elucidating true organismal phylogeny. Marker incongruence also is significant to the evolutionist because it may provide insights into the differences in the evolutionary biology of organellar versus nuclear genes (as well as differences among nuclear genes), the role of selection, linkage and recombination in controlling the frequency and spatial distribution of introgressed genes, and the effects of introgression on the maintenance of species differences.

In this paper, we list some of the best examples of molecular marker incongruence from both hybrid zones and phylogenetic trees in plants. General patterns emerging from this tabulation are discussed, and possible explanations are briefly summarized. In addition, recent studies of differential introgression and its mechanistic basis in wild sunflowers of the genus *Helianthus* are reviewed in detail. Results from these studies appear to explain patterns of marker discordance in *Helianthus*, and possibly many other groups of plants as well.

Table 1. Examples of phylogenetic incongruence resulting from hybridization and introgression

Taxa	Marker[1]	Pattern[2]	References
Andira humilis	C	P	Pennington 1995
Andira vermifuga	C	P	Pennington 1995
Andira sp. nov.	C	P	Pennington 1995
Argyroxiphium grayanum	C	C, P	Baldwin *et al.* 1990
Brassica napus	C, N, M	H, P	Erickson *et al.* 1983; Palmer 1988; Palmer *et al.* 1983; Song *et al.* 1990; Song & Osborn 1992
Brongniartia lupinoides	C	C	Dorado & Rieseberg 1992
Brongniartia molliculas	C	C	Dorado & Rieseberg 1992
Dioscorea cayenensis	C, R	H	Terauchi *et al.* 1992
Disa tripetaloides	C	P	Parker & Koopowitz 1993
Draba corymbosa	[C], [R], I	H	Brochman *et al.* 1992
Draba lactea	[C], [R], I	H	Brochman *et al.* 1992
Dubautia scabra	C	P, C	Baldwin *et al.* 1990
Glycine latifolia	C	P	Doyle *et al.* 1990b
Glycine microphylla	C	P	Doyle *et al.* 1990b
Glycine tabacina	C	H	Doyle *et al.* 1990a,b,c
Gossypium aridium	C	C	Wendel & Albert 1992
Gossypium barbadense	C, I	C	Percy & Wendel 1990
Gossypium bickii	C, R, [I]	D	Wendel *et al.* 1991
Gossypium cunninghamii	C	C	Wendel & Albert 1992
Gossypium gossypiodes	C, R	D	Wendel *et al.* 1995
Gossypium hirsutum	C, I	C	Brubaker *et al.* 1993
Helianthus annuus	C, [I]	C, P	Rieseberg *et al.* 1991a
Helianthus annuus subsp. *texanus*	C, R	H	Rieseberg *et al.* 1990a
Helianthus anomalus	C, R, [I]	P, H	Rieseberg 1991
Helianthus debilis subsp. *cucumerifolius*	C, R	P, H	Rieseberg *et al.* 1990a, 1991a, 1991b
Helianthus deseritcola	C, R, [I]	P, H	Rieseberg 1991
Helianthus neglectus	C, [R], [I]	C	Rieseberg *et al.* 1990b
Helianthus paradoxus	[C], R, I	H	Rieseberg *et al.* 1990b; Dorado *et al.* 1992
Helianthus petiolaris ssp. *fallax*	C, R	C, P	Rieseberg *et al.* 1991a
Helianthus petiolaris ssp. *petiolaris*	C, R	C, P	Dorado & Rieseberg, 1992; Rieseberg *et al.* 1991a
Hemionitis pinnatifida	C, I	H	Ranker *et al.* 1989
Heuchera grossularifolia	C, I, R	H	Wolf *et al.* 1990
Heuchera hallii	C, R	P	Soltis *et al.* 1991a
Heuchera micrantha	C, R	P	Soltis *et al.* 1991a
Heuchera nivalis	C, R	P	Soltis *et al.* 1991a
Heuchera parviflora	C, R	P	Soltis *et al.* 1991a
Heuchera	C, R	D	Kuzoff *et al.* 1993; Soltis & Kuzoff 1995
Ipomopsis aggregata susbsp. *aggregata*	C	C, P	Wolf *et al.* 1993
Ipomopsis aggregata subsp. *weberi*	C	C, P	Wolf *et al.* 1993
Ipomopsis tenuituba	C	P	Wolf *et al.* 1993
Iris nelsonii	C, I, N	H	Arnold 1993; Arnold *et al.* 1990b, 1991
Melaleuca alternifolia	C	C	Butcher *et al.* 1995
Microseris douglasii	C, [N]	C	Roelofs & Bachmann 1995
Paeonia banatica	R	H	Sang *et al.* 1995
Paeonia broteri	R	H	Sang *et al.* 1995
Paeonia cambessidesii	R	H	Sang *et al.* 1995

