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# Molecular Data and the Dynamic Nature of Polyploidy

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**ABSTRACT:** During the past decade, molecular techniques have provided a wealth of data that have facilitated the resolution of several controversial questions in polyploid evolution. Herein we have focused on several of these issues: (1) the frequency of recurrent formation of polyploid species; (2) the genetic consequences of multiple polyploidizations within a species; (3) the prevalence and genetic attributes of autopolyploids; and (4) the genetic changes that occur in polyploid genomes following their formation.

Molecular data provide a more dynamic picture of polyploid evolution than has been traditionally espoused. Numerous studies have demonstrated multiple origins of both allopolyploids and autopolyploids. In several polyploid species studied in detail, multiple origins were found to be frequent on a local geographic scale, as well as during a short span of time. Molecular data strongly suggest that recurrent formation of polyploid species is the rule, rather than the exception. In addition, molecular data indicate that recurrent formation of polyploids has important genetic consequences, introducing considerable genetic variation from diploid progenitors into polyploid derivatives.

Molecular data also suggest a much more important role for natural autopolyploids than has been historically envisioned. In contrast to the longstanding view of autopolyploidy as being rare, molecular data continue to reveal steadily increasing numbers of well-documented autoploids having tetrasomic or higher-level polysomic inheritance. Although autopolyploidy undoubtedly occurs much less frequently than allopolyploidy in natural populations, it nonetheless has been a significant evolutionary mechanism. Molecular data also provide compelling genetic evidence that contradicts the traditional view of autopolyploidy as being maladaptive. Electrophoretic studies have revealed three important attributes of autopolyploids compared to their diploid progenitors: (1) enzyme multiplicity, (2) increased heterozygosity, and (3) increased allelic diversity. Genetic variability is, in fact, typically substantially higher in autopoloids than in their diploid progenitors. These genetic attributes of autopolyploids are due to polysomic inheritance and provide strong genetic arguments for the potential success of autopolyploids in nature.

In addition to providing numerous important insights into the formation of polyploids and the immediate genetic consequences of polyploidy, molecular data also have been used to study the subsequent evolution of polyploid genomes. Common hypotheses on the subsequent evolution of polyploid genomes include (1) gene silencing, eventually leading to extensively diploidized polyploid genomes; (2) gene diversification, resulting in regulatory or functional divergence of duplicate genes; and (3) genome diversification, resulting in chromosomal repatterning. Compelling, but limited, genetic evidence for all of these factors has been obtained in molecular analyses of polyploid species. The occurrence of these processes in polyploid genomes indicates that polyploid genomes are plastic and susceptible to evolutionary change.

In summary, molecular data continue to demonstrate that polyploidization and the subsequent evolution of polyploid genomes are very dynamic processes.

**KEY WORDS:** polyploidy, multiple origins, gene silencing, genome diversification.

## I. INTRODUCTION

Polyploid evolution has been the source of considerable interest and controversy for nearly half a century. There have been numerous reviews of diverse aspects of polyploid evolution, including types or categories of polyploids,[18,53,80,96,164,165] ecological and evolutionary attributes,[37,79,165,166] polyploidy as a speciation mechanism,[79,165,166] genetic consequences of polyploid evolution,[7,22,25,49,50,79,132,145] ancient polyploidy,[51,58,155,166,178] and mode of formation of polyploids.[30,31,53]

In a very short time, molecular data have provided a wealth of new insights into polyploid evolution. Isozyme and DNA approaches have

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become standard tools in addressing the parentage of polyploids (reviewed in References 26, 151, and 155) and have proven invaluable in distinguishing between possible examples of auto- and allopolyploidy. Concomitantly, molecular approaches have provided critical data regarding the genetic consequences of polyploid evolution. For example, numerous studies have revealed permanent genetic hybridity or fixed heterozygosity in alloploids, as well as increased heterozygosity in autoploids due to polysomic inheritance.[21,24,25,132,144,145] The possible biochemical and genetic benefits that result from polyploid genetic systems have similarly been well reviewed.[7,21,24–26,79,132,145]

Several recent reviews have espoused the virtues of a molecular approach to the study of polyploid evolution.[151,152,155] These reviews have focused largely on the advantages of using DNA markers in determining the parentage of polyploids and assessing the possibility of multiple origins of polyploids. Also reviewed were the advantages of molecular data in elucidating possible instances of ancient polyploidy.

During the past several years, molecular data have allowed significant progress in understanding the mechanistic and evolutionary aspects of polyploidy. Herein we concentrate on the following: (1) the high frequency of recurrent formation (multiple origin) of polyploid species; (2) the genetic and evolutionary consequences of multiple polyploidizations within species; (3) the prevalence and genetic attributes of autopolyploids; and (4) the genetic changes that occur in polyploid genomes following their formation. In discussing these topics, we rely on molecular data from enzyme electrophoresis, restriction site analysis of cpDNA and rDNA, RFLP analysis of nuclear genomes, gene sequencing, and RAPD markers.

## II. FREQUENCY OF MULTIPLE ORIGINS OF POLYPLOIDS

Perhaps the most important contribution of molecular data to the study of polyploid evolution in plants is the documentation that a single polyploid species may be formed recurrently. Although polyploidy has long been considered a major force in plant evolution,[51,80,164–166] polyploid taxa have traditionally been viewed as possessing fundamentally different genetic characteristics than diploids. Because polyploidization was considered a rare process, each polyploid species typically was thought to have had a single origin, initially resulting in genetic uniformity across all individuals of the species (however, see Ownbey and McCollum[107]). Furthermore, because of the "buffering" of multiple genomes (or homologs in autoploids), mutation and recombination are less effective at constructing new adaptive complexes in polyploids than in diploids.[166] Reflecting this line of thinking, Stebbins[166] suggested that the primary mechanism by which polyploids could enlarge their gene pools was through acquisition of genetic diversity from related diploid species through hybridization. The genetic uniformity of a polyploid species, coupled with its reduced capacity for molding new genotypes, led to the extreme view of polyploid species as evolutionary dead ends.[176,177]

We argue in this section of our review that multiple origins of polyploids are the rule and not the exception. Furthermore, recurrent polyploidization has occurred with great frequency in several species studied in detail, both during short time spans and in small geographic areas. Thus, numerous molecular studies indicate that substantial genetic variation may be introduced into polyploids from genetically different parental populations.

Over 40 examples of multiple origins of polyploid taxa have been reported (Table 1), including both autoploids and alloploids, although the latter by far predominate. Table 1 includes one bryophyte and numerous pteridophytes and angiosperms, a majority of which were documented in the past 3 years. Several lines of molecular data have documented multiple origins of polyploids (Table 1), including isozyme data from enzyme electrophoresis, restriction site analysis of chloroplast DNA (cpDNA) and nuclear rDNA, RFLP analysis of nuclear DNA, and, most recently, RAPDs. Before considering the examples of recurrent polyploidization further, we first review briefly the molecular data that were used in the documentation of these events.

Most of the examples of multiple polyploid formation given in Table 1 were documented using isozymes, reflecting, in part, the fact that isozymes have been available as genetic markers

**TABLE 1**
**Examples of Recurrent Formation of Polyploids Based on Molecular Data**

| Taxon | Ploidy | Type of polyploid | Number of origins[a] | Evidence for multiple origins | Ref. |
|---|---|---|---|---|---|
| Bryophytes | | | | | |
| *Plagiomnium medium* | 4× | Alloploid | Minimum of 4 | Isozymes | 197, 198 |
| Pteridophytes | | | | | |
| *Asplenium adiantum-nigrum* | 4× | Alloploid | Minimum of 7 | Isozymes | 126, 127 |
| *A. bradleyi* | 4× | Alloploid | | Isozymes | 188, 189 |
| *A. pinnatifidum* | 4× | Alloploid | | Isozymes | 188, 189 |
| *Cystopteris laurentiana* | 4× | Alloploid | | Isozymes | Haufler et al. (unpublished) |
| *C. tennesseensis* | 4× | Alloploid | Minimum of 6 | Isozymes | Haufler et al. (unpublished) |
| *Dryopteris campyloptera* | 4× | Alloploid | | Isozymes | Werth (unpublished data) |
| *D. cristata* | 4× | Alloploid | | Isozymes | Werth (unpublished data) |
| *Hemionitis pinnatifida* | 4× | Alloploid | Minimum of 5 | Isozymes | 125 |
| *Pellaea wrightiana* | 4× | Alloploid | | Isozymes | 194 |
| *Polypodium calirhiza* | 4× | Alloploid | | cpDNA | Haufler et al. (unpublished) |
| *P. hesperium* | 4× | Alloploid | | cpDNA, isozymes | Haufler et al. (unpublished) |
| *P. interjectum* | 4× | Alloploid | | Isozymes | Haufler (unpublished) |
| *P. virginianum* | 4× | Alloploid | | cpDNA, isozymes | 16, Haufler et al. (unpublished) |
| *Polystichum californicum* | 4× | Alloploid | Maybe | Isozymes | 159 |
| *P. scopulinum* | 4× | Alloploid | Maybe | Isozymes | 159 |
| Angiosperms | | | | | |
| *Aegilops triuncialis* | 4× | Alloploid | | cpDNA | 104 |
| *Antennaria neodioica* | 6× | Alloploid | | Isozymes | 8, 9, Bayer (personal communication |
| *A. parlinii* | 6× | Alloploid | | Isozymes | 8, 9 |
| *A. rosea* | 4× | Alloploid | | Isozymes | 8, Bayer (personal communication) |
| *Brassica juncea* | 4× | Alloploid | | Nuclear RFLPs | 160 |
| *B. napus* | 4× | Alloploid | Minimum of 4 | cpDNA, nuclear RFLPs | 39, 65, 109, 160, 161 |
| *Dactylis glomerata* | 4× | Alloploid | | cpDNA, isozymes | 88, 89 |
| *Draba cacuminum* | 8× | Alloploid | Minimum of 3 | Isozymes | 13 |
| *D. corymbosa* | 16× | Alloploid | | Isozymes | 12 |
| *D. lactea* | 6× | Alloploid | | rDNA, isozymes | 12 |
| *D. norvegica* | 6× | Alloploid | Probably 13 | isozymes | 12 |
| *Glycine tabacina* race $BBB_2B_2$ | 4× | Alloploid | | cpDNA | 33–35 |
| *G. tomentella* $T_1$ race | 4× | Alloploid | | cpDNA | 33 |
| *Heuchera grossulariifolia* | 4× | Autoploid | Minimum of 3 | cpDNA, isozymes | 196 |
| *H. micrantha* | 4× | Autoploid | Minimum of 3 | cpDNA, isozymes | 153 |

