The Role of Hybridization in Plant Speciation

Pamela S. Soltis[1] and Douglas E. Soltis[2]

The Genetics Institute, [1]Florida Museum of Natural History, and [2]Department of Botany, University of Florida, Gainesville, Florida 32611; email: psoltis@flmnh.ufl.edu; dsoltis@botany.ufl.edu

Annu. Rev. Plant Biol. 2009. 60:561–88

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

This article's doi: 10.1146/annurev.arplant.043008.092039

1543-5008/09/0602-0561$20.00

Key Words

polyploidy, genome duplication, angiosperms

Abstract

The importance of hybridization in plant speciation and evolution has been debated for decades, with opposing views of hybridization as either a creative evolutionary force or evolutionary noise. Hybrid speciation may occur at either the homoploid (i.e., between two species of the same ploidy) or the polyploid level, each with its attendant genetic and evolutionary consequences. Whereas allopolyploidy (i.e., resulting from hybridization and genome doubling) has long been recognized as an important mode of plant speciation, the implications of genome duplication have typically not been taken into account in most fields of plant biology. Recent developments in genomics are revolutionizing our views of angiosperm genomes, demonstrating that perhaps all angiosperms have likely undergone at least one round of polyploidization and that hybridization has been an important force in generating angiosperm species diversity. Hybridization and polyploid formation continue to generate species diversity, with several new allopolyploids having originated just within the past century or so. The origins of polyploid species—whether via hybridization between species or between genetically differentiated populations of a single species—and the immediate genetic consequences of polyploid formation are therefore receiving enthusiastic attention. The time is therefore right for a review of the role of hybridization in plant speciation.

Contents

Clade: a monophyletic group; an ancestor and all of its descendants

Hybridization: crossing between species or between genetically differentiated races or populations of the same species

Homoploid hybrid speciation: the origin of a new species through hybridization of two species of the same ploidy (e.g., two diploid species)

Allopolyploidy: polyploidy involving interspecific hybridization

INTRODUCTION

Approximately half a million species of green plants—the clade that encompasses green algae and land plants—have been recognized scientifically. Of these, more than half are angiosperms (198), although estimates of the number of angiosperm species vary from 350,000 to 400,000 (e.g., P. Raven in Reference 80). The causes and nature of plant speciation are therefore important for understanding the origin and maintenance of a large proportion of the world's biodiversity (estimates of total species numbers range from 1 million to 10 million species).

Plants exhibit diverse speciation mechanisms and modes of reproductive isolation (66, 97, 98, 159, 192, 193). Whereas earlier investigators (e.g., 66, 193) emphasized the dramatic differences between plants and animals with regard to population dynamics and speciation, recent work has illustrated many noteworthy similarities. For example, gene flow in plants appears to be higher than traditionally maintained and may be very similar to estimates for animals (134). Furthermore, many plant species appear to be reproductively isolated and therefore meet the same criterion for species recognition as do animals (see below and Reference 160). Nonetheless, despite some underappreciated similarities in speciation in plants and animals, there are also important differences. Most notable is the high frequency of hybridization and its role in speciation. Hybridization—typically considered to represent crossing between species—has been extended to include crossing between genetically divergent populations or races within a species (e.g., 17, 72), and we follow this broad definition here. Hybridization has often been viewed as a creative force in evolution (e.g., 13, 17). To understand plant speciation, the origin of many adaptations, and the maintenance of plant diversity, we therefore need a renewed emphasis on the processes of species formation through hybridization. Here we review the field of hybrid speciation and offer suggestions for fertile research.

Extreme Reticulation: Ancient and Recent Polyploidy

A significant portion of speciation events in plants involves hybridization, in contrast to most other clades, in which speciation is divergent. Such hybridization results in a phylogenetic net, rather than a classic bifurcating tree. Hybrid speciation can occur either at the same ploidal level (homoploid hybrid speciation) or much more commonly via allopolyploidy (speciation via hybridization and genome doubling) (**Figure 1**). [Even autopolyploidy—genome doubling within a species—may typically involve hybridization between populations of the same species (e.g., 181)]. Hybridization seems to be a ubiquitous feature of green plant evolution, although it is particularly pronounced in angiosperms and ferns (178, 212).

Whereas both homoploid hybridization and allopolyploidy can be potential sources of new species, allopolyploidy appears to be much more common than homoploid hybrid

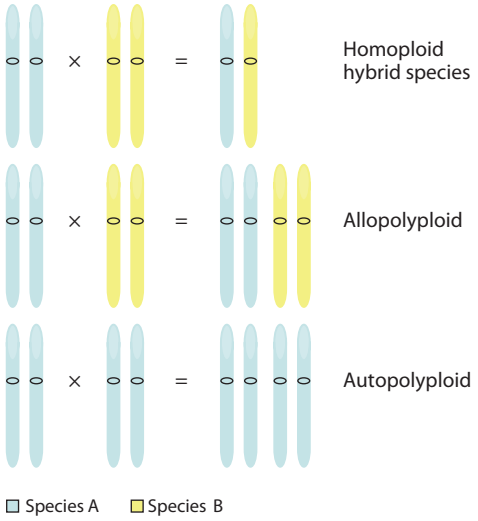


Figure 1

The origins of species via homoploid hybridization and polyploidy, as represented by a single pair of chromosomes in each diploid parental species, A and B. A homoploid hybrid species (here, a diploid) arises through hybridization of species A and B; the resultant diploid hybrid species has one chromosome complement (here, one chromosome) of each parental species. Allopolyploid formation is similar in that species A and B hybridize; however, unlike homoploid hybrid speciation, allopolyploid formation involves chromosome doubling. The resultant allopolyploid species combines the entire nuclear genomes of both parental species. Autopolyploid formation also involves chromosome doubling and may occur via crossing of genetically differentiated diploid individuals from the same or different populations of a single species. As illustrated here, crossing occurs between two individuals of the same species; chromosome doubling yields the autopolyploid. Note that in both allopolyploid and autopolyploid formation, chromosome doubling may occur either before or after the crossing event takes place.

speciation for reasons discussed by Stebbins (193) and Grant (66) and summarized most recently by Rieseberg & Willis (159). Homoploid hybrid species may have greatly reduced fitness in early generation hybrids, whereas this may not be the case in early generation allopolyploids (despite a possible sterility bottleneck in polyploids; 97). Furthermore, genome doubling reduces or eliminates the possibility of the new polyploid backcrossing with its parents—such is not the case for a homoploid hybrid. For these reasons, formation of a new species via allopolyploidy is more likely than through homoploid hybridization.

Genomic studies indicate that many, if not most, angiosperms are ultimately of ancient polyploid origin. Thus, the intertwined processes of hybridization and genome doubling have been significant in generating species diversity. These processes that have shaped much of angiosperm species diversity continue to the present, with allopolyploid species having arisen during the past century (see below). In the following paragraphs we review both the extent of ancient polyploidy in the angiosperms and several examples of allopolyploids that have arisen within the past 150 years.

Autopolyploidy: polyploidy that arises within a single species, although it may involve crossing between genetically differentiated populations

Ancient polyploidy. Although genomic studies are clear in identifying many examples of ancient polyploidy, distinguishing between ancient allopolyploidy on the one hand and ancient autopolyploidy followed by genomic diploidization on the other hand may be difficult. However, given that allopolyploidy is more common than autopolyploidy, extrapolating into the past suggests that most ancient polyploid events were allopolyploid. Ancient episodes of polyploidy have clearly played major roles in angiosperm evolution as well as in the evolution of ferns and lycopods (66, 194, 212). Inference of ploidy in angiosperms from chromosome numbers and hypothesized breaks between diploid and polyploid base numbers yielded estimates of 30–35% (193), to nearly 50% (40, 62, 66, 135), to as high as 70–80% (62, 102). In contrast, Masterson (118) used leaf guard cell size in fossil and extant taxa to estimate that 70% of all angiosperms have experienced polyploidy in their evolutionary history. On the basis of genomic evidence, even these older values underestimate the frequency of ancient polyploidy in the angiosperms.

Genomic studies have completely altered our view of the frequency of polyploidy in angiosperms. The question is no longer "What proportion of angiosperms are polyploid?" but

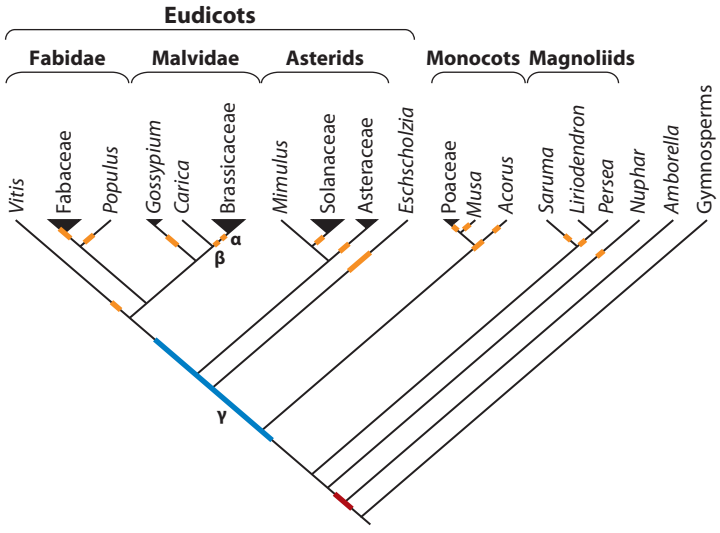


Figure 2

Whole-genome duplication (WGD) events in angiosperm evolution, inferred from complete genome sequences or other genome-level data. Each colored bar represents a separate WGD. Note that the elongated blue bar along the spine of the tree indicates that the position of this WGD is not clear from current data. However, a WGD unites either all eudicots or all core eudicots, or possibly monocots + eudicots. Likewise, the red bar near the base of the tree indicates that either all angiosperms share a WGD or all extant angiosperms except *Amborella* share a WGD. Additional data are needed for *Amborella* to resolve the placement of this WGD and determine whether or not the origin of the angiosperms coincided with a WGD. Orange bars indicate more WGD events that characterize smaller groups of species. Greek letters refer to duplications described by Bowers et al. (Reference 24). Modified with permission from Reference 176.

"How many episodes of polyploidy characterize any given lineage?" (see References 32 and 176). Complete sequencing of the nuclear genome has revealed evidence of ancient polyploidy throughout angiosperms and in other eukaryotes. All plant nuclear genomes sequenced to date show evidence of ancient genome duplication (176, 199) (**Figure 2**): *Arabidopsis* (24, 173, 209), *Oryza* (143), *Populus* (206), *Vitis* (79, 208), and *Carica* (130). A growing body of evidence suggests that the common ancestor of *Vitis*, *Populus*, *Arabidopsis*, and *Carica* was an ancient hexaploid that arose after the split between monocots and eudicots. Following this paleopolyploidy event, subsequent genome duplications occurred within Brassicales (leading to *Arabidopsis*) and in the lineage leading to *Populus* (Salicaceae).

In the absence of complete genome sequences, ESTs (expressed sequence tags) are an important source of genomic data that can be used to infer occurrences of ancient genome duplication (22, 39, 168). ESTs can be analyzed following Lynch & Conery (109) to estimate whether an ancient polyploid event may have occurred as well as its approximate age. Using this approach, ancient polyploidy has been identified in a number of crops, including *Zea* (maize), *Glycine* (soybean), and *Gossypium* (cotton), among others, with multiple whole-genome duplications in Fabaceae, Solanaceae, and Poaceae (22, 168). Likewise, ancient polyploidy is evident in several lineages of basal angiosperms: *Nuphar* (water lilies; Nymphaeaceae), *Persea* (avocado; Lauraceae), *Liriodendron* (tulip tree; Magnoliaceae), and *Saruma* (Aristolochiaceae) as well as the basal monocot *Acorus* (sweet flag; Acoraceae) and the basal eudicot *Eschscholzia* (California poppy; Papaveraceae) (39). *Nuphar* may in fact exhibit signatures of two ancient duplications, the older of which may be the oldest duplication so far discovered in flowering plants (39). Surprisingly, despite evidence for extensive polyploidy throughout the angiosperms, *Amborella*, the sister to all other extant angiosperms, does not show evidence of ancient polyploidy. Although *Amborella* has a high chromosome number ($2n = 26$), few duplicate gene pairs were detected in an initial survey of 10,000 ESTs (39); even with a greatly expanded data set, there is still no evidence for genome duplication in *Amborella* (176). However, lack of evidence for polyploidy in analyses of duplicate gene pairs does not rule out the possibility of ancient polyploidy, of which the signature may have been erased.

Recent polyploidy. Several plant species have originated via polyploidy within just the past 150 years: *Spartina anglica* (6, 7, 76, 116, 117) (**Figure 3**), *Senecio cambrensis* (**Figure 4**) and *S. eboracensis* (1, 163), *Cardamine schultzii* (207), and *Tragopogon mirus* and *T. miscellus* (139, 183) (**Figure 5**). These systems represent important evolutionary models because they formed

very recently, their parentage is known with certainty, and in several, the polyploid formed multiple times, permitting one to ask whether evolution is repetitive. Studies on the genetic consequences of allopolyploidy in these recently formed polyploid species reveal some significant similarities as well as differences. In *Spartina anglica*, allopolyploidy resulted in few changes in genome structure, but there is evidence for changes in methylation or epigenetic reprogramming (6, 7, 164). In contrast, in recently formed allotetraploids in both *Senecio* (1, 73, 74) and *Tragopogon* (119, 183, 200; R. Buggs, A. Doust, J. Tate, J. Koh, K. Soltis, F. Feltus, A. Paterson, P. Soltis & D. Soltis, unpublished data), evidence exists for major genetic changes, including loss of homeologs and DNA sequence as well as changes in DNA expression, including tissue-specific expression. These recently formed natural polyploids are unique evolutionary models that afford the opportunity to compare the consequences of polyploidization with results for better-studied genetic models, such as *Arabidopsis* (e.g., 32), *Gossypium* (cotton; 2, 3), *Oryza* (rice; e.g., 223), and *Brassica* (e.g., 60), and with synthetic polyploids (e.g., 60, 84, 105, 189).