Table 1. *Continued*

Taxa	Marker[1]	Pattern[2]	References
Paeonia clusii	R	H	Sang *et al.* 1995
Paeonia coriaceae	R	H	Sang *et al.* 1995
Paeonia emodi	R	H	Sang *et al.* 1995
Paeonia mascula ssp. *hellenica*	R	H	Sang *et al.* 1995
Paeonia mascula ssp. *mascula*	R	H	Sang *et al.* 1995
Paeonia mlokosewitschi	R	H	Sang *et al.* 1995
Paeonia peregrina	R	H	Sang *et al.* 1995
Paeonia rhodia	R	H	Sang *et al.* 1995
Paeonia russi	R	H	Sang *et al.* 1995
Paeonia sterniana	R	H	Sang *et al.* 1995
Paeonia turcica	R	H	Sang *et al.* 1995
Paeonia wittmanniana	R	H	Sang *et al.* 1995
Persea americana var. *guatemalensis*	C, R	D, H	Furnier *et al.* 1990
Pinus banksiana	[C], [I], M	C	Dong & Wagner 1993; Wagner *et al.* 1987; Wheeler & Guries 1987
Pinus densata	C, [I]	H	Wang *et al.* 1990; Wang & Szmidt 1990, 1994
Pinus radiata	C	C	Hong *et al.* 1993
Pinus sylvestris var. *sylvestriformis*	C, I	H	Szmidt & Wang 1993
Pisum sativum	C	P	Palmer *et al.* 1985
Plantago major subsp. *major*	C	C	Hooglander *et al.* 1993
Plantago major subsp. *pleiosperma*	C	C	Wolff & Schaal 1992
Populus nigra	C, R	D	Smith & Sytsma 1990
Psilactis brevilingulata	C, R, [I]	D	Morgan 1993
Quercus alba	C, [R]	P	Whittemore & Schaal 1991
Quercus macrocarpa	C, [R]	P	Whittemore & Schaal 1991
Quercus michauxii	C, [R]	P	Whittemore & Schaal 1991
Quercus petraea	C	C (?)	Petit *et al.* 1993
Quercus pubescens	C	C (?)	Petit *et al.* 1993
Quercus robur	C	C (?)	Petit *et al.* 1993
Quercus stellata	C, R	P	Whittemore & Schaal 1991
Salix melanopsis	C, I	C	Brunsfeld *et al.* 1991, 1992
Salix taxifolia	C, I	D	Brunsfeld *et al.* 1991, 1992
Senecio cambrensis	[C], R, I	H	Ashton & Abbott 1992; Harris & Ingram 1992
Senecio flavus	C, I	C	Liston & Kadereit 1996
Sorghum bicolor	C, N	D	Aldrich & Doebley 1992
Streptanthus glandulosus	C, I	C, D	Mayer & Soltis 1994
Streptanthus glandulosus ssp. *secundus*	C, I	C, D	Mayer & Soltis 1994
Styrax americanum var. *americanum*	C, R	C	Fritsch 1995
Taraxacum officinale	C, R	D	King 1993
Tellima grandiflora	C, R	P, D	Soltis *et al.* 1991b; Soltis & Kuzoff 1995
Tripsacum andersonii	C, R	C, D	Larson & Doelbley 1994; Talbert *et al.* 1990
Tripsacum dactyloides	C, [R]	P	Larson & Doebley 1994
Tripsacum zopilotense	C, [R]	P	Larson & Doebley 1994
Zea perennis	C	C	Doebley 1989

[1]C=chloroplast DNA, R=nuclear ribosomal DNA, I=isozymes, M=mitochondrial DNA, N=nuclear markers other than rDNA (RAPDS, RFLPs). Markers in brackets have no bearing on conflict.
[2]C=morphologically 'pure' individuals contain markers characteristic of another species, H=population or taxon combines markers from two other species, P=species is polyphyletic or paraphyletic with respect to marker, D=phylogenetic analysis of different datasets differ in their placement of a taxon.

PATTERNS OF MARKER INCONGRUENCE

Theoretical and experimental studies (reviewed in Avise 1989; Rieseberg & Soltis 1991; Doyle 1992; Kadereit 1994) have demonstrated that incongruence among gene trees or between gene trees and organismal phylogenies can result from a variety of factors, including sampling error, convergence, evolutionary rate heterogeneity, phylogenetic sorting and hybridization and introgression. However, the most common source of phylogenetic incongruence in plants appears to be hybridization and introgression. In fact, Rieseberg & Soltis (1991) compiled a list of 37 examples of phylogenetic discordance due to cytoplasmic introgression. Of these, 28 were considered convincing. Since then 61 additional cases of phylogenetic incongruence due to cytoplasmic introgression have been reported, as well as several cases where the phylogenetic discordance is attributable to introgression of nuclear markers. These additional reports are listed in Table 1, along with the original 28 'convincing' examples from Rieseberg & Soltis (1991). However, because the plant molecular phylogenetic literature has grown so rapidly in the past 5 years, it is unlikely that Table 1 is comprehensive.

Although most of the examples in Table 1 clearly represent instances of nuclear or cytoplasmic introgression, several cases of incongruence appear to result from the biphyletic origin of diploid taxa (e.g. *Gossypium bickii*; Wendel *et al.* 1991) or the multiple origins of allopolyploids (e.g. *Glycine tabacina*; Doyle *et al.* 1990a, 1990b, 1990c). In addition, in several instances it is difficult to rule out alternative explanations for the incongruence observed such as phylogenetic sorting or the retention of ancestral polymorphism.

There are two major results from the expanded dataset that were not evident from the Rieseberg & Soltis (1991) listing. First, because many of the original examples were derived from crop plants and their wild relatives, it was not clear whether the high frequency of cytoplasmic introgression reported for these taxa would be characteristic of species groups not manipulated by humans. The current listing indicates that phylogenetic discordance due to introgression is a common feature of both wild and domesticated plant groups. Secondly, the original list only included examples where phylogenetic discordance was attributable to cpDNA introgression, whereas the current list includes many examples where phylogenetic incongruence results from introgression of nuclear markers. We predict that these examples will grow rapidly over the next decade as the nuclear genome is exploited more frequently in phylogenetic studies.

In addition to listing examples of phylogenetic incongruence, we also examined marker discordance in plant hybrid zones (Table 2). We define hybrid zone rather broadly here to include hybrid swarms and mosaic hybrid zones, as well as classic tension zones. Although we employ a broad definition of hybrid zones, we have restricted Table 2 to 16 hybrid zones where incongruence in the spatial distribution of molecular markers has been reported. We recognize that incongruence is often observed between morphological markers or between morphological and molecular markers (Rieseberg & Ellstrand 1993) in hybrid zones, but wish to avoid additional ambiguities often associated with phenotypic data.

One of the striking observations from Table 2 is that it includes most studies of hybrid zones that have employed more than one molecular marker. This provides confirmation for the prediction of Rieseberg & Ellstrand (1993) that 'as more molecular data become available, it is likely that their spatial distributions in hybrid swarms will prove to be increasingly idiosyncratic relative to morphological markers and relative to each other'.