**TABLE 1 (continued)**
**Examples of Recurrent Formation of Polyploids Based on Molecular Data**

| Taxon | Ploidy | Type of polyploid | Number of origins[a] | Evidence for multiple origins | Ref. |
|---|---|---|---|---|---|
| *Microseris heterocarpa* | 4× | Alloploid | | cpDNA | 179 |
| *Musa* × *paradisiaca* AAB | 3× | Alloploid | Maybe | rDNA | 73 |
| *Musa acuminata* AAA | 3× | Autoploid | Maybe | rDNA | 73 |
| *Oryza malampuzhaensis* | 4× | Alloploid | Maybe | Nuclear RFLPs | 180 |
| *O. minuta* | 4× | Alloploid | Maybe | Nuclear RFLPs | 180 |
| *O. punctata* | 4× | Alloploid | Maybe | Nuclear RFLPs | 180 |
| *Senecio cambrensis* | 6× | Alloploid | 2–4 | Isozymes | 4 |
| *Solanum tuberosum* ssp. *andigena* | 4× | Alloploid | Minimum of 3 | cpDNA | 63<br>64 |
| *Tragopogon mirus* | 4× | Alloploid | 7–11 | rDNA, isozymes, RAPDs | 117, 158, Plunkett et al. (unpublished) |
| *T. miscellus* | 4× | Alloploid | 9–13 | cpDNA, isozymes, RAPDs | 117, 148, Plunkett et al. (unpublished) |
| *Triticum dicoccoides* | 4× | Alloploid | | cpDNA | 104 |
| *Triticum* Emmer wheats | 4× | Alloploid | | mtDNA | 170 |
| Dinkel wheats | 6× | Alloploid | | mtDNA | 170 |
| Timopheevi wheats | 4× | Alloploid | | mtDNA | 170 |
| *Turnera ulmifolia* var. *elegans* | 4× | Autoploid | | Isozymes | 138 |

[a] Unless otherwise stated, the minimum number of origins is two.

much longer than the other above-noted methodologies. In addition, however, isozymes provide a series of readily scorable, single-gene markers with a high probability of allozyme polymorphism for testing hypotheses of polyploid origins (see reviews by Crawford,[22,25,26] Weeden and Wendel,[182] and Gottlieb[48]). Despite their usefulness in revealing the origins of polyploids, isozyme data also are plagued by limitations. As several authors have noted,[12,13,125,159] the presence of different fixed patterns among polyploid populations does not necessarily imply multiple origins. Very important in terms of estimating the number of multiple polyploid events is the fact that initial segregation among progeny from a highly heterozygous raw polyploid and the subsequent fixation by inbreeding, drift, or selection (or a combination thereof) could lead to different populations possessing different fixed heterozygous genotypes. Thus, it is possible to overestimate the prevalence of multiple polyploidization events with isozyme data. Some investigators have attempted to incorporate this limitation into their estimates, whereas others simply equate each multilocus genotype with a separate polyploid origin.

In contrast to isozymes, cpDNA restriction site data may be less likely to reveal multiple origins of polyploids because of the conservative rate of evolution of the chloroplast genome (see reviews by Palmer,[110] Palmer et al.,[111] and Clegg et al.[19]). That is, this approach is less likely to reveal polymorphism within a parental species (see Soltis et al.[155] for a review of intraspecific cpDNA variation), an essential feature of a genetic marker found to be useful in the study of multiple polyploidizations from different parental populations. Furthermore, because the chloroplast genome typically is uniparentally inherited, only the contribution of one parent (the maternal parent in most angiosperms) is analyzed. Therefore, cpDNA data are less likely to reveal the

frequency of polyploid events. Nonetheless, cpDNA restriction site data have revealed many instances of recurrent polyploidization (Table 1). cpDNA data are particularly powerful from an evolutionary standpoint when they reveal that different polyploid populations have incorporated the chloroplast genome of each of the two different parents. That is, separate polyploid events occurred involving parental species A and B; in some instances A was the maternal parent, while in others, B was the maternal parent. This has been documented in *Tragopogon miscellus,*[148] *Brassica napus*,[161] and *Polypodium hesperium*, *P. virginianum* and *P. calirhiza* (Haufler et al., unpublished).

Genetic markers also may be derived through the amplification of random DNA segments with single primers of arbitrary nucleotide sequence.[183,192] Typically referred to as RAPD markers (random amplified polymorphic DNA[192]), this approach is a rapid, efficient, and relatively inexpensive method for identifying plant genotypes. Although not yet used frequently in this regard, RAPD markers may be particularly valuable in documenting multiple origins of polyploids.

The large number of examples of multiple origins of polyploids reported in Table 1 is noteworthy in several respects. First, many of the examples given were revealed despite the investigation of only a few populations of the polyploid species. For example, only five populations of the wide-ranging tetraploid *Asplenium bradleyi* were investigated with enzyme electrophoresis,[188,189] but convincing evidence of multiple origins was provided. In *P. calirhiza,* the two populations analyzed possessed divergent cpDNAs, reflecting two separate polyploidization events (Haufler et al., unpublished). Only three populations of the newly arisen allotetraploid *Senecio cambrensis* are known, yet Ashton and Abbot[4] provided strong evidence of separate origins of two of these populations. The small sample sizes of many polyploid species reflect the fact that the goal of the molecular investigation was to ascertain the percentage of the species, rather than to test for the possibility of recurrent formation of that polyploid. In fact, most of the examples in Table 1 are based on six or fewer polyploid populations.

Few of the polyploids listed in Table 1 were examined exhaustively using several different molecular methodologies. In fact, most of the examples of multiple origins (Table 1) generally were revealed in studies that involved a single molecular approach. Recurrent origins are more likely to be revealed via a comprehensive approach involving a combination of nuclear (e.g., allozymes, nuclear RFLPs, rDNA) and cytoplasmic markers (cpDNA, mtDNA).[151,159] That is, any of these nuclear or cytoplasmic markers can potentially reveal multiple origins, as is clearly seen in Table 1; however, when nuclear and cytoplasmic markers are used in concert, the number of separate origins documented can increase over that revealed by a single approach. For example, cpDNA restriction site data revealed multiple origins in *T. miscellus,* whereas rDNA data, as well as an initial investigation of allozymes,[132] did not.[151,154] Conversely, allozymes and rDNA data documented multiple origins of *T. mirus,* whereas cpDNA data did not reveal recurrent formation of this tetraploid.

Although a few polyploids have been thoroughly studied both in terms of population number and methodologies, recurrent origins have nonetheless been documented in numerous species. We therefore suggest that the prevalence of this evolutionary event has been grossly underestimated; only recently have the frequency of recurrent formation of polyploids and the evolutionary implications of this phenomenon been appreciated.

Molecular data not only demonstrate numerous examples of the recurrent formation of polyploid taxa (Table 1), but also strongly suggest that multiple origin is the rule, rather than the exception, in polyploid evolution. It is noteworthy, for example, that whereas numerous molecular studies have documented multiple origins of polyploids, few have argued convincingly for a single origin of a polyploid species. It is, of course, easier to demonstrate conclusively multiple origins of a polyploid than a single origin. Nonetheless, in the vast majority of instances in which a polyploid taxon has been investigated in any detail using molecular approaches, evidence for multiple origins has been forthcoming.

