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HYBRID ORIGINS OF PLANT SPECIES

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ABSTRACT

The origin of new homoploid species via hybridization is theoretically difficult because it requires the development of reproductive isolation in sympatry. Nonetheless, this mode is often and carelessly used by botanists to account for the formation of species that are morphologically intermediate with respect to related congeners. Here, I review experimental, theoretical, and empirical studies of homoploid hybrid speciation to evaluate the feasibility, tempo, and frequency of this mode. Theoretical models, simulation studies, and experimental syntheses of stabilized hybrid neospecies indicate that it is feasible, although evolutionary conditions are stringent. Hybrid speciation appears to be promoted by rapid chromosomal evolution and the availability of a suitable hybrid habitat. A selfing breeding system may enhance establishment of hybrid species, but this advantage appears to be counterbalanced by lower rates of natural hybridization among selfing taxa. Simulation studies and crossing experiments also suggest that hybrid speciation can be rapid—a prediction confirmed by the congruence observed between the genomes of early generation hybrids and ancient hybrid species. The frequency of this mode is less clear. Only eight natural examples in plants have been rigorously documented, suggesting that it may be rare. However, hybridization rates are highest in small or peripheral populations, and hybridization may be important as a stimulus for the genetic or chromosomal reorganization envisioned in founder effect and saltational models of speciation.

INTRODUCTION

Hybridization may have several evolutionary consequences, including increased intraspecific genetic diversity (2), the origin and transfer of genetic adaptations

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(2, 93), the origin of new ecotypes or species (42, 102), and the reinforcement or breakdown of reproductive barriers (27, 55, 77). Although the frequency and importance of these outcomes are not yet clear in either plants or animals, a critical body of data is now available for assessing the mechanistic basis and frequency of one of these—the origin of new species. The last comprehensive review of this topic in relation to plants was Grant's (42) monograph "Plant Speciation." Grant listed six mechanisms by which the breeding behavior of hybrids could be stabilized, thus providing the potential for speciation:

1. asexual reproduction;
2. permanent translocation heterozygosity;
3. permanent odd polyploidy;
4. allopolyploidy;
5. the stabilization of a rare hybrid segregate isolated by postmating barriers;
6. the stabilization of a rare hybrid segregate isolated by premating barriers.

The first three of these mechanisms generate flocks of clonal or uniparental microspecies that span the range of morphological variability between the parental species. Sexual reproduction among microspecies is limited or absent, making it difficult to discuss their origin and evolution in the context of sexual isolation and speciation. By contrast, the latter three mechanisms generate sexual derivatives and therefore have the potential to give rise to new biological species.

This review focuses on the origin of sexual, homoploid hybrid species (mechanisms 5 and 6), (but see 50, 89 for reviews of polyploidy in plants). After clarification of concepts and terminology, the historical basis of our current understanding of hybrid speciation is reviewed. This is followed by examination of the frequency of natural hybridization and an exploration of experimental and theoretical studies that test the feasibility of homoploid hybrid speciation. Once the feasibility of this mode of speciation has been established, I briefly critique the methods used for identifying homoploid hybrid species in nature and then focus on those examples of hybrid speciation that are well established. Finally, I discuss promising areas for future research and possible approaches that may facilitate studies of this mode.

WHAT IS A HYBRID SPECIES?

Both "hybrid" and "species" can have several meanings for evolutionary biologists. The term hybrid can be restricted to organisms formed by cross-fertilization between individuals of different species, or it can be defined more broadly as the offspring between individuals from populations "which are distinguishable on the basis of one or more heritable characters" (44). I prefer this broader definition of hybrids, as it provides greater flexibility in usage. Nonetheless, in this review, I focus on hybrids formed by crosses between species.

The term species has a much wider variety of definitions, ranging from concepts based on the ability to interbreed to those based on common descent. Mayr's (59) biological species concept—"species are groups of interbreeding natural populations which are reproductively isolated from all other such groups"—is perhaps the most widely accepted of these. Although I have previously expressed concern about the limitations of this concept (73), its emphasis on reproductive isolation does offer a straightforward approach to the study of speciation (20). Moreover, the evolution of reproductive barriers is particularly crucial to the successful origin of new hybrid species; otherwise, the new hybrid lineage will be swamped by gene flow with its parents. Thus, the focus of this review is on the evolution of reproductive isolation between new hybrid lineages and their parents.

HISTORICAL PERSPECTIVE

The hypothesis that new species may arise via hybridization appears to have originated with Linnaeus (58; cited in 84), who wrote "it is impossible to doubt that there are new species produced by hybrid generation.... For thence it appears to follow, that the many species of plants in the same genus in the beginning could not have been otherwise than one plant, and have arisen from this hybrid generation." This represents a modification of the orthodox view of special creation, which asserted that all existing species were created by the hand of God and which denied the existence of constant hybrids (15). However, Linnaeus' observations were limited to F_1 hybrids, and he was unaware of potential difficulties with his hypothesis such as segregation and sterility.

Rigorous experimental study of plant hybridization was initiated by Joseph Kölreuter in 1760 and led to two critical discoveries (84). First, Kölreuter found that a hybrid from *Nicotiana paniculata* × *N. rustica* produced no seeds—the first "botanical mule." As a result, Kölreuter concluded that hybrid plants are produced only with difficulty and are unlikely to occur in nature in the absence of human intervention or disturbance to the habitat. Second, Kölreuter and his successor, Carl Gartner, discovered that later generation hybrids tended to revert back to the parental forms, thus refuting the existence of constant hybrids and supporting the orthodox view of special creation (84). The views of Kölreuter and Gartner on the lack of constancy of hybrids (although not necessarily on creation) were held by most other prominent botanical hybridizers during the eighteenth and nineteenth centuries, including Charles Darwin, John

Goss, Thomas Laxton, Patrick Shireff, Gregor Mendel, Charles Naudin, and WO Focke (84).

Nonetheless, reports of constant hybrids continued to arise. For instance, Herbert (49) noted that hybrid varieties of plants sometimes preserve themselves almost as distinctly as species. These reports, although controversial, were taken seriously by prominent botanists such as Mendel (61), who emphasized in his *Pisum* paper: "This feature is of particular importance to the evolutionary history of plants, because constant hybrids attain the status of *new species*" (emphasis in the original). Naudin (66) also recognized the possibility that hybrid characters may become fixed in later generations and that this may facilitate species formation. This represented the first explicit recognition of the possibility that later-generation hybrids may become stabilized and thus foreshadowed modern models of hybrid speciation.

The potential role of hybridization in species formation was taken a step further by Anton Kerner (51), who recognized the important role of habitat in governing hybrid species establishment. Kerner realized that although hybrids were frequently formed in nature, their successful establishment required suitable open habitat that was not occupied by the parental species. This was a significant contribution, as ecological divergence plays an important role in current models of hybrid speciation. However, Kerner restricted his discussion to fertile hybrids, thus ignoring the sterility problems associated with many hybrids. Furthermore, like Linnaeus, Kerner failed to recognize the potential problem of segregation, and it was not until the rediscovery of Mendel's work in the early portion of the twentieth century that the old problem of hybrid speciation could be stated correctly—what is the mechanism by which a new fertile and constant hybrid lineage could arise and become reproductively isolated from its parents?

The first major contribution to this problem was made by Winge (103), who postulated that a fertile and constant hybrid species could be derived instantaneously by the duplication of a hybrid's chromosome complement (i.e. allopolyploidy). This hypothesis was quickly confirmed experimentally in a variety of plant species, and allopolyploidy is now recognized to be a prominent mode of speciation in flowering plants and ferns (89, 93).

