American Journal of Botany 82(7): 944–953. 1995.

INVITED SPECIAL PAPER

THE ROLE OF HYBRIDIZATION IN EVOLUTION: OLD WINE IN NEW SKINS[1,2]

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Perhaps no one has had a greater influence on the development of hybridization theory than Dr. Ledyard Stebbins. However, his contributions have not been restricted to his writings or lectures alone. Many of you have marveled at the great authority with which Dr. Stebbins identified the hybrid status, parentage, and ploidal level of plants in the field or on herbarium sheets. He didn't seem to need all the modern techniques that the current generation of botanists is both blessed and burdened with. For example, I remember a short field trip with Dr. Stebbins in the fall of 1984 to look at some sunflower hybrids near Davis. The plants were dead, only a few dried leaves, stalks, and floral heads remained, yet he confidently identified parental, hybrid, and backcrossed generations. Ironically, after 2 yr of careful molecular studies, I essentially confirmed his initial observations (Rieseberg, Soltis, and Palmer, 1988). Thus, I came to the conclusion that he was able to see things in plants that escaped less careful or less knowledgeable observers such as myself.

The interest of the botanical community in plant hybridization appears to be somewhat cyclical. From the late 1940s through the middle 1960s, much of the morphological and cytological variation in plant populations was attributed to introgressive hybridization (e.g., Anderson, 1949; Stebbins, 1959). Over the next two decades, these interpretations were viewed with greater skepticism (Barber and Jackson, 1957; Gottlieb, 1972; Heiser, 1973; Doebley, 1984), and I think appropriately so, leading to perhaps less interest in hybridization and its evolutionary role. In the early 1990s, however, there appears to have been a renewed interest in hybrids, stimulating several recent reviews (e.g., Abbot, 1992; Arnold, 1992, 1994; Rieseberg and Brunsfeld, 1992; Rieseberg and Wendel, 1993; Strauss, 1994). This resurgence in interest has led to a reevaluation of some of the central tenets or dogmas of hybridization theory (e.g., Rieseberg and Ellstrand, 1993; Arnold and Hodges, in press), as well as to the origin of some new ideas and approaches to the study of hybrids (e.g., Whitham, 1989; Rieseberg et al., 1993).

The purpose of this paper is to discuss some general misconceptions that many botanists and zoologists appear to hold about hybrids, as well as to present several of the most exciting recent discoveries in the area of plant hybridization. Finally, I will discuss what we know now about the "evolutionary role" of hybridization in plants and what kinds of studies are needed to achieve a satisfactory answer to this question. Throughout the paper, my comments will be restricted to diploid hybrids rather than allopolyploids since several excellent reviews of polyploid evolution have recently been published (e.g., Hilu, 1993; Soltis and Soltis, 1993).

MISCONCEPTIONS ABOUT HYBRIDS

The study of hybridization has been hampered in the past by the belief in certain simplistic rules or tenets that more careful study suggests are at least partially misleading or wholly inaccurate.

Hybrids are morphologically intermediate—One of the most common misconceptions is that hybrids are typically morphologically intermediate between their parents. The problem with this view is clearly illustrated in a recent review by Rieseberg and Ellstrand (1993), who compiled a list of 46 studies that report morphological character expression in hybrids. The list included 32 examples of character expression in first-generation hybrids, nine examples from later generation hybrids, and four examples of diploid hybrid speciation. For each hybrid, the number of intermediate, parental, and extreme characters was determined. The studies analyzed were not parallel in terms of their treatment of hybrids. For example, several of the data sets were taken from cladistic studies, where quantitative variation may be partitioned into discrete classes. Thus, partially intermediate character states might have been scored as parental due to the lack of an intermediate state for that character (e.g., McDade 1990). In other instances, means and/or ranges of values were given for hybrids rather than absolute values. Some studies tended to emphasize quantitative traits, particularly those interested in hybrid identification or morphological genetics, whereas other studies, particularly phylogenetic ones, tended to emphasize qualitative characters. Finally, some studies employed floral characters only, others emphasized vegetative characters, whereas others reported on both floral and vegetative characters. Given these caveats, Rieseberg and Ellstrand (1993) note that the results from this compilation must be interpreted cautiously.

Nonetheless, the survey clearly indicates that hybrids are no more likely to display intermediate character states than parental ones (Table 1; also see Wilson, 1992). A possible explanation for the high proportion of parental characters expressed in hybrids is that many morpholog-

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[1] Manuscript received 11 October 1994; revision accepted 18 January 1995.

The author thanks Jerry Gastony for suggestions on the title and Rick Noyes for helpful comments on the manuscript. The research on sunflowers discussed in this paper has been supported by grants from the NSF and USDA.

[2] Ledyard Stebbins lecture presented at the 45th AIBS annual meeting at Knoxville, TN.

TABLE 1. Morphological character expression in plant hybrids (Rieseberg and Ellstrand, 1993). *N* refers to the number of characters surveyed.

Hybrid genealogical class	Percentage intermediate characters	Percentage parental characters	Percentage extreme characters
First-generation hybrids ($N = 1{,}353$)	44.6	45.2	10.2
Later generation hybrids ($N = 372$)	—	—	30.6
Hybrid species ($N = 319$)	35.3	48.7	16.0

ical differences differentiating closely related species are under simple genetic control (Hilu, 1983; Gottlieb, 1984). Thus, the expression of parental vs. intermediate character states in hybrids will depend on the nature of the genetic control of a particular character, as well as interactions with the environment.

Perhaps the most striking result from this survey, however, was the high frequency of "extreme" or novel characters observed in hybrids. Over 10% of morphological characters in primary hybrids are extreme, and over 30% are extreme in later generation hybrids (Table 1). Several explanations have been put forward to account for the expression of novel or extreme characters in hybrids including 1) an increased mutation rate in hybrids; 2) the complementary action of new combinations of normal alleles; 3) unexpressed (or expressed) alleles may be placed under a new pattern of regulation, as has been suggested to explain novel floral pigmentation in *Clarkia gracilis* (Gottlieb and Ford, 1988); 4) the fixation of recessive alleles present in the heterozygous form in the parents; and 5) reduced developmental stability (Wagner, 1962; Levin, 1970; Grant, 1975).