Polyploid species for which molecular data suggest the possibility of only a single origin

include the ferns *Dryopteris celsa,*[187] *D. carthusiana* (Werth, unpublished data), *A. ebenoides,*[188,189] *Cystopteris utahensis* (Haufler, unpublished), *Polystichum californicum* and *P. scopulinum,*[159] and the angiosperms *Tolmiea menziesii*[147,154] and *Krigia virginica.*[70] However, for most of these species, the data suggesting only a single origin are not overwhelming. In most cases, only a single molecular approach was applied, and in many studies, the number of populations sampled was low. In *K. virginica,* the evidence suggesting a single origin involves cpDNA restriction site data for four tetraploid populations and seven diploid populations. It would be of interest to examine populations of this tetraploid with nuclear markers, such as allozymes. Similarly, only allozyme data were used in the analyses of *D. celsa, D. carthusiana,* and *A. ebenoides,* and only two populations of the latter were investigated.

For some of these species, the data available are simply inconclusive. In *T. menziesii,* cpDNA data for 30 diploid and autotetraploid populations provided no unambiguous evidence of multiple origins.[154] Diploid and tetraploid populations share three or four alleles at each of six polymorphic isozyme loci, suggesting either that the autotetraploid was formed via crossing of genetically different diploids, or that autopolyploidy did occur more than once.[147] Similarly, for *Polystichum scopulinum* and *P. californicum,* both allozyme and cpDNA restriction site data were used. cpDNA data indicated that all populations of each allotetraploid were identical to only one of the diploid parents. For both allotetraploids, allozyme studies revealed seven different multilocus genotypes. Thus, for both tetraploids, it is possible that some or all of these allozyme patterns reflect discrete origins, or they may have resulted from a single polyploidization event that involved highly heterozygous diploid gametes, followed by genetic segregation and interpopulational divergence.[159] The difficulty in documenting that a polyploid may have had a single origin also is illustrated by the recently formed alloploid *Spartina anglica.*[128] Isozyme electrophoresis and seed protein electrophoretic profiles revealed almost no genetic variation in this alloploid. However, the diploid parents also are characterized by low levels of genetic variation. Thus, genetic data are ambiguous: *S. anglica* may have originated only once or multiple times from genetically uniform parents.[128]

Perhaps the strongest evidence for a single origin of a polyploid species can be made for the ferns *C. utahensis* and *D. celsa. C. utahensis* is genetically highly uniform throughout the southwestern U.S., as is one of its parents, *C. bulbifera.* The second parent, *C. reevesiana,* is highly variable genetically. Thus, if multiple origins involving different populations of *C. reevesiana* had occurred, one would expect more of this genetic variation to have been incorporated into the tetraploid *C. utahensis* (Haufler, personal communication). Werth[187] similarly makes a strong genetic argument for a single origin of *D. celsa.*

Werth,[186] using molecular data, determined that polyploid ferns of multiple origins outnumber those of a single origin by at least two to one. However, although the examples given of multiple origins are for the most part convincing, several of the examples of single origins for ferns are tentative at best, involving only a few polyploid populations. Nonetheless, Werth's data for ferns generally parallel those for all land plants presented here. That is, molecular data argue convincingly that multiple origins of polyploids are much more frequent than single origins.

Not only do molecular studies indicate that recurrent formation of polyploids is the rule, these data also demonstrate that for some polyploid species, recurrent formation has occurred with remarkable frequency. For most of the polyploids given in Table 1, the data available suggest that a particular polyploid originated at least twice. However, many of these examples did not involve broad surveys; the goal was not to document multiple origins per se, but in most cases simply to examine the parentage of a given polyploid. Thus, those polyploid species studied in detail with the specific goal of tracing the frequency of polyploid formation provide important evolutionary insights. These examples are discussed in more detail later to illustrate the frequency with which multiple polyploid formation can occur.

*A. adiantum-nigrum* has a highly scattered worldwide distribution. Only a few populations are known from North America and Hawaii, with the majority of populations located in Eurasia. Ranker et al. (unpublished) have documented a

minimum of seven unique origins of this species, based primarily on the isozyme analysis of its limited Hawaiian and North American range; only one Eurasian population has so far been sampled. Another wide-ranging species that has been studied extensively is the allopolyploid moss *Plagiomnium medium,* which has a circumboreal distribution. Wyatt et al.[198] have analyzed allozymically 14 populations of this tetraploid from the U.S. (California, Oregon, New York, Maine, and Michigan), Sweden, Norway, and Finland and 29 populations of the two parental diploids. Wyatt et al. suggest a minimum of four separate origins of *P. medium.* With much of the geographic ranges of these two wide-ranging species unsampled, and only allozyme evidence so far available, the possibility of much higher estimates of independent formation of each of these polyploid species seems likely.

Perhaps more remarkable are polyploid species of restricted geographic distributions for which molecular data indicate a high frequency of independent origins. The allotetraploid fern *Hemionitis pinnatifida* is distributed from southern Mexico to Costa Rica. Based on allozyme and cpDNA data for only three populations of this species, Ranker et al.[125] proposed that tetraploid individuals were synthesized *de novo* at least five times. Similarly, allozyme data for three populations suggest at least three origins of the narrow endemic *Draba cacuminum* (Brassicaceae), an octoploid from Scandinavia.[12] Haufler et al. (Reference 59 and personal communication) suggests at least six origins of the narrowly distributed fern *C. tennesseensis:* furthermore, Haufler (personal communication) indicates that this polyploid continues to form. Other allotetraploids of even more restricted geographic distribution are *Tragopogon mirus* and *T. miscellus,* which have formed between 7 to 11 and 9 to 13 times, respectively, in a small region of eastern Washington and adjacent Idaho (Reference 117 and unpublished data). Because these species originated during the past 60 years,[106] they offer a model system for the study of the recurrent synthesis of polyploids and are discussed in more detail later.

Some of the molecular studies indicating a high frequency of independent polyploid origins are particularly important because of their broader implications for certain taxonomic groups and floras. For example, Brochmann et al.[12–14] examined the parentage and multiple origins of several polyploids in *Draba* from the Nordic area. In these studies, multiple origins not only were documented for the narrow endemic *Draba cacuminum,* noted earlier, but also for several more widely distributed species: *D. norvegica* (6 times), *D. lactea* (6 times), and *D. corymbosa* (16 times). These studies are noteworthy in several respects. First, multiple origins were documented in a small geographic area using a relatively small number of populations. For example, based on allozyme data for only 10 Nordic populations of *D. norvegica,* Brochmann et al.[13] suggested that the likely number of independent origins of these palnts is 13. Secondly, the allozyme variation of *D. norvegica* was not correlated with the geographical origin of the polyploid populations, implying that populations occurring within the same general area have had independent origins. Furthermore, even plants of *D. norvegica* occurring within the same local area and assigned to the same population do not necessarily have the same alloploid origin. For example, in a population covering a few square meters in northern Norway, two very different fixed electrophoretic phenotypes were found, which almost certainly represent two distinct alloploid lineages. This does not imply, however, that these different phenotypes arose *in situ;* rather, once formed, subsequent migration may have brought them into contact at this site. Finally, the Nordic populations analyzed of *D. norvegica, D. lactea,* and *D. corymbosa* and their progenitors represent only a small fraction of the geographic distributions of these widely distributed species.[13] *D. norvegica* has an amphiatlantic distribution, whereas both *D. lactea* and *D. corymbosa* are circumpolar. Thus, it seems likely that these polyploids have formed numerous times in different geographic areas. Brochmann et al.[13] suggest that this process of repeated alloploidization offers an explanation for the taxonomic complexity in *Draba.* This may be a common pattern in *Draba,* which contains 16 commonly recognized arctic-alpine species, 13 of which are polyploid and known for their vexing taxonomic problems. On a broader scale, these data for arc-

tic-alpine *Draba* polyploids may typify polyploids of the arctic flora, a flora characterized by a high frequency of polyploidy.[37]

For most polyploids, the time of origin is, of course, unknown. Those few polyploids for which the time of origin is well documented therefore represent model systems for the study of polyploid evolution. Within just the past 150 years or less, four new allopolyploids have arisen in nature: *Tragopogon mirus* and *T. miscellus*,[106] *Spartina anglica*,[68] and *Senecio cambrensis*.[133] All four of these species have been investigated with molecular approaches. For *Senecio cambrensis*, *T. mirus*, and *T. miscellus*, molecular data clearly indicate multiple origins during very short time frames. Furthermore, the genetic data have important implications regarding genetic diversity in newly formed alloploids. Because of their great importance to the topic of multiple polyploidization, all four of these examples of recent polyploid evolution are reviewed here.