Table 2. Hybrid zone studies where incongruence in the spatial distribution of species-specific molecular markers was detected

Taxa	Markers[1]	Pattern[2]	References
Aesculus pavia/*A. sylvatica*/*A. flava*	I	N	dePamphilis & Wyatt 1990
Asclepias exaltata/*A. syriaca*	I	N	Wyatt & Broyles 1992
Cypripedium candidum/*C. pubescens*	I	N	Klier *et al.* 1991
Gaillardia pulchella	I, N	N	Heywood 1986
Gossypium arboreum/*G. herbaceum*	I, N	N	Wendel *et al.* 1989
Gossypium barbadense/*G. hirsutum*	C, I, N	C	Brubaker *et al.* 1993; Percy & Wendel 1990; Wendel *et al.* 1992
Helianthus annuus subsp. *texanus*/ *H. debilis* subsp. *cucumerifolius*	C, R	C	Rieseberg *et al.* 1990a, 1991a, 1991b
Helianthus annuus/*H. petiolaris*	C, R	C	Dorado *et al.* 1992
Iris fulva/*I. hexagona*/*I. brevicaulis*	R, C, N, I	C	Arnold *et al.* 1990a,b, 1991, 1992
Melaleuca alternifolia	C		
Penestemon centranthifolius/ *P. sect. Specabilis*	I	N	Wolfe & Elisens 1993
Phlox drummondii/*P. cuspidata*	I	N	Levin 1975
Picea glauca/*P. engelmannii*/*P. sitchensis*	M, C, R	C	Sutton *et al.* 1991, 1994
Pinus contorta/*P. banksiana*	I, C, M	C, N	Dong & Wagner 1993; Wagner *et al.* 1987; Wheeler & Guries 1987
Populus fremontii/*P. angustifolia*	N, M	C	Keim *et al.* 1989; Paige *et al.* 1991; Paige & Capman 1993
Rhododendron canescens/*R. flammeum*	C, I	C	Kron *et al.* 1993
Yucca shidigera/*Y. baccata*	C, I, N	C	Hanson 1993

[1]C=chloroplast DNA, R=nuclear ribosomal DNA, I=isozymes, M=mitochondrial DNA, N=nuclear DNA markers other than rDNA.
[2]C=incongruence in the spatial distribution of species-specific cytoplasmic markers relative to nuclear markers, N=incongruence in the spatial distribution of species-specific nuclear markers.

It also is noteworthy that the majority of examples listed in Table 2 involve incongruence between cytoplasmic and nuclear markers. As will be discussed in more detail below, this is not surprising given the typically low nucleotide substitution rate, asymmetric inheritance and low effective number of alleles characteristic of plant cytoplasmic genomes (Birky *et al.* 1989). Of these factors, the asymmetric (often maternal) inheritance of cytoplasmic genomes is most critical because patterns of pollen and seed dispersal may differ dramatically, resulting in different patterns of introgression for nuclear versus cytoplasmic markers (e.g. Arnold *et al.* 1991).

In eight of the 16 hybrid zones listed in Table 2, spatial patterns of nuclear markers differed as well. Again, this is not surprising given that selection, linkage and genetic drift are likely to affect different markers in different ways (see Rieseberg & Ellstrand 1993; also below).

From a phylogenetic perspective, hybrid zones can be viewed as a likely source of phylogenetic incongruence. In fact, several of the phylogenetic studies listed in Table 1 prompted detailed studies of the hybrid zones (Table 2) responsible for the phylogenetic discordance observed. Thus, hypotheses for differential cytoplasmic versus nuclear

introgression or for the idiosyncratic distribution of taxon-specific markers in hybrid zones often also account for incongruence in the phylogenetic distribution of these markers. Potential explanations have been put forward by many authors (e.g. Smith & Sytsma 1990; Rieseberg & Soltis 1991; Wendel *et al.* 1991; Whittemore & Schaal 1991) and are summarized below. Many of these are interrelated and not mutually exclusive. The first eight hypotheses apply primarily to differential introgression between cytoplasmic and nuclear genes, whereas the final three hypotheses explain the idiosyncratic distributions of species-specific markers from both the cytoplasmic and nuclear genomes.

POTENTIAL EXPLANATIONS FOR THESE PATTERNS

(1) The effective number of alleles hypothesis. The often uniparental inheritance, vegetative segregation and low mutation rate of plant cytoplasmic genomes results in organelle genes having an effective number of alleles that is much lower than for nuclear genes (Birky *et al.* 1989). Thus, organelle variation will be influenced more strongly by genetic drift than nuclear variation and will tend to be distributed among rather than within populations or species. As a result, hybrid or introgresive populations are less likely to be polymorphic for parental cytoplasmic variants than for parental nuclear alleles, but more likely to be fixed for a foreign organelle than foreign nuclear allele.

(2) The dispersal hypothesis. Assuming maternal inheritance, organelle genes will be distributed by seeds only, whereas nuclear genes may be dispersed by both seeds and pollen (Birky 1988). Thus, in the absence of selection, dispersal should be less for organelle genes than nuclear genes, and hybrid zone width for cytoplasmic genes should be narrower than for nuclear genes. These differences will be largely dependent on dispersal distances, with the greatest differences in zone width occurring when dispersal via pollen is much greater than that via seed. On the other hand, hybrid zone width for organelle and nuclear genes should be roughly equivalent when seed dispersal distances are equal or greater than those of pollen (e.g. wind-dispersed seeds).

(3) The asymmetry hypothesis. Many plant hybrids are only possible, or at least much more frequent, with one of the parental species as the maternal parent (e.g. Keim *et al.* 1989). This may lead to cytoplasmic introgression in only one direction, whereas bidirectional nuclear introgression would still be possible.

(4) The minority hypothesis. Intraspecific pollen often appears to out-compete interspecific pollen in mixed pollen loads (e.g. Smith 1968, 1970; Kiang & Hamrick 1978; Carney *et al.* 1994). When this occurs, hybrid formation is often rare and limited to populations where flowering individuals of one species are in a minority (Arnold *et al.* 1993). The pollen load delivered to the minority species would consist primarily or entirely of foreign pollen and thus be more likely to result in the formation of hybrids. The consequences of the 'minority' hypothesis are similar to those of the 'asymmetry' hypothesis since the minority species will serve as the maternal parent of hybrids far more frequently than the majority species.

(5) The hybrid male sterility hypothesis. Male sterility in first generation hybrids and first or second generation backcrosses could quickly lead to the transfer of a foreign

cytoplasm in the absence of significant nuclear exchange (Aubert & Solignac 1990; Dorado *et al.* 1992).

(6) The founder event hypothesis. Hybrid founder events in combination with hybrid male sterility probably account for many of the cases of differential cytoplasmic versus nuclear introgression observed in hybrid zones and phylogenetic trees (e.g. Gyllensten & Wilson 1987; Dorado *et al.* 1992). A possible scenario might involve the introduction of a hybrid seed into a pure population of the paternal species. Due to hybrid male sterility, the foreign cytoplasm might become fixed in the population without significant nuclear gene introgression. Alternatively, in a dioecious species, the immigration of a small number of females into populations of another species would be an effective mechanism for differential cytoplasmic introgression (Aubert & Solignac 1990).