Although it is difficult to determine which of these phenomena is responsible for any given hybrid trait, the high frequency of extreme characters in hybrids supports the view of hybridization as a source of variability upon which selection can act. From a systematic perspective, these data suggest that rather than being morphologically intermediate, hybrids are actually a mosaic of parental, intermediate, and extreme characters. Thus, some traits are more likely to reveal hybrid ancestry than others.

Hybrids are uniformly less fit than their parents—There is also a widespread view that hybrids are uniformly less fit than the parental species. This belief appears to derive from observations of sterility or semisterility in F_1 hybrids between many animal and plant species (e.g., Grant, 1964; Heiser et al., 1969). However, we also know that many plant hybrids are fully fertile (e.g., Hardin, 1975). Moreover, even in those instances where F_1s are partially sterile, full fertility is often achieved in later hybrid generations (e.g., Heiser, 1947).

TABLE 3. Abundance of parasites and herbivores in hybrid zones (Strauss, 1994).

Number of cases	Abundance of pests on hybrids
32	> Than either parent
24	> Than one parent
6	< Than either parent
28	= To one parent
27	= To both parents

Unfortunately, there are few detailed studies in the literature that carefully examine the fitness of hybrids. However, the results from these few studies, as well as the fitness parameters measured, are listed in Table 2 (also see Arnold and Hodges, in press). In almost all cases, only a subset of fitness components was reported. Nonetheless, based on those fitness components measured, hybrids may be more fit than both parental species (*Oryza*), less fit than either parent (*Helianthus*), intermediate in fitness (*Artemisia, Eucalyptus, Iris*), or equivalent in fitness to both parents (*Phlox*). Thus, it appears that hybrids are not always less fit than their parents.

Several other studies have indirectly measured hybrid fitness by studying the abundance of parasites and herbivores in hybrid zones (Table 3). In general, the results are similar to direct estimates of hybrid fitness in that there is great diversity in the patterns of abundance of herbivores, pathogens, and parasites in hybrid zones (Strauss, 1994).

Hybrids exhibit character coherence—Another commonly held view is that parental characteristics often remain associated or correlated in segregating hybrid progenies (Anderson, 1949; Clausen and Hiesey, 1958). In fact, these character associations (or coherence) were proposed by Anderson to be a diagnostic feature of natural hybrid populations (Anderson, 1949) and led to the widespread use of pictorial scatter diagrams for diagnosing hybrids and introgressants in natural and artificial populations. Anderson believed that character coherence was the result of both physical linkage (which inhibited the break-up of parental character combinations in hybrids) and selection against plants with recombinant phenotypes.

The Andersonian theory of character coherence has been questioned, however, on the basis of several theoretical, experimental, and descriptive studies (Dempster, 1949; Goodman, 1966; Wall, 1970; Grant, 1979; Ford and Gottlieb, 1989, 1990). For example, Goodman (1966)

TABLE 2. Relative fitness of plant hybrids. Numerous examples of reduced hybrid fitness due to sterility or semisterility are not included.

Taxon	Relative fitness of hybrids	Fitness measurement	Reference
Artemisia	> One parent	Herbivore attack, fecundity	Graham et al., 1994
Eucalyptus	> One parent	Reproductive parameters	Potts and Reid, 1985, 1990
Helianthus	< Both parents	Pollen stainability, seed set	Heiser, 1947, 1949
Iris	> One parent	Shade tolerance	Bennett, 1989; Bennett and Grace, 1990
Oryza	> Both parents	Vigor, tiller number	Langevin, Clay, and Grace, 1990
Phlox	= To both parents	Reproductive parameters	Levin and Schmidt, 1985

TABLE 4. Character correlations in hybrid populations (Grant, 1979).

Species pair	Percentage of character pairs with significant correlations ($P < 0.05$)
Aquilegia formosa × *A. pubescens*	0.0%
Gilia latiflora × *G. leptantha*	9.5%
Iris fulva × *I. hexagona*	70%
Opuntia edwardsii × *O. phaeacantha*	45%, 18%, 9.1%[a]
Oxytropis albiflora × *O. lambertii*	0.0%

[a] Character correlations were determined for three hybrid populations of *Opuntia edwardsii* × *O. phaeacantha.*

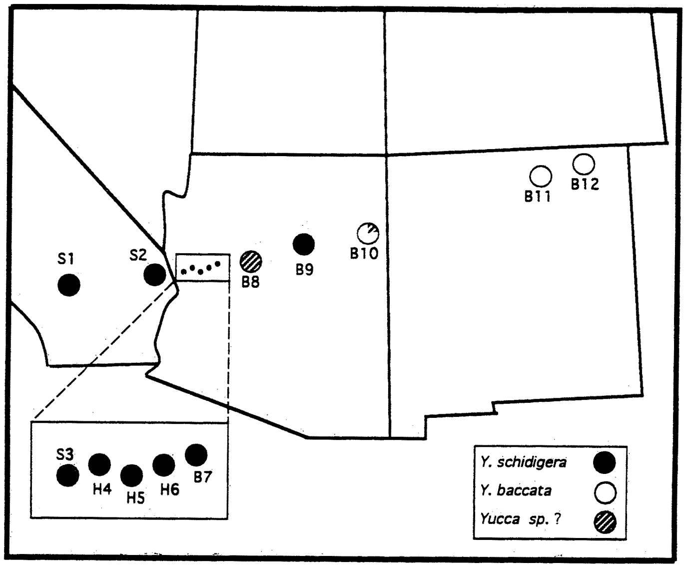


Fig. 1. Distribution of chloroplast DNA haplotypes in a hybrid zone between *Yucca shidigera* and *Y. baccata.* Populations are classified with respect to morphological characteristics. Reprinted from Hanson and Rieseberg (in press).

suggested that character coherence in natural populations is often overestimated because of the inclusion of parental species individuals in the analysis. He further demonstrated the artifactual nature of these estimates by comparing values of character coherence in artificially constructed hybrid populations of cotton that varied with regard to the presence or absence of parental species individuals. Predictably, character coherence was low in segregating populations that lacked parental individuals, whereas high levels of coherence were observed when even a small number of parental individuals was included in the population sample.