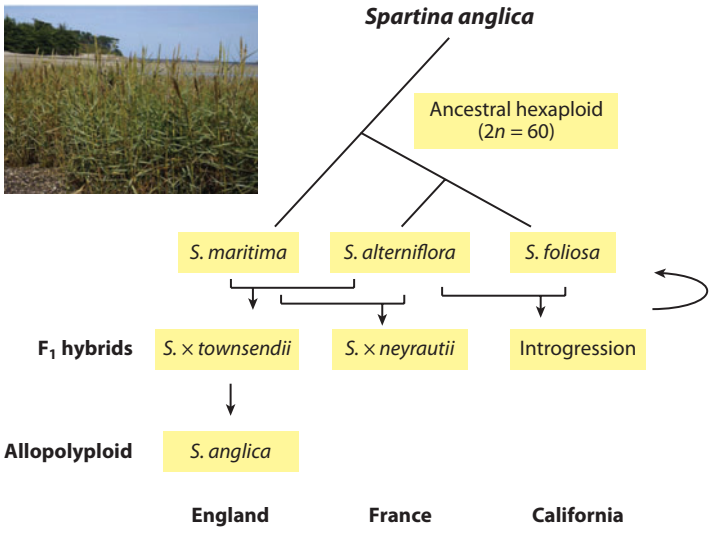


Figure 3

Examples of recent allopolyploids: *Spartina anglica*. Introduction of *S. alterniflora* outside its native range has resulted in hybridization with local species in England, France, and California. Hybridization has led to hybrid species formation (*S. X townsendii* and *S. X neyrautii*, both from *S. maritima* and *S. alterniflora*), polyploid formation (*S. anglica*), and introgression (*S. alterniflora* X *S. foliosa*, denoted by curved arrow). Modified from Reference 7; photo contributed by M. Ainouche.

SPECIES CONCEPTS

A discussion of speciation requires that we step—however trepidatiously—into the realm of species concepts. The issue of when two entities should be considered distinct species has been a longstanding controversy, particularly for plants (reviewed in References 66, 97, 216, and 220). In fact, the uncertainty of what constitutes a plant species may well have slowed progress in the study of some aspects of plant speciation (159, 184). It is beyond the scope of this review to cover species concepts in detail; however, without a coherent definition of a species, it is impossible to determine when a new one has arisen. Comprehensive reviews of species concepts are given elsewhere (18, 19, 37, 41, 58, 59, 66, 97, 155, 203, 216). Rather than rely on the adage that "a species is whatever a good taxonomist says it is," we review some of the more prominent concepts below and return eventually to the issue of hybridization and species.

The morphology-based taxonomic species concept—an assemblage of morphologically similar individuals that differs from other such assemblages (66)—continues to be widely used in plants. Although practical for taxonomic purposes, this system is subjective—the amount of difference that "is worthy of species rank cannot be prescribed objectively" (66). Different taxonomists may have different criteria and emphasize different characters. Adherence to this concept has certainly been an impediment to the recognition of the importance of autopolyploid speciation, in which autopolyploids, even those formed via some degree of hybridization, very closely resemble their diploid progenitors (see below and Reference 184).

The biological species concept (122), which maintains that a species is a "a group of interbreeding (or potentially interbreeding) populations that are reproductively isolated

Homeologs: chromosomes (and the genes that reside on them) in an allopolyploid that are similar due to shared evolutionary descent; for example, chromosome pair 1 of parental species A is homeologous to chromosome pair 1 of parental species B in allopolyploid species AB

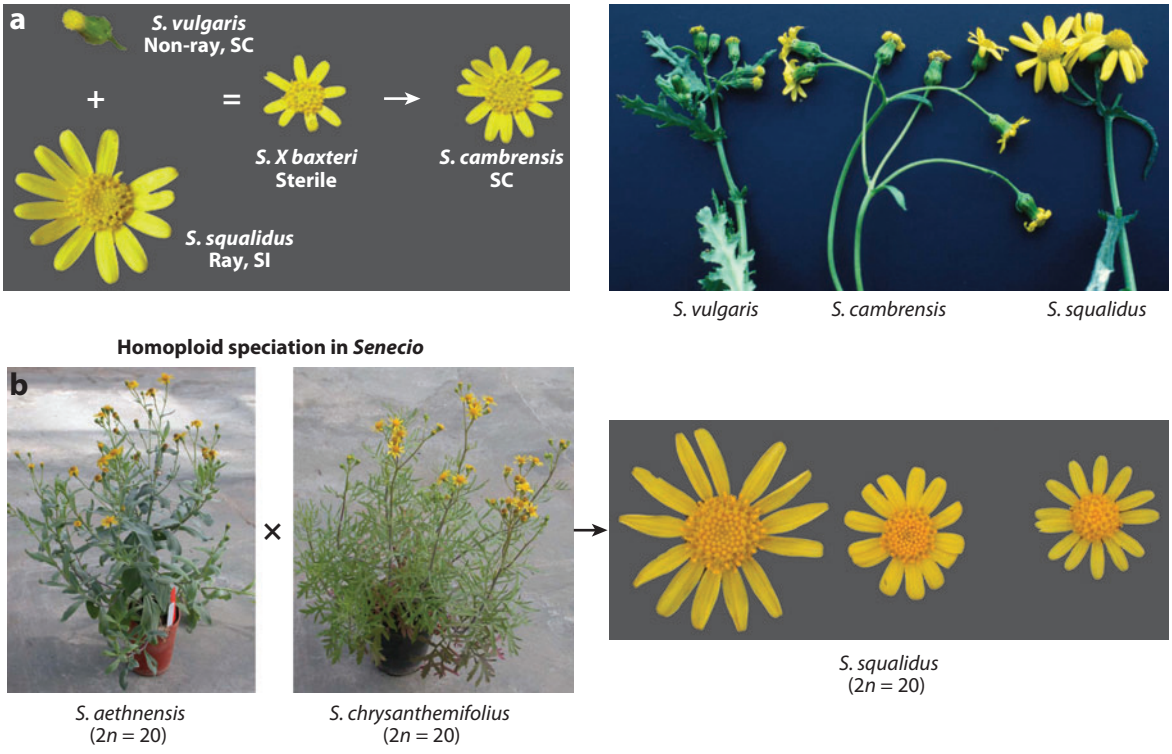


Figure 4

(*a*) Examples of recent allopolyploids: *Senecio cambrensis*. (*b*) Homoploid hybrid speciation occurs in *Senecio* as well: *S. squalidus*. Photos contributed by S. J. Hiscock. SC, self-compatible; SI, self-incompatible.

from other such groups," remains the prevailing view of species in animals (e.g., 36, 37) and has long played a major role in views of plant species as well. However, the application of the biological species concept is difficult in plants because of frequent hybridization and asexual reproduction. In part because of frequent hybridization between plant species and coupled with theoretical arguments against the biological species concept, many plant systematists have abandoned the biological species concept (e.g., 20, 42, 48, 52, 82, 125, 132, 136). However, the biological species concept has recently had a modest resurgence in popularity (e.g., 160, 167), due, at least in part, to the interpretation that some hybridization between species should not dictate that only a single species be recognized (e.g., 37). Despite this renewed pragmatism and empirical work linking morphological similarity to reproductive cohesion (and the converse) (160), many systematists continue to oppose the biological species on theoretical grounds (i.e., the feature uniting members of a biological species—intercrossability—is generally a symplesiomorphy, a shared, ancestral trait).

The evolutionary species concept (174, 218, 219) recognizes ancestral-descendant sequences of populations that evolve separately from other such lineages and have their own ecological niches, evolutionary tendencies, and historical fates. Limited hybridization can be accommodated by the evolutionary species concept as long as the hybridizing species do not merge (66, 174). Likewise, the origin of a new species via homoploid hybridization or allopolyploidy would yield a new evolutionary lineage with its own evolutionary tendencies and historical fate. Again, as long as the parental lineages remain intact, such hybrid speciation

causes no problems for the evolutionary species concept.

The widespread acceptance of phylogenetic approaches prompted the development of several phylogenetic species concepts. Following the phylogenetic species concept (sensu Reference 38; the diagnosability species concept, Reference 82), a species "is the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent." Likewise, species are considered "the minimal elements of hierarchic descent systems" (41). Operationally, species are defined as "the smallest aggregation of (sexual) populations or (asexual) lineages diagnosable by a unique combination of character states" (42, 136, 217). Under a second phylogenetic species concept (48, 131, 133; the apomorphic species concept, Reference 82), species are recognized on the basis of monophyly and are defined as "the least inclusive taxon recognized in a formal phylogenetic classification" (133; but see also References 48 and 131). Variants on these phylogenetic concepts have also been proposed (e.g., 19, 41, 45), but discussion of them is beyond the scope of this review. Application of either the diagnosable or apomorphic (sensu Reference 82) species concepts to hybrid-derived lineages is not straightforward. The diagnosable species concept may be difficult to apply in cases of hybrids and polyploids in which a (new) derivative species cannot be diagnosed as distinct from its progenitors. Recognition of a hybrid/polyploid species based on monophyly is likewise problematic; although the new hybrid/polyploid is a new evolutionary lineage, it may not appear as such in a phylogenetic tree, unless provisions for incorporating hybridization events are in place. Furthermore, recurrent formation of allopolyploid "species" raises questions about the strict monophyly of most allopolyploids: under the apomorphic species concept, should each polyploid lineage of independent origin be considered a separate species, particularly in the absence of evidence on crossing relationships with and among populations of separate origin?

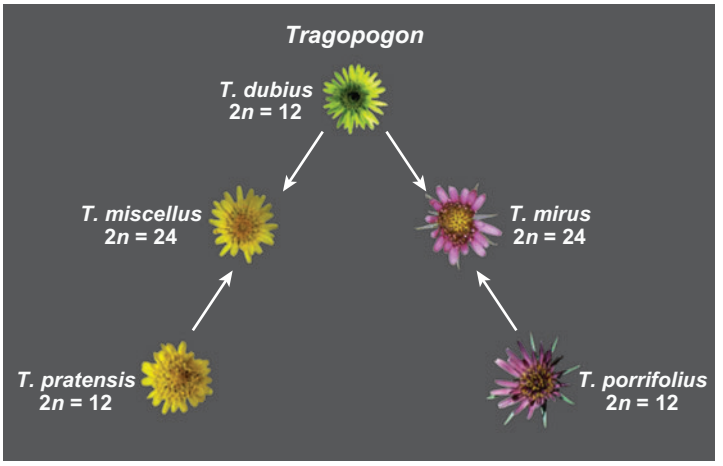


Figure 5

Examples of recent allopolyploids: *Tragopogon mirus* and *T. miscellus*.

HOMOPLOID HYBRID SPECIATION

The laws of inheritance allow for predictions about the genetic properties of a hybrid species at either the diploid or polyploid level. Here we review the expectations for a diploid hybrid under a range of scenarios for the mode of origin of the hybrid and then consider how empirical data support or deviate from these expectations. Similar discussion for polyploid species follows (see below).

A typical expectation of hybridization is additivity, but what exactly does additivity mean? From the perspective of quantitative genetics underlying polygenic traits, this is the additive genetic variance, and in a hybrid between inbred lines, additivity will result in some sort of intermediate phenotype, barring dominance or epistasis (see Reference 53). From the joint perspective of morphology and classical systematics, additivity in a hybrid has been typically equated with morphological intermediacy. However, morphological analyses of natural and artificial hybrids clearly indicate that a range of morphological outcomes is possible: from characters that are identical to those of one parent, to those that are truly intermediate, to those that are identical to the second parent, to novel traits, with intergradations among these conditions (124, 151). Likewise, both theory

Hybrid swarm: results when two (or more) species interbreed extensively; includes parental species, F_1, F_2, and later-generation hybrids, and backcrosses to one or both parental species

Introgressive hybridization/ introgression: the transfer of genetic material from one species to another through hybridization and backcrossing

(63) and analyses of flavonoid chemistry (100, 101) support the hypotheses of both additivity and novelty in hybrids.

If we turn to patterns exhibited by molecular markers, we do not, by definition, see intermediacy, but the expectation of additivity remains. For example, consider the simple case of two parental individuals belonging to species A and B, respectively, and fixed for alternative alleles at all loci. In this case, the F_1 hybrid AB would show complete additivity at all loci. But does that mean the hybrid species AB should be expected to exhibit strict additivity at all loci? The answer is: only if the hybrid species reproduced asexually, through either vegetative reproduction or some other method of asexual propagation. This form of hybrid species has been recognized by many systematists, for example in characterizing hybrids in the genus *Rubus* (Rosaceae; blackberries and raspberries; e.g., 8). If the F_1 were fertile and led to an F_2, the F_2 would not show additive patterns, but instead the genes would follow the laws of segregation and independent assortment. These combined processes could produce an array of genotypes spanning from one parental genotype to the other (the F_1) and various novel genotypes.

Under these simplistic conditions, molecular markers and minimal analyses could probably identify with confidence likely cases of hybrid speciation. However, let us consider a more real-life situation: Both parental individuals are heterozygous at some or all loci (although they do not share alleles), so a sample of the parental species compared with the hybrid may show some differences. These differences may or may not be extensive, but they could interfere with the recognition of a hybrid as a true hybrid and with the identification of a species' progenitors.

A more complicated scenario is that the parents share alleles at some loci, which is not unlikely if they are closely related species (e.g., 51). The result may be that the hybrid is additive of the parental alleles at some loci, but the pattern may not be clear at other loci because of the parents' shared alleles; thus, the expectation of additivity is a little more difficult to evaluate under this situation.