By contrast, the feasibility of hybrid speciation in the absence of ploidal change remained unsolved until it was addressed by Müntzing (65). He postulated that the sorting of chromosomal rearrangements in later-generation hybrids could, by chance, lead to the formation of new population systems that were homozygous for a unique combination of chromosomal sterility factors (Figure 1). The new hybrid population would be fertile, stable, and at the same ploidal level as its parents, yet partially reproductively isolated from both parental species due to a chromosomal sterility barrier. Although early authors

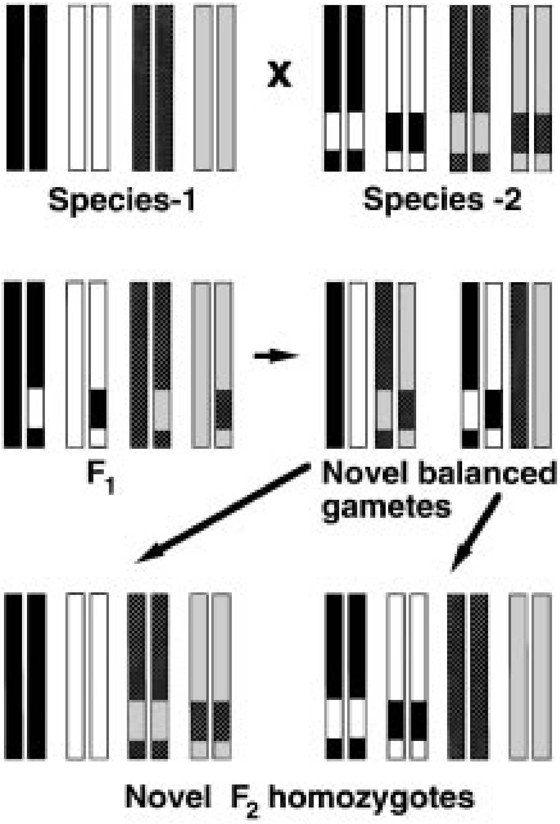

Figure 1 Simple chromosomal model for the initial stages of recombinational speciation. The two parental species have the same diploid chromosome number ($2n = 8$), but differ by two reciprocal translocations. The first generation hybrid will be heterozygous for the parental chromosomal rearrangements and will generate 16 different classes of gametes with respect to chromosome structure (not shown). Twelve of these will be unbalanced and presumably inviable due to deletions and/or insertions. The remaining four will be balanced and viable. Two of the four will recover parental chromosome structures, whereas the final two will have recombinant karyotypes. If selfed, a small fraction of F_2 individuals will be recovered that possess a novel homokaryotype. These F_2s will be fertile and stable but will be at least partially intersterile with the parental species.

focused on chromosomal rearrangements (33, 65), it is clear that the sorting of genic sterility factors should generate similar results. Thus, current models incorporate both genic and chromosomal sterility factors. Modern contributions to the study of this process, termed recombinational speciation (39), include rigorous experimental and theoretical tests of the model as well as the gradual accumulation of well-documented case studies from nature.

Concomitant with the development of the recombinational speciation model was the growing recognition that new hybrid species might become isolated from their parents by premating barriers rather than hybrid sterility or inviability. In fact, this view was implicit in Kerner's (51) account of the hybrid origin of *Rhodendron intermedium*, which appears to be partially isolated from its parents due to soil preferences and the behavior of pollinators. More recently, Grant (38) presented a model for sympatric speciation in flowering plants based on flower constancy of pollinators and then suggested that hybridization might be as likely as mutation in generating the new floral structures required for speciation. Unfortunately, rigorous experimental or theoretical studies of this hypothesis have not been conducted. Nonetheless, a number of empirical studies have identified hybrid taxa that are isolated by premating barriers alone, and new recombinational species are typically isolated from the parental species by both premating and postmating barriers.

THE FREQUENCY OF INTERSPECIFIC HYBRIDIZATION

Data on naturally occurring plant hybrids are quite extensive, and several compilations illustrate the extent of hybridization in nature. Perhaps the most comprehensive listing is Knobloch's (52) compilation of 23,675 putative examples of interspecific or intergeneric hybrids, but this figure must be interpreted with caution. Some of the hybrids appear to be fanciful, and many known hybrids have been omitted (92). Moreover, Knobloch included both natural and artificial hybrids, and it is not clear what fraction of this listing comprises natural hybrids.

A more reliable indicator of the frequency of hybridization comes from a recent review of five biosystematic floras (28). The frequency of natural hybrids when compared to the total number of species in the flora ranged from approximately 22% for the British flora to 5.8% for the intermountain flora of North America, with an average of 11% over all five floras. Assuming a similar frequency of natural hybrids worldwide, this would suggest a worldwide total of 27,500 hybrid combinations among the 250,000 described plant species. Although this is a sizable number, it may represent a substantial underestimate; many hybrids have gone undetected due to inadequate systematic attention to certain groups in certain floras.

Natural hybrids also were found to be unevenly distributed taxonomically (28). Only 16%–34% of plant families and 6%–16% of genera have one or more reported hybrids. Thus, contemporary hybridization may not be as common or ubiquitous as believed, but appears to be concentrated in a small fraction of families and genera. Notably, the life-history characteristics significantly associated with these hybridizing genera include perennial habit, outcrossing breeding systems, and asexual reproductive modes that allow stabilization of hybrid reproduction.

The estimates provided above indicate that the rate of natural hybrid formation in plants is sufficiently high to provide ample opportunity for homoploid hybrid speciation. Although hybrid speciation seems most likely to be important in family or genera with high rates of contemporary hybridization, even rare hybridization events can be evolutionarily important, as a single, partially fertile, hybrid individual can suffice as the progenitor of a new evolutionary lineage (28).

THEORY

Models

Homoploid hybrid speciation is unusual because not only does it involve hybridization between taxa at the same ploidal level, but it also represents a type of sympatric speciation, as the parental species must co-occur geographically to produce hybrids. Chromosomal models for this process were proposed by Stebbins (94) and Grant (39) and can be summarized as follows (Figure 1):

1. Two parental species are distinguished by two or more separable chromosomal rearrangements.
2. Their partially sterile hybrid gives rise via segregation and recombination to new homozygous recombinant types for the rearrangements.
3. The recombinant types are fertile within the line but at least partially sterile with both parents.

Grant (39) also noted that the formation of new structural homozygotes in the progeny of a hybrid is more likely under conditions of inbreeding than of outbreeding. This leads to the prediction that recombinational speciation should be more common in selfing species than outcrossers.

A more general model has recently been proposed by Templeton (98) that incorporates both chromosomal and genic incompatibility and recognizes the important roles of selection and ecological divergence. Moreover, this model can be applied to the stabilization of hybrid segregates isolated by either premating or postmating barriers. There are four critical steps in Templeton's model:

1. Hybridization is followed by inbreeding and hybrid breakdown due to chromosomal or genic incompatibilities.

2. Hybrid segregates with the highest fertility or viability are favored by selection. (This differs from the model of Grant & Stebbins, who emphasized the role of chance in generating novel homokaryotypes).

3. A new hybrid genotype may become stabilized if it becomes reproductively isolated from the parental species. Otherwise, it will be overcome by gene flow with its parents. Presumably, reproductive isolation will evolve as a by-product of selection for increased viability or fertility, rather than by selection for particular chromosomal rearrangements (although see 86).