Evidence from natural hybrid populations has been critically analyzed as well. Grant (1979) estimated character correlations for eight hybrid populations from six species using data from the hybrid fraction of the population only (Table 4). Coherence was present in some populations but not others. In addition, characters correlated in one hybrid population might not be correlated in other populations involving the same species cross. Thus, it now appears that character correlations in hybrid populations are the exception rather than the rule.

It is noteworthy that the idiosyncratic relationship among morphological characters also appears to be the norm for the relationships of morphological to molecular characters, as well as for the relationship among molecular characters (reviewed in Rieseberg and Ellstrand, 1993). For example, studies of a hybrid zone between two species of *Yucca* (Hanson and Rieseberg, in press), revealed little or no correlation between the distribution of morphological characters, chloroplast DNA (cpDNA) haplotypes (Fig.1), and diagnostic RAPD markers.

Hybrids disrupt phylogenetic trees—A final misconception about hybrids involves their impact on the topology of phylogenetic trees. Most methods of phylogenetic reconstruction assume hierarchical rather than reticulate patterns of evolution. Thus, for groups with taxa of hybrid origin, these methods cannot generate a correct phylogeny. Furthermore, it has been suggested that the presence of hybrids may distort hypothesized relationships among related nonhybrid taxa, and thus possibly limit the utility of standard phylogenetic approaches in plants (Hull, 1979; Cronquist, 1987).

Fortunately, empirical tests of the effects of hybrids on phylogenetic trees do not confirm these predictions, except under certain conditions. The most comprehensive studies have been conducted in the Central American species of the genus *Aphelandra* (McDade, 1990, 1992). McDade generated first-generation hybrids between species in this group and then conducted a series of phylogenetic analyses to test the impact of hybrids on phylogenetic trees. When hybrids between closely related species were included in the phylogenetic analyses, there typically were minor or no changes in topology (Fig. 2). However, the inclusion of hybrids between distantly related species did result in the predicted breakdown in cladistic structure and major topological changes (Fig. 2).

For phylogenists, the good news then is that the inclusion of hybrids in a phylogenetic analysis will not necessarily disrupt the placement of nonhybrid taxa, at least if the hybrid was generated by reasonably closely related species. The bad news is that because hybrids may not disrupt phylogenetic trees, there may be little detectable signal to allow the diagnosis of hybrids in phylogenetic trees.

For example, Rieseberg and Morefield (in press) tested the effectiveness of parsimony criteria for identifying artificially synthesized or previously documented hybrid species in *Gilia* and *Helianthus,* respectively. This was accomplished using the computer program RETICLAD (Morefield, 1992). RETICLAD determines the increase or decrease in parsimony required for all possible hybrid combinations among terminal taxa in a phylogenetic tree. RETICLAD also tests the character state distribution of the putative hybrid and its parents against expected frequencies for five null hypotheses. Thus, for each data set analyzed by Rieseberg and Morefield (in press), the relative likelihoods of the most parsimonious hybrid hypotheses were tested using RETICLAD.

The combined phylogenetic and probabilistic approach was effective in terms of identifying *Gilia* hybrid derivatives, suggesting that there may be some validity to these methods. However, the build-up of numerous morphological apomorphies in the *Helianthus* hybrid species resulted in a decrease rather than the predicted increase in parsimony for each of the hybrid hypotheses. Likewise, the character patterns produced were more similar to those predicted for random data than to those predicted for a hybrid origin. Chemical data performed considerably better than the morphological data, but the large increase in parsimony predicted was not realized. In contrast, the use of molecular phylogenetic data was effective for identifying hybrid species in *Helianthus.* Theoretical considerations, however, suggest that many hybrid lineages will be cryptic to this approach as well due to sampling error and the age of the reticulation event. Thus, the accurate reconstruction of phylogenies for groups with reticulate evolutionary histories may be difficult or sometimes impossible, at least with current phylogenetic methods.

In summary, hybrids typically are not morphologically intermediate, but rather are a mosaic of parental, intermediate, and novel characters. Likewise, hybrids may be less, more, or equally fit relative to the parents. Character coherence appears to be the exception rather than the rule. Finally, hybrids between closely related lineages are unlikely to cause major disruptions in the topology of phylogenetic trees.

RECENT ADVANCES

The last several years have also seen a number of advances with regard to the study of hybridization, as well as an invigorating infusion of new data. Below, I discuss in more detail three advances that have been of particular interest to me.

Discovery of frequent cytoplasmic introgression—One of the most exciting discoveries from the young field of molecular systematics is the many unexpected instances of hybridization and introgression detected in molecular phylogenetic studies. Most commonly, populations are characterized by the morphology or nuclear markers of one species, but have the chloroplast genome of another species or genus.

One of the best examples is from the genus *Gossypium,* where comparison of a cpDNA-based phylogenetic tree with the current classification of the genus revealed several areas of incongruence (Fig. 3; Wendel and Albert, 1992). Most commonly, species with the morphology of one section had the chloroplast genome of another section, suggestive of cytoplasmic introgression or hybrid speciation. It is noteworthy that a detailed population level study of one of these putative examples, *G. bickii,* employing both nuclear and cytoplasmic markers, confirmed the hybridization hypothesis (Wendel, Stewart, and Rettig, 1991).