Perhaps the most real-life scenario involves the formation of the hybrid species from a hybrid swarm that was originated by heterozygous parents. With this mode of formation, F_1s, F_2s, later-generation progeny, and possibly even backcrosses may all have contributed to the formation of a stabilized hybrid derivative, and strict additivity is not to be expected. Under this scenario, a hybrid species should share approximately half of its alleles with each parent; thus, it is additive in a very loose sense, but certainly not in the way in which an F_1 formed from two completely genetically differentiated parents is additive. If multiple origins of the species have occurred from genetically differentiated parental populations (typically associated with hybrid species at the polyploid level rather than the diploid level, but possible nonetheless for a diploid hybrid species), the additivity is even looser. Furthermore, depending on the age of the hybrid species, it may or may not have had time for the origin of novel alleles through point mutations (e.g., 63). Thus, several processes may contribute to patterns that are not strictly additive, even in a species that in fact was formed through hybridization.

Gallez & Gottlieb (61) described the genetic expectations of a hybrid species from a populational perspective. First, a hybrid species is expected to show additivity at a single locus, with alleles derived from the two parents. In addition, additivity is expected at the populational level when alleles from the different parents are combined across loci (e.g., locus A has alleles from parent 1 and locus B has alleles from parent 2, with perhaps locus C having alleles from both parents). These predictions were formulated for use with allozyme markers, but they remain valid for both sequence-based and microsatellite-based alleles.

Homoploid hybrid speciation appears to occur rarely, but is also difficult to detect, with perhaps only 20 good examples in the literature (reviewed in Reference 68). The best-studied examples of diploid hybrid species in plants are found in *Helianthus* and *Iris*. Examples from both genera have been studied for decades. The concept of introgressive hybridization

(or introgression)—that is, interspecific hybridization followed by backcrossing to one or both parental species, with the ultimate transfer of genetic material between species—was first described on the basis of patterns of hybridization in *Iris* (161; see also References 11, 12, 14, 16, and 17 and references therein). The stabilized hybrid derivative *I. nelsonii* was later recognized as a three-way hybrid among *I. brevifolia*, *I. hexagona*, and *I. fulva*. Patterns of genetic variation in *I. nelsonii* are complex (e.g., 15), but the contributions of the three proposed parental species are all evident.

Helianthus annuus, the widespread cultivated sunflower, has hybridized with other species of *Helianthus* all across North America (e.g., 75, 154, 158). At least three species have formed through hybridization between *H. annuus* and *H. petiolaris*, which is also fairly widespread, at least in the American Southwest. *H. anomalus*, *H. deserticola*, and *H. paradoxus*, all diploid hybrid derivatives from the same parents, exhibit genetic contributions of the two parents, but not in the most explicit combinations. In addition, all these species have adapted to extreme, but different, habitats: *H. deserticola* occurs in the Great Basin in Nevada, Utah, and northern Arizona; *H. anomalus* is found on sand dunes in Utah and northern Arizona; and *H. paradoxus* is restricted to saline wetlands in western Texas and New Mexico (157). Most spectacularly, all have undergone parallel chromosomal evolution; all share the same prominent rearranged segments of the genome relative to their parents, and these rearrangements have also occurred in synthetic hybrids between *H. annuus* and *H. petiolaris*. Both the causes of this parallel evolution and its consequences remain under study, but clearly demonstrate that hybridization and hybrid speciation may have far-reaching and unanticipated effects.

POLYPLOIDY

Types of Polyploids

How many types of polyploids should be recognized and how to characterize have has long been debated (e.g., 34, 40, 66, 192, 193) and are reviewed elsewhere (e.g., 177, 201). In brief, two general types of polyploids have historically been recognized: those involving the multiplication of one chromosome set and those resulting from the merger of structurally different chromosome sets. Kihara & Ono (86) used the terms autopolyploidy (auto = same) and allopolyploidy (allo = different), respectively, to distinguish between these two types. This approach was later employed by other early workers (34, 40, 66, 135). However, despite long-term efforts to categorize polyploids as either auto- or allopolyploids, a continuum of polyploid types clearly exists in nature; some polyploids simply cannot be easily placed in either of these two groups (102, 193). Stebbins (192, 193) referred to polyploids that comprise slightly differentiated chromosome sets as segmental allopolyploids. Such polyploids likely resulted through chromosome doubling associated with hybridization between parental individuals that bear complements that differ, for example, in the arm length of one pair of chromosomes. Hence, researchers have debated for more than 70 years about the types of polyploids that should be recognized in nature and the proper definitions of autopolyploidy and allopolyploidy.

True genomic allopolyploids are typically derived from hybridization between two (or more) distantly related species and thus combine divergent genomes with chromosome complements that are unable to pair with each other. In contrast, strict autopolyploids result from genome doubling within a single individual or by crossing between different plants or populations within a species, which involves the production and merger of unreduced (diploid) gametes from genetically and chromosomally similar individuals. [Because an autopolyploid may arise via crossing between genetically different individuals (179, 195), the concept of hybridization may extend to autopolyploids as well (17, 72), and we therefore include autopolyploidy in this review.] Thus, an autotetraploid will contain four copies of each chromosome (all four are homologs), whereas

Fixed heterozygosity: nonsegregating heterozygosity, usually due to the additivity of divergent parental genomes in an allopolyploid; may also arise following gene duplication and divergence in a diploid

an allotetraploid will contain two of each pair of the counterpart chromosomes derived from two different species (homeologous chromosomes). In (most) allopolyploids, inheritance patterns are therefore disomic because multiple copies of the same genetic loci are not present. In contrast, a strict autotetraploid will likely have tetrasomic inheritance. However, although tetrasomic inheritance is a useful tool for recognizing autopolyploids, it is not an absolute marker of autopolyploidy because a given polyploidy may have some loci with disomic inheritance and others with tetrasomic inheritance (184, 196; see below).

Polyploidy has long been recognized as a major force in plant evolution (e.g., 34, 40, 107, 118, 135, 192, 193, 194). Following the work of Stebbins (191, 192, 193, 194), Clausen and coworkers (34), and Grant (e.g., 65, 66), polyploidy became a major focus of biosystematic research. Botanists have long appreciated that polyploid lineages may show complex relationships with each other and their diploid ancestors (reviewed in References 159 and 184). The past 10 to 15 years have witnessed a dramatic resurgence in the study of polyploidy (e.g., see Reference 91; also reviewed in References 4, 5, 185, 201, and 214), with renewed interest in the mechanisms of polyploid formation and establishment (77, 147, 148); the frequency of recurrent polyploidization (e.g., 195, 200); the ecological effects of plant polyploidy (e.g., 204, 205); and the genetic, epigenetic, chromosomal, and genomic consequences of polyploidization (e.g., 4, 5, 24, 49, 60, 90, 92, 104, 106, 138, 144, 149, 199). Recent research has resulted in major modifications to many of the traditional tenets of polyploid evolution (e.g., 3, 49, 138, 185).

Recent research has also confirmed that polyploidy is not limited to plants; it has also played a major role in the evolution of other eukaryotes (67, 112, 113). Two episodes of polyploidy are hypothesized for the common ancestor of vertebrates (57, 114, 128, 137, 141, 190). Polyploidy has also been important in the subsequent evolution of amphibians (21), as well as salmonids and other fish (10, 44, 89, 127). The genomes of yeast and other *Saccharomyces* were anciently duplicated (50, 85, 222).

Genetic Expectations for an Allopolyploid Species

As for a diploid hybrid species, the fundamental genetic expectation for an allotetraploid (and other allopolyploids) is additivity of the parental genotypes. However, unlike a fertile diploid hybrid with segregating parental alleles, an allotetraploid will sequester its parental genetic variation into its component genomes. Thus, some genetic diversity in an allotetraploid will segregate and some will not. That is, genetic variation on homologous chromosomes (i.e., those that pair, contributed from the same parent species) will segregate, whereas genetic variation on homeologous chromosomes (i.e., similar chromosomes derived from the two parents) will not. Let us consider the simplest scenario: that of two homozygous parents, fixed for alternative alleles at all loci. If these two parents gave rise to an allotetraploid via the combined processes of hybridization and chromosome doubling (see References 147 and 148 for reviews of polyploid formation), the allotetraploid would be homozygous at all loci within a parental genome and heterozygous at all homeologous loci contributed by the two parents. In other words, the allotetraploid would exhibit complete additivity of the parental genes and would appear heterozygous at all homeologous loci. Note, however, that there is no segregating variation under this scenario. Because the parental individuals were homozygous at all loci, this homozygosity is maintained in the allotetraploid; even though the homologous chromosomes are segregating, they do not bear different alleles and thus there is no segregating variation. Furthermore, the fixed heterozygosity contributed by the parental individuals does not segregate because it is borne on homeologous, rather than homologous, chromosomes. This heterozygosity results from the divergence of the parental alleles at homeologous loci and appears similar to the

heterozygosity of an F_1, except that it does not segregate.

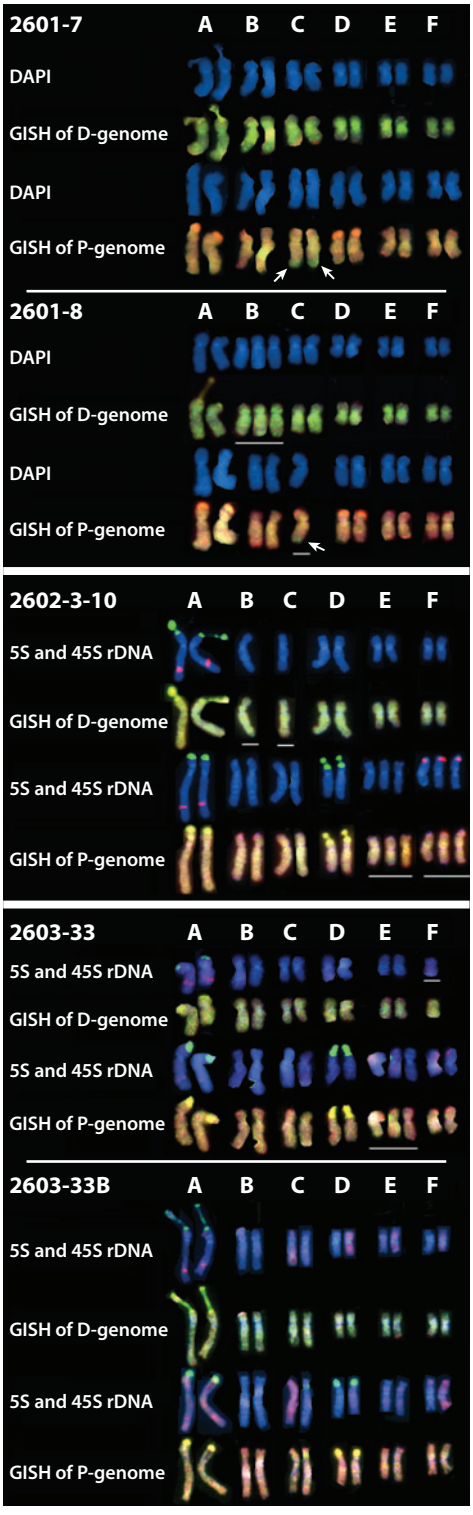
Depending on the mode of formation, true heterozygosity may have been introduced into the first allotetraploid individual, thus generating a more complex scenario than that described above. If we continue to assume that the parents have completely different alleles, rather than homozygosity at all loci within the contributed genome of each parent, as above, here segregating allelic variation occurs between homologs for at least some loci. Furthermore, nonsegregating fixed heterozygosity will still be maintained at homeologous loci, combining the genotypes of the parents.

Finally, the most complex scenario—and that most likely to reflect patterns of genetic variation in natural populations of allotetraploids—incorporates the role of multiple origins of polyploids in shaping the genetic diversity of polyploid individuals, populations, and species. It is now widely recognized that nearly all polyploid species comprise populations of independent formation from genetically distinct progenitor populations (see, e.g., Reference 201). If each of several constituent populations of an allotetraploid species of multiple origin had the genetic attributes described above (i.e., both segregating variation and fixed heterozygosity), then each allotetraploid population would have its own set of genotypes and all populations would be genetically distinct. However, with even limited gene flow among populations, novel genotypes could result from crossing between genetically different individuals, followed by segregation and independent assortment of genetic variants (182). The result would be a highly diverse allotetraploid species, with more genetic diversity than could possibly be incorporated into it via a single origin. Thus, in contrast to classical interpretations of polyploids as genetically depauperate and potential evolutionary dead ends, polyploids of multiple origin, especially those with interpopulational gene flow, are expected to maintain levels of genetic diversity—and therefore evolutionary potential—comparable to that of their diploid progenitors.

Polyploid Evolution: Rapid and Diverse Changes

Flowering plants exhibit remarkable genome plasticity (90), yet the merger of two genomes in a common nucleus should be viewed as a major upheaval at the nuclear and cellular levels—that is, as a sudden, violent disruption. However, angiosperm genomes tend to tolerate this merger, but with the effect of "genomic shock" (35). The genomic interactions that occur following interspecific hybridization are among the causes of genomic shock, which is essentially a response to severe stress (123). Newly formed polyploid genomes subsequently undergo movement of transposable elements and rapid changes in genome size, genome structure (e.g., insertions, deletions, translocations), and epigenetic control. Polyploidy may therefore result in a dramatic "restructuring of the transcriptome, metabolome and proteome" (90).

On the basis of studies of synthetic and natural polyploids, these changes may begin to occur almost immediately postpolyploidization (e.g., 60, 73, 84, 87, 103, 119, 189, 213; see also References 4, 5, 49, 91, 183, 201, and 214). For example, structural changes in synthetic wheat and *Brassica* occur within the first few generations (60, 84, 189). In natural populations of *Tragopogon*, major structural changes, including translocations and trisomy/monosomy, are apparent in new synthetic polyploids of *Tragopogon* and in natural populations that cannot represent more than 30–40 generations since polyploidy formation (103) (**Figure 6**). The dynamism of polyploid genomes is also manifested by changes in gene silencing, DNA methylation, and tissue-specific gene expression (e.g., 4, 5, 32, 138). Polyploidy clearly plays a major role in modifying global patterns of gene expression (2, 73, 138, 197) and may be a major source of developmental novelty in polyploid systems (49). All these processes are sources of novel genetic variation and as such can play a major role in the evolutionary success of polyploids (49, 90). These rapid changes are the fuel of polyploid evolution. As a result of genetic and genomic changes, individuals may arise with a



modified phenotype and ecological preferences and hence are able to exploit new niches or to outcompete progenitor species.