The perennial salt marsh grass *Spartina anglica* arose in Hampshire, England, in the late 19th century; the earliest specimens were collected in 1892. The parents of this alloploid are *S. maritima*, a native of British salt marshes, and *S. alterniflora*, native to the U.S. and introduced into Britian by shipping around 1816.[68,128] The allotetraploid has spread widely in Britian and also has been successfully introduced in areas around the world. Electrophoretic analyses of allozymes and seed proteins revealed that *S. anglica* is genetically depauperate.[128] The comparison of 261 cloned lines from 14 populations revealed only 1 rare variant for GOT. However, both parental species also maintain very low levels of genetic variation; hence, the lack of variation in *S. anglica* is the result of either a single polyploid origin or multiple allopolyploid events from uniform parents.[128]

*Senecio cambrensis* (Compositae) is an allohexaploid, the parents of which are *S. vulgaris* and *S. squalidus* (reviewed by Ashton and Abbott[4]). Contact between the parental species did not occur until after the escape of *S. squalidus*, a species introduced into Britian from the Oxford Botanic Garden in approximately 1910. The new allohexaploid, *S. cambrensis*, was first discovered in 1948 near Wrexham, North Wales. Thus, the time of origin of *S. cambrensis* is between 1910 and 1948. In Wales, *S. cambrensis* is now common in Wrexham, as well as the small towns in the vicinity. Other populations of the hexaploid were discovered more recently in areas well-removed from the initial area of origin: Mochdre, North Wales (discovered in 1966) and Edinburgh, Scotland (discovered in 1982). Ashton and Abbott[4] surveyed allozyme variation in the three locations where *S. cambrensis* occurs: Wrexham and Mochdre in Wales, and Edinburgh, Scotland. Their data indicate separate origins of *S. cambrensis* in Wales and Scotland. Furthermore, the possibility exists that the two populations in Wales represent separate lineages of independent origins. However, the pattern of variation in Wales also could have arisen following segregation and founder effects or selection.

*Tragopogon* (Compositae) provides two of the best known and most thoroughly studied examples of recent polyploid speciation: *T. mirus* and *T. miscellus*.[106] These two allotetraploids originated very recently, probably just within the past 50 to 60 years, in the Palouse region of eastern Washington and adjacent Idaho (Figure 1). The diploid progenitors of the two polyploids (*T. dubius*, *T. porrifolius*, and *T. pratensis*) are native to the Old World and were introduced into North America and are now widely naturalized. According to Ownbey,[106] *T. porrifolius* and *T. pratensis* were first collected in the Palouse as early as 1916; *T. dubius* was first collected from the region in 1928. *T. dubius* is by far the most common, occurring in waste places, fields, and along roadsides throughout much of North America, whereas *T. porrifolius* and *T. pratensis* are uncommon and confined to waste places and lawns in towns of the Palouse and a few other geographic areas.

The parentage of the two alloploids has been well documented based on numerous experimental approaches:[11,106–108,132,148,151,158] the parents of *T. mirus* are *T. dubius* and *T. porrifolius;* those of *T. miscellus* are *T. dubius* and *T. pratensis* (Figure 2). The distribution of *Tragopogon* in the Palouse resembles a model of island biogeography in many respects. The tetraploids, as well as their diploid progenitors, are typically confined to the small towns that typify the Palouse region and are absent from the large tracts of surrounding agricultural land. The occurrence of tetra-

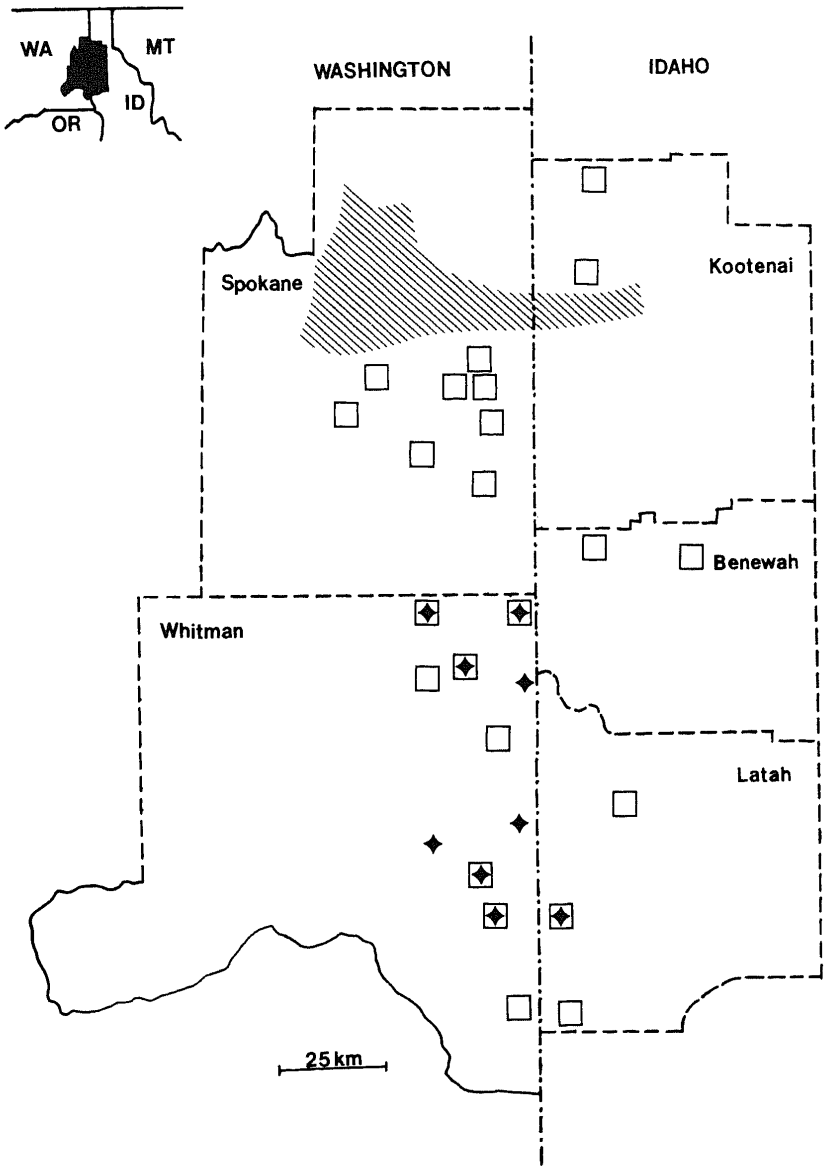


**FIGURE 1.** Distribution map of the allotetraploid *Tragopogon* species within five counties of eastern Washington and adjacent Idaho. Populations of *Tragopogon mirus* are indicated by stars, whereas populations of *Tragopogon miscellus* are indicated by open squares. Localities containing both species possess both symbols. The cross-hatched area indicates the continuous range of *Tragopogon miscellus* in the vicinity of Spokane, WA. (From Novak, S. J., Soltis, D. E., and Soltis, P. S., *Am. J. Bot.*, 78, 1586, 1991. With permission.)

ploids and diploids in several of the towns in eastern Washington and adjacent Idaho suggested the possibility of multiple origins on a local geographic scale. Based on various lines of evidence, including cytology, flavonoid chemistry, and morphology, Ownbey and co-workers suggested that *T. mirus* and *T. miscellus* arose independently at least three and two times, respectively, in the Palouse region. More recently, *T. mirus* and *T. miscellus* were reported from Arizona,[15] and *T. miscellus* was observed in Sheridan, WY, and Gardiner, MT (Ownbey, unpublished data), raising the possibility of multiple origins on an even larger geographic scale.

Molecular analyses of most populations of *T. mirus* and *T. miscellus* known before 1990 from the Palouse region documented the occurrence of multiple allopolyploid events in this small

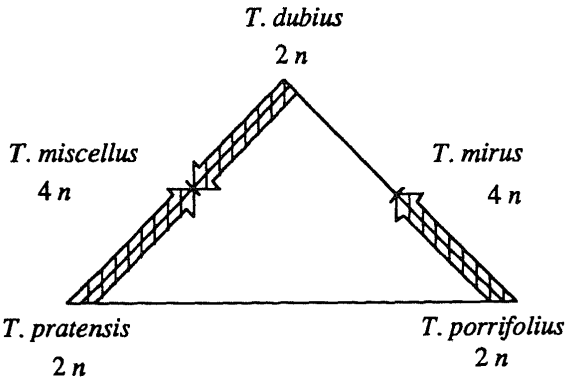


**FIGURE 2.** Parentage and reciprocal origins of tetraploid Tragopogons. Shaded lines indicate diploid(s) contributing chloroplast to the tetraploids.

geographic area during a very short time span. Using allozyme data, Roose and Gottlieb[132] suggested that *T. mirus* had at least three separate origins in the Palouse; electrophoretic data were inconclusive, however, with regard to the possibility of multiple origins of *T. miscellus*. Using cpDNA restriction site data, Soltis and Soltis[148] demonstrated two independent origins of *T. miscellus*. Populations from Pullman, WA, have *T. dubius* as the maternal parent; all other populations have *T. pratensis* as the maternal parent. Significantly, plants resulting from these two separate origins differ morphologically. Those plants of *T. miscellus* having *T. pratensis* as the maternal parent have short ligules; those plants having *T. dubius* as the maternal parent have long ligules. These two types of *T. miscellus* may differ ecologically. Long-liguled *T. miscellus* seems to prefer wetter sites than short-liguled *T. miscellus* (personal observation). A subsequent analysis of rDNA variation did not reveal multiple origins of *T. miscellus*, but did demonstrate unequivocally two origins of *T. mirus*.[158]

Both *T. mirus* and *T. miscellus* have increased substantially in both geographic range and numbers, just in the Palouse region, during the past 40 years.[103] In particular, *T. miscellus* is much more widespread in the Palouse than previously thought. For example, the studies of Roose and Gottlieb[132] and Soltis and Soltis[148] involved five general locations of *T. miscellus*, considered to represent all locations of this new tetraploid in the Palouse. Based on a broad survey of towns conducted in 1990, Novak et al.[103] discovered, however, that *T. miscellus* actually occurred in 38 locations throughout the Palouse. *T. mirus* also was more widespread than previously believed. Both Roose and Gottlieb[132] and Soltis and Soltis[148,158] sampled all five of the known locations of this species. Novak et al.,[103] in their survey of 90 localities representing nearly all towns in the Palouse, found *T. mirus* in 4 new localities, yielding a total of 9 sites for this species.