4. Once a hybrid genotype becomes stabilized, it must co-exist with one or both parents or occupy a new ecological niche. Either outcome requires ecological divergence.

Templeton emphasized two factors that appear to facilitate this mode. First, he argues that the evolution of reproductive barriers between the stabilized hybrid genotype and its parents could be facilitated by rapid chromosomal evolution. The presence of chromosomal rearrangements in a segregating population can lead to further chromosomal breakage (81, 86), particularly if accompanied by inbreeding (56). Moreover, there is substantial evidence that genic mutation rates also increase in hybrid populations (13). The elevated chromosomal and genic mutation rates in hybrid populations are often referred to as hybrid dysgenesis, and it now appears to be the rule rather than the exception in hybrid populations (13). The rate of chromosomal evolution should also be enhanced by inbreeding and/or population subdivision because both will reduce effective population sizes and increase the fixation of novel chromosomal rearrangements through drift.

A second factor that Templeton considered even more critical to rates of speciation via this mode is the availability of suitable habitat. The importance of habitat for the establishment and success of hybrids was previously recognized by Kerner (51) and Anderson (2). Kerner commented on the critical role of "open habitats" for the establishment of hybrids, whereas Anderson emphasized the importance of habitat disturbance for facilitating breakdown of premating reproductive barriers between previously isolated parental species, and for providing suitable habitat for hybrid segregates, which often diverge ecologically from both parents (2). Moreover, as is discussed in detail later in this review, most bona fide hybrid species are found in habitats that are extreme relative to the parental species (71, 100), implying a critical role for habitat availability and ecological divergence in homoploid hybrid speciation.

Simulation Studies

The only detailed quantitative study of the feasibility and dynamics of hybrid speciation was by McCarthy et al (60) who focused on the strict chromosomal or recombinational model and used computer simulations to test how various parameters affect rates of establishment and spread of recombinational species in a spatially structured environment. The model simulated breeding in a hybrid zone between two hermaphroditic plant species with nonoverlapping discreet generations and no seed dormancy. The effects of five parameters were tested: (*a*) fertility of F_1s, (*b*) relative fitnesses of stabilized derivatives, (*c*) selfing rate, (*d*) number of chromosomal differences between the parental species, and (*e*) hybrid zone interface length (this parameter increases the number of hybrid matings per generation).

Most of the findings were intuitive and consistent with the simple genetic models of Grant (39) and Stebbins (94). In general, the process was facilitated by increased F_1 fertility, a great selective advantage for recombinant types, high selfing rates, a small number of chromosomal differences between the parental species, and a long hybrid zone interface. However, recombinational speciation was possible even with F_1 fertilities as low as 0.018, although the number of generations required for the new species to become established was high. Likewise, outcrossing retarded the speciation process, but given a sufficiently high selective advantage for the stabilized derivatives ($\alpha = 2.0$), the process became feasible even under conditions of obligate outcrossing. Similarly, the process was slowed but not ruled out by increased numbers of chromosomal rearrangements differentiating the parental species or a short hybrid zone interface.

In addition to establishing the feasibility of the recombinational speciation model, the simulations also revealed several new insights into the dynamics of the process. In particular, hybrid speciation was “punctuated”: long periods of hybrid zone stasis were followed by abrupt transitions in which the selected type became established and rapidly displaced the parental species. Apparently, the critical factor in this process is the number of individuals of the optimal recombinant type, as mates of their own kind are scarce when numbers are low. This leads to a feedback effect as increased numbers of the optimal type leads to an increased chance of finding a similar mate, which in turn increases the number of optimal types in the next generation, and so forth.

Given the critical effect of the number of optimal type individuals, it is perhaps not surprising that the spatial distribution of these individuals is also important. Optimal recombinant–type individuals that are clumped are more likely to mate than those that are evenly dispersed. Thus, it appears that high selfing but low dispersal rates should facilitate this mode.

An important parameter in this model is the relative fitnesses of the new recombinant types. To implement selection in their model, the homoploid hybrid derivative was assumed to have a new, better-adapted combination of genes, resulting in a selective advantage over all other types (both hybrids and parentals). Although an increasing number of studies suggest that a subset of hybrid genotypes may be more fit than the parental genotypes in certain habitats (reviewed in 7), none has measured lifetime fitness. Thus, the validity of this assumption is untested. Also, it seems unlikely that the hybrid genotypes would be more fit than the parentals in all habitats in the hybrid zone. However, the occurrence of new hybrid species in habitats in which neither parent can survive (103) does suggest that particular hybrid genotypes may be more fit than their parents in novel habitats (see below).

Other Considerations

All of these discussions have assumed that the establishment of the novel hybrid type will occur in sympatry with both parents. Although hybrid speciation must be initiated in sympatry, Charlesworth (18) argued that this mode is most likely when “a group of hybrid plants colonize a new locality and are by chance spatially or ecologically isolated from the parental species.” Thus, hybrid founder events might be viewed as foci of speciation. The possibility that a hybrid derivative might be stabilized in parapatry or allopatry should not be seen as minimizing the importance of the development of reproductive barriers. As the hybrid derivative becomes established and expands its geographic distribution, it most likely will come back in contact with its parents. Presumably, the existence of reproductive barriers will allow it to survive the challenge of sympatry.

The similarity of homoploid hybrid speciation to saltational (56) and founder effect models of speciation (16, 59) has previously been recognized (37, 71). Rieseberg (71) suggested that intra- or interspecific hybridization might be a stimulus for the chromosomal repatterning envisioned by Lewis’s saltational model. Similarly, Grant & Grant (37) argued that hybridization is more likely than founder events to generate the genetic reorganization proposed in founder effect models. Theoretical studies indicate that for founder events to provide significant isolation, founding populations must contain high levels of genetic load, which is subject to strong epistatic selection (12, 98). Populations with these characteristics are instantaneously created by hybridization (79).

The small, peripheral populations emphasized in the saltational and founder models also are those most prone to hybridization. In general, the smaller the population the larger the relative proportion of foreign pollen, seeds, or spores (27). Moreover, because pollinators spend more time periods foraging in large than small populations, the proportion of interpopulational and presumably interspecific matings increases in the latter (27). Peripheral populations also are likely to experience higher levels of foreign reproductive propagules because the proportion of potential interspecific mates is likely to be greater at the boundary of a species range. These observations provide additional support for an important role for hybrid founder events in speciation.

ESTIMATES OF HYBRID FITNESS

If homoploid hybrid speciation occurs in sympatry, the fitness of the new hybrid lineage must play a critical role. If the new hybrid lineage is more fit than either parent in all habitats, as assumed in the McCarthy et al (60) model, it quickly replaces the parental species. However, if the fitness advantage of the hybrid lineage is restricted to a divergent habitat, as assumed in Templeton's (98) model, then it must co-exist with the parental species. Finally, if the new hybrid lineage is less fit in both parental and divergent habitats, it cannot be maintained in sympatry.

Hybrid fitness should be less immediately critical in hybrid founder events, as the potential for gene flow or competition with the parental species is reduced. However, once established, the new hybrid lineage is likely to expand its range and come back in contact with the parental species. At this point, both reproductive isolation and fitness become critical as the hybrid lineage could be eliminated by either gene flow or competition.