Polyploidy and Diversification

Given the many suggested benefits of polyploidy (e.g., 96, 97) and the often proposed relationship of genome duplication to speciation (110, 111, 215), a major question remains: Are rates of diversification higher in polyploid lineages than in diploid groups (because of either increased rates of speciation, decreased rates of extinction, or both)? Identifying ancient polyploid events in angiosperm phylogeny provides the opportunity to assess the correspondence between inferred genome duplication events and large diversifications. That is, anecdotal data suggest that polyploid lineages are successful, but a statistical association of polyploidy and species richness has not been rigorously tested. To address this question, Soltis and coworkers (176) compared species richness in clades that are ancient polyploids with sister clades that are not. Using this approach, polyploidy certainly appears to have been a major driving force behind the diversification of the angiosperms.

Figure 6

Rapid chromosomal change in *Tragopogon mirus*. Genomic/fluorescence in situ hybridization (GISH/FISH) and 4′,6-diamidino-2-phenylindole (DAPI) karyotypes of mitotic chromosomes of naturally occurring plants of tetraploid *T. mirus*. Fluorochrome colors are as follows: Yellow/green is fluorescein isothiocyanate (FITC), digoxigenin-labeled probes; orange/red is Cy3, biotin-labeled probes; blue/purple is DAPI staining. Each karyotype is shown with DAPI staining, sometimes also simultaneously labeled for 45S rDNA (*yellow*) or 5S rDNA (*red*), and after GISH with *T. dubius* (*green*) and *T. porrifolius* (*red*) total genomic DNA probes. Plant 2601-7-translocation at end of chromosome C is shown (*arrows*). Also shown are plant 2601-8-trisomy for B in the D genome (contributed from *T. dubius*) and monosomy for C in the P genome (contributed from *T. porrifolius*), possible translocation at the end of chromosome C (see arrow), and plant 2602-3-10-monosomy for the D genome for chromosomes B and C.

For example, comparisons of diversification rates suggest that genome doubling may have led to a dramatic increase in species richness in several angiosperm lineages (see Reference 176), including Poaceae (22, 115, 143, 168) (**Figure 7**), Solanaceae (168), Fabaceae (88, 146), Cleomaceae (170), and Brassicaceae (130). The importance of polyploid events in diversification may be even more profound at deeper levels in the angiosperm tree. Polyploidy may be associated with the origin of the eudicots and perhaps even the origin of the angiosperms (29, 43, 176).

Conditions That Favor Polyploidization Versus Hybidization

What conditions favor the formation of polyploids? What conditions favor the formation of homoploid hybrid species? Plant biologists have long maintained that the divergence between diploid parents impacts the likelihood that those species will successfully produce a polyploid derivative. That is, closely related diploids are less likely to form a polyploid than are more divergent congeneric diploid species. Digby (47) and Clausen & Goodspeed (33) demonstrated that successful allopolyploids could be derived more easily than homoploid hybrids from distantly related parents (reviewed in Reference 28). In fact, a high degree of differentiation between diploid parents was considered much more likely to lead to polyploidization than to stabilization of a homoploid hybrid (34, 40). More recently, Grant (66) considered chromosomal repatterning between diploids to be a particularly important feature that would promote allopolyploid formation.

The distance of the relationship between diploid parents and the likelihood of those parents to form polyploid versus homoploid hybrid species has been reconsidered in light of molecular data. On the basis of sequence divergence in the internal transcribed spacers (ITS) of nuclear ribosomal DNA as a proxy for overall genetic (and presumably phylogenetic) divergence, Chapman & Burke (31) found that

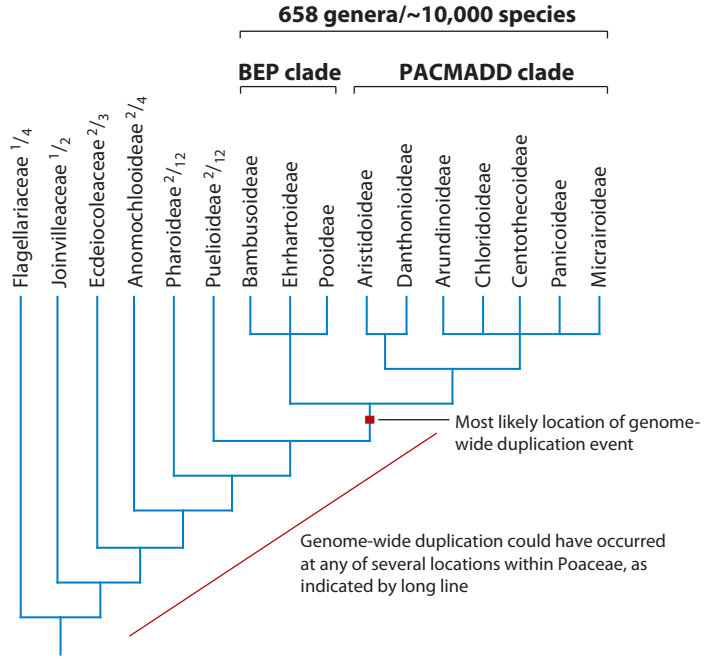


Figure 7

Possible placement of an inferred genome duplication event detected in Poaceae (143). PACCMAD clade = Panicoideae, Arundinoideae, Centothecoideae, Chloridoideae, Micraioideae, Aristidoideae, and Danthonioideae; BEP clade = Ehrhartoideae, Bambusoideae, and Pooideae. Figure modified with permission from Reference 176.

parents of allopolyploids were typically twice as divergent as the parents of homoploid hybrid species. Buggs and coworkers (28), in contrast, assessed phylogenetic distance directly through the use of phylogenetic trees and found instead that the phylogenetic distance between the progenitors of polyploids is on average no different from what would be expected by chance—that is, there was an equal probability of polyploid formation between all diploid members of a genus. However, the phylogenetic distance between parents that form homoploid hybrids was less than the null expectation of hybridization at random (28). These analyses suggest that the different patterns of parental divergence between polyploids and homoploid hybrids are the result of homoploid hybrid formation occurring only between parents of low divergence, rather than allopolyploids occurring only between parents of high divergence.

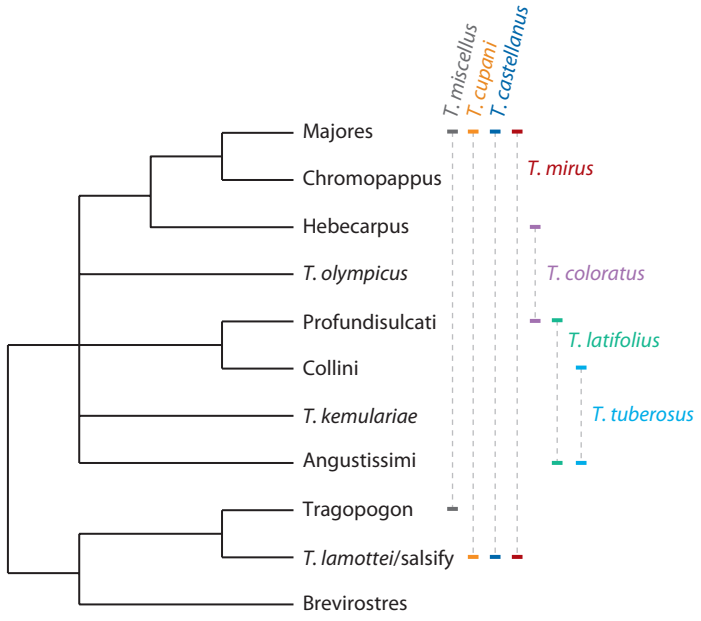


Figure 8

Phylogenetic tree showing relationships among major clades of *Tragopogon* (and those few species that do not fall into these clades) (based on Reference 121); species listed at the right (*various colors*) are polyploids and dashed lines indicate their parentage at the clade level. *T. cupani*, *T. castellanus*, and *T. mirus* were all derived from diploid parents that belong to the same clades. Note that allopolyploids in *Tragopogon* form between both close and distant relatives (see References 28 and 120).

In fact, the data now available suggest that polyploid formation is not highly impacted by the phylogenetic distance of the parental species, as seen for individual genera for which comprehensive phylogenies are available and for which the parents of the polyploids are known (reviewed in Reference 28); an example from *Tragopogon* is given in **Figure 8** (120, 121). However, the roles that genetic and/or chromosomal distances may play—because they are not always coupled with molecular phylogenetic distance—require continued study.

Chromosomal races: populations or groups of populations of a species that differ in chromosome number or arrangement at either the diploid or polyploid level

Autopolyploidy

Although autopolyploidy may result from genome doubling within a single individual, most natural autopolyploids likely formed via some degree of hybridization, involving, for example, individuals of genetically differentiated populations (179, 195). For this reason, we consider autopolyploid speciation relevant to the current discussion. Reviews of polyploidy and speciation by Grant (66), Stebbins (193, 194), Wagner (210, 211), and others make it clear that most researchers considered autopolyploidy to be rare: Stebbins considered only *Galax aphylla* (now *G. urceolata*) to be a clear-cut autopolyploid. Furthermore, autopolyploidy was not thought to result in the formation of new species. The phrase autopolyploid speciation is, in fact, rarely used in the literature (e.g., 147, 177, 181, 197). Only Lewis (102), among his contemporaries, appears to have recognized that autopolyploidy played a major evolutionary role.

Müntzing (135) long ago recognized that chromosomal races within species likely represented distinct evolutionary lineages: In nearly all 58 examples of chromosome races he investigated, the autopolyploid was morphologically distinct from its diploid parent and the chromosome races were reproductively isolated. Thus, Müntzing had compelling evidence for cryptic autopolyploid species but followed the trend of that time and lumped each example into one species with its diploid progenitor. Likewise, most autopolyploids continue to be named with their diploid progenitor, despite differences that would qualify them as distinct species following any of a number of species concepts (e.g., biological, taxonomic, diagnosable, apomorphic, and evolutionary). Thus, distinct species status is warranted for many, if not most, autopolyploids (184), as in *Zea perennis* and *Z. diploperennis* (78) and *Tolmiea menziesii* and *T. diplomenziesii* (83) (**Figure 9**).

Today, allopolyploidy is considered to be far more widespread than autopolyploidy, but is that an accurate assessment? Because autopolyploidy may be difficult to detect (175, 184, 221), its frequency may still be underestimated and underappreciated. Given that the rate of autotetraploid formation may be high, as high as the genic mutation rate, at least in some groups, many autopolyploids should be expected in nature (147). If this hypothesis is true, then hybridization among populations, leading to

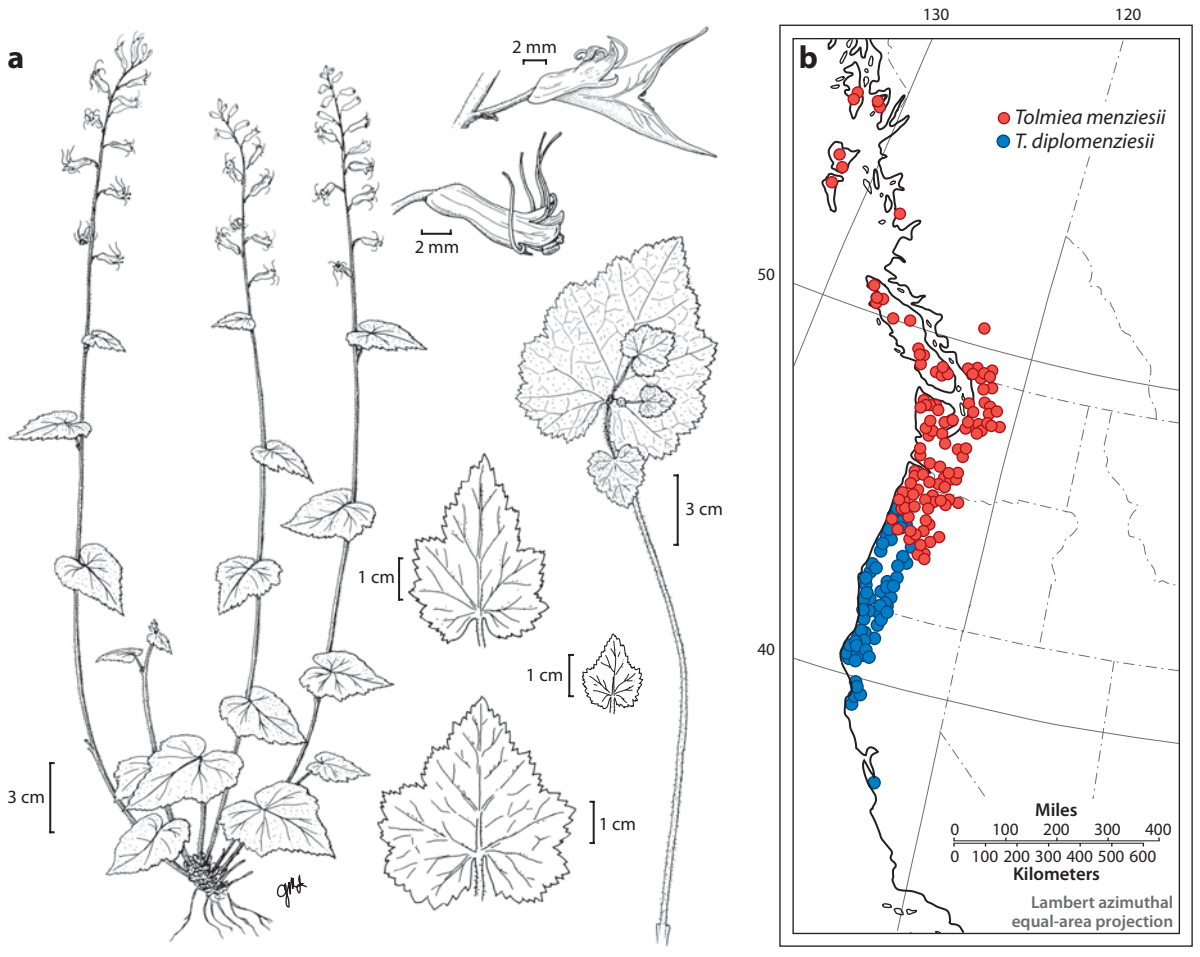


Figure 9

Autopolyploid speciation in *Tolmiea*, showing (*a*) the recently described *Tolmiea diplomenziesii* (83) and (*b*) the allopatric distributions of *T. menziesii* ($2n = 28$; *red circles*) and *T. diplomenziesii* ($2n = 14$; *blue circles*). Modified from Reference 83.

autopolyploids, may rival allopolyploidy in frequency.