(7) The semigamy hypothesis. Wendel *et al.* (1991) suggest that the process of semigamy, where gamete fusion occurs without nuclear fusion, could result in the fixation of the nuclear genome of a male donor into a foreign cytoplasm in a single generation. This phenomenon has been reported for cotton (Turcotte & Feaster 1967) and may partly explain patterns of differential cytoplasmic exchange among wild cotton species (Wendel *et al.* 1991).

(8) The cytoplasm–nuclear combination hypothesis. Differences in relative fitness among cytoplasm–nucleus combinations also may lead to differential introgression (Wendel *et al.* 1991). For example, if the foreign cytoplasm/native nuclear combination has a slight advantage through female fitness, the foreign cytoplasm could quickly replace the native one (Frank 1989). Alternatively, the foreign cytoplasm may confer a competitive advantage regardless of its nuclear counterpart.

(9) The selection/linkage hypothesis. Studies of hybrid zones have revealed that the permeability of species barriers is largely dependent on selection, linkage and recombination (Barton & Hewitt 1983, 1985). Advantageous or neutral alleles will be slowed from introgressing 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 & Jones 1983; Powell 1983; Whittemore & Schaal 1991). Selection against loci scattered throughout the nuclear genome in concert with linkage could greatly reduce overall nuclear gene flow (Whittemore & Schaal 1991). This hypothesis is most plausible if epistatic interactions within the nuclear genome are strong. The rapid elimination of the donor parent nuclear genome in interspecific backcrosses in several genera (e.g. Stephens 1949; Rick 1963) appears to provide strong evidence for this hypothesis.

These same forces may largely account for differences in patterns of nuclear gene introgression as well. For example, recent linkage-map-based studies of introgression in sunflower (below) reveal that markers within or adjacent to chromosomal rearrangements (which are highly disadvantageous in hybrids) introgressed at very low frequencies or not at all. By contrast, rates of marker introgression in collinear genomic regions were much more variable, dependent apparently on physical linkage with advantageous or disadvantageous loci or on epistatic interactions among unlinked loci.

(10) The genetic drift hypothesis. Like the first nine explanations, genetic drift is most likely to lead to discordance in the spatial distributions between cytoplasmic and nuclear genes because of the large difference in effective numbers of alleles. Nonetheless, it is clear that chance also plays a major role in generating the idiosyncratic distribution of nuclear markers in plant hybrid zones and probably phylogenetic trees.

Genetic drift may be most critical in sorting polymorphisms following hybridization. Spatial sorting of parental markers typically follows range expansion of hybrid or introgressed populations (e.g. Dorado *et al.* 1992) or the movement of hybrid zones (e.g. Hanson 1993). Similarly, given sufficient time, the branching of lineages following hybridization may lead to phylogenetic sorting of parental markers (e.g. Rieseberg 1991). Both phenomena may lead to marker incongruence in phylogenetic trees.

(11) The gene conversion hypothesis. Molecular mechanisms such as gene conversion have the potential to introduce alleles into a new genetic background without introducing other parts of the genome (Harrison 1990). Gene conversion has been used to explain introgressive patterns of rDNA variation in grasshoppers (Arnold *et al.* 1988), as well the introduction of alien markers into sunflower without concomitant transfer of closely linked genes (below).

Obviously, the hypotheses listed above are not mutually exclusive. Thus, several of these are typically required to explain patterns of discordance in any particular instance, as will be illustrated below from our work on hybridization in wild sunflowers.

DIFFERENTIAL INTROGRESSION IN *HELIANTHUS*

Patterns in nature

Molecular phylogenetic study of *Helianthus* section *Helianthus*, which comprises the annual members of the genus, has generated numerous examples of marker incongruence. For example, a phylogenetic tree based on nuclear ribosomal DNA (nrDNA) (Rieseberg 1991) was topologically very similar to a morphologically based phylogenetic tree for the group (Schilling & Heiser 1981), and suggested relationships agreed in almost all respects to a published taxonomy for the group (Heiser *et al.* 1969). In contrast to the nrDNA tree, a cpDNA-based tree for the group (Rieseberg *et al.* 1991a) did not resemble any previous phylogenetic hypothesis for the group. In general, species geographically most proximal were most closely related in terms of cpDNA. All four polytypic species were polyphyletic in the cpDNA tree. Moreover, cpDNA haplotype was discordant with nrDNA genotype and morphological appearance for populations or individuals of six taxa.

The discordance reported between the cpDNA and nuclear-based trees in *Helianthus* was largely attributed to hybridization and introgression (Rieseberg 1991; Rieseberg *et al.* 1991a), although phylogenetic sorting could not be ruled out in several cases (Beckstrom-Sternberg *et al.* 1991). Nonetheless, several detailed studies of the geographic distribution of taxon-specific molecular markers were undertaken in areas where the geographic range of two species overlapped (mosaic hybrid zones) or where marker incongruence was detected in the phylogenetic study (Rieseberg *et al.* 1990a, *et al.* 1991b; Dorado *et al.* 1992).

Patterns of marker incongruence were also observed in the hybrid zone studies. Again, extensive cpDNA introgression was detected, but little introgression was

observed for nrDNA or morphological characters. For example, 71% of *H. debilis* spp. *cucumerifolius* individuals sampled from throughout its range had the cpDNA haplotype of the common sunflower *H. annuus*, but nuclear introgression was detected in less than 8% of the plants (Rieseberg *et al.* 1991b). Similarly, 97% of *H. petiolaris* individuals sampled from Southern California displayed cpDNA restriction site profiles of *H. annuus*, but only 1% had nrDNA markers of the latter species (Dorado *et al.* 1992).

Two different explanations were put forward to account for the differential cytoplasmic vs. nuclear gene flow observed in these studies. The high frequency of the *H. annuus* cytotypes in *H. debilis* ssp. *cucumerifolius* populations (Rieseberg *et al.* 1991b) was hypothesized to result from the greater abundance of *H. annuus* in the area of sympatry. This would result in a proportionately greater introduction of *H. annuus* achenes into *H. debilis* ssp. *cucumerifolius* populations than vice versa. The integrity of the nuclear genome would be maintained by selection against foreign nuclear genes coupled with selection. By contrast, Dorado *et al.* (1992) suggested that the presence of the *H. annuus* cpDNA haplotype in *H. petiolaris* populations from Southern California was more probably the result of stochastic events than selection. Specifically, they suggested that, due to the recent introduction of both species to Southern California, the pattern of cytoplasmic introgression observed was probably best accounted for by a hybrid founder event and subsequent range expansion of the introgressed individuals.