Note that the primary concern is with the average fitnesses of fertile, stabilized hybrid lineages, not F_1s or early segregating hybrid classes. Nonetheless, fitness estimates of early generation hybrids can provide insights into the likelihood of homoploid hybrid speciation and can suggest possible fitness expectations for stabilized hybrid derivatives. It also is useful to distinguish between the average fitness of a genealogical class of hybrids and the fitnesses of particular genotypes (V Grant, personal communication). Finally, as has been stressed by Arnold (5), the interaction between habitat and fitness must be recognized.

The average viability and fertility of early hybrid generations (e.g. F_2s, F_3s, etc) is predicted to be lower than that of the parental species due to the break-up of adaptive gene combinations (24). This is generally what is found, particularly for species with strong postmating reproductive barriers. Well-characterized examples include *Zauschneria* (19), *Layia* (19), *Gilia* (40), and *Helianthus* (45). This makes sense because hybridizing species would merge if the average fitness of the hybrids were greater than that of the parents. The fact that most plant hybrid zones are limited in extent also implies that hybrids are on average less fit than their parents (21), at least in parental habitats. Although a number of recent studies have described the replacement of populations of rare taxa by hybrid swarms (14, 55, 77), this appears to be due to genetic swamping by a numerically larger congener rather than high average hybrid fitness.

On the other hand, there are several examples in which the average fitness of a particular class or classes of hybrids appears to be equivalent to or to exceed that of their parents, at least for those fitness parameters measured (reviewed in 7). For example, *Artemisia* hybrids were more developmentally stable and had higher seed germination rates than either parent (31, 35), and *Iris* (29) and *Oryza* (53) hybrids had higher vegetative growth rates than their parents in parental or hybrid habitats. Unfortunately, none of these studies measured lifetime fitness, so whether they represent valid exceptions to the general rule of reduced average hybrid fitness is unclear. Nonetheless, enhanced hybrid fitness does seem plausible if postmating isolating barriers are weak and the hybrids occupy a novel habitat (30, 63), or if environmental conditions change (2, 36). These conditions are common in hybridizing plant species, so rank-order fitness estimates that occasionally favor hybrids should not be unexpected.

Even if the average fitness of a hybrid generation is lower than that of the parental species, this does not rule out the possibility that a particular hybrid genotype might be more fit than either parent, particularly in novel or "hybrid" habitats. Although lifetime fitness estimates are not yet available for individual hybrids, indirect evidence is accumulating that particular hybrid genotypes may be more fit than their parents in hybrid habitats. For example, significant genotype-habitat associations are often reported for hybrid swarms (2, 23, 68, 95). Presumably, this indicates that a selective advantage accrues for certain hybrid genotypes when found in favorable habitats, although these correlations could also result from historical factors (16). Likewise, studies that describe fertility, viability, or other fitness parameters in hybrids almost invariably report the presence of a small fraction of hybrid genotypes that are more fit than parental individuals, even if the hybrids on average exhibit reduced fitness (40, 45). These observations are supported by genetic studies which suggest that a small fraction of gene combinations may be favorable in interspecific hybrids, perhaps allowing them to colonize previously unoccupied adaptive peaks. For instance, 5% of the interspecific gene combinations tested in sunflower interspecific hybrids were favorable (79). Finally, the occurrence of hybrid species in habitats in which neither parent can survive (71) does suggest that assumptions of greater fitness of certain hybrid genotypes in novel habitats are realistic.

EXPERIMENTAL STUDIES

Experimental Verification of the Recombinational Speciation Model

Most experimental studies of homoploid hybrid speciation have focused on the recovery of fertile recombinant types following hybridization between chromosomally differentiated parents. A relevant early study in the genus *Crepis* was conducted by Gerassimova (33), who crossed two *Crepis tectorum* lines that differed by two reciprocal translocations. Selfing of a semisterile F_1 hybrid resulted in the recovery of a fertile F_2 plant that was homozygous for both translocations and, as a result, semisterile with the parental lines. Although this study demonstrated that fertile, recombinant individuals can be derived by hybridization between chromosomally divergent parents, conclusions that can be drawn from this experiment are limited due to the small number of generations analyzed and the weakness of the sterility barriers among the parental taxa and their hybrid derivative. These limitations were corrected in a series of comprehensive experiments involving *Elymus* (94), *Nicotiana* (87), and *Gilia* (40, 41).

Snyder (88) suggested that some of the morphologically diverse microspecies assigned to the selfing grass species *Elymus glaucus* might in fact result from introgression between ancestral *E. glaucus* and two related species known to hybridize in nature, *Sitanion jubatum* and *S. hystrix.* To test this hypothesis, Stebbins (94) generated several F_1 hybrids between microspecies of *E. glaucus* and either *S. jubatum* or *S. hystrix.* Although the vast majority of F_1 florets did not produce seeds (>99.99%), a small number of seeds were generated by four F_1s. In three cases, the F_1 seeds were not useful because the offspring had either recovered the morphology of their maternal parent, had undergone polyploidization, or were sterile. However, a single seed from the fourth F_1 appeared to result from a backcross toward *E. glaucus*. This plant had a seed fertility of 30% and was selfed for two generations. The resulting progeny were vigorous and had normal seed fertility (88%–100%). Moreover, crosses with the original *E. glaucus* parent indicated almost complete reproductive isolation; pollen fertility in the progeny of these crosses ranged from 0% to 3%.

These experiments not only verified the plausibility of Snyder's hypothesis, they also indicated that the origin of homoploid hybrid species need not be restricted to a selfing reproductive mode, particularly when the F_1 hybrids are highly sterile. This is important because backcross progeny are typically more easily generated and more fertile than self- or sib-crosses in early hybrid generations. In addition, the parental species used in these crosses have no apparent chromosomal differences, suggesting that it was the assortment of genic rather than chromosomal sterility factors that resulted in the isolation of the artificial neospecies.

Although the *Elymus* experiment appears to have involved unintentional selection for fertility, the first direct test of the effects of selection on the genetic isolation and morphological divergence of interspecific hybrids was conducted by Smith & Daly (87) in *Nicotiana.* They generated interspecific hybrids between the large-flowered *N. sanderae* and small-flowered *N. langsdorffii.*

Individual F_1 plants with large, intermediate, and small flowers were used to initiate three self-pollinating hybrid lineages, which were then subjected to selection for flower size (large, intermediate, and small, respectively) over 10 generations. By the tenth generation, all lines bred true for both floral (selected) and vegetative (unselected) morphological characters, and statistical analyses revealed that the three lines were separable from each other and from the parental species on the basis of either type of character. Investigation of reproductive barriers also revealed that each selected line was isolated from its parents by one or more genetic barriers such as crossability, meiotic aberration frequency, and pollen abortion. These results indicated that morphological divergence and genetic isolation can arise following strong selection in self-fertilizing hybrid populations.

The most convincing experimental validation of the recombinational speciation model comes from a series of elegant studies (40, 41) involving hybrids of *Gilia malior* × *G. modocensis*. The two species are selfing annual tetraploids with a relatively high chromosome number ($2n = 36$). First generation hybrids are highly sterile, with pollen and seed fertility of 2% and 0.007%, respectively; abnormal meiotic pairing suggests that this reduction in fertility is due to structural chromosomal differences between the parental genomes. To generate fertile and meiotically normal hybrid lines, the most fertile and viable plants were artificially selected from each generation, thus augmenting natural selection on the same traits. Although early generation plants were weak and partially sterile (hybrid breakdown), vigor and fertility improved rapidly. By the F_8 and F_9, full vigor, normal chromosomal pairing, and full fertility had been recovered in three hybrid lineages or branches. Branch I and branch III each possessed a new combination of morphological and cytogenetic features (40), whereas branch II reverted largely to the *G. modocensis* parent both morphologically and in terms of crossability (41). As in the case of *Elymus*, the two recombinant *Gilia* lineages were strongly isolated from their parents (4%–18% pollen fertility). This is concordant with theoretical expectations that the strength of genetic isolation between hybrid derivatives and their parents should be strongly correlated with barrier strength between the parents themselves (39).