Given the substantial increase in the number of known locations in the Palouse for both *T. miscellus* and *T. mirus*, Plunkett et al. (Reference 117 and in preparation) undertook a genetic analysis of populations of these two tetraploids using data from enzyme electrophoresis, cpDNA, and rDNA. Allozyme data revealed eight and four multilocus genotypes, respectively, in *T. miscellus* and *T. mirus*. When molecular data sets are combined, they suggest a minimum of 9 and a maximum of 13 independent origins of *T. miscellus* in the Palouse; a minimum of 7 and a maximum of 11 are suggested for *T. mirus*. For *T. miscellus*, as noted previously, each diploid parent has acted as the maternal parent in these origins; for *T. mirus*, in contrast, *T. porrifolius* has consistently been the diploid parent despite recurrent formation of the tetraploid.

Thus, molecular data for *T. mirus* and *T. miscellus* have indicated an abundance of multiple origins of these allotetraploids. Furthermore, these origins have been very frequent on a very local geographic scale. The total area involved encompasses only five counties in eastern Washington and adjacent Idaho, an area of approximately 14,000 km$^2$. The extent to which multiple origins can occur on a very local geographic scale is illustrated further by the fact that molecular data suggest recurrent formation of *T. mirus* just within the small town of Pullman. It will be of interest to expand these molecular studies to a larger geographic scale to include populations of *T. mirus* and *T. miscellus* from Flagstaff, AZ, and *T. miscellus* from Sheridan, WY, and Gardiner, MT. Undoubtedly, evidence of additional independent origins of these two tetraploids will be forthcoming. Finally, the molecular data for *Tragopogon* illustrate frequent polyploidization on a very short time scale (50 to 60 years). If we extrapolate from the *Tragopogon* data to any two parental species and their derivative polyploids and consider the potential

for contact between parental populations over a much larger geographic scale and a much larger time frame, the likelihood of numerous polyploidization events must, in most cases, be very high.

## III. GENETIC AND EVOLUTIONARY CONSEQUENCES OF MULTIPLE POLYPLOIDIZATIONS

The frequent recurrence of polyploidization has important evolutionary implications. Genetic evidence clearly suggests that polyploids are much more dynamic systems than formerly envisioned. The view of polyploidy actually acting to "retard...evolutionary progress at the gene level,"[166] as well as the more extreme concept of polyploids as evolutionary dead ends,[176,177] are contradicted by evidence of the recurrent formation of polyploids from genetically different diploid individuals. This process of multiple polyploidization would certainly enrich the total gene pool of the polyploid species, particularly given the frequency with which these events may occur. Furthermore, molecular data also provide evidence for significant gene flow across ploidal levels through semifertile hybrids between diploids and polyploids (e.g., see References 29, 83, 89, and 112; also C. Brochmann, unpublished data), a mechanism adding yet another dynamic aspect to polyploid evolution.

Once genetic variation is introduced into polyploid species, molecular evidence also indicates that dynamic evolutionary processes continue to act at the polyploid level. Genetic segregation among progeny from a heterozygous raw polyploid and subsequent fixation of these new genotypes by inbreeding, drift, or selection (or a combination thereof) also can increase the array of genotypes present in polyploid populations and may have contributed to the genetic diversity present within some polyploid species.[13,125,159] In addition, new polyploids may be able to generate genetic diversity following recombination between parental genomes (homologous chromosome pairing).[4,132]

Furthermore, for several polyploid species (e.g., species of *Draba* [Reference 13] and *Tragopogon* [Reference 117 and Plunkett et al., in preparation]), individuals with multilocus genotypes indicative of separate origins co-occur in the same local area as a result of migration. That different genotypes resulting from independent polyploidizations come into contact obviously affords the opportunity for subsequent recombination and the production of new genotypes, further contributing to the dynamic nature of polyploid gene pools.

With these various processes acting in concert, the amount of genetic diversity present in polyploids, as revealed by molecular investigations, is remarkably high. The tetraploid Tragopogons, which are only 50 to 60 years old, illustrate this point well. *T. mirus* possesses four multilocus electrophoretic genotypes and two distinct rDNA genotypes; *T. miscellus* exhibits eight multilocus electrophoretic genotypes and two distinct chloroplast genomes. Similarly, Brochmann et al.[13] observed a very high number of multilocus genotypes in a small geographic area in polyploid *Draba* species: both *D. norvegica* and *D. lactea* exhibited 13 multilocus genotypes in 10 populations; *D. corymbosa* possessed 19 multilocus patterns in 11 populations. Thus, the genetic diversity of many polyploid species appears to be quite high, in striking contrast to the traditional view of polyploids as being genetically uniform.

Previous molecular studies have revealed the well-known ability of alloploids to exhibit fixed heterozygosity due to the combination of divergent genomes (e.g., see Roose and Gottlieb;[132] reviewed in Gottlieb[49,50] and Crawford[22,24–26]). As a result, these plants exhibit enzyme multiplicity, i.e., the presence of two divergent genomes enables the alloploid to produce all of the enzymes produced by each parent, as well as novel enzymes. Although autoploids do not exhibit fixed heterozygosity, they have greatly increased levels of heterozygosity at individual gene loci due to polysomic inheritance (reviewed in Soltis and Rieseberg[145] and Soltis and Soltis[147]); like alloploids, autoploids also exhibit enzyme multiplicity. It has often been suggested that these genetic attributes might provide both auto- and alloploids with greater genetic and biochemical flexibility than that of their diploid progenitors and may contribute to the success of both types of polyploids in nature.[7,79,132,145,147–150] Because

many polyploid species also exhibit high levels of genetic diversity at individual gene loci, more than just fixed heterozygosity and enzyme multiplicity need to be considered in evaluating the success of polyploids in nature. That is, the high level of segregating genetic variation in polyploids, resulting in part from recurrent polyploidization, also may be an important factor that contributes to the success of these plants in natural populations.

Polyploid species exhibit not only nuclear genetic variation, but also cytoplasmic diversity. cpDNA restriction site studies have revealed reciprocal origins of some alloploids (e.g., *T. miscellus* [Reference 148]; *Polypodium hesperium, P. virginianum,* and *P. calirhiza* [Haufler et al., unpublished]; and *B. napus* (Song and Osborn, in preparation). In some instances, the cytoplasmic origin of an allotetraploid may be complex. *B. napus* has been derived from at least four independent hybridization events, each with a different maternal parent.[161] *B. campestris* (= *B. rapa*) is the maternal parent of some accessions, whereas *B. oleracea* is the maternal parent of other accessions. Adding to the complexity is the fact that most accessions of cultivated *B. napus* apparently derived from a cross in which the common ancestor of *B. rapa* and *B. oleracea* was the maternal parent. In *T. miscellus,* the profound effects of divergent cytoplasms are reflected in an obvious morphological difference that accompanies this cytoplasmic difference. When *T. dubius* is the maternal parent (and cytoplasmic donor), the resulting *T. miscellus* has long ligules; when *T. pratensis* is the maternal parent, *T. miscellus* has short ligules. Such a morphological difference could perhaps affect the pollination biology of these plants. These different types of *T. miscellus* may be different physiologically as well. The long-liguled *T. miscellus* seems to occur in different microsites than those in which short-liguled *T. miscellus* occurs (personal observation).

A good example of the extent to which reciprocal origins of polyploids can occur is represented by the *Polypodium vulgare* complex. This complex comprises five allopolyploids, three of which are known to have reciprocal origins, *P. calirhiza, P. hesperium,* and *P. virginianum* (Figure 3) (Haufler et al., unpublished). In *P. hesperium,* the two different cytoplasmic forms appear to have very different geographic distributions. One cytoplasmic form occurs along the northwestern coast of North America and in the southwestern U.S., whereas the reciprocal cytoplasmic form occurs in the interior northwest (Washington, Idaho, and Montana). Whether this reflects chance or underlying cytoplasmically based differences is unknown. However, these results further demonstrate an additional genetic component of polyploid species that must be considered in future studies aimed at elucidating the success of polyploids in natural populations.

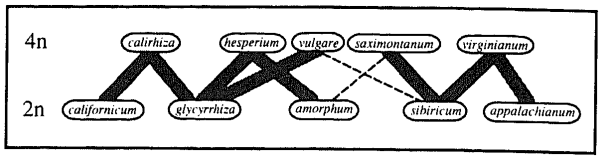


**FIGURE 3.** Chloroplast inheritance in tetraploid *Polypodium* species. Shaded lines indicate diploid(s) contributing chloroplasts to the tetraploids. (From Haufler, unpublished.)