The situation in *Gossypium* is not uncommon. For example, Rieseberg and Soltis (1991) list 37 potential examples of "chloroplast capture" through hybridization and introgression, and I suspect that detailed surveys of the current literature might yield over 100 potential examples. In fact, most phylogenetic studies of cpDNA that employ multiple population samples appear to include at least one example of cytoplasmic introgression.

These data not only suggest that hybridization is phylogenetically widespread, but also have important implications for the interpretation of gene trees, for sampling strategies employed in phylogenetic studies, and for combining data sets in phylogenetic studies. For example, perhaps due in part to the high frequency of cytoplasmic introgression in plants, most systematists now recognize the distinction between gene and species trees and typically employ more than one gene for phylogenetic analysis. Hence, the recent spate of phylogenetic studies that employ variation from both cpDNA and the internal transcribed spacer of nuclear ribosomal genes. Unfortunately, the implications of these data in terms of the need for more extensive population sampling have been largely disregarded. Likewise, the emphasis for combining data sets in phylogenetic analysis has been on searching for character incongruence, whereas the high frequency of reticulation suggests that incongruence in **species placement** due to hybridization is a more critical consideration when combining data sets.

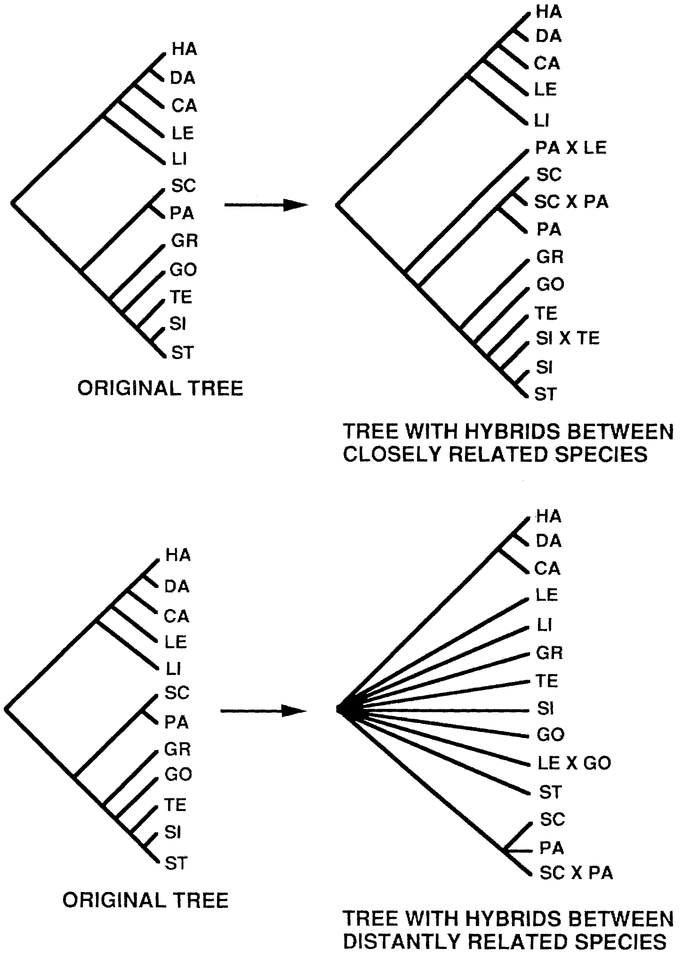
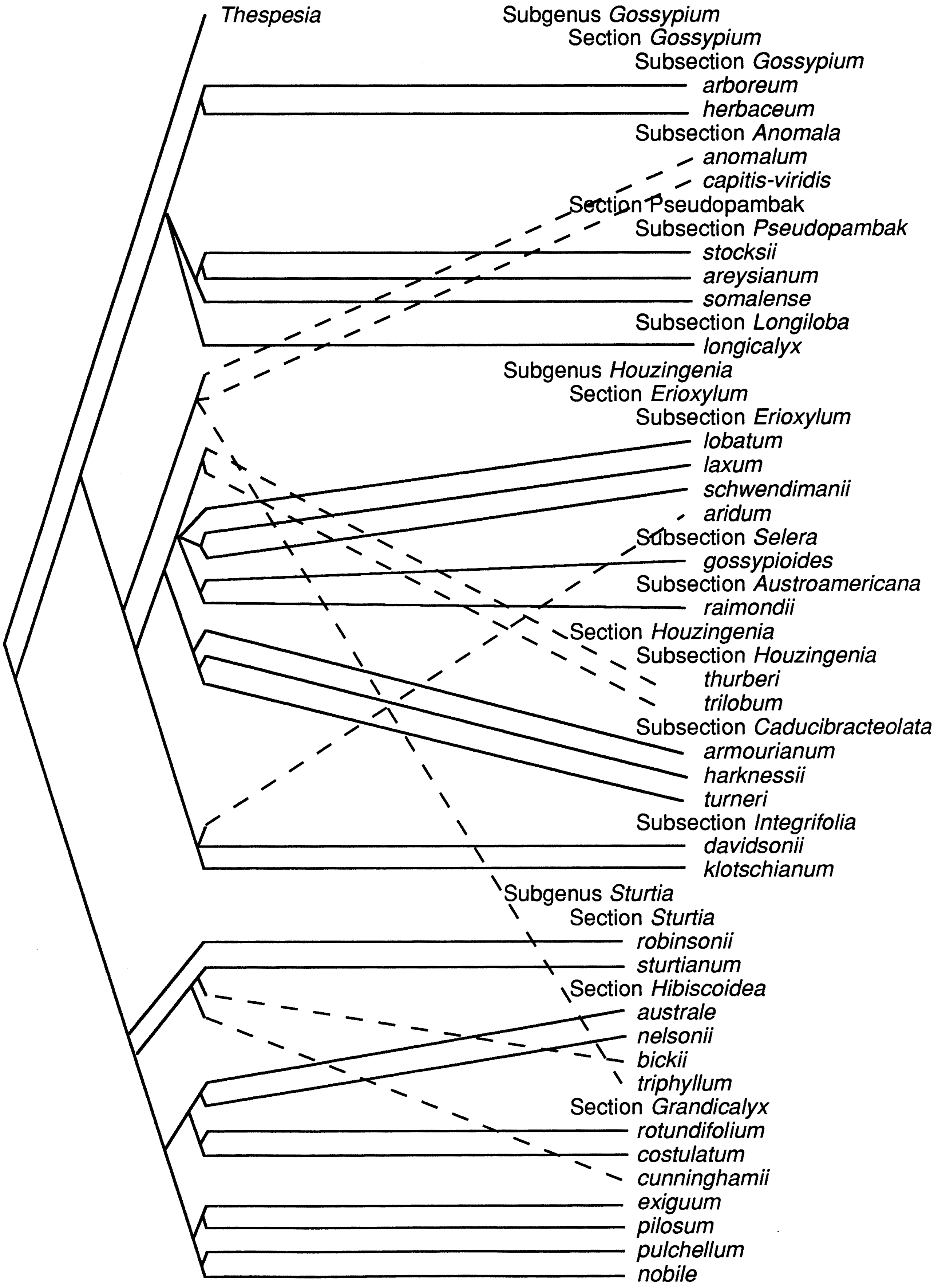