The Role of Gene Interactions in Hybrid Speciation

The crossing experiments discussed above demonstrate that fertile, stable hybrid lines can arise via crosses between both weakly and strongly isolated species and that these new lines may be morphologically divergent relative to the parents. However, these studies tell us little about the forces governing the merger of differentiated parental species genomes or whether the results from these experimental studies are readily extrapolated to speciation in nature. To address these questions, Rieseberg et al (79) compared the genomic composition of experimentally synthesized hybrid lineages (*Helianthus annuus* × *H. petiolaris*) with that of an ancient hybrid species, *H. anomalus* (71). Interactions among genes that affect hybrid fitness and, indirectly, hybrid genomic composition were detected by analyzing parental marker segregation in the synthesized lineages.

Three hybrid lineages were synthesized: lineage I, P-F_1-BC_1-BC_2-F_2-F_3; lineage II, P-F_1-F_2-BC_1-BC_2-F_3; and lineage III, P-F_1-F_2-F_3-BC_1-BC_2. Crosses were performed by applying pooled pollen from all plants from a given generation to stigmas of the same individuals—a strategy that facilitates natural selection for increased fertility. Fifty-six or 58 progeny from the final generation of each hybrid lineage were then surveyed for 197 mapped *H. petiolaris* markers. The marker surveys were used to estimate the genomic composition of each of the 170 hybrid progeny individually and each of the three hybrid lineages cumulatively.

Comparison of the genomic composition of the ancient hybrid and synthetic hybrid lineages revealed that although generated independently, all three synthesized hybrid lineages converged to nearly identical gene combinations, and this set of gene combinations was recognizably similar to that found in *H. anomalus*. Concordance in genomic composition between the synthetic and ancient hybrids suggests that selection rather than chance largely governs hybrid species formation. Because the synthetic hybrid lineages were generated in the greenhouse rather than under natural conditions, congruence in genomic composition appears to result from fertility selection rather than selection for adaptation to a xeric habitat. This conclusion is supported by the rapid increase in fertility observed in the three hybrid lineages; average pollen fertility increased from 4% in F_1s to over 90% in the fifth generation hybrids. Congruence in genomic composition also implies that the genomic structure and composition of hybrid species may be essentially fixed within a few generations after the initial hybridization event and remain relatively static thereafter. This observation is concordant with the experimental studies described above and simulation studies (60) that suggest a rapid tempo for hybrid speciation.

Analysis of patterns of parental marker distributions in the experimental hybrids also allowed insights into the genetic processes governing hybrid genomic composition. The two parental species differ by a minimum of ten interchromosomal translocations and inversions (Figure 2), and hybrid plants heterozygous for one or more of these rearrangements exhibit reduced fertility (Figure 2). Because all backcrosses in the synthetic hybrids were in the direction of *H. annuus*, selection against chromosomally heterozygous individuals appears to have greatly reduced the frequency of *H. petiolaris* chromosomal fragments in the rearranged portion of the genome—an observation that holds for both the synthetic and ancient hybrid lineages. However, chromosomal

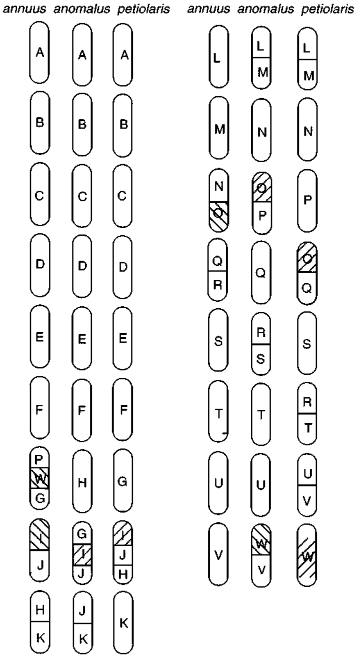

Figure 2 Linkage relationships between *Helianthus annuus*, *H. petiolaris*, and their putative hybrid derivative, *H. anomalus*, as inferred from comparative linkage mapping (81). Lines of shading within linkage groups indicate inversions.

rearrangements alone cannot explain the concordance in genomic composition between the synthesized and ancient hybrids as significant congruence is observed for both collinear and rearranged linkage groups.

Gene interactions also appear to play an important role in controlling hybrid genomic composition. Evidence for gene interactions comes from two sources: marker frequencies and associations. Most *H. petiolaris* markers (71% to 85%) introgressed at significantly lower than expected frequencies in the synthesized hybrids, suggestive of unfavorable interactions between loci tightly linked to these markers and *H. annuus* genes. By contrast, favorable interspecific gene interactions are implied by the significantly higher than expected rates of introgression observed for 5% to 6% of *H. petiolaris* markers. Concordance of marker frequency across the three synthesized hybrid lineages further suggests that these interactions remain largely constant regardless of hybrid genealogy.

Analyses of associations among segregating parental markers allow detection of specific interactions among chromosome segments that affect hybrid fertility, rather than the general interactions inferred from the frequency data. The rationale for this approach is that selection will favor the retention of genes that interact favorably. The signature of this epistatic selection should be detectable by positive associations or correlations among markers linked to these interacting genes. Likewise, genes that interact negatively should be detectable by negative associations among markers linked to these genes.

To test for these interactions, Rieseberg et al (79) analyzed all unlinked introgressed markers for significant two-way and three-way associations. The results from these analyses were compelling. Ten or more significant two-way associations were observed in each of the three synthetic hybrid lineages. In the more powerful three-way analysis, 21, 29, and 15 significant three-way associations were observed, generating complex epistatic webs. It is noteworthy that even though very stringent significance levels were employed in this analysis ($\alpha \leq 0.0001$), many of the same two- and three-way associations were observed in multiple hybrid lineages. Because the hybrid lineages were generated independently, selection rather than drift must account for these shared associations. Moreover, markers with epistatic interactions were more often found in all three lineages than markers lacking epistasis, suggesting that these interactions influence hybrid genomic composition.

The convergence of the synthetic and ancient hybrid sunflower lineages toward a similar set of gene combinations also suggests that hybrid speciation may be more repeatable than previously believed (21). However, if this is the case, one might ask why, in contrast to the sunflower results, the experimentally synthesized *Nicotiana* (87) and *Gilia* hybrid lineages (40, 41) diverged from each other in terms of morphology and cytogenetics. There are several possible reasons for this. In *Nicotiana*, diversifying selection was employed, essentially guaranteeing the generation of divergent lineages. Moreover, in both *Nicotiana* and *Gilia*, lineages were initiated and maintained by self-pollination of single, selected individuals—thus ensuring a major role for drift. By contrast, population sizes of 20 or more were maintained in *Helianthus*, plants were outcrossed, and natural fertility selection was allowed to proceed via pollen pooling. Another critical factor is that two generations of backcrossing toward *H. annuus* were employed in the generation of all three synthetic sunflower hybrid lineages. This appears to have resulted in the retention of that subset of *H. petiolaris* chromosomal fragments that interacted in a neutral or positive manner with the *H. annuus* genetic background. Backcrossing toward *H. annuus* also is likely to have occurred during the formation of the ancient hybrid species, *H. anomalus*, as backcrosses in this direction are more fertile and easily produced than other genotypic classes (48).