## IV. FREQUENCY AND GENETIC CONSEQUENCES OF AUTOPOLYPLOID EVOLUTION

In only the past 5 years, molecular data have provided a dramatic reshaping of longstanding views regarding autopolyploid evolution. Traditionally, autopolyploid evolution was viewed as maladaptive (e.g., see Stebbins;[165,166] see review by Levin[79]). Stebbins[166] stated, for example, that "chromosome doubling by itself is not a help but a hindrance to the evolutionary success of higher plants." In part, this view was based on the assumption that autopolyploidy is accompanied by chromosome pairing difficulties, resulting in the frequent formation of multivalents and a concomitant reduction in pollen and seed fertility.[165] Because of its presumed adverse evolutionary consequences, autopolyploidy was considered extremely rare in natural populations of plants. Clausen et al.[18] recognized *Galax urceolata* (referred to at that time as *G. aphylla), Biscutella laevigata,* and *Zea perennis* as the only clear-cut examples of naturally occurring auto-

**TABLE 2**
**Examples of Tetrasomic or Higher Level Polysomic Inheritance in Natural Populations**

| Taxon | Inheritance | Evidence | Ref. |
|---|---|---|---|
| *Allium nevii* | Tetrasomic | Isozymes | 130 |
| *Chrysanthemum morifolium* | Hexasomic | Morphology | 74 |
| *Dactylis glomerata* | Tetrasomic | Isozymes | 85, 86 |
| *Dahlia variabilis* | Tetrasomic | Morphology | 75 |
| *Haplopappus spinulosus* | Tetrasomic | Isozymes | 55 |
| *Heuchera grossulariifolia* | Tetrasomic | Isozymes | 195 |
| *H. micrantha* | Tetrasomic | Isozymes | 150 |
| *Lotus corniculatus* | Tetrasomic | Cyanogenic markers, isozymes | 27, 124 |
| *Lythrum salicaria* | Tetrasomic | Morphology | 42, 43 |
| *Maclura pomifera* | Tetrasomic | Isozymes | 135, Schnabel et al. (unpublished) |
| *Medicago falcata* | Tetrasomic | Morphology, isozymes | 119, 163, 168 |
| *M. sativa* | Tetrasomic | Isozymes | 119, 163 |
| *Pachycereus pringlei* | Tetrasomic | Isozymes | Murawski et al. (unpublished) |
| *Phleum pratense* | Hexasomic | Morphology | 102 |
| *Solanum tuberosum* | Tetrasomic | Morphology, isozymes | 66, 91, 123 |
| *Tolmiea menziesii* | Tetrasomic | Isozymes | 144 |
| *Turnera ulmifolia* var. *elegans* | Tetrasomic | Isozymes | 137, 138 |
| *T. ulmifolia* var. *intermedia* | Tetrasomic | Morphology, isozymes | 137, 138 |
| *Vaccinium corymbosum* | Tetrasomic | Isozymes | 72 |

polyploids. Stebbins,[164,165] in contrast, considered only *G. urceolata* (= *G. aphylla*) to be a clear example of autopolyploidy in natural populations. Although recent workers stressed that autopolyploidy played a much more important role in nature (e.g., see Levin[79] and Lewis,[80] who listed 20 examples of naturally occurring autopolyploids), the view of autopolyploidy as being rare and maladaptive has prevailed, deterring the investigation of autopolyploid evolution.[145]

Although there has been controversy considering the definition of autopolyploidy (see review by Soltis and Rieseberg[145]), most agree that autotetraploids should be characterized by tetrasomic inheritance, or higher-level polysomic inheritance in the case of higher-level autopolyploids. The classification of polyploids widely followed today was proposed by Stebbins,[164] who recognized three major types based on genetic and cytogenetic criteria: autopolyploids, allopolyploids, and segmental allopolyploids. Following Stebbins, "autopolyploids usually are characterized by the presence of multivalents at meiosis, [and] of tetrasomic ratios...". Inheritance data therefore provide the strongest and least equivocal mechanism for the identification of an autopolyploid. However, inheritance studies using morphological characters have historically been limited by the rarity of single-gene polymorphisms.[72] As a result, tetrasomic inheritance has rarely been documented in naturally occurring autotetraploid plants. Notable exceptions include the yellow ground color of the flower of *Dahlia variabilis*[75] and cyanogenic markers in *Lotus corniculatus*.[27] Hexasomic inheritance was shown in *Phleum pratense* using seedling coloration.[102]

In contrast to morphological traits, isozyme markers provide an alternative source of genetic polymorphism that is easily scored and interpreted. As a result, isozyme electrophoresis has greatly facilitated the study of polysomic inheritance in plants. We found 19 examples of tetrasomic or hexasomic inheritance (Table 2), most documented within the past 5 years, using isozyme markers. Thus, a rapidly growing data base suggests that polysomic inheritance is more common than previously believed; the importance of this genetic system in natural populations was certainly underestimated in the past.

Many additional examples of autopolyploids have been reported based on the identity, or near identity, of the polyploid to a single diploid (putative progenitor) species using molecular markers such as allozymes and/or cpDNA restriction

site variation. This list of recently documented putative autoploids is large and includes *Coreopsis grandiflora* var. *longipes*,[23] *Galax urceolata*,[38] *Galium pusillum*,[134] *Plantago media*,[173,174] *Thinopyrum flaccidifolium*,[99] *Zea perennis* (Reference 32 and Doebley, personal communication), several species of *Najas*,[171] and two members of the *Pellaea glabella* complex.[45] Despite the fact that these and many other putative autopolyploid taxa have not been examined for polysomic inheritance, the increasing number of putative autoploids (see Lewis[80] for a partial listing) further points to the potential importance and prevalence of autopolyploidy in natural populations. Although autopolyploidy certainly is not nearly as common as allopolyploidy, it nonetheless appears to be of major evolutionary importance.

Molecular data also provide compelling genetic evidence to contradict the traditional view of autopolyploidy as maladaptive. Data from enzyme electrophoresis have revealed three important attributes of autopolyploids compared to their diploid progenitors: (1) enzyme multiplicity, (2) increased heterozygosity, and (3) increased allelic diversity. Several recent studies have shown that individual autotetraploid plants may maintain three or four alleles at a single locus, whereas a single diploid plant can, of course, only maintain one or two alleles per locus. In autotetraploid *Tolmiea menziesii,* for example, 30% of the 678 tetraploid plants collected in nature exhibited 3 or 4 alleles at 1 or more loci.[147] As a result of this increased number of alleles per locus, enzyme multiplicity is evident in autopolyploid plants. For example, for a dimeric enzyme such as PGI or TPI, an autotetraploid plant can produce more types of hybrid enzymes (heterodimers) at a given locus than can a heterozygous diploid. A heterozygous diploid plant can produce a maximum of three enzymes at a locus specifying a dimeric enzyme: two parental enzymes plus a single heterodimer. At that same locus, an autotetraploid having three alleles can produce six enzymes (three parental enzymes plus three heterodimers), and an autotetraploid with four alleles can produce ten enzymes (four parental enzymes plus six heterodimers) (reviewed in Soltis and Rieseberg[145]). The presence of multiallelic genotypes and enzyme multiplicity has been reported for several autopolyploids, including *Tolmiea menziesii*,[147] *Heuchera grossulariifolia*,[196] *H. micrantha*,[101] *Medicago sativa* and *M. falcata*,[119] *Coreopsis grandiflora* var. *longipes*,[23] *Solanum tuberosum*,[91] and *Allium nevii*.[130,131]

Levels of genetic variability in autopolyploid species clearly are influenced by a number of features including breeding system, frequency of multiple origins, and historical factors such as genetic bottlenecks. However, autotetraploids should theoretically exhibit higher levels of genetic variability than their diploid progenitors simply due to tetrasomic inheritance.[97] Empirical studies demonstrate that genetic variability is typically substantially higher in autotetraploids compared to their diploid progenitors (Table 3). In five of the six examples given in Table 3, values of $P$ (proportion of polymorphic loci), $H_o$ (observed heterozygosity), and $A$ (mean number of alleles per locus) are significantly higher in autotetraploids than in their diploid progenitors. In *Dactylis glomerata,* for example, heterozygosity within diploid populations ranged from 8 to 31% (average 17%), whereas in tetraploids the range was 41 to 50% (average 43%). In *Tolmiea menziesii,* heterozygosity also was significantly higher in autotetraploid populations compared to diploid populations: observed heterozygosity was 0.070 and 0.237 in diploid and tetraploid *Tolmiea,* respectively.[147] Similar results have been observed in other comparisons of autotetraploids and their diploid progenitors, such as *H. micrantha*,[101] *H. grossulariifolia*,[196] and *Turnera ulmifolia* var. *elegans*.[138] Although it had been predicted that increased heterozygosity should characterize autopolyploids,[7,52,79,100,164,165] only recently have these genetic features been documented in natural populations (Table 3). In addition to increased heterozygosity, autotetraploids also exhibit increased allelic diversity (Table 3). For example, in *D. glomerata* the mean number of alleles per locus and per population is more than twice as high in tetraploid populations compared to diploid populations (reviewed in Lumaret[87]). Significantly higher values of $A$ also have been observed in comparisons of autotetraploid and diploid cytotypes in *Tolmiea menziesii, H. grossulariifolia,* and *Turnera ulmifolia* var. *elegans* (Table 3). Values of $P$ are similarly higher in most autotetraploids (Table 3). The only study in which genetic variation was not higher in an