Fig. 2. Cladograms of Central American *Aphelandra* species. Redrawn from McDade (1992).

Access to an unlimited number of markers—Accompanying molecular phylogenetic studies have been technological advances that have provided students of hybridization with an almost unlimited number of markers. These molecular markers have greatly enhanced our ability to detect and quantify introgression, as well as to more precisely identify different genealogical classes of hybrids (e.g., Keim et al., 1989). In addition to the many advantages afforded by the availability of large numbers of markers, detailed genetic linkage maps may be generated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Subsection Anomala
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Section Pseudopambak
Subsection Pseudopambak
stocksii
areysianum
somalense
Subsection Longiloba
longicalyx
Subgenus Houzingenia
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aridum
Subsection Selera
gossypioides
Subsection Austroamericana
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trilobum
Subsection Caducibracteolata
armourianum
harknessii
turneri
Subsection Integrifolia
davidsonii
klotschianum
Subgenus Sturtia
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robinsonii
sturtianum
Section Hibiscoidea
australe
nelsonii
bickii
triphyllum
Section Grandicalyx
rotundifolium
costulatum
cunninghamii
exiguum
pilosum
pulchellum
nobile

for the hybridizing species so that the genomic location and linear order of the markers can be determined. The maps can then be compared to identify changes in gene order and by inference, structural changes in the genomes of the hybrids. Moreover, the mapped molecular markers can be used to assess the genomic contribution of parental taxa to suspected hybrid species or introgressant populations (e.g., Rieseberg et al., 1993).

An example of the use of mapped molecular markers for the study of hybrids comes from work in my laboratory, where we have generated genetic linkage maps for the putative diploid hybrid species, *H. anomalus,* and its two putative parents, *H. annuus* and *H. petiolaris* (Rieseberg,1991; Rieseberg et al., 1993; Rieseberg, Desrochers, and Youn, 1995). Comparison of gene order among the three species revealed a number of different structural relationships. Six linkage groups were co-linear for all three species, whereas the remaining 11 linkages were not conserved in terms of gene order. The two parental species, *H. annuus* and *H. petiolaris,* differ by at least ten separate chromosomal rearrangements including three inversions and a minimum of seven interchromosomal translocations (Fig. 4).

The putative hybrid, *H. anomalus,* shared the linkage arrangement of one parent or the other for four of the 11 rearranged linkage groups. However, several unique arrangements or sterility factors were observed as well. In fact, a minimum of three chromosomal breakages, three fusions, and one duplication are required to achieve the *H. anomalus* genome from its parents. It is noteworthy that all seven novel rearrangements in *H. anomalus* involve linkage groups that are structurally divergent in the parental species, suggesting that structural differences may induce additional chromosomal rearrangements upon recombination.

In addition to generating genomic maps for each species, we also attempted to determine the parental origin of each locus mapped in the putative hybrid, *H. anomalus.* This was done by surveying four natural populations from each parental species for loci/alleles mapped in *H. anomalus* (Rieseberg et al., 1993; Rieseberg, Desrochers, and Youn, 1995). Analysis of the parental origin of each locus revealed that *H. anomalus* retained 56% of the *H. annuus* genome and 44% *H. petiolaris* genome, but this difference was not statistically significant ($P > 0.05$). In addition, the parental linkage groups were not transmitted or retained intact, but generally were interspersed within individual *H. anomalus* linkage groups (Fig. 5). Thus, diploid hybrid speciation is indeed recombinant as predicted by theory (Grant, 1981). This was not the case, however, for three large linkage blocks, which appear to have been transmitted intact from *H. petiolaris* and are speculated to have been protected from recombination in the initial hybrid. However, this pattern may also be a sampling artifact. Nonetheless, as noted by Rieseberg et al. (1993), these data do suggest that diploid hybrid species may be able to retain different portions or proportions of their parental species' genomes, perhaps providing them with greater flexibility than allopolyploid species in terms of optimizing their genomes for a new ecological niche. Likewise, these same characteristics may permit the production of several different diploid hybrid species from the same two parents, as appears to have happened in *Helianthus* (Rieseberg, 1991).