Polyploidy at the Population Level: Unanswered Questions

Recent research has provided many new insights into the genetic and genomic consequences of polyploidy, but many unanswered questions remain, ranging from establishment and ecological attributes to genetic and epigenetic effects. Doyle and coworkers (49) recently summarized a suite of critical questions that surround polyploidy and hybridization, with a focus on issues of gene expression, epigenetics, and the fate of duplicate genes. Here, in contrast, we emphasize unanswered questions at the population level. Our list of unanswered questions is not comprehensive, but instead addresses issues related to mode of formation, establishment, and evolutionary rules. We hope others will add to this short list and develop new avenues in polyploid research.

What are the most frequent mechanisms of polyploidization? The mechanisms by which

natural polyploid organisms most frequently form remain unclear. In plants, formation via the union of unreduced gametes has been viewed as more likely than somatic doubling (46, 71). However, it is unclear how frequent a one-step process of formation is, involving fusion of unreduced egg and unreduced pollen, relative to a two-step process that involves formation of a triploid intermediate via fusion of one normal haploid gamete (e.g., a typical, haploid egg) with an unreduced gamete (e.g., unreduced or diploid pollen) (147). The latter mechanism, involving a triploid step toward tetraploid formation via fusion of a triploid gamete with a normal, unreduced gamete, is referred to as a triploid bridge. Evidence suggests that this pathway may be an important step in the formation of some polyploids (26, 77, 147), but does a triploid pathway actually predominate in natural systems, or do polyploids more often form instantly via the fusion of two unreduced gametes?

Given the important role attributed to unreduced gamete formation in the polyploidization process, it is noteworthy that numerous fundamental questions regarding the frequency, causes, and importance of unreduced gamete formation remain unanswered (e.g., 147). As reviewed by Ramsey & Schemske (147), on the basis of the response to selection for unreduced gametes in crops (e.g., 142, 202), plant populations may possess heritable genetic variation for the capacity to produce unreduced gametes. Are some diploid parental genotypes more likely to produce unreduced gametes than others? If so, are those same genotypes more likely than others to be involved in polyploidization events? Furthermore, what is the impact of the environment on unreduced gamete formation? There may be an environmental component to unreduced gamete formation (reviewed in Reference 147; see also References 126 and 166), suggesting that polyploids may be more likely to form in some portions of the progenitor species' range than in others. However, this topic has not been pursued in a rigorous fashion for decades and never with regard to its evolutionary consequences.

How do new polyploids establish and then persist in natural populations? Several researchers have examined rates and mechanisms of polyploid formation (e.g., 147, 165). Others have examined various factors that might influence establishment (e.g., 26, 108, 145, 148, 205). Theoretical papers have explored the fate of autotetraploids that arise within populations of their diploid parents (54, 56, 94, 162). Allopolyploids, as in the case of homoploid hybrids, are generally thought to occupy intermediate habitats between those of their diploid progenitors. However, evidence supporting or refuting this hypothesis is limited. Future studies are needed to evaluate how hybrids and allopolyploids overcome their numerical disadvantage and become established.

What factors favor auto- versus allopolyploid formation? Autopolyploidy seems prevalent in several angiosperm families, such as Saxifragaceae (reviewed in Reference 184) and Cactaceae (55, 70). In these families, most, if not all, of the documented polyploids seem to be autopolyploids. Other genera and families seem to produce exclusively allopolyploids (e.g., *Nicotiana*, *Brassica*). Some families appear to harbor both mechanisms (e.g., Poaceae). What factors determine whether autopolyploids or allopolyploids form? Is chromosome size a factor, or chromosomal structure? Would small chromosomes be less likely to form multivalents that promote chromosome pairing abnormalities? Saxifragaceae, Crassulaceae, and Cactaceae all have small chromosomes, but there are groups with small chromosomes that do not form autopolyploids. This is an area for which basically nothing is known.

Do multiple origins produce lineages with differing evolutionary trajectories? Although multiple origins of polyploid species are common (181, 187, 188), the long-term evolutionary impact of repeated formations is unclear. Recurrent formations from genetically distinct diploid parental populations can introduce genetic variation into a polyploid species (e.g., 171, 182). It is less clear, however, if this

genetic variation influences ecological or life-history attributes that may have a subsequent impact on evolutionary trajectory.

Werth & Windham (215) provocatively suggested that if alternative homeologs were silenced across an allopolyploid genome in different polyploid individuals (reciprocal silencing), reproductive isolation and incipient speciation could result. Lynch & Force (110) independently elaborated on this same hypothesis. Of particular interest are the fates of polyploid populations of independent origin, which, because of their formation from genetically divergent parents, may be initiated with very distinct genetic and cytogenetic signatures (182). The suggestion that different populations or lines of an allopolyploid might be reproductively isolated actually has a long history in the literature. Well before Werth & Windham (215), early investigators proposed that small changes in chromosomes might be responsible for reproductive isolation between allopolyploid populations (e.g., 34).

Some evidence suggests that repeated formations can lead to distinct lineages of incipient or even cryptic species. *Tragopogon miscellus* has formed reciprocally (139, 180) (**Figure 10**), yielding a dramatic difference in floral morphology and possible reproductive isolation between lineages. Rigorous crossing experiments between different populations have not been conducted, but Ownbey & McCollum's (140) crosses between individuals from reciprocally formed populations of *T. miscellus* failed, suggesting the possibility of reproductive barriers between populations of this newly formed species. Molecular cytogenetic data indicate substantial chromosomal change in *T. miscellus* (and *T. mirus*), including translocations, inversions, and trisomy/monosomy (103). If different populations have different chromosomal attributes, reduced fertility or even reproductive isolation between those populations could result. The extent of reproductive isolation among independently formed polyploid populations remains unknown.

Figure 10

The reciprocally formed short-liguled (*T. pratensis* ♀ × *T. dubius* ♂) and long-liguled (*T. dubius* ♀ × *T. pratensis* ♂)*Tragopogon miscellus*: possible cryptic allopolyploid species? Note difference in inflorescence morphology. Crosses between the short- and long-liguled forms failed (140), suggesting possible reproductive isolation and the possibility of very local cryptic speciation at the allopolyploid level. Ligules are the strap-like ray flowers found in the capitulum (flower heads) of most species of Compositae.

Many cryptic allopolyploid (as well as autopolyploid) species may also be present in the arctic flora, where polyploid species are well known for their taxonomic complexity (27). For example, in arctic *Draba* (Brassicaceae), hybrids from crosses within populations were largely fertile (63%), but the fertility of interspecific crosses was low—only 8% of the hybrids from crosses within and among geographic regions (Alaska, Greenland, Svalbard, and Norway) were fertile. However, the frequent intraspecific crossing barriers observed are not accompanied by any obvious morphological or ecological differentiation, indicating that numerous cryptic biological species have arisen within each taxonomic species despite their origin in the Pleistocene (69).

Does evolution repeat itself? Evolutionary biologists have long wondered if evolution would repeat itself, given the chance. Gould (64) suggested that if we could replay the evolutionary tape of life on Earth, it would play differently—"history involves too much chaos" and too many chance events are involved for the evolutionary process to be repetitive on a broad scale. However, is this true on a finer scale? Are certain aspects of the polyploidy process actually hard-wired? Preservation of duplicated gene copies following genome duplication appears to be nonrandom, with specific functional categories preferentially retained (23) and

Reciprocal silencing: silencing of reciprocal homeologs in a polyploid genome; e.g., for some genes the homeolog of parental species A is silenced and at other genes the homeolog of parental species B is silenced

reduplicated in subsequent polyploidizations (30, 172). Independent whole-genome duplications in the ancestors of *Arabidopsis*, *Oryza* (rice), *Saccharomyces* (yeast), and *Tetraodon* (pufferfish) have been followed by convergent fates of many gene families (144). Using identically produced synthetic lines of *Brassica napus*, Gaeta and coworkers (60) showed evidence for repeatability of the evolutionary process. Some of the same changes in expression, homeolog loss, and structural changes occurred repeatedly across 50 separate polyploid lines. In *Tragopogon*, populations of independent origin show evidence of both repeated gene loss and stochasticity (200; R. Buggs, A. Doust, J. Tate, J. Koh, K. Soltis, F. Feltus, A. Paterson, P. Soltis & D. Soltis, unpublished data). That is, some loci seem to be retained as duplicates in populations of multiple origin while others tend to undergo loss of one parental homeolog. Furthermore, other loci show no pattern—they are retained as duplicates in some populations and undergo homeolog loss in others. Collectively, these observations indicate that there certain principles may exist that govern the fates of gene and genome duplications.

Does parental genotype matter in polyploidization? Are certain diploid parental genotypes more likely to contribute to the formation of a given polyploid than others? Limited evidence suggests that parental genotype may matter in polyploid formation. For example, populations of a given species (or individuals in a population) may differ in their ability to produce unreduced gametes (see above; reviewed in Reference 147). If unreduced gamete formation is an essential component of polyploidization and this ability is genetically controlled, with some individual plants producing significantly more unreduced gametes than others, then clearly parental genotype could play a major role in the outcome of polyploidization.

Microsatellite data for populations of the recently and recurrently formed allopolyploids *Tragopogon mirus* and *T. miscellus* likewise point to differences among diploids in their likelihood of forming polyploids. Only a few of the numerous genotypes detected in populations of the diploid *T. dubius* have actually been involved in polyploid formation (V. Symonds, P. Soltis & D. Soltis, unpublished data), raising the possibility that these genotypes are more likely to hybridize, more likely to produce unreduced gametes, or more likely to produce a functional polyploid when paired with either *T. pratensis* or *T. porrifolius*—or some combination of the above. Further research is clearly needed on the factors that contribute to the formation of polyploids.

ISOLATING MECHANISMS

Plant species are prevented from interbreeding by both premating and postmating isolating mechanisms. Although some treatments categorize such barriers as intrinsic or extrinsic, we prefer the pre- versus postmating characterization because (*a*) it is descriptive of the processes and forces at play and (*b*) to some extent, even those features typically considered extrinsic, such as ecological barriers, are controlled by the genetics and features of the plants themselves and are therefore fundamentally intrinsic. Here we briefly review the major types of isolating mechanisms before considering how the breakdown of such barriers may result in hybridization. For further discussion of isolating mechanisms in plants, we refer readers to both recent reviews (25, 81, 112, 113, 152, 153, 156, 169) and classic overviews (e.g., 66, 93, 95).

Premating isolating mechanisms may be broadly geographic or ecological, such that potentially interbreeding individuals do not have the opportunity to mate. For wind-pollinated plants that do not maintain postmating barriers, this distance between either geographically or ecologically isolated individuals is fundamentally important for keeping species separate. Pollinators play a major role in isolating species, and detailed analyses of pollinator preferences and effectiveness demonstrate the relative roles of pollinator specificity and other barriers in maintaining species

boundaries. As in the case of pollinator specificity, differences in flowering phenology may prevent interbreeding between species or incipient species. Even individuals without genetic or chromosomal incompatibilities may be isolated phenologically, ultimately allowing the accumulation of postmating isolation. Finally, mechanical differences may prevent successful mating in plants with highly specialized pollination mechanisms, such as milkweeds and orchids in which pollinia (structures bearing aggregates of thousands of pollen grains) must fit appropriately into receptive structures for pollination to be effected.

In contrast, many mechanisms may prevent the formation or ultimate establishment of hybrids, even if mating has in fact occurred. These barriers span the earliest phase of postmating (i.e., pollen-stigma incompatibility and pollen tube competition) to the establishment and perpetuation of hybrid individuals in natural populations. Following successful pollen germination, isolation can occur at any of a number of stages: e.g., pollen tube competition, zygotic or embryonic mortality, and hybrid inviability at any point until reproduction. If a hybrid survives until reproduction, it may be confronted by either partial or complete sterility through a variety of causes—most prominent are genetic or chromosomal barriers that lead to meiotic irregularities (see Reference 66). However, even if a hybrid survives and reproduces, subsequent generations may be increasingly less fit (Reference 66 and references therein)—a phenomenon referred to as hybrid breakdown. Even viable, fertile hybrids may be effectively isolated over longer timescales when an appropriate niche for the hybrid is unavailable, forcing the hybrid into parental habitats where it may be less fit. In addition, purely demographic processes may prevent the establishment of a hybrid-derived population in a manner similar to the minority cytotype exclusion described for new polyploids (56). In other words, a new hybrid, whether diploid or polyploid, may have trouble finding a mate because it occurs in low frequency. With failed mating opportunities due to low abundance, the hybrid or hybrid derivative can be excluded from the mixed population with one or both parental species. Repeated exclusion in this manner over multiple generations may actually serve as a mechanism that maintains the integrity of the parental species and provides a long-term barrier to interspecific mating.