**TABLE 3**
**Measures of Genetic Variation within Populations of Diploids and Autotetraploids Compared Using Enzyme Electrophoresis**

| Taxon | $P$ | | $H_o$ | | $A$ | | Ref. |
|---|---|---|---|---|---|---|---|
| | 2× | 4× | 2× | 4× | 2× | 4× | |
| *Tolmiea menziesii* | 0.240 | 0.408 | 0.070 | 0.237 | 3.0 | 3.75 | 147 |
| *Heuchera grossulariifolia* | 0.238 | 0.311 | 0.058 | 0.159 | 1.35 | 1.55 | 196 |
| *H. micrantha* | 0.240 | 0.383 | 0.074 | 0.151 | 1.41 | 1.64 | 101 |
| *Dactylis glomerata* | 0.70 | 0.80 | 0.17 | 0.43 | 1.51 | 2.36 | 84, 85, 87 |
| *Turnera ulmifolia* var. *elegans* | 0.459[a] | 0.653 | 0.11[a] | 0.42 | 2.20[a] | 2.56 | 138 |
| *T. ulmifolia* var. *intermedia* | 0.459 | 0.201 | 0.11 | 0.07 | 2.20 | 2.00 | 138 |

*Note:* $P$ = proportion of polymorphic loci; $H_o$ = mean observed heterozygosity; $A$ = mean number of alleles per polymorphic locus.

[a] Shore[138] compared both *Turnera ulmifolia* var. *elegans* (4×) and var. *intermedia* (4×) to var. *intermedia* (2×).

autotetraploid than in its diploid progenitor involved *Turnera ulmifolia* var. *intermedia* (Table 3). In this taxon, values of $P$ and $H_o$ were actually significantly lower in the autotetraploid than in the diploid, whereas values of $A$ were not significantly different. In contrast, autotetraploid *T. ulmifolia* var. *elegans* has significantly higher values of $P$, $H_o$, and $A$ compared to diploid populations. The autotetraploid populations of *T. ulmifolia* var. *intermedia* examined were restricted to islands, and the resulting founder effect following long-distance dispersal to the islands, as well as subsequent inbreeding, could account for low levels of genetic variation in island populations of this taxon.[138]

Polyploids may have a fitness advantage over their diploid progenitors due to their increased biochemical diversity.[79] In allopolyploids, this increased biochemical diversity is achieved via "fixed heterozygosity," which results from the addition of divergent genomes and promotes enzyme multiplicity. First documented in the recent allotetraploids *Tragopogon mirus* and *T. miscellus*,[132] fixed heterozygosity has been a standardly observed feature of allopolyploids (see reviews by Crawford[22,24–26]). Molecular data for autopolyploids indicate that they too have increased biochemical diversity compared to their diploid progenitors. However, rather than exhibiting fixed heterozygosity, autoploids achieve this biochemical diversity and enzyme multiplicity through increased heterozygosity and allelic diversity due to tetrasomic inheritance. Thus, rather than the traditional view of autopolyploidy as maladaptive, molecular data provide strong genetic arguments for the potential success of autopolyploids in nature.

## V. GENOME EVOLUTION IN POLYPLOIDS

Although molecular data have provided important insights into the parentage, formation, and immediate genetic consequences of polyploidy, much less is known about the subsequent evolution of polyploid genomes following their formation. Several suggestions have been proposed regarding the evolution of polyploid genomes. Following a polyploid event, the genome is expected to experience gene silencing.[51,105]

Given sufficient time, the number of genes expressed in a polyploid could be sufficiently reduced to return the polyploid to a level of expression similar or identical to that of the diploid ancestor, i.e., the polyploid would become genetically diploidized.[51,56,78,105] Leipoldt and Schmidtke[78] suggest that this diploidization process also would involve chromosomal rearrangement. Others have noted that the polyploid process provides an abundance of redundant genes that can subsequently undergo mutation and potentially evolve new functions.[105,135a] This latter view holds that polyploidy has enormous potential for bringing about subsequent evolutionary change. In recent years, molecular data have provided compelling evidence for gene silencing and chromosomal rearrangements in polyploids. Examples of these processes are provided in the following sections.

## A. Gene Silencing

Direct evidence for the silencing of duplicate genes in polyploids (of fairly recent origin) has been obtained for several angiosperms and ferns. We stress here that "gene silencing" actually includes several different types of mutations: those that prevent transcription and those that involve transcription and translation of a nonfunctional protein. Most studies to date that have provided evidence for gene silencing involve enzyme electrophoresis, and it typically was not possible to differentiate between these two possible types of gene silencing.

One of the most frequently cited examples of loss of duplicate gene expression in plants involves tetraploid *Chenopodium*.[193] Null alleles were observed at each of two duplicated leucine aminopeptidase (LAP) loci that resulted from allotetraploidy. Genetic analyses demonstrated that single-banded LAP phenotypes in tetraploid *Chenopodium* species are the result of homozygosity for null alleles. Furthermore, loss of duplicate gene expression for LAP apparently occurred independently in several different taxa. Several other polyploid species exhibit apparent gene silencing. Isozyme studies of hexaploid wheat, *Triticum aestivum,* indicate that only a small percentage of the triplicate isozyme structural genes produced by polyploidy have been silenced.[54] Nonetheless, strong evidence for gene silencing was provided for one locus (representing the B genome) of the β-*Amy-1* set. Another possible example of gene silencing in wheat involves the isozyme locus *Ep*-1. Evidence for the silencing of a duplicate TPI locus was obtained in *Clarkia breweri,*[113] and gene silencing also may have occurred in *Z. mays* (maize), with two types of null alleles at a duplicate TPI locus, *Tpi-4*.[185]

Convincing evidence for gene silencing was recently provided for *Pellaea rufa,* an allotetraploid fern species.[46] Gastony demonstrated progressive silencing of the gene for cytosolic PGI in one ancestral genome of this relatively recently formed allotetraploid, resulting in a completely diploidized electrophoretic phenotype for this enzyme. Many other isozyme studies also suggest silenced gene expressioon in other allotetraploid ferns, although the evidence is not as convincing as that provided for *Pellaea rufa.* These include several species of *Asplenium,*[189] *Polypodium virginianum,*[16] *P. vulgare* (Haufler, personal communication), and *Polystichum californicum.*[159] Additional examples of gene silencing revealed by enzyme electrophoresis have been proposed for tetraploid fish.[2,41,81]

Gene silencing also can be documented indirectly by comparing the distribution of duplicate gene expression to the presumed phylogenetic relationships of the species involved. Comparison of the distribution of a duplicated gene for plastid phosphoglucomutase (PGM) in diploid species of *Clarkia* with interspecific relationships based on morphology and a cpDNA-based phylogeny suggests two separate silencing events for duplicated PGM, one each in *C. concinna* and *C. lassenensis.*[156,167] Using a similar approach, Weeden et al.[181] suggested that silencing of duplicated cytosolic phosphoglucoisomerase (PGI) has occurred many times in the Leguminosae. Gene silencing has apparently occurred in many tribes, including Vicieae, Trifolieae, and Cicereae, and also numerous times at lower taxonomic levels.[181]

Werth and Windham[190] have greatly extended the concept of gene silencing to propose a model of allopatric speciation at the polyploid level based on different patterns of gene silencing

in populations from different geographic areas. These workers base their model on a process of "reciprocal silencing," that is, the possibility that allopatric populations of a single allotetraploid species may undergo silencing of the same gene, but in different parental genomes. Because hybrids between two such reciprocally silenced genotypes would result in some reduction in the viability of gametophytic progeny, these workers suggest that if reciprocal silencing at enough genes occurs, intersterility will ultimately result.

Isozyme data clearly indicate, therefore, that gene silencing can occur. A more direct analysis of the evolutionary processes occurring at the gene level in polyploids is offered, however, by investigations of the actual structure of individual genes. Although few comparisons of this type have been conducted, several studies reveal the unique opportunities such analyses provide for studying molecular evolution in polyploids.

A model study in this regard is provided by Lee and Verma,[76] who determined the structure and chromosomal arrangement of leghemoglobin (Lb) genes in *Phaseolus vulgaris* (kidney bean), a diploid having $n = 11$. The gene organization for Lb genes in *P. vulgaris* was compared with that found in two other legume species, *Glycine max* (cultivated soybean) and *G. soya* (wild soybean). Both of these species of *Glycine* have $n = 20$ and are considered to represent ancient polyploids. Through the isolation and sequencing of Lb genes, Lee and Verma[76] traced the possible evolutionary divergence in the structure and arrangement of Lb genes in these three species (Figure 4). Their results suggest three major evolutionary events leading to soybean Lb genes. Beginning with a single primordial globin gene (Figure 4), the following occurred.