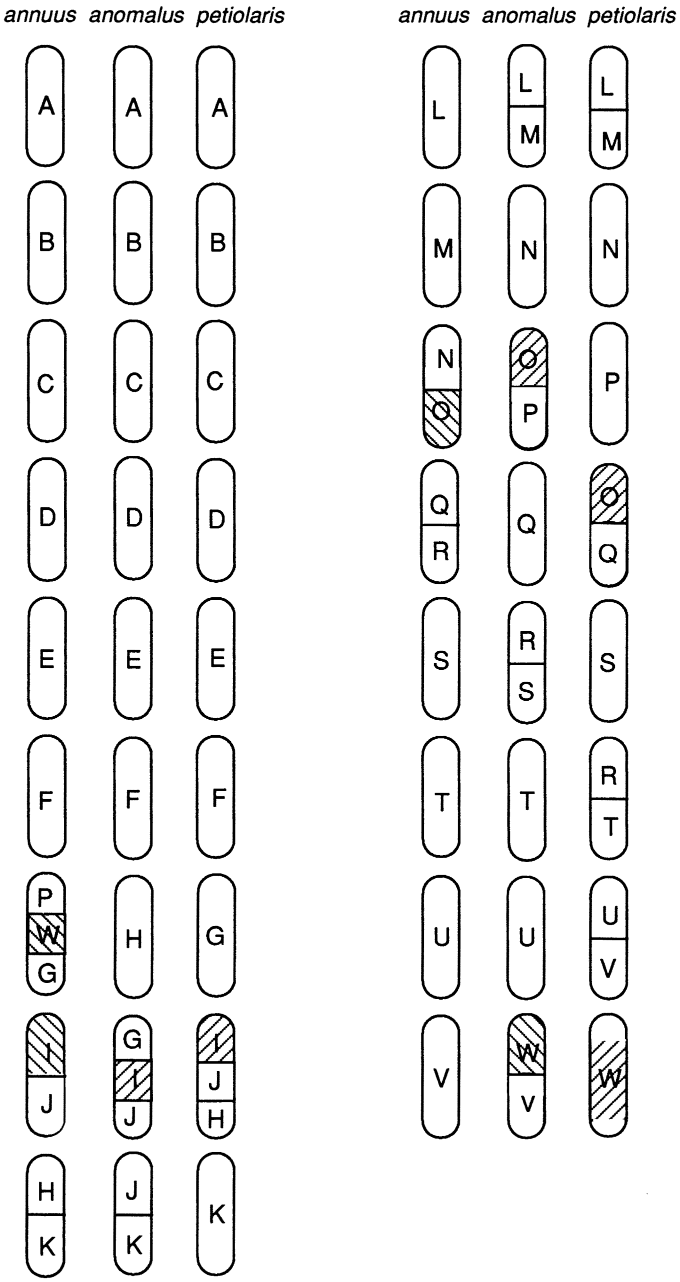

Fig. 4. Hypothesized chromosomal structural relationships between the putative hybrid species, *Helianthus anomalus* and its putative parents, *H. annuus* and *H. petiolaris,* based on comparative genetic linkage mapping. Hatched lines indicate regions with inversions.

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Fig. 3. Heuristic comparison of cpDNA-based phylogeny of 34 diploid species of *Gossypium* (on left) with the current classification of the genus (on right). Redrawn from Wendel and Albert (1992).

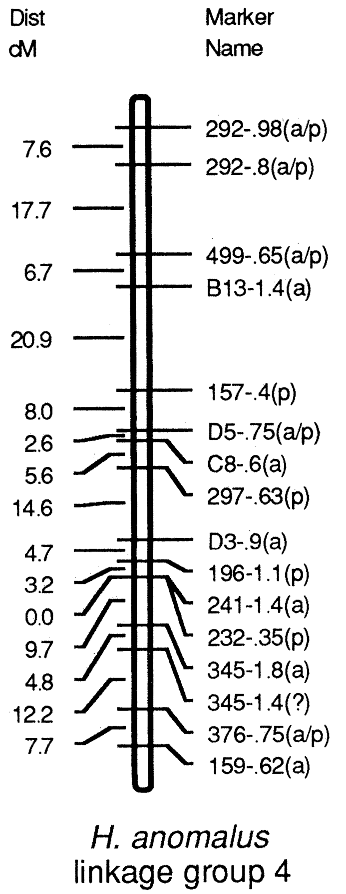

Fig. 5. Graphical genotype for one of seventeen linkage groups in the putative hybrid species, *Helianthus anomalus.* Letters in parentheses following each marker name indicate its putative parental origin: a = *H. annuus,* p = *H. petiolaris,* a/p = *H. annuus* or *H. petiolaris,* and u = unique to *H. anomalus.*

These maps can also be used to monitor introgression on a chromosome by chromosome basis (Doebley and Wendel, 1989; Rieseberg and Wendel, 1993). For example, in *Helianthus,* these linkage maps have been used to study the effects of chromosomal rearrangements on gene introgression in a backcrossed progeny of *H. annuus* × *H. petiolaris* (Rieseberg, unpublished data). This was accomplished by generating "graphical genotypes" for 58 introgressed individuals. Graphical genotypes are similar to cytological karyotypes in describing the entire genome in a single image, but different in that the genomic constitution and parental derivation for all points on the genome are depicted in the graphical genotype (Young and Tanksley, 1989).

The chromosomal structural differences between *H. annuus* and *H. petiolaris* had a significant impact on introgression ($P < 0.001$); 40% of the nonrearranged portion of the genome was transferred in at least one of the 58 individuals, whereas only 2.4% of the genome from rearranged linkages was transferred, and that portion was found in fewer than 10% of the introgressed plants. Thus, chromosomal sterility barriers appear to be extremely efficient at reducing or eliminating introgression in rearranged chromosomal segments or adjacent regions.