NATURAL HYBRID SPECIES

The Difficulty of Unambiguous Documentation

The experimental and theoretical studies discussed above indicate that homoploid hybrid speciation is a workable process under artificial conditions and may therefore occur in nature. Nevertheless, the actual extent of this mode of speciation in nature is unclear. This is not due to a lack of proposed examples in the taxonomic literature. In fact, there are few monographs that do not invoke hybridization to account for the origin of at least one or two morphologically intermediate or mosaic taxa (48, 67, 90). However, it is well known that morphological intermediacy can arise from forces other than hybridization. Dobzhansky (24), for example, recognized that intermediacy could arise by convergent morphological evolution. He also noted that remnants of the ancestral population from which two species differentiated might have the appearance of hybrids—an early and explicit recognition of plesiomorphy (the retention of primitive characteristics). Other authors have expressed skepticism concerning the use of quantitative phenotypic data to identify hybrids in the absence of information regarding the genetic basis of the characters being scored (10, 34, 47).

In many studies, morphological information has been augmented with evidence from secondary chemistry, ecological, and geographic data, and/or the production of synthetic hybrids that resemble the natural hybrids. Each of these approaches has its strengths, but as with morphological data, it is often difficult to determine whether intermediacy for chemical characters, ecological distribution, and/or geographic range actually result from hybridity. And putative examples of hybrid species based on these traditional biosystematic data sets often cannot be verified with molecular evidence (64, 74, 80, 91, 104).

Molecular markers represent a more powerful tool for identifying hybrid taxa (82), but even this approach can generate ambiguous results. As with morphological characters, a taxon can share molecular markers of related taxa due to the joint retention of alleles following speciation in a polymorphic ancestor (symplesiomorphy). This phenomenon has also been referred to as lineage sorting when discussed in the context of gene lineage data (8). As a result, it is much easier to reject the hypothesis of hybrid origin than to confirm it with molecular data sets. The use of multiple loci (32), linked markers (25), and gene lineage data (9, 69, 74) greatly enhances the probability of distinguishing between symplesiomorphy and hybridization. For example, if a putative hybrid species possessed multiple, linked markers of potential parents, and/or additivity for diagnostic parental markers at multiple loci, the probability that this situation could be attributed to symplesiomorphy or convergence is minimized. Likewise, hybridization becomes an increasingly probable explanation for shared alleles of identical sequence as species divergence times increase (43).

Even if evidence in favor of hybridity is unambiguous, this does not mean it had anything to do with speciation. For example, discordant organellar and nuclear phylogenies apparently due to hybridization are being reported with increasing frequency (83), but the evolutionary outcome of most of these ancient cases of hybridization appears to be introgression rather than hybrid speciation. The distinction between introgression and hybrid speciation can be difficult as well (101, 104). Incongruence between cytoplasmic- and nuclear-based phylogenetic trees, for example, suggest that hybridization played a role in the evolution of a wild species of cotton, *Gossypium bickii* (101). However, it is not clear whether this ancient hybridization event was important in its origin, as *G. bickii* does not appear to have a biparental nuclear genome typical of hybrid species.

Case Studies

A survey of the botanical literature identified more than 50 putative examples of homoploid hybrid species representing over 20 families of seed plants. However, only 17 examples have been rigorously tested with molecular markers. In my judgment, homoploid hybrid speciation has been convincingly documented in eight of these cases (Table 1), whereas in the remaining nine cases, hybrid speciation was disproved (78, 80, 91, 104) or the molecular marker data were ambiguous with regard to hybrid origin (1, 22, 64, 101).

HYBRID SPECIES ISOLATED BY POSTMATING BARRIERS The classification of hybrid species by their mode of reproductive isolation is somewhat arbitrary as most probably have both postmating and premating barriers. Premating barriers are especially ubiquitous as all hybrid species appear to have diverged ecologically from their parents. Nonetheless, the examples discussed in this section do differ from their parents by chromosomal or genic sterility factors, whereas those in the next section appear to be isolated primarily due to habitat differences (Table 1).

The first application of molecular methods to the study of homoploid hybrid speciation was in *Stephanomeria* (Compositae) (32). The hybrid species, *S. diegensis*, and its parents, *S. exigua* and *S. virgata*, are self-incompatible annuals with the same diploid chromosome number ($2n = 16$). The parental species are widespread and largely allopatric, but they do co-occur and hybridize in Southern California. First generation hybrids are semisterile (14% pollen viability), apparently due to chromosomal structural differences between the parental species.

Stephanomeria diegensis is an abundant native of coastal southern California and morphologically is an “amalgam” of its parents. Analyses of 20 isozyme

Table 2 Confirmed examples of homoploid hybrid species in flowering plants

Taxon	Evidence[a]	Isolating mechanisms[b]	Growth form	Breeding system	Pollination syndrome	Reference
Helianthus anomalus	C,I,L,M,N,P	E,G	annual herb	outcrossing	animal	71
Helianthus deserticola	C,I,M,N,P	E,G	annual herb	outcrossing	animal	71
Helianthus paradoxus	C,I,M,N,P	E,G	annual herb	outcrossing	animal	71, 74
Iris nelsonii	C,I,M,N,P	E,G	annual herb	outcrossing	animal	4, 6
Peaonia emodi	N	E	perennial herb	outcrossing	animal	85
Peaonia species group	N	E	perennial herb	outcrossing	animal	85
Pinus densata	I,M,P	E	tree	outcrossing	wind	99, 100
Stephanomeria diagensis	C,I,M	E,G	annual herb	outcrossing	animal	32

[a] C = cytological or crossing studies; I, isozymes; L, linkage mapping; M, morphology; N, nuclear markers; P, plastid markers.

[b] E = ecogeographic isolation (premating); G, isolation due to genic or chromosomal sterility factors (postmating).

loci revealed that *S. diegensis* was a composite of the genes of *S. exigua* and *S. virgata* and had only one very rare unique allele. Furthermore, artificial F_1 hybrids between *S. diegensis* and its parents averaged 1% to 2% pollen viability, and thus they are significantly less fertile than those between the parental species. Rapid chromosomal evolution may have facilitated the speciation process as has been shown for *Helianthus* (below).

Molecular studies of the annual *Helianthus* species of section *Helianthus* have identified three species that appear to be derived via this mode: *H. anomalus*, *H. deserticola*, and *H. paradoxus* (71, 72, 74, 75, 79, 81). Although morphologically distinctive and allopatric, all three species appear to be derived from the same two parents (*H. annuus* and *H. petiolaris*) as they combine parental allozymes and nuclear ribosomal repeat units and share the chloroplast DNA (cpDNA) haplotype of one or both parental species. Like their parents, the three hybrids are self-incompatible and have a haploid chromosome number of 17. They differ from the parental species, however, in terms of geographic distribution and habitat preferences. *Helianthus paradoxus* is endemic to saline brackish marshes in west Texas, whereas *H. anomalus* and *H. deserticola* are xeric species restricted to the Great Basin desert of the southwestern United States. By contrast, the parental species are widespread throughout the central and western portion of the United States, with *H. annuus* found primarily in mesic soils and *H. petiolaris* in dry, sandy soils. Artificial crossing experiments indicate that all three hybrid species are semisterile with their parents, apparently due to chromosomal sterility barriers (below).