Given all these potential isolating mechanisms that typically keep species separate, it is perhaps surprising that plant species hybridize at all, or if they hybridize, that they establish. However, given the common reports of interspecific hybrids (even if they do not lead to species formation) and the high frequency of allopolyploidy within the angiosperms, these isolating mechanisms routinely fail. A slight change in the behavior of a pollinator, a small shift in flowering time, a minute chromosomal mutation that improves meiotic pairing and thereby increases fertility—any of these could be sufficient to allow hybridization and the possible establishment of hybrid individuals. And once hybridization occurs, any of several avenues may be followed: the persistence of a small hybrid lineage, the development of a hybrid swarm, the origin of a new homoploid hybrid species, the origin of an allopolyploid species, or the extinction of the hybrid.

Introduced species may be particularly instructive regarding the breakdown of isolating mechanisms. For example, an introduced plant species will likely not be accompanied by its native pollinator(s). Survival of the introduced species depends on the services of other pollinators, some of which may visit close relatives of the introduced species, thereby effecting hybridization. We suspect that one of the reasons that hybridization between *Tragopogon* species in North America is so high relative to hybridization in the native range of Eurasia is that in North America, the three introduced species are all visited by the same set of generalist pollinators that do not discriminate among the inflorescences of the three species (L. Cook, D. Soltis & P. Soltis, unpublished data). Furthermore, placement of a species into

Phenology: the response of an organism to seasonal/climatic cues; for example, the flowering phenology of related species may differ based on their responses to climatic stimuli

Minority cytotype exclusion: the difficulty of establishment of a low-frequency chromosomal type within a population of a different chromosomal type, for example, of a polyploid within a diploid population

Allopatric: nonoverlapping geographic distributions; allopatric speciation occurs when diverging populations are geographically isolated from each other

Vicariance: the separation of a group of organisms by a geographic barrier, often resulting in speciation

a new area via human introduction may lead to changes in phenology, with different cues triggering flowering and perhaps leading to overlap of flowering times with a native species and the opportunity for hybridization. Moreover, the disturbance that allows for the establishment of an introduced species to begin with may provide just the right opportunities for hybridization and subsequent establishment of a hybrid. Through studies of hybrid formation involving introduced species, we may be able to learn more about how isolating mechanisms break down between native species.

Given the putative importance of disturbance in generating and establishing hybrids, the potential effects of climate change cannot be ignored. A warmer climate can lead—and is leading to—changes in distribution, bringing related (and therefore potentially hybridizable) species into contact where once they were not, and changes in phenology, allowing hybridization when it could not have occurred before (e.g., 129). The role of climate change on rates of hybridization and the breakdown of species integrity, as well as the ecological effects, merits further study.

CONCLUSIONS

Hybridization, in the form of homoploid hybrid speciation and polyploidy, has contributed extensively to angiosperm diversity. Whereas allopatric speciation via vicariance, long-distance dispersal and founding, and peripheral isolation may collectively be responsible for many, if not most, speciation events, the evolutionary signature of hybridization is rampant. Particularly important is the discovery that most angiosperm genomes are fundamentally polyploid, with varying degrees of diploidization. The ramifications of this knowledge should permeate plant evolutionary biology, from population genetics to quantitative genetics to systematics to ecology—in addition to genetics. Perhaps our century of population genetic theory needs modification to accommodate the likelihood of homeologous loci, even in a presumed (and genetic) diploid species. Likewise, quantitative genetic models do not adequately reflect the typical plant genome as we are beginning to understand it. Systematists, who have a longer tradition of considering hybridization and its consequences, nonetheless need to adjust the scale by which they measure the frequency of polyploidy in particular, perhaps by an order of magnitude if all angiosperms really are of polyploid origin. The effect(s) of a hybrid genome, whether diploid or polyploid, has not received sufficient attention from ecologists, and perhaps the time is right for delving further into the ecological consequences of polyploidy. Models of both ancient and recent polyploidization have been described, allowing for appropriate selection of species with questions for future research.

The tremendous role that hybridization and polyploidy have played in angiosperm diversification also has implications for conservation (e.g., 9, 99, 150, 186). Understanding both the threats that hybridization may pose to rare species and the beneficial ways it may lead to their rescue has become more crucial in a world in which species are introduced to distant areas of the globe and others come into contact because of range alterations associated with climate change. Both situations provide opportunities for hybridization with species for which isolating mechanisms may not have evolved and can threaten the genetic and ecological properties of native species. Thus, studies of the processes leading to hybridization and polyploidization, along with analyses of the consequences of hybridization—at all levels of biological organization—are strongly encouraged.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Lily Ainouche and Simon Hiscock for sharing images and information on *Spartina* and *Senecio*, respectively, and the late Yoong Lim for the beautiful karyotype photographs of *Tragopogon*. Work in the authors' laboratories was supported in part by NSF grants MCB-034637 and DEB-0614421.

LITERATURE CITED

1. Abbott RJ, Lowe AJ. 2004. Origins, establishment and evolution of new polyploid species: *Senecio cambrensis* and *S. eboracensis* in the British Isles. *Biol. J. Linn. Soc.* 82:467–74
2. Adams KL. 2007. Evolution of duplicate gene expression in polyploid and hybrid plants. *J. Hered.* 98:136–41
3. Adams KL, Wendel JF. 2004. Exploring the genomic mysteries of polyploidy in cotton. *Biol. J. Linn. Soc.* 82:573–81
4. Adams KL, Wendel JF. 2005. Allele-specific, bidirectional silencing of an alcohol dehydrogenase gene in different organs of interspecific diploid cotton hybrids. *Genetics* 171:2139–42
5. Adams KL, Wendel JF. 2005. Polyploidy and genome evolution in plants. *Curr. Opin. Plant Biol.* 8:135–41
6. Ainouche ML, Baumel A, Salmon A. 2004. *Spartina anglica* C. E. Hubbard: a natural model system for analysing early evolutionary changes that affect allopolyploid genomes. *Biol. J. Linn. Soc.* 82:475–84
7. Ainouche ML, Salmon A, Baumel A, Yannic G. 2004. Hybridization, polyploidy and speciation in *Spartina* (Poaceae). *New Phytol.* 161:165–72
8. Alice LA, Eriksson T, Eriksen B, Campbell CS. 2001. Hybridization and gene flow between distantly related species of *Rubus* (Rosaceae): evidence from nuclear ribosomal DNA internal transcribed spacer region sequences. *Syst. Bot.* 26:769–78
9. Allendorf FW, Leary RF, Spruell P, Wenburg JK. 2001. The problems with hybrids: setting conservation guidelines. *Trends Ecol. Evol.* 16:613–22
10. Allendorf FW, Thorgaard GH. 1984. Tetraploidy and the evolution of salmonid fishes. In *The Evolutionary Genetics of Fishes*, ed. BJ Turner, pp. 1–53. New York: Plenum Press
11. Anderson E. 1949. *Introgressive Hybridization*. New York: Wiley
12. Anderson E, Hubricht L. 1938. Hybridization in *Tradescantia*. III. The evidence for introgressive hybridization. *Am. J. Bot.* 25:396–402
13. Anderson E, Stebbins GL. 1954. Hybridization as an evolutionary stimulus. *Evolution* 8:378–88
14. Arnold ML. 1992. Natural hybridization as an evolutionary process. *Annu. Rev. Ecol. Evol. Syst.* 23:237–61
15. Arnold ML. 1993. *Iris nelsonii*: origin and genetic composition of a homoploid hybrid species. *Am. J. Bot.* 80:577–83
16. Arnold ML. 1994. Natural hybridization and Louisiana irises. *BioScience* 44:141–47
17. Arnold ML. 1997. *Natural Hybridization and Evolution*. New York: Oxford Univ. Press
18. Baum D. 1992. Phylogenetic species concepts. *Trends Ecol. Evol.* 7:1–2
19. Baum D, Donoghue MJ. 1995. Choosing among "phylogenetic" species concepts. *Syst. Bot.* 20:560–73
20. Baum D, Shaw KI. 1995. Genealogical perspectives on the species problem. In *Experimental Approaches to Plant Systematics*, ed. PC Hoch, AG Stephenson, pp. 289–303. Monogr. Syst. Bot. Mo. Bot. Gard., Vol. 53). St. Louis, MO: Mo. Bot. Gard.
21. Beçak ML, Beçak W. 1998. Evolution by polyploidy in Amphibia: new insights. *Cytogenet. Genome Res.* 80:28–33
22. Blanc G, Wolfe KH. 2004. Widespread paleopolyploidy in model plant species inferred from age distributions of duplicate genes. *Plant Cell* 16:1667–78
23. Blanc G, Wolfe KH. 2004. Functional divergence of duplicated genes formed by polyploidy during *Arabidopsis* divergence. *Plant Cell* 16:1679–91
24. Bowers JE, Chapman BA, Rong JK, Paterson AH. 2003. Unravelling angiosperm genome evolution by phylogenetic analysis of chromosomal duplication events. *Nature* 422:433–38
25. Brady KU, Kruckeberg AR, Bradshaw HD Jr. 2005. Evolutionary ecology of plant adaptatin to serpentine soils. *Annu. Rev. Ecol. Evol. Syst.* 36:243–66

26. Bretagnolle F, Thompson JD. 1995. Tansley review no. 78. Gametes with the somatic chromosome number: mechanisms of their formation and role in the evolution of autopolyploid plants. *New Phytol.* 129:1–22
27. Brochmann C, Brysting AK, Alsos IG, Borgen L, Grundt HH, et al. 2004. Polyploidy in Arctic plants. *Biol. J. Linn. Soc.* 82:521–36
28. Buggs RJA, Soltis PS, Mavrodiev EV, Symonds VV, Soltis DE. 2008. Does phylogenetic distance between parental genomes govern the success of polyploids? *Castanea* 73:74–93
29. Buzgo M, Soltis PS, Kim S, Soltis DE. 2005. The making of the flower. *Biologist* 52:149–54
30. Chapman BA, Bowers JE, Feltus FA, Paterson AH. 2006. Buffering of crucial functions by paleologous duplicated genes may contribute cyclicality to angiosperm genome evolution. *Proc. Natl. Acad. Sci. USA* 103:2730–35
31. Chapman MA, Burke JM. 2007. Genetic divergence and hybrid speciation. *Evolution* 61:1773–80
32. Chen ZJ. 2007. Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. *Annu. Rev. Plant Biol.* 58:377–406
33. Clausen RE, Goodspeed TH. 1925. Interspecific hybridization in *Nicotiana*. II. A tetraploid *Glutinosa-tabucam* hybrid, an experimental verification of Winge's hypothesis. *Genetics* 10:278–84
34. Clausen J, Keck DD, Hiesey WM. 1945. Experimental studies on the nature of species. II. Plant evolution through amphiploidy and autoploidy, with examples from the Madiinae. *Carnegie Inst. Wash.* Publ. 564
35. Comai L, Madlung A, Josefsson C, Tyagi A. 2003. Do the different parental 'heteromes' cause genomic shock in newly formed allopolyploids? *Philos. Trans. Biol. Sci.* 358:1149–55
36. Coyne JA. 1992. Genetics and speciation. *Nature* 355:511–15
37. Coyne JA, Orr HA. 2004. *Speciation*. Sunderland, MA: Sinauer Associates
38. Cracraft C. 1983. Species concepts and speciation analysis. *Ornithology* 1:159–87
39. Cui L, Wall PK, Leebens-Mack JH, Lindsay BG, Soltis DE, et al. 2006. Widespread genome duplications throughout the history of flowering plants. *Genome Res.* 16:738–39
40. Darlington CD. 1937. *Recent Advances in Cytology*. Philadelphia: Blakiston
41. Davis JI. 1997. Evolution, evidence, and the role of species concepts in phylogenetics. *Syst. Bot.* 22:373–403
42. Davis JI, Nixon KC. 1992. Populations, genetic variation, and the delimitation of phylogenetic species. *Syst. Biol.* 41:421–35
43. De Bodt S, Maere S, Van de Peer Y. 2005. Genome duplication and the origin of angiosperms. *Trends Ecol. Evol.* 20:591–97
44. de Boer JG, Yazawa R, Davidson WS, Koop BF. 2007. Bursts and horizontal evolution of DNA transposons in the speciation of pseudotetraploid salmonids. *BMC Genomics* 8:422
45. De Queiroz K, Donoghue MJ. 1988. Phylogenetic systematics and the species problem. *Cladistics* 4:317–38
46. deWet JMJ. 1980. Origins of polyploids. In *Polyploidy-Biological Relevance*, ed. WH Lewis, pp. 3–15, New York: Plenum Press
47. Digby L. 1912. The cytology of *Primula kewensis* and of other related *Primula* hybrids. *Ann. Botany* 26:357–88
48. Donoghue MJ. 1985. A critique of the biological species concept and recommendations for a phylogenetic alternative. *Bryologist* 88:172–81
49. Doyle JJ, Flagel LE, Paterson AH, Rapp RA, Soltis DE, et al. 2008. Evolutionary genetics of genome merger and doubling in plants. *Annu. Rev. Genet.* 42:443–61
50. Dujon B, Sherman D, Fischer G, Durrens P, Casaregola S, et al. 2004. Genome evolution in yeasts. *Nature* 430:35–44
51. Edwards CE, Lefkowitz D, Soltis DE, Soltis PS. 2008. Combined phylogenetic analysis of heterozygous nuclear loci: an example using *Conradina* and related mints (Lamiaceae). *Int. J. Plant Sci.* 169:579–94
52. Ehrlich PR, Raven PH. 1969. Differentiation of populations. *Science* 165:1228–32
53. Falconer DS. 1981. *Introduction to Quantitative Genetics*, 2nd ed., New York, NY: Longman
54. Felber F. 1991. Establishment of a tetraploid cytotype in a diploid population: effect of relative fitness of the cytotypes. *J. Evol. Biol.* 4:195–207