Although these explanations may be largely correct, understanding their mechanistic basis requires detailed study of the reproductive barriers isolating wild sunflower species. Recent studies of two of these isolating barriers, interspecific pollen competition and chromosomal structural differentiation, provide empirical evidence for several mechanisms capable of generating marker discordance in sunflower hybrid zones and, eventually, phylogenetic trees.

Interspecific pollen competition

Interspecific pollen competition was one of the first reproductive isolating mechanisms to be described in plants. For example, Darwin (1859, p. 84) writes that 'if you bring on the same brush a plant's own pollen and pollen from another species, the former will have such a prepotent effect that it will invariably and completely destroy, as has been shown by Gärtner, any influence from the foreign pollen'. Although Darwin clearly considered pollen competition to be an important isolating mechanism in plants, it has received surprisingly little attention since then. Nonetheless, there are several recent studies that suggest that the importance Darwin placed on interspecific pollen competition was well-founded (Smith 1968, 1970; Heiser *et al.* 1969; Kiang & Hamrick 1978; Arnold *et al.* 1993; Carney *et al.* 1994).

One of the best examples of the role of interspecific pollen competition as a reproductive barrier is in the genus *Iris* (Arnold *et al.* 1993; Carney *et al.* 1994; Carney *et al.* 1996). They showed that when mixed loads of *Iris fulva* or *I. hexagona* pollen were applied to stigmas of either species, intraspecific pollen was much more likely to fertilize ovules than interspecific pollen. Thus, they suggest that hybrid formation in *Iris* is likely to be relatively rare and often restricted to populations where flowering individuals of one species are in a quantitative minority. This would create a situation where the pollen load delivered to the minority species would consist almost or entirely of foreign pollen and thus be more likely to result in the production of hybrids.

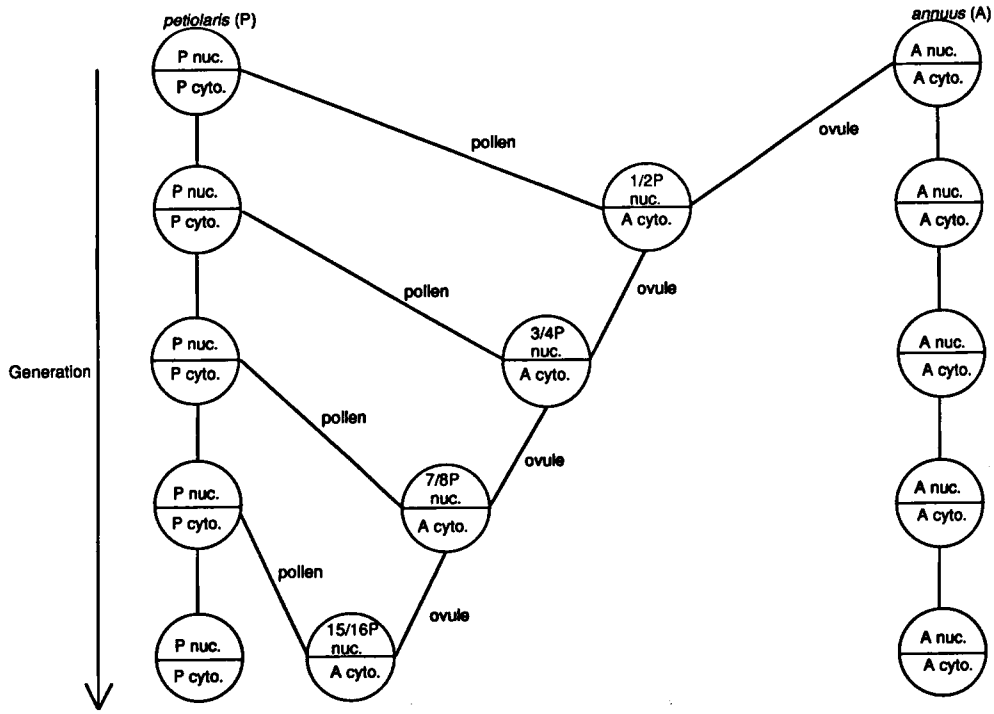


Fig. 1. Hypothetical scenario for cytoplasmic introgression in a population of *H. petiolaris* following the introduction of a single individual of *H. annuus*. Due to pollen competition, *H. annuus* serves as the maternal parent (ovule). Male sterility in first and later generation hybrids and backcrosses quickly leads to the production of plants that have the cytoplasm of *H. annuus*, but whose nuclear genes are predominately those of *H. petiolaris*. Redrawn from Rieseberg *et al.* (1995a).

A similar phenomenon has been reported for artificial crosses between *Helianthus annuus* and *H. petiolaris* (Rieseberg *et al.* 1995a). The number of hybrids produced from mixed intra- and interspecific pollen loads was significantly less than expected ($P<0{\cdot}01$), regardless of the maternal parent. However, hybrids were significantly more frequent with *H. annuus* rather than *H. petiolaris* as the maternal parent ($P<0{\cdot}01$).

The presence of interspecific pollen competition in sunflower and other species is significant because it may explain patterns of differential cytoplasmic versus nuclear introgression reported in many plant groups. For example, in *Helianthus*, several wild sunflower species, including *H. petiolaris*, appear to have captured the cytoplasm of the common sunflower, *H. annuus* (Rieseberg *et al.* 1991a; Dorado *et al.* 1992). Due to pollen competition, hybridization is most likely to take place when a single individual of *H. annuus* is introduced into a population (Fig. 1). In addition, as the minority taxon, it will nearly always be the female parent of the hybrid. Male sterility in hybrids and introgressants could quickly lead to the presence of individuals carrying the cytoplasm of the minority species and the nuclear genes of the majority species. Continuing this scenario, individuals from the 'hybrid founder population' could expand their geographic distribution, leading to the differential patterns of cytoplasmic versus nuclear introgression observed in many plant hybrid zones, including several in *Helianthus* (e.g. Dorado *et al.* 1992).