To test the hypothesis that rapid karyotypic evolution (98) can facilitate the development of reproductive isolation between a new hybrid lineage and its parents, genetic linkage maps were generated for *H. annuus*, *H. petiolaris*, and one of their hybrid derivatives, *H. anomalus*. Gene order comparisons revealed that 6 of the 17 linkage groups were co-linear among all three species, whereas the remaining 11 linkages were not conserved in terms of gene order (Figure 2). The two parental species, *H. annuus* and *H. petiolaris,* differed by at least 10 separate structural rearrangements, including three inversions, and a minimum of seven interchromosomal translocations. The genome of the hybrid species, *H. anomalus*, was extensively rearranged relative to its parents (Figure 2). For 4 of the 11 rearranged linkages, *H. anomalus* shared the linkage arrangement of one parent or the other. For the remaining seven linkages, however, unique linkage arrangements were displayed. 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. Similar increases in chromosomal mutation rates following hybridization have previously been reported in grasshoppers (86).

To reduce gene flow, chromosomal structural differences must enhance reproductive isolation. This does appear to be the case in *H. anomalus*, in which first generation hybrids with its parents are partially sterile, with pollen stainabilities of 1.8%–4.1% (*H. annuus*) and 2%–58.4% (*H. petiolaris*) (17, 46). Meiotic analyses revealed multivalent formations and bridges and fragments suggesting that chromosomal structural differences are largely responsible for hybrid semisterility (17). Thus, the rapid karyotypic evolution inferred from these mapping data does satisfy genetic models for speciation through hybrid recombination (98).

Thus far, all of the examples of hybrid speciation have involved two parental species, but there is no particular reason why additional species cannot be involved. An example comes from the Louisiana irises where *Iris nelsonii* contains genetic markers from *I. fulva*, *I. hexagona*, and *I. brevicaulis* (4, 6). Speciation appears to have occurred very recently, as individual plants cannot be unequivocally distinguished from either *I. fulva* or certain hybrid genotypes from contemporary hybrid zones. However, taken as a whole, the genetic make-up of *I. nelsonii* does differ significantly from parental and contemporary hybrid populations, and the species is stable and distinct in terms of morphology, ecological preference, and karyotype.

HYBRID SPECIES ISOLATED BY PREMATING BARRIERS Good examples of homoploid hybrid species that are isolated by premating barriers only are difficult to find. One possible example is *Rhodendron intermedium,* which appears to be partially isolated from its parents due to soil preferences and the behavior of pollinators (51), but Kerner's hypothesis has not been tested using modern methods. More recently, Grant (38) presented a model for sympatric speciation in flowering plants based on flower constancy of pollinators and suggested that hybridization might be as likely as mutation in generating the new floral structures. Straw (96) postulated that two species of *Penstemon* might represent homoploid hybrid species that were derived via this manner. However, molecular studies do not support this hypothesis (104).

This mode is well-documented in Asian pines. Closely related species of *Pinus* hybridize frequently, and interspecific hybrids often are fertile and vigorous. Nonetheless, species remain distinct, apparently due to habitat isolation. Although several Asian pine species are thought to be of hybrid origin (62), the only example that has been analyzed rigorously is the putative origin of *P. densata* from *P. tabulaeformis* and *P. yunnanensis.* The three species have different ecological requirements, with *P. densata* endemic to high mountain elevations where neither of its putative parents is found. The geographic distribution of *P. densata* overlaps slightly with both parents, but the parents themselves are allopatric. Isozyme studies indicate that *P. densata* does combine the allozymes of its putative parents (100). However, analyses of cpDNA variation revealed the presence of three cpDNA haplotypes in *P. densata* (99). Two were identical to those found in the putative parents, but the third could not be found in extant Asian pine species. As a result, Wang et al (99) concluded that *P. densata* may have been derived via hybridization between two extant and one extinct Asian pine species.

A final study that may provide insights into the evolutionary potential of homoploid hybrid speciation concerns the genus *Paeonia* (85). Additivity of individual nucleotide positions in the internal transcribed spacer region of nuclear ribosomal genes implied a hybrid origin of a single species, *P. emodi*, as well as that of an entire lineage of ten species. The latter discovery is important because it suggests that homoploid hybrid species are not evolutionary deadends, but can found dynamic and speciose lineages.

THE BIOLOGY OF HOMOPLOID HYBRID SPECIES

The eight confirmed examples of hybrid speciation include one tree, three perennial herbs, and four annual herbs (Table 1). Although this sample is too small to make valid generalizations about homoploid hybrid speciation, one surprising result is that all have an outcrossing breeding system. The presence of outbreeding is unexpected, because hybrid speciation is predicted to occur more readily in highly inbreeding populations (42, 60, 98). Likewise, the high proportion of annual species of confirmed hybrid origin is unusual as hybridization appears to be more frequent in perennials.

All of the proposed and confirmed hybrid species differ from their parental species in habitat preference. This is expected, of course, as two species cannot occupy the same niche. What is unexpected, however, is that the habitat occupied by the hybrid taxa is often novel or extreme rather than intermediate relative to that of the parental species. Examples include the high altitude habitat of *Pinus densata* (100) and the xeric or marshy habitats of the three *Helianthus* hybrid species (71).

Morphologically, confirmed hybrid species exhibit a large proportion of extreme or novel characteristics when compared with their parents (34, 70, 76, 78). At least some of this morphological divergence appears to arise as a direct result of hybridization, as studies of synthetic hybrids from 33 plant genera reveal that over 10% of morphological characteristics are extreme in first generation hybrids and greater than 30% are extreme in later generation hybrids (76). This phenomenon is often referred to as transgressive segregation (97), and it appears to be the rule rather than the exception in segregating progenies from

Table 2 Isozyme variability in confirmed hybrid species (Hd) and their parents (Pt)

Taxon	Percentage of loci polymorphic[a]	Mean no. of alleles per locus	Mean heterozygosity[b]	Reference
Helianthus:				
annuus (Pt)	23.5	1.3	0.065	72
anomalus (Hd)	17.6	1.2	0.069	72
deserticola (Hd)	5.0	1.1	0.022	72
paradoxus (Hd)	5.9	1.1	0.027	72
petiolaris (Pt)	35.3	1.5	0.123	72
Iris:				
brevicaulis (Pt)	54.0	1.7	0.167	6
fulva (Pt)	41.5	1.4	0.122	6
hexagona (Pt)	43.6	1.6	0.152	6
nelsonii (Hd)	38.0	1.5	0.134	6
Pinus:				
densata (Hd)	61.5	2.5	0.210	100
tabulaeformis (Pt)	53.8	2.8	0.195	100
yunnanensis (Pt)	46.2	2.2	0.169	100
Stephanomeria:				
diegensis (Hd)	36.3	2.3	0.082	32
exigua (Pt)	33.6	2.6	0.098	32
virgata (Pt)	34.0	2.6	0.109	32

[a] For *Helianthus*, *Iris*, and *Stephanomeria*, a locus was considered polymorphic if the frequency of the most common allele did not exceed 0.99, whereas for *Pinus*, a frequency of 0.95 was used to define a polymorphic locus.

[b] For *Helianthus*, *Iris*, and *Pinus*, values are for mean expected heterozygosity, whereas for *Stephanomeria*, values represent observed mean heterozygosity per individual.

interspecific crosses (76). Botanists have speculated that the morphological and ecological novelty created by hybridization might allow hybrid populations to spread onto previously unoccupied adaptive peaks (3, 5, 54, 93).