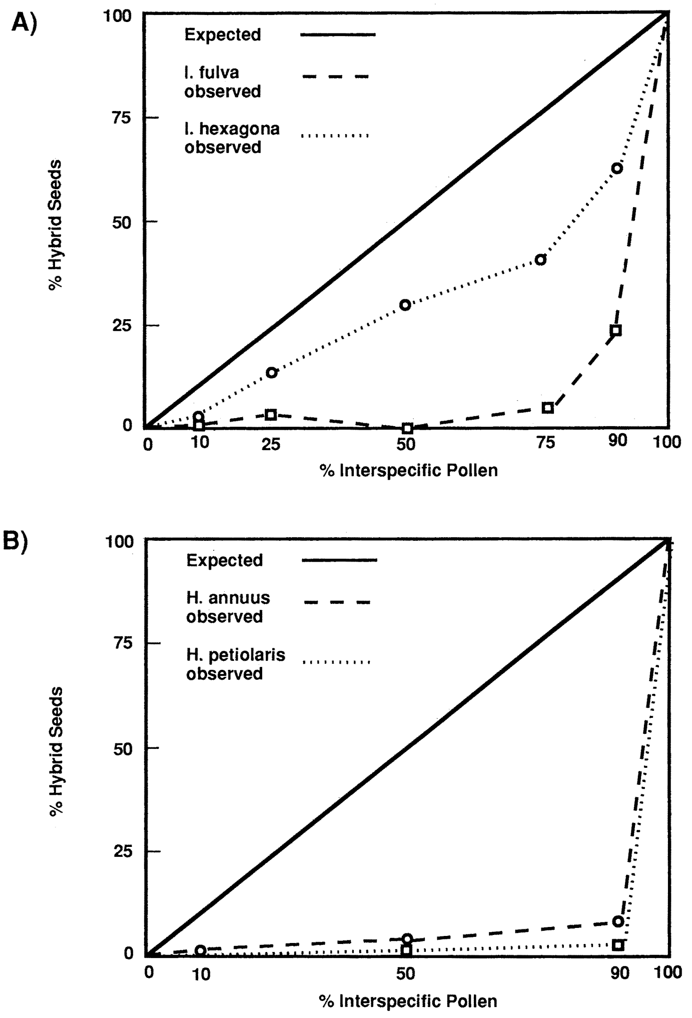

Fig. 6. Percentage hybrid seed generated from different intraspecific : interspecific pollen ratios in *Iris* (A) and *Helianthus* (B). Redrawn from Carney et al., 1994; Rieseberg, Desrochers, and Youn, 1995.

These data suggest that although evolutionists usually view isolating barriers as protecting the entire genome from interspecific gene flow, certain isolating barriers, such as chromosomal structural differences, may resist gene flow selectively. Theoretically, this might allow the maintenance of species differences in the face of extensive introgression, such as appears to be the case for many plant groups.

Reconsideration of interspecific pollen competition—The final data set I want to discuss is more ecological in nature and involves one of the mechanisms that appears to play a major role in controlling the formation of hybrids in many plant groups: interspecific pollen competition. The possibility that selective fertilization by intraspecific pollen in mixed pollen loads might function as a reproductive isolating barrier was first suggested by Darwin (1859, p. 85) who noted that ". . . a plant's own pollen is always prepotent over foreign pollen." This observation has been shown to hold true in species pairs from several plant groups including *Datura* (Buchholz et al., 1935), *Haplopappus* (Smith, 1968, 1970), perennial *Helianthus* species