Chromosomal structural differences

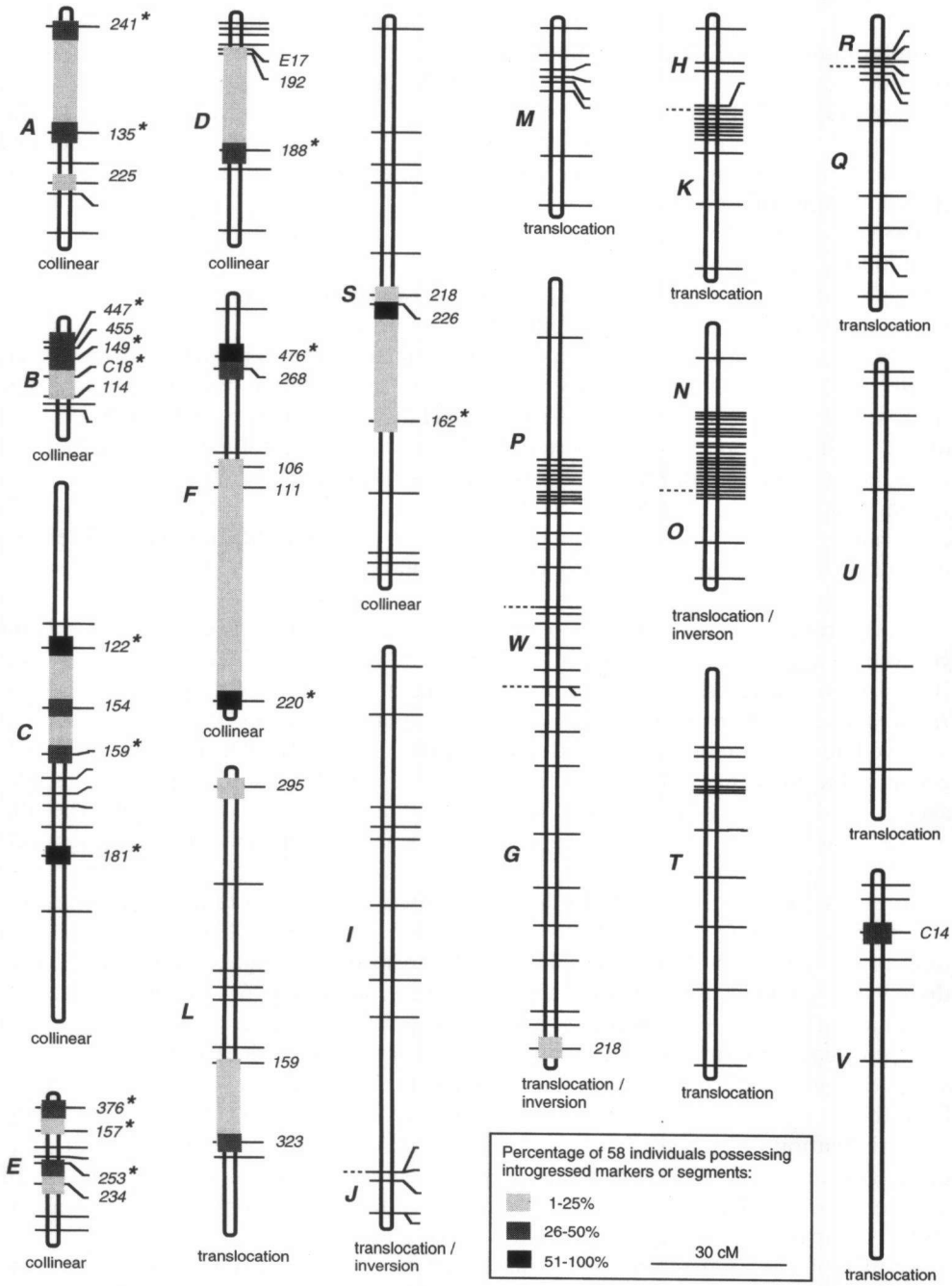
Chromosomal structural differences are thought to serve as important reproductive isolating mechanisms among sympatric *Helianthus* species. First generation hybrids are semisterile, apparently due to meiotic abnormalities; meiotic preparations reveal complex multivalent formations and bridges and fragments, suggestive of chromosomal translocations and inversions. These observations have been confirmed by comparative linkage mapping studies (Rieseberg *et al.* 1995c). For example, *H. petiolaris* and *H. annuus*, two common annual sunflowers, are known to differ by a total of ten chromosomal rearrangements including seven interchromosomal translocations and three inversions (Riseberg *et al.* 1995c).

Theory suggests that chromosomal structural changes have the potential to generate the kinds of marker incongruence often observed in hybrid zones, and that eventually appear in phylogenetic trees. This is because most chromosomal rearrangements have the effect of reducing recombination between rearranged linkages and, as a consequence, rates of introgression within those regions of the genome (Hanson 1959a, 1959b). In some instances, the effective reduction in recombination appears to result from selection against recombinant gametes (Hanson 1959a, 1959b) leading to lower hybrid fertility, whereas in other cases actual decreases in recombination frequency are observed, without loss of fertility. Thus, markers within the rearranged portion of the genome are unlikely to introgress, or if they do, at a rate much lower than that in the collinear genomic region. Cytoplasmic genomes will, of course, be immune to the effects of rearrangements and their introgression should not be limited by chromosomal structural divergence.

To determine whether chromosomal structural differences might play a role in generating marker incongruence in *Helianthus*, Riseberg *et al.* (1995b) generated a BC_2F_3 (two backcrosses followed by two generations of selfing) progeny between *H. annuus* and *H. petiolaris*. Fifty-eight individuals from this progeny array were surveyed for 197 random amplified polymorphic DNA (RAPD) markers of known genomic location (Rieseberg *et al.* 1995b). Based on *H. annuus* map distances, the average distance between markers was approximately 6·5 cM (Fig. 2). Of the 197 markers surveyed, 31 (15·7%) were observed in at least one of the 58 introgressed individuals (Fig. 2).

To assess whether the overall, collinear, and non-collinear patterns of introgression observed in *Helianthus* differed from what would be expected if there were no barriers to introgression, we performed simulations of unrestricted introgression for each of the three views of the genome. Because markers were separated by 6·5 cM on average and the actual rates of introgression were assessed from BC_2F_3 generation plants, significant linkage disequilibrium was detected among only a few tightly linked loci. Hence, in each simulation we sampled at random, the proportion of markers expected to introgress from the total markers in a portion of the genome (Table 3) for each of 58 individuals. This stimulation was repeated 100 times for the entire, collinear and non-collinear parts of the genome. The simulations allowed us to generate null-hypothesis distributions of the proportions of markers that should introgress into a given proportion of individuals and to compare our actual results with those of the null hypothesis.