Earlier workers predicted that hybrid taxa would be more variable genetically and have greater evolutionary potential than their parental species because they would combine the alleles of both parents (3, 39, 93). Although this is a reasonable argument, it is not supported by data from confirmed hybrid species (Table 2). The three *Helianthus* hybrid species exhibited lower levels of genetic diversity than either parent as measured by estimates of percentage polymorphic loci, mean number of alleles per locus, and mean heterozygosity (Table 2). *Pinus densata* was slightly more variable genetically than either parent, whereas *Stephanomeria diegensis* and *Iris nelsonii* were roughly equivalent to their parents in terms of variability (Table 2). The lower-than-predicted levels of diversity in the *Helianthus* hybrids may indicate that a small number of parental individuals was involved in their origin, possibly via hybrid founder events as discussed earlier.

CONCLUSIONS AND FUTURE DIRECTIONS

Satisfactory understanding of any mode of speciation requires answers to the following questions: Is the mode theoretically possible? Is there evidence for it in nature? How does it occur? Under what evolutionary conditions is it most likely? How quickly does it occur? And how frequent is it?

Most of these questions can be answered adequately for homoploid hybrid speciation. As discussed above, experimental and theoretical data indicate that this mode is feasible, and molecular marker data provide convincing evidence for its operation in nature. Likewise, the evolutionary processes accompanying or facilitating this mode of speciation are well understood. Important components such as the sorting of genic and chromosomal sterility factors, rapid chromosomal evolution, strong fertility and viability selection, and ecological divergence have been verified by both theoretical and experimental studies. Many of the critical ecological parameters that promote this mode of speciation have also been identified: the availability of suitable hybrid habitat, a selective advantage for hybrids in a hybrid habitat, and a long hybrid zone interface, which enhances the number of hybrid matings per generation. However, the observation that confirmed hybrid species are outbreeding (Table 1) contradicts theoretical studies indicating that rates of hybrid speciation should increase with selfing (60). Perhaps the advantage of selfing for hybrid species establishment is counterbalanced by lower rates of natural hybridization among selfing lineages.

Less evidence is available concerning the tempo and frequency of this mode. However, both experimental and theoretical data point to a rapid tempo of speciation. For example, fertile and stable hybrid segregants can often be obtained after only a few generations of hybridization, and simulation studies suggest that hybrid speciation is punctuated: Long periods of hybrid zone stasis are followed by the rapid establishment and growth of the new hybrid lineage (62). Congruence in genomic composition of synthetic and ancient hybrid species also suggests that hybrid genomes are likely to be stabilized quickly, with little change thereafter (79). Possibly, the tempo of speciation in natural hybrid species could be tested by analyzing the sizes of parental chromosomal fragments. Due to recombination, fragment sizes should decline in a predictable manner over time (11), perhaps making it feasible to estimate the number of generations of hybridization required to stabilize a hybrid species genome.

Estimating the frequency of hybrid speciation in nature is more speculative. Only eight examples in plants have been rigorously documented (Table 1), and even fewer in animals (26), suggesting that this mode may be rare. However, these low numbers may be an artifact of the difficulty of detecting and rigorously documenting homoploid hybrid species, particularly if the hybridization events are ancient. A much larger number of hybrid species have been proposed, and phylogenetic studies continually uncover unexpected cases of ancient hybridization in many evolutionary lineages (83). Although attempts to estimate the frequency of homoploid hybrid speciation are probably premature, hybridization may play a major role as the creative stimulus for speciation in small or peripheral populations. Hybridization rates appear to be highest in populations with these characteristics (28), and hybridization may be a more plausible mechanism than population bottlenecks for generating the genetic or chromosomal reorganization proposed in founder effect or saltational models of speciation (37, 71).

Although substantial progress has been made in studying this mode, much remains to be understood. A major gap in our knowledge relates to the origin of homoploid hybrid species that are isolated from their parents by premating barriers only. Empirical data indicate that species have arisen in this manner, but experimental and theoretical studies have focused on the strict recombinational model that involves the sorting of genic and chromosomal sterility factors. Because hybrid speciation is both reticulate and rapid, it is particularly amenable to experimental manipulation and replication. Thus, it should be feasible experimentally to synthesize new homoploid hybrid species isolated by premating barriers only. The design of these experimental studies could be informed by theoretical studies that identify parameters critical to this mode.

Another important issue relates to the fitness of hybrid genotypes. The ecological divergence required by the hybrid speciation model implies that the average fitness of the new hybrid lineage must exceed that of the parental species in hybrid habitat. This does not mean that early generation hybrids are more fit on average than their parents, but it does imply the existence of interspecific gene combinations that convey a fitness advantage in hybrid habitats. This hypothesis could be tested by comparing the lifetime fitnesses of individual hybrid and parental genotypes in hybrid habitats. Presumably, later generation hybrid segregants resulting from several generations of habitat and fertility selection would be most likely to exhibit a fitness advantage. The value of these experiments would be enhanced if the genomic composition of the hybrids was known (79), so that the effects of particular gene combinations on fitness could be determined. An alternative approach would be selection experiments that compare the responses of hybrid and parental populations to selective regimes approximating those expected in hybrid habitats (57).

Questions also remain concerning the genetic processes by which new hybrid species arise: 1. How do hybrid populations move to a new adaptive peak? Sunflower genetic mapping experiments suggest that this is accomplished primarily by selection rather than drift, and that selection can act directly on gene combinations as well as all individual genes. However, it is not clear whether this result is generalizable to hybrid lineages in which selection is less intense. Detailed mapping studies of synthetic hybrid lineages from less divergent species crosses may be required to address this question satisfactorily. 2. Do postmating reproductive barriers in new hybrid species arise primarily by the sorting of preexisting parental sterility factors or via the high genic and chromosomal mutation rates characteristic of hybrid populations? This question could be addressed by comparing the locations of quantitative trait loci (QTL) contributing to postmating reproductive barriers between the parental species with those isolating the hybrid species from its parents. If the "sterility QTL" in the hybrid taxon are a subset of those found in its parents, then the sorting hypothesis would be accepted. The presence of unique sterility QTL in the hybrid taxon would be more difficult to interpret, as this could be attributed either to rapid evolution during speciation or to divergent evolution following speciation. 3. What fraction of the morphological and ecological novelty observed in hybrid species is created by hybridization versus divergent evolution following speciation? As with the previous question, the critical issue here is to elucidate the direct role of hybridization in the speciation process, in this case with respect to the origin of morphological and ecological novelty. Although this question could be answered by comparing the morphological and ecological characteristics of synthetic and natural hybrid species, these comparisons have yet to be made.

Finally, I want to emphasize the continuing importance of molecular phylogenetic studies that identify and document natural hybrid species, as these studies are critical to reliable generalizations about the frequency and evolutionary significance of this mode. If designed well, these studies not only provide a means for "cleansing" the literature of oft-cited but incorrect examples of hybrid speciation, but they also provide an efficient strategy for testing large numbers of plant and animal groups for the existence of hybrid species (71). Clearly, studies that generate trees for multiple, unlinked loci and sample several populations per species will be most successful. The use of multiple loci is of particular importance as five independent gene trees are required to achieve 95% confidence that a given reticulation event will be detected (78). Greater phylogenetic resolution also will increase the chance of detecting ancient and possibly speciose hybrid lineages.

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