55. Fleming TH, Maurice S, Hamrick JL. 1998. Geographic variation in the breeding system and the evolutionary stability of trioecy in *Pachycereus pringlei* (Cactaceae). *Evol. Ecol.* 12:279–89
56. Fowler NL, Levin DA. 1984. Ecological constraints on the establishment of a novel polyploid in competition with its diploid progenitor. *Am. Nat.* 124:703–11
57. Furlong RF, Holland PWH. 2004. Polyploidy in vertebrate ancestry: Ohno and beyond. *Biol. J. Linn. Soc.* 82:425–30
58. Futuyma DJ. 1998. *Evolution*. 3rd ed. Sunderland, MA: Sinauer Associates
59. Futuyma DF. 2005. *Evolution*. 4th ed. Sunderland, MA: Sinauer Associates
60. Gaeta RT, Pires JC, Iniguez-Luy F, Leon E, Osborn TC. 2007. Genomic changes in resynthesized *Brassica napus* and their effect on gene expression and phenotype. *Plant Cell* 19:3403–17
61. Gallez GP, Gottlieb LD. 1982. Genetic evidence for the hybrid origin of the diploid plant *Stephanomeria diegensis*. *Evolution* 36:1158–67
62. Goldblatt P. 1980. Polyploidy in angiosperms: monocotyledons. In *Polyploidy: Biological Relevance*, ed. WH Lewis, pp. 219–39. New York: Plenum Press
63. Golding GB, Strobeck C. 1983. Increased number of alleles found in hybrid populations due to intragenic recombination. *Evolution* 37:17–29
64. Gould SJ. 1994. The evolution of life on earth. *Sci. Am.* 271:85–86
65. Grant V. 1963. *The Origins of Adaptations*. New York: Columbia Univ. Press
66. Grant V. 1981. *Plant Speciation*, 2nd ed. New York: Columbia Univ. Press
67. Gregory TR, Mable BK. 2005. Polyploidy in animals. In *The Evolution of the Genome*, ed. TR Gregory, pp. 428–501. NY: Academic
68. Gross BL, Rieseberg LH. 2005. The ecological genetics of homoploid hybrid speciation. *J. Hered.* 96:241–52
69. Grundt HH, Kjølner S, Borgen L, Rieseberg LH, Brochmann C. 2006. High biological species diversity in the Arctic flora. *Proc. Nat. Acad. Sci. USA* 103:972–75
70. Hamrick JL, Nason JD, Fleming TH, Nassar JM. 2002. Genetic diversity in columnar cacti. In *Columnar Cacti and Their Mutualists: Evolution, Ecology and Conservation*, ed. TH Fleming, A Valiente-Banuet, pp. 122–33. Tucson: Univ. Arizona Press
71. Harlan JR, de Wet JMJ. 1975. On O. Winge and a prayer: the origins of polyploidy. *Bot. Rev.* 41:361–90
72. Harrison RG. 1990. Hybrid zones: windows on evolutionary process. *Oxf. Surv. Evol. Biol.* 7:69–128
73. Hegarty M, Barker G, Wilson I, Abbott RJ, Edwards KJ, Hiscock SJ. 2006. Transcriptome shock after interspecific hybridization in *Senecio* is ameliorated by genome duplication. *Curr. Biol.* 16:1652–59
74. Hegarty MJ, Hiscock SJ. 2008. Genomic clues to the evolutionary success of polyploid plants. *Curr. Biol.* 18:R435–44
75. Heiser CB. 1949. Studies in the evolution of the sunflower species *Helianthus annuus* and *H. bolanderi*. *Univ. Calif. Publ. Bot.* 23:157–96
76. Hubbard JCE. 1965. Spartina marshes in southern England. VI. Patterns of invasion in Poole Harbour. *J. Ecol.* 53:799–813
77. Husband BC. 2004. The role of triploid hybrids in the evolutionary dynamics of mixed-ploidy populations. *Biol. J. Linn. Soc.* 82:537–46
78. Iltis HH, Doebley JF, Guzmán R, Pazy B. 1979. *Zea diploperennis* (Gramineae): a new teosinte from Mexico. *Science* 203:186–88
79. Jaillon O, Aury JM, Noel B, Policriti A, Clepet C, et al. French-Italian Public Consort. Grapevine Genome Charact. 2007. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* 449:463–67
80. Jarvis C. 2007. *Order Out of Chaos: Linnaean Plant Names and Their Types*. London: Linn. Soc. Lond.
81. Johnson SD, Steiner KE. 2000. Generalization versus specialization in plant pollination systems. *Trends Ecol. Evol.* 15:140–43
82. Judd WS, Campbell CS, Kellogg EA, Stevens PF, Donoghue MJ. 2002. *Plant Systematics, Phylogenetic, and Approach*. 2nd ed. Sunderland, MA: Sinauer Associates
83. Judd WS, Soltis DE, Soltis PS. 2007. *Tolmiea diplomenziesii*: a new species from the Pacific Northwest and the diploid sister taxon of the autotetraploid *T. menziesii* (Saxifragaceae). *Brittonia* 59:217–25

84. Kashkush K, Feldman M, Levy AA. 2002. Gene loss, silencing and activation in a newly synthesized wheat allotetraploid. *Genetics* 160:1651–59
85. Kellis M, Birren BW, Lander ES. 2004. Proof and evolutionary analysis of ancient genome duplication in the yeast *Saccharomyces cerevisiae*. *Nature* 428:617–24
86. Kihara H, Ono T. 1926. Chromosomenzahlen und systematische Gruppierung der Rumex-Arten. *Z. Zellforsch. Mikrob. Anat.* 4:475–481
87. Kovarik A, Pires JC, Leitch AR, Lim KY, Sherwood AM, et al. 2005. Rapid concerted evolution of nuclear ribosomal DNA in two tragopogon allopolyploids of recent and recurrent origin. *Genetics* 169:931–44
88. Lavin M, Herendeen P, Wojciechowski M. 2005. Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the Tertiary. *Syst. Biol.* 54:575–94
89. Le Comber SCL, Smith C. 2004. Polyploidy in fishes: patterns and processes. *Biol. J. Linn. Soc.* 82:431–2
90. Leitch AR, Leitch IJ. 2008. Genomic plasticity and the diversity of polyploid plants. *Science* 320:481–83
91. Leitch AR, Soltis DE, Soltis PS, Leitch IJ, Pires JC. 2004. Biological relevance of polyploidy: ecology to genomics. *Biol. J. Linn. Soc.* 82:4 (Special issue)
92. Leitch IJ, Bennett MD. 2004. Genome downsizing in polyploid plants. *Biol. J. Linn. Soc.* 82:651–63
93. Levin DA. 1971. The origin of reproductive isolating mechanisms in flowering plants. *Taxon* 20:91–113
94. Levin DA. 1975. Minority cytotype exclusion in local plant populations. *Taxon* 24:35–43
95. Levin DA. 1978. The origin of isolating mechanisms in flowering plants. *Evol. Biol.* 11:185–317
96. Levin DA. 1983. Polyploidy and novelty in flowering plants. *Am. Nat.* 122:1–25
97. Levin DA. 2000. *The Origin, Expansion and Demise of Plant Species*. New York: Oxford Univ. Press
98. Levin DA. 2002. *The Role of Chromosomal Change in Plant Evolution*. New York: Oxford Univ. Press
99. Levin DA, Francisco-Ortega J, Jansen RK. 1996. Hybridization and the extinction of rare plant species. *Conserv. Biol.* 10:10–16
100. Levy M, Levin DA. 1974. Novel flavonoids and reticulate evolution in the *Phlox pilosa-P. drummondii* complex. *Am. J. Bot.* 61:156–67
101. Levy M, Levin DA. 1975. The novel flavonoid chemistry and phylogenetic origin of *Phlox floridana*. *Evolution* 29:487–99
102. Lewis WH. 1980. Polyploidy in species populations. In *Polyploidy: Biological Relevance*, ed. WH Lewis, pp. 103–144, New York: Plenum
103. Lim KY, Soltis DE, Soltis PS, Tate J, Matyasek R, et al. 2008. Rapid chromosome evolution in recently formed polyploids in *Tragopogon* (Asteraceae). *PLoS ONE* 3e:3353
104. Liu B Wendel JF. 2003. Epigenetic phenomena and the evolution of plant allopolyploids. *Mol. Phylogenetics Evol.* 29:365–79
105. Liu B, Vega JM, Segal G, Abbo S, Rodova H, Feldman M. 1998. Rapid genomic changes in newly synthesized amphiploids of *Triticum* and *Aegilops*. I. Changes in low-copy noncoding DNA sequences. *Genome* 41:272–77
106. Liu Z, Adams KL. 2007. Expression partitioning between genes duplicated by polyploidy under abiotic stress and during organ development. *Curr. Biol.* 17:1669–74
107. Löve A, Löve D. 1949. The geobotanical significance of polyploidy. I. Polyploidy and latitude. *Port. Acta Biol. Ser. A* 1949:273–352
108. Lumaret R, Guillerm J-L, Delay J, Ait Lhaj Louffi A, Izeo J, Jay M. 1987. Polyploidy and habitat differentiation in *Dactylis glomerata* L. from Galicia (Spain). *Oecologia* 73:436–46
109. Lynch M, Conery JS. 2000. The evolutionary fate and consequences of duplicate genes. *Science* 290:1151–55
110. Lynch M, Force A. 2000. The probability of duplicate gene preservation by subfunctionalization. *Genetics* 154:459–73
111. Lynch M, O'Hely M, Walsh B, Force A. 2001. The probability of fixation of a newly arisen gene duplicate. *Genetics* 159:1789–1804
112. Mable BK. 2003. Breaking down taxonomic barriers in polyploidy research. *Trends Plant Sci.* 8:582–90
113. Mable BK. 2004. Why polyploidy is rarer in animals than in plants: myths and mechanisms. *Biol. J. Linn. Soc.* 82:453–66
114. Makalowski W. 2001. Are we polyploids? A brief history of one hypothesis. *Genome Res.* 11:667–70

115. Malcomber ST, Kellogg EA. 2005. *SEPALLATA* gene diversification: brave new whorls. *Trends Plant Sci.* 10:427–35
116. Marchant CJ. 1967. Evolution in *Spartina* (Gramineae). I. The history and morphology of the genus in Britain. *J. Linn. Soc.* 60:1–24
117. Marchant CJ. 1968. Evolution in *Spartina* (Gramineae). II. Chromosomes, basic relationships and the problem of *Spartina* X *townsendii* agg. *J. Linn. Soc.* 60:381–409
118. Masterson J. 1994. Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. *Science* 264:421–23
119. Matyasek R, Tate JA, Lim YK, Šrubařova H, Koh J, et al. 2007. Concerted evolution of rDNA in recently formed tragopogon allotetraploids is typically associated with an inverse correlation between gene copy number and expression. *Genetics* 176:2509–19
120. Mavrodiev EV, Soltis PS, Soltis DE. 2008. Putative parentage of six Old World polyploids in *Tragopogo*n L. (Asteraceae: Scorzonerinae) based on ITS, ETS, and plastid sequence data. *Taxon* 57:1215–32
121. Mavrodiev EV, Tancig M, Sherwood AM, Gitzendanner MA, Rocca J, et al. 2005. Phylogeny of *Tragopogon* L. (Asteraceae) based on internal and external transcribed spacer sequence data. *Int. J. Plant Sci.* 166:117–33
122. Mayr E. 1942. *Systematics and the Origin of Species*. New York: Columbia Univ. Press
123. McClintock B. 1984. The significance of the responses of the genome to challenge. *Science* 226:792–801
124. McDade L. 1990. Hybrids and phylogenetic systematics. I. Patterns of character expression in hybrids and their implications for cladistic analysis. *Evolution* 44:1685–1700
125. McDade L. 1995. Hybridization and phylogenetics. In *Experimental Approaches to Plant Systematics*, ed. PC Hoch, AG Stephenson, pp. 305–31. (Monogr. Syst. Bot. Mo. Bot. Gard., Vol. 53), St. Louis, MO: Mo. Bot. Gard.
126. McHale NA. 1983. Environmental induction of high frequency 2n pollen formation in diploid *Solanum*. *Can. J. Genet. Cytol.* 25:609–15
127. McKay SJ, Trautner J, Smith MJ, Koop BF, Devlin RH. 2004. Evolution of duplicated growth hormone genes in autotetraploid salmonid fishes. *Genome* 47:714–23
128. McLysaght A, Hokamp K, Wolfe KH. 2002. Extensive genomic duplication during early chordate evolution. *Nat. Genet.* 31:200–4
129. Miller-Rushing AJ, Primack RB. 2008. Global warming and flowering times in Thoreau's Concord: a community perspective. *Ecology* 89:332–41
130. Ming R, Hou S, Feng Y, Yu Q, Dionne-Laporte A et al. 2008. The draft genome of the transgenic tropical fruit tree papaya (*Carica papaya* Linnaeus). *Nature* 452:991–96
131. Mishler BD. 1985. The morphological, developmental, and phylogenetic basis of species concepts in bryophytes. *Bryologist* 88:207–14
132. Mishler BD, Donoghue MJ. 1982. Species concepts: a case for pluralism. *Syst. Zool.* 31:491–503
133. Mishler BD, Theirot EC. 2000. The phylogenetic species concept (sensu Mishler and Theirot): monophyly, apomorphy, and phylogenetic species concepts. See Ref. 216, pp. 44–54
134. Morjan CL, Rieseberg LH. 2004. How species evolve collectively: implications of gene flow and selection for the spread of advantageous alleles. *Mol. Ecol.* 13:1341–56
135. Müntzing A. 1936. The evolutionary significance of autopolyploidy. *Hereditas* 21:263–378
136. Nixon KC, Wheeler QD. 1990. An amplification of the phylogenetic species concept. *Cladistics* 6:211–23
137. Ohno S. 1970. *Evolution by Gene Duplication*. New York: Springer-Verlag
138. Osborn TC, Pires JC, Birchler JA, Auger DL, Chen ZJ, et al. 2003. Understanding mechanisms of novel gene expression in polyploids. *Trends Genet.* 19:141–47
139. Ownbey M. 1950. Natural hybridization and amphiploidy in the genus *Tragopogon*. *Am. J. Bot.* 37:487–99
140. Ownbey M, McCollum GD. 1953. Cytoplasmic inheritance and reciprocal amphiploidy in *Tragopogon*. *Am. J. Bot.* 40:788–96
141. Panopoulou G, Poustka AJ. 2005. Timing and mechanism of ancient vertebrate genome duplications—the adventure of a hypothesis. *Trends Genet.* 21:559–67
142. Parrott WA, Smith RR. 1986. Recurrent selection for 2*n* pollen formation in red clover. *Crop Sci.* 26:1132–35