Results of the simulations are summarized in Table 3. Even under worst case conditions, i.e. allowing the maximum number of markers to introgress into a particular proportion of individuals, all portions of the genome had significantly higher numbers of markers that failed to introgress at all (entire genome: $G=2169$, $P<<0·0001$; collinear


241*
A
135*
225
collinear
D
E17
192
188*
collinear
447*
455
149*
C18*
B
114
collinear
476*
268
F
106
111
220*
collinear
122*
154
C
159*
181*
collinear
295
L
159
323
translocation
S
218
226
162*
collinear
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218
translocation / inversion
N
O
translocation / inverson
T
translocation
U
translocation
C14
V
translocation
Percentage of 58 individuals possessing introgressed markers or segments:
1-25%
26-50%
51-100%
30 cM

Table 3. Observed and expected proportions and numbers (in parentheses) of markers expected to introgress into 0%, 1–25%, 26–50% and >50% of individuals

Percentage of individuals in which markers introgressed	Entire genome (197 markers)		Collinear portion (58 markers)		Non-collinear portion (139 markers)	
	observed	expected	observed	expected	observed	expected
0%[1]	0·85 (167)	0·0009 (0)	0·57 (33)	0·0006 (0)	0·96 (134)	0·0008 (0)
1–25%	0·07 (13)	0·9662 (190)	0·17 (10)	0·9755 (57)	0·02 (3)	0·9656 (134)
26–50%	0·06 (12)	0·0329 (6)	0·17 (10)	0·0239 (1)	(0·01) (2)	0·0336 (5)
>50%	0·03 (5)	<<0·0001 (0)	0·09 (5)	<<0·0001 (0)	0·0 (0)	<<0·0001 (0)

[1]Expected values were calculated using the mean standard deviation calculated from the standard deviations of 100 simulations in which there were no barriers to introgression. Details on the simulations are available from the authors.

portion: $G=420$, $P<<0·0001$; non-collinear portion: $G=1871$, $P<<0·0001$). Nonetheless, a much higher proportion of markers from non-collinear genomic regions failed to introgress (96·4%) than of markers from collinear portions of the genome (56·9%). In contrast, the collinear portion of the genome had significantly higher proportions of markers introgressing into >25% of individuals ($G=76·4$, $P<0·0001$), whereas the numbers of markers from non-collinear genomic regions introgressing into >25% of individuals did not differ significantly from expectation ($G_1=0·104$, $P>0·5$). Finally, five markers from the collinear portion of the genome introgressed into >50% of the individuals when none were expected to. These results suggest (1) the non-collinear portion of the genome is well-protected from introgression by translocations and inversions between *H. annuus* and *H. petiolaris*, (2) some segments of the collinear portion of the genome are also protected from introgression although the mechanism is unclear and (3) other segments of the collinear portion of the genome appear to introgress preferentially relative to expectations.

The low frequency of introgression for many markers in the collinear portion of the genome suggests that genic factors, as well as chromosomal structural rearrangements, may affect rates of introgression in *Helianthus*. That is, selection against *H. petiolaris* genes in concert with linkage, may have reduced or eliminated introgression in parts of the genome not protected by structural changes. Possibly, this results from co-adapted

Fig. 2. Composite graphical; genotype for 58 individuals from a BC_2F_3 progeny of *H. annuus* × *H. petiolaris* (modified from Rieseberg *et al.* 1995b). The graphical genotype is based on the 1084 cM genomic map of the recipient species, *H. annuus* (Rieseberg *et al.* 1995c), which has been extended by approximately 290 cM due to the occurrence of several *H. petiolaris* markers outside currently mapped regions of *H. annuus*. Letters at the left of each linkage group designate major linkage blocks and indicate their relationship to homologous linkages in *H. petiolaris* (Rieseberg *et al.* 1995c). Chromosomal structural differences between the species are indicated at the base of each linkage group. Horizontal lines indicate the genomic location of the 197 *H. petiolaris* RAPD markers surveyed, with an average distance of 6·5 cM between markers based on *H. annuus* map distances. The percentages of individuals carrying a particular introgressed marker or putative introgressed chromosomal segment are indicated by black or grey bars within linkage groups. Because most of the RAPD markers are dominant, we often were unable to determine whether the *H. petiolaris* markers or chromosomal segments were present in the homozygous or heterozygous condition. Markers with epistatic interactions are indicated by asterisks.

gene complexes that resist being broken. Alternatively, areas of the collinear portion of the genome that are protected from introgression may be so because of small-scale chromosomal inversions and translocations that were not detected by the mapping study.

However, why certain markers or chromosomal segments introgressed at higher than predicted rates is not as easily explained. Strong selection for *H. petiolaris* co-adapted gene complexes and linkage may at least partly account for the patterns of introgression observed. If this explanation is correct, significant associations should be observed among loci that are not physically linked. This 'co-adaptive fitness epistasis' should be detectable as two-way, three-way and higher-order associations among unlinked markers, that are themselves tightly linked physically with loci that influence hybrid fitness and thus the genomic composition of hybrids. To test for these interactions (Rieseberg *et al.* 1996), we analysed the locus × progeny array from each hybrid lineage for significant negative and positive associations between every two-way and three-way combination of unlinked loci (Fig. 3). Analyses of higher-order interactions were precluded by the number of combinatorial possibilities.

The following equation was used to compute the test statistic (ρ) for two-way epistatic interactions among unlinked loci (N_{loci}=number of unlinked loci; N_{progeny}=number of progeny tested; and $\text{locus}_{n,p}$=0 if the *H. petiolaris* marker is absent and 1 if present):

$$\rho_{i \times j} = \frac{\sum_{p=1}^{N_{progeny}} \prod_{n=i,j} locus_{n,p}}{\sqrt{\sum_{p=1}^{N_{progeny}} locus_{i,p} \times \sum_{p=1}^{N_{progeny}} locus_{j,p}}}, \text{ where } 0 \leqslant \rho_{i \times j} \leqslant 1 \qquad (1)$$

Equation (1) can be generalized to N-way epistatic interactions:

$$\rho_{i \times \ldots \times Nloci} = \frac{\sum_{p=1}^{N_{progeny}} \prod_{n=1}^{N_{loci}} locus_{n,p}}{\sqrt[Nloci]{\prod_{n=1}^{N_{loci}} \sum_{p=1}^{N_{progeny}} locus_{n,p}}}, \text{ where } 0 \leqslant \rho_{i \times j} \leqslant 1 \qquad (2)$$

Significance for each two- or three-way association was tested by comparing ρ_{observed} with ρ_{expected} as computed by bootstrap randomization of the observed data (N=10 000, Fig. 3).