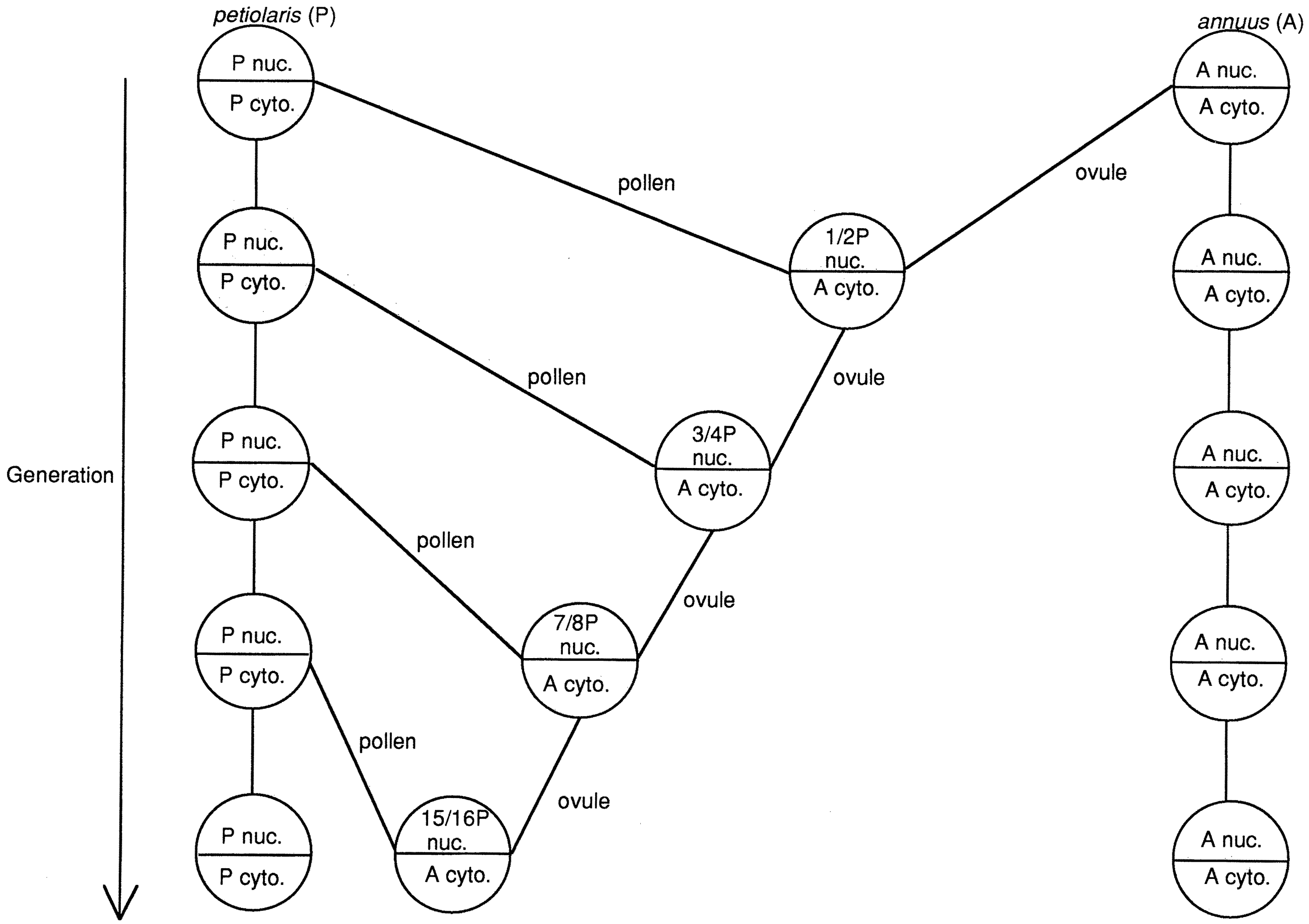

Fig. 7. Hypothetical scenario for cytoplasmic introgression in a population of *H. petiolaris* following the introduction of a single individual of *H. annuus*. Due to pollen competition, *H. annuus* serves as the maternal parent (ovule). Male sterility in first- and later generation hybrids and backcrosses quickly leads to the production of plants that have the cytoplasm of *H. annuus*, but whose nuclear genes are predominately those of *H. petiolaris*.

(Heiser et al., 1969), *Mimulus* (Kiang and Hamrick, 1978), and *Iris* (Arnold, Hamrick, and Bennett, 1993; Carney, Cruzan, and Arnold, 1994); although in *Mimulus* the prepotency of conspecific pollen was only observed with *M. guttatus* as the seed parent.

The most detailed studies to date, however, have been conducted in the genus *Iris* by Arnold and co-workers (Arnold, Hamrick, and Bennett, 1993; Carney, Cruzan, and Arnold, 1994). 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 (Fig. 6). 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.

We have observed a similar phenomenon when artificial crosses between *Helianthus annuus* and *H. petiolaris* were conducted using different mixtures of intra- and interspecific pollen loads (Rieseberg, Desrochers, and Youn, 1995; Fig. 6). The number of hybrids produced was significantly less than expected ($P < 0.01$), regardless of the maternal parent. However, hybrids were significantly more frequent with *H. annuus* than with *H. petiolaris* as the maternal parent ($P < 0.01$).

The discovery of interspecific pollen competition in sunflower and other species is also important because it may explain patterns of differential cytoplasmic vs. 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., 1991; Dorado, Rieseberg, and Arias, 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. 7). In addition, as the minority taxon, it will inevitably 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 vs. nuclear introgression observed in many plant hybrid zones, including several in *Helianthus* (e.g., Dorado, Rieseberg, and Arias, 1992).

THE ROLE OF HYBRIDIZATION IN PLANT EVOLUTION

We have discussed some of the misconceptions about hybrids and presented some exciting new data, but the question suggested by the title of the paper has not been addressed directly. That is, what is the evolutionary role of hybridization in plants?

In Stebbins' seminal 1959 paper he suggested that a high degree of genetic variability was required for major evolutionary advances. Because of the slow mutation rate, Stebbins argued that genetic recombination was the most likely source of this variation. This recombination-generated diversity could be maximized, he felt, by hybridization between populations with different adaptive norms.

Unfortunately, we still cannot confirm or refute this proposed role for hybridization. The molecular phylogenetic data as well as studies of the geographical distribution of molecular markers have confirmed Stebbins' assertions that hybridization is both geographically and phylogenetically widespread. However, it's not clear whether the frequent hybridization events recorded in the evolutionary history of many plant groups are an accidental consequence of the evolutionary history of those lineages and have little significance other than to befuddle taxonomists, or whether they are indicative of a larger role for hybridization in the origin and evolution of species.

There is some evidence recently generated by my lab and others that suggests that hybrid swarms and hybrid zones can serve as founding populations for new species (e.g., Gallez and Gottlieb, 1982; Arnold, Buckner, and Robinson, 1991; Rieseberg, 1991), but whether this is a common mode of speciation is unclear. Likewise, the few studies of hybrid fitness available provide insufficient data to evaluate Stebbin's hypothesis. It seems, therefore, that we still need more data, and the data needs to be more critically obtained and analyzed. In particular, we need:

1) Molecular phylogenetic studies that employ multiple gene trees and sample multiple populations and individuals. This comparative phylogenetic approach is sensitive in that numerous unexpected cases of hybridization can be detected, powerful in that relatively ancient hybridization events can be discovered, and global in the sense that all species in a group are examined.

2) More data on the biology of different genealogical classes of hybrids. In particular we need detailed and well-designed studies of the fitness of different genealogical classes of hybrids in a variety of different habitats, including parental, intermediate, as well as novel habitats. We need to know why hybrids are formed, under what circumstances they survive, and what factors control the dispersal of introgressed plants or introgressed genes.

3) To employ a larger number of markers in studies of hybridization and pay more attention to their chromosomal location and linkage relationships. These types of studies will not only allow us to more precisely identify different genealogical classes of hybrids, but will also increase our understanding of the genetic mechanisms accompanying or facilitating hybrid speciation and introgression.

4) To accept the reality of reticulate evolution in plants and invest greater energy toward the development of phylogenetic algorithms that consider reticulate phylogeny.

With the completion of these studies, hopefully we will be able to confirm Stebbins' hypothesis of a significant role for hybridization in evolution, and to be able to do so with the confidence and authority with which the original hypotheses were made.

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