143. Paterson AH, Bowers JE, Chapman BA. 2004. Ancient polyploidization predating divergence of the cereals, and its consequences for comparative genomics. *Proc. Natl. Acad. Sci. USA* 101:9903–8
144. Paterson AH, Chapman BA, Kissinger JC, Bowers JE, Feltus FA, Estil JC. 2006. Many gene and domain families have convergent fates following independent whole-genome duplication events in *Arabidopsis*, *Oryza*, *Saccharomyces* and *Tetraodon*. *Trends Genet.* 22:597–602
145. Petit RJ, Bretagnolle CF, Felber F. 1999. Evolutionary consequences of diploid-polyploid hybrid zones in wild species. *Trends Ecol. Evol.* 14:306–11
146. Pfeil BE, Schlueter JA, Shoemaker RC, Doyle JJ. 2005. Placing paleopolyploidy in relation to taxon divergence: a phylogenetic analysis in legumes using 39 gene families. *Syst. Biol.* 54:441–54
147. Ramsey J, Schemske DW. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annu. Rev. Ecol. Evol. Syst.* 29:467–501
148. Ramsey J, Schemske DW. 2002. Neopolyploidy in flowering plants. *Annu. Rev. Ecol. Evol. Syst.* 33:589–639
149. Rapp RA, Wendel JF. 2005. Epigenetics and plant evolution. *New Phytol.* 168:81–91
150. Rhymer JM, Simberloff D. 1996. Extinction by hybridization and introgression. *Annu. Rev. Ecol. Evol. Syst.* 27:83–109
151. Rieseberg LH. 1995. The role of hybridization in evolution: old wine in new skins. *Am. J. Bot.* 82:944–53
152. Rieseberg LH. 1997. Hybrid origins of plant species. *Annu. Rev. Ecol. Evol. Syst.* 28:359–89
153. Rieseberg LH. 2001. Chromosome rearrangements and speciation. *Trends Ecol. Evol.* 16:351–58
154. Rieseberg LH. 2006. Hybrid speciation in wild sunflowers. *Ann. Mo. Bot. Gard.* 93:34–48
155. Rieseberg LH, Brouillet L. 1994. Are many plant species paraphyletic? *Taxon* 43:21–32
156. Rieseberg LH, Carney SE. 1998. Plant hybridization. *New Phytol.* 140:599–624
157. Rieseberg LH, Kim S-C, Randell RA, Whitney KD, Gross BL, et al. 2007. Hybridization and the colonization of novel habitats by annual sunflowers. *Genetica* 129:149–65
158. Rieseberg LH, Van Fossen C, Desrochers A. 1995. Hybrid speciation accompanied by genomic reorganization in wild sunflowers. *Nature* 375:313–16
159. Rieseberg LH, Willis JH. 2007. Plant speciation. *Science* 317:910–14
160. Rieseberg LH, Wood TE, Baack EJ. 2006. The nature of plant species. *Nature* 440:524–27
161. Riley HP. 1938. A character analysis of colonies *of Iris fulva*, *Iris hexagona* var. *giganticaerulea* and natural hybrids. *Am. J. Bot.* 25:727–38
162. Rodriguez DJ. 1996. A model for the establishment of polyploidy in plants. *Am. Nat.* 147:33–46
163. Rosser EM. 1955. A new British species of Senecio. *Watsonia* 3:228–32
164. Salmon A, Ainouche ML, Wendel JF. 2005. Genetic and epigenetic consequences of recent hybridization and polyploidy in *Spartina* (Poaceae). *Mol. Ecol.* 14:1163–75
165. Sato T, Maciera M, Lumaret R, Jacquard P. 1993. Flowering characteristics and fertility of interploidy progeny from normal and 2*n* gametes in *Dactylis glomerata* L. *New Phytol.* 124:309–19
166. Sax K. 1936. The experimental production of polyploidy. *J. Arnold Arboretum* 17:153–59
167. Schemske DW. 2000. Understanding the origin of species. *Evolution* 54:1069–73
168. Schlueter JA, Dixon P, Granger C, Grant D, Clark L, et al. 2004. Mining EST databases to resolve evolutionary events in major crop species. *Genome* 47:868–76
169. Schluter D. 2001. Ecology and the origin of species. *Trends Ecol. Evol.* 16:372–80
170. Schranz ME, Mitchell-Olds T. 2006. Independent ancient polyploidy events in the sister families Brassicaceae and Cleomaceae. *Plant Cell* 18:1152–65
171. Segraves KA, Thompson JN, Soltis PS, Soltis DE. 1999. Multiple origins of polyploidy and the geographic structure of *Heuchera grossulariifolia*. *Mol. Ecol.* 8:253–62
172. Seioghe C, Gehring C. 2004. Genome duplication led to highly-selective expansion of the *Arabidopsis thaliana* proteome. *Trends Genet.* 20:461–64
173. Simillion C, Vandepoele K, Van Montagu MCE, Zabeau M, Van de Peer Y. 2002. The hidden duplication past of *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 99:13627–32
174. Simpson GG. 1961. *Principles of Animal Taxonomy*. New York: Columbia Univ. Press
175. Soltis DE. 1984. Autopolyploidy in *Tolmiea menziesii* (Saxifragaceae). *Am. J. Bot.* 71:1171–74
176. Soltis DE, Albert VA, Leebens-Mack J, Bell CD, Paterson AH, et al. 2008. Polyploidy and angiosperm diversification. *Am. J. Bot.* In press

177. Soltis DE, Rieseberg LH. 1986. Autopolyploidy in Tolmiea menziesii (Saxifragaceae): Genetic insights from enzyme electrophoresis. *Am. J. Bot.* 73(2):310–18
178. Soltis DE, Soltis PS. 1989. Polyploidy, breeding systems, and genetic differentiation in homosporous pteridophytes. In *Isozymes in Plant Biology*, ed. DE Soltis, PS Soltis, pp. 241–258. Portland: Dioscorides Press
179. Soltis DE, Soltis PS. 1989. Genetic consequences of autopolyploidy in *Tolmiea* (Saxifragaceae). *Evolution* 43:586–94
180. Soltis DE, Soltis PS. 1989. Allopolyploid speciation in *Tragopogon*: insights from chloroplast DNA. *Am. J. Bot.* 76:1119–24
181. Soltis DE, Soltis PS. 1993. Molecular data faciliate a reevaluation of traditional tenets of polyploid evolution. *Crit. Rev. Plant Sci.* 12:243–73
182. Soltis DE, Soltis PS. 1999. Polyploidy: recurrent formation and genome evolution. *Trends Ecol. Evol.* 14:348–52
183. Soltis DE, Soltis PS, Pires JC, Kovarik A, Tate J, Mavrodiev E. 2004. Recent and recurrent polyploidy in *Tragopogon* (Asteraceae): cytogenetic, genomic and genetic comparisons. *Biol. J. Linn. Soc.* 82:485–501
184. Soltis DE, Soltis PS, Schemske DW, Hancock J, Thompson J, et al. 2007. Autopolyploidy in angiosperms: Have we grossly underestimated the number of species? *Taxon* 56:13–30
185. Soltis DE, Soltis PS, Tate JA. 2004b. Advances in the study of polyploidy since *Plant Speciation*. *New Phytol.* 161:173–91
186. Soltis PS, Gitzendanner MA. 1999. Molecular systematics and the conservation of rare species. *Conserv. Biol.* 13:471–83
187. Soltis PS, Soltis DE. 1991. Multiple origins of allopolyploidy in *Tragopogon mirus* (Compositae): rDNA evidence. *Syst. Bot.* 16:407–413
188. Soltis PS, Soltis DE. 2000. The role of genetic and genomic attributes in the success of polyploids. *Proc. Natl. Acad. Sci. USA* 97:7051–57
189. Song K, Lu P, Tang K, Osborn TC. 1995. Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. *Proc. Natl. Acad. Sci. USA* 92:7719–23
190. Spring J. 2002. Genome duplication strikes back. *Nat. Genet.* 31:128–29
191. Stebbins GL. 1940. The significance of polyploidy in plant evolution. *Am. Nat.* 74:54–66
192. Stebbins GL. 1947. Types of polyploids: their classification and significance. *Adv. Genet.* 1:403–429
193. Stebbins GL. 1950. *Variation and Evolution in Plants*. New York: Columbia Univ. Press
194. Stebbins GL. 1971. *Chromosomal Evolution in Higher Plants*. London: Addison-Wesley
195. Stebbins GL. 1985. Polyploidy, hybridization, and the invasion of new habitats. *Ann. Mo. Bot. Garden* 72:824–32
196. Stift M, Berenos C, Kuperus P, van Tienderen PH. 2008. Segregation models for disomic, tetrasomic and intermediate inheritance in tetraploids: A general procedure applied to Rorippa (yellow cress) microsatellite data. *Genetics* 179:2113–23
197. Stupar RM, Hermanson PJ, Springer NM. 2007. Nonadditive expression and parent-of-origin effects identified by microarray and allele-specific expression profiling of maize endosperm. *Plant Physiol.* 145:411–25
198. Takhtajan A. 1997. *Diversity and Classification of Flowering Plants*. New York: Columbia Univ. Press
199. Tang HB, Bowers JE, Wang XY, Ming R, Alam M, et al. 2008. Synteny and collinearity in plant genomes. *Science* 320:486–88
200. Tate JA, Ni ZF, Scheen AC, Koh J, Gilbert CA, et al. 2006. Evolution and expression of homeologous loci in *Tragopogon miscellus* (Asteraceae), a recent and reciprocally formed allopolyploid. *Genetics* 173:1599–611
201. Tate JA, Soltis DE, Soltis PS. 2005. Polyploidy in plants. In *The Evolution of the Genome*, ed. TR Gregory, pp. 371–426. San Diego: Elsevier Academic
202. Tavoletti S, Mariani A, Veronesi F. 1991. Phenotypic recurrent selection for 2*n* pollen and 2*n* egg production in diploid alfalfa. *Euphytica* 57:97–102
203. Templeton AR. 1989. The meaning of species and speciation: a genetic perspective. In *Speciation and Its Consequences*, ed. D Otte, JA Endler, pp. 3–27. Sunderland: Sinauer Associates

204. Thompson JN, Cunningham BM, Segraves KA, Althoff DM, Wagner D. 1997. Plant polyploidy and insect/plant interactions. *Am. Nat.* 150:730–43
205. Thompson JN, Nuismer SL, Merg K. 2004. Plant polyploidy and the evolutionary ecology of plant/animal interactions. *Biol. J. Linn. Soc.* 82:503–510
206. Tuskan GA, DiFazio S, Jansson S, Bohlmann J, Grigoriev I, et al. 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* 313:1596–604
207. Urbanska KM, Hurka H, Landolt E, Neuffer B, Mummenhoff K. 1997. Hybridization and evolution in *Cardamine* (Brassicaceae) at Urnerboden, Central Switzerland: biosystematic and molecular evidence. *Plant Syst. Evol.* 204:233–56
208. Velasco R, Zharkikh A, Troggio M, Cartwright DA, Cestaro A, et al. 2007. A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. *PLoS ONE* 2:e1326
209. Vision TJ, Brown DG, Tanksley SD. 2000. The origins of genomic duplications in *Arabidopsis*. *Science* 290:2114–17
210. Wagner WH Jr. 1970. Biosystematics and evolutionary noise. *Taxon* 19:146
211. Wagner WH Jr. 1983. Reticulistics: the recognition of hybrids and their role in cladistics and classification. In *Advances in Cladistics 2*, ed. NI Platnick, VA Funk, pp. 63–79, New York: Columbia Univ. Press
212. Wagner WH Jr, Wagner FS. 1980. Polyploidy in pteridophytes. In *Polyploidy: Biological Relevance*, ed. WH Lewis, pp. 199–214. New York: Plenum
213. Wang J, Tian L, Lee HS, Wei NE, Jiang H, et al. 2006. Genomewide nonadditive gene regulation in *Arabidopsis* allotetraploids. *Genetics* 172:507–17
214. Wendel JF, Doyle JJ. 2004. Polyploidy and evolution in plants. In *Diversity and Evolution in Plants*, ed. RJ Henry, pp. 97–117. Wallingford, UK: CABI Publishing
215. Werth CR, Windham MD. 1991. A model for divergent, allopatric speciation of polyploid pteridophytes resulting from silencing of duplicate-gene expression. *Am. Nat.* 137:513–21
216. Wheeler QD, Meier R, eds. 2000. *Species Concepts and Phylogenetic Theory: A Debate*. New York: Columbia Univ. Press
217. Wheeler QD, Platnick NI. 2000. The phylogenetic species concept (sensu Wheeler and Platnick). See Ref. 216, pp. 55–69
218. Wiley EO. 1978. The evolutionary species concept reconsidered. *Syst. Zool.* 27:17–26
219. Wiley EO, Mayden RL. 2000. The evolutionary species concept. See Ref. 216, pp. 70–89
220. Wilson RA, ed. 1999. *Species: New Interdisciplinary Essays*. Cambridge: MIT Press
221. Wolf PG, Soltis DE, Soltis PS. 1990. Chloroplast-DNA and electrophoretic variation in diploid and autotetraploid *Heuchera grossulariifolia* (Saxifragaceae). *Am. J. Bot.* 77:232–44
222. Wolfe KH, Shields DC. 1997. Molecular evidence for an ancient duplication of the entire yeast genome. *Nature* 387:708–13
223. Yu J, Wang J, Lin W, Li S, Li H, et al. 2005. The genomes of *Oryza sativa*: a history of duplications. *PLoS Biol.* 3:e38

Annual Review of Plant Biology
Volume 60, 2009

Contents