Ten significant ($\alpha \leqslant 0{\cdot}0001$; Fig. 3) two-way associations were observed among the introgressed markers, whereas $<<1$ were expected by chance, given the total number of pairwise comparisons. In the more powerful three-way analysis, we observed 21 three-way associations ($\alpha \leqslant 0{\cdot}0001$; Fig. 3), whereas none were expected by chance. Thus, a complex web of genetic epistasis was revealed, involving 15 (48%) of the *H. petiolaris* loci retained in the experimental hybrids and seven of the 17 *Helianthus* linkage groups (Figs 1, 2). Moreover, because much of the *H. petiolaris* genome was eliminated from the hybrid lineages in early generations not analysed here, the epistasis we report only represents that subset of *H. petiolaris* co-adapted gene complexes that have neutral or favourable interactions with the *H. annuus* genomic background.

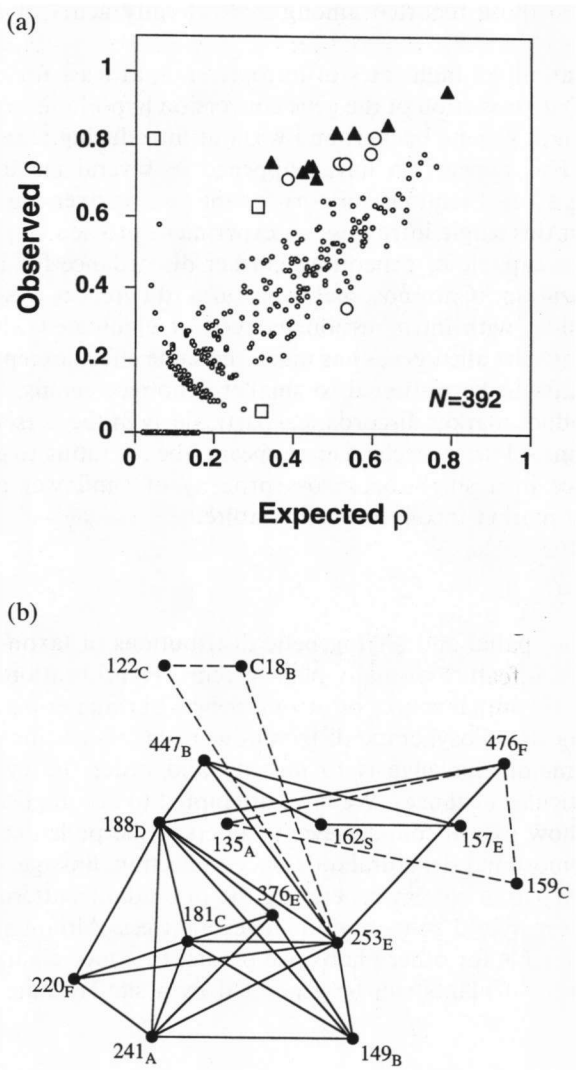

Fig. 3. Epistatic interactions in the BC_2F_3 progeny array. (a). Scatter plots of observed and expected ρs for two-way epistatic interactions: ▲ = $P<0{\cdot}0001$; ○ = $P\leqslant 0{\cdot}001$; □ = $P\leqslant 0{\cdot}01$; o = NS. Symbols (▲, ○, □) above the non-significant interactions are positive associations, and symbols below are negative associations. Positive associations occur when unlinked *H. petiolaris* markers appear together within individuals of the progeny array more often than would be expected by chance, suggesting non-additive, positive fitness effects when these markers appear together. By contrast, negative associations occur when unlinked *H. petiolaris* markers appear together less often than would be expected by chance, suggesting non-additive negative fitness effects when these markers appear together. These negative associations appear to reflect poor interactions between *H. petiolaris* markers in a *H. annuus* genetic background. (b). Web of three-way epistatic interactions of $P\leqslant 0{\cdot}0001$ for BC_2F_3 progeny array as indicated by triangles connecting three unlinked markers. Marker designations indicate primer number and linkage block (cf. Fig. 2). Positive associations are indicated by solid lines and negative by dashed lines. Positive three-way associations are as described above for two-way associations, except that three, rather than two, *H. petiolaris* markers are involved. Negative three-way associations may consist of negative two-way associations only, or a combination of negative and positive two-way associations.

Analyses of negatively-selected markers might reveal additional evidence for epistatic interactions such as those reported among male sterility genes in *Drosophila* (Cabot *et al.* 1994).

Another explanation for high rates of introgression, at least for certain markers, is gene conversion. One prediction of the gene conversion hypothesis is that alleles may be introduced into a new genetic background without introducing closely linked markers (Harrison 1990). This appears to have happened in several instances in *Helianthus* (Fig. 2), providing at least tenuous support for the gene conversion model.

The results from this single introgression experiment provide empirical evidence for several mechanisms capable of generating marker discordance for four to five generations of hybridization. Chromosomal structural differences result in differential genomic permeability, with introgression reduced or eliminated within non-collinear regions. Selection against alien genes has much the same effect, except that resistance to introgression appears to be restricted to smaller genomic regions. Selection for alien genes also will produce marker discordance, particularly in the presence of epistasis, as will gene conversion. All these mechanisms appear to be operating to generate molecular marker discordance in a single backcross progeny of sunflower and may partially explain patterns of marker incongruence in nature.

CONCLUSIONS

Incongruence in the spatial and phylogenetic distributions of taxon-specific molecular markers is a common feature of many plant groups. Hybridization and introgression often appear to be the initial source of incongruence, but numerous other factors play a role in generating the idiosyncratic distributions of taxon-specific markers following hybridization. A major challenge is to identify and order the evolutionary factors responsible in particular instances. We have attempted to accomplish this in sunflower and demonstrate how hybrid founder events, interspecific pollen competition, hybrid male sterility, chromosomal structural differences, selection, linkage, epistasis, and gene conversion all may play a role in generating the discordant patterns of introgression reported in sunflower hybrid zones and phylogenetic trees. Although comparable data sets are not yet available for other plants, we predict that molecular marker incongruence in many groups of plants will be generated by a similar suite of ecological and genetic factors.

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