1. Two gene duplication events occurred at the diploid level, producing a locus having four Lb genes. This condition is present in the diploid, *P. vulgaris*.
2. A truncated gene was generated (LbT), probably through unequal crossing over, following the divergence of *P. vulgaris* and the diploid ancestor of *Glycine*.
3. Genome duplication (polyploidy) took place in the ancestors of *G. max* and *G. soya* resulting in two Lb loci, each with four genes, and two LbT loci.
4. Finally, a deletion (12 kb) in one of the Lb loci resulted in the loss of two Lb genes from *G. max;* only one functional gene and a pseudogene ($\Psi 2$) remain.

Thus, the study of Lee and Verma demonstrated two important processes in the genome evolution of polyploids: gene silencing and the occurrence of relatively large (12 kb) deletion events. Interestingly, in terms of gene function, whereas only one major Lb is produced in the diploid *P. vulgaris,* several different Lbs seem to be present in *Glycine* species. Variability and differences in temporal expression of Lbs in *Glycine* suggest possible distinct roles for each gene.

Another possible example of changes in gene structure associated with polyploid evolution involves the fern *Polystichum munitum.* Pichersky et al.[116] demonstrated the presence of multiple defective copies of chlorophyll *a/b*-binding (CAB) protein genes in this species that may be the result of gene silencing following ancient polyploidy. Ferns and their allies present a particularly intriguing situation in the study of genome evolution in polyploids and are discussed in more detail later.

## B. Extensive Genome Diploidization

Allozyme and molecular genetic data certainly document the likelihood of gene silencing. Whether wholesale gene silencing can occur to the extent envisioned by some,[51,57] ultimately resulting in a diploidized polyploid, is a major question in polyploid evolution. One difficulty in addressing this question involves the obvious paradox that the more extensively the process of diploidization has occurred, the more difficult it will be to document. Nonetheless, recently obtained molecular data strongly suggest the possibility of gene silencing on a massive scale.

Some of the earliest data to suggest that silenced copies of duplicate genes may be present at many loci were obtained for polyploid fish. As reviewed by Li,[81] isozyme data for tetraploid fishes indicate that, in every enzyme system examined, loss of gene expression has occurred in at least one species. Furthermore, some of these fish species may have lost expression of 70% or more of their duplicate genes. This would seem

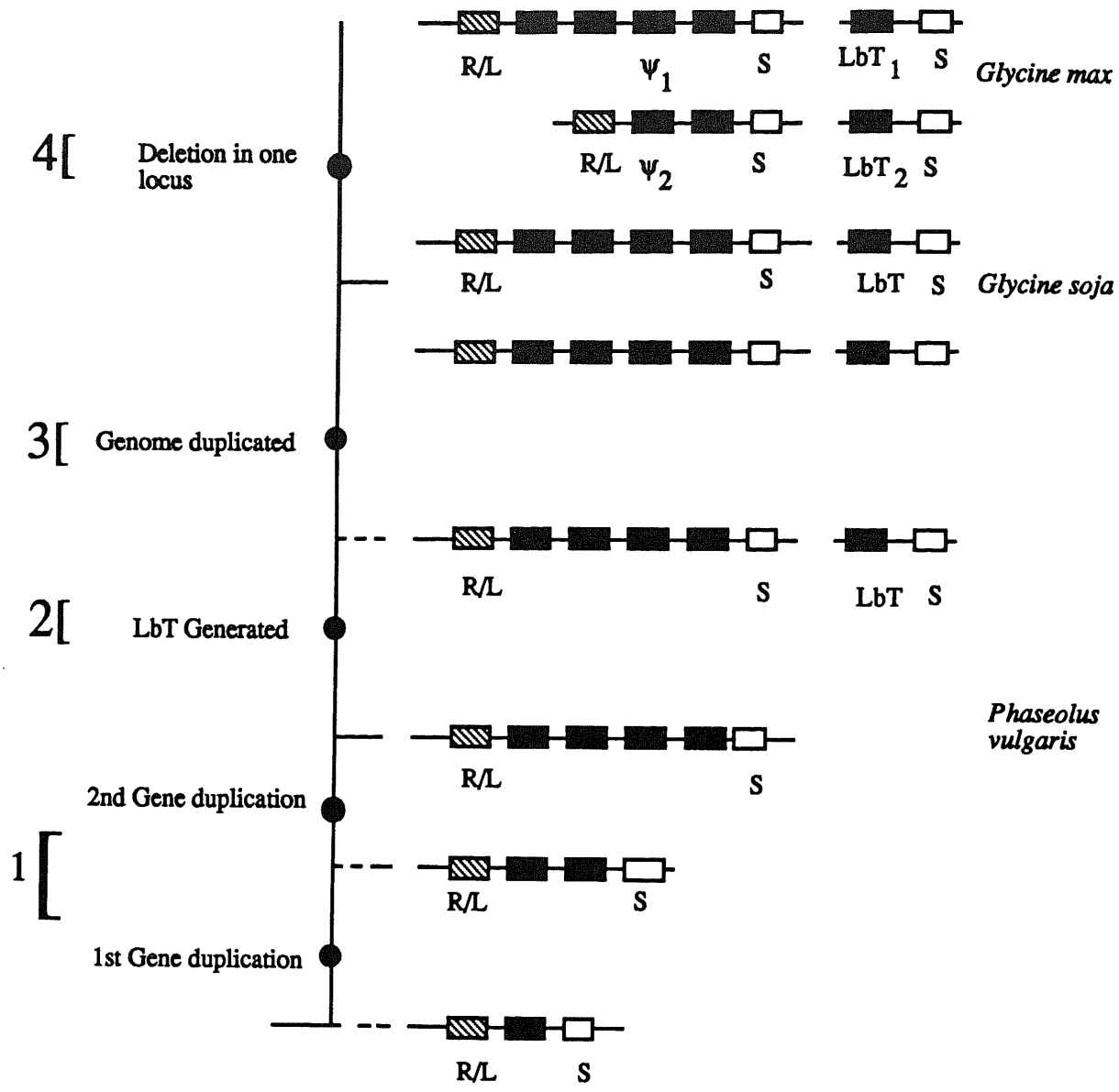


**FIGURE 4.** Phylogeny of the leghemoglobin (Lb) loci. The chromosomal arrangements deduced from this study revealed possible events that could have occurred through evolutionary time to lead to the leghemoglobin organization in soybean. R/L = flanking sequence at 5′ end; S = flanking sequence at 3′ end; LbT = truncated gene that was formed after divergence of *Phaseolus* and ancestor of *Glycine max* and *G. soya*. The large numbers 1, 2, 3, and 4 refer to points of discussion in text of this review. (Modified from Lee, J. S., and Verma, D. P., *EMBO J.*, 3, 2745, 1984.)

to suggest that loss of duplicate genes is not harmful or, at worst, has only mildly deleterious effects.[6,81] Bailey et al.[6] also note that loss of duplicate gene expression in fishes has apparently occurred in a random fashion among the species involved.

Pteridophytes (ferns and fern allies) undoubtedly represent the most intriguing group of plants with regard to the possibility of wholesale genetic diploidization (see reviews by Haufler,[56,57] Soltis et al.,[155] and Soltis and Soltis[149]). Because most lineages of homosporous pteridophytes have extremely high chromosome numbers compared to those of heterosporous pteridophytes, seed plants, and bryophytes, they have been considered by some to represent ancient polyploids.[71,178] The mean basic chromosome number of homosporous pteridophytes is $n = 55$, whereas that for angio-

sperms is approximately $n = 16$. However, other data suggest the possibility of an alternative explanation. Enzyme electrophoretic studies of ferns and other pteridophytes have revealed only diploid isozyme expression in these plants, despite their extremely high chromosome numbers.[56,57,142–144,157] These results for all major lineages of homosporous pteridophytes having high chromosome numbers are made more intriguing by isozyme data for woody angiosperms having high chromosome numbers. Angiosperm families such as Magnoliaceae, Lauraceae, Calycanthaceae, and Salicaceae similarly have been considered ancient polyploids; furthermore, several of these angiosperm families are regarded as very old. Significantly, however, polyploid gene expression was demonstrated in isozyme surveys of these families.[152] In addition, critical analyses of karyotypic data failed to reveal convincing evidence of multiple chromosome sets in homosporous pteridophytes.[36] As a result, homosporous pteridophytes either (1) are not truly ancient polyploids, in which case they may have achieved high chromosome numbers through aneuploidy, or (2) they represent ancient polyploid lineages that have experienced extensive diploidization (reviewed in Soltis and Soltis[149]).

Molecular genetic data for ferns, while still incomplete, begin to favor hypothesis (2) noted above. If pteridophytes are of ancient polyploid origin, they should possess multiple, nonfunctional copies of genes, particularly those regarded as "single-copy" in diploid angiosperms. In contrast, if these plants are true diploids, they should possess approximately the same number of copies of genes as observed in diploid angiosperms. Significantly, multiple copies of defective genes encoding the CAB proteins have been detected in the genome of the isozymically diploid fern *Polystichum munitum* ($n = 41$).[116] In angiosperms, CAB genes occur as a small gene family of 3 to 16 copies;[115] only a single defective CAB gene has been detected in the scores of CAB genes analyzed in flowering plants,[114] whereas a majority of copies detected in *P. munitum* were defective. Thus, the presence of numerous defective CAB genes in *P. munitum* supports the hypothesis of ancient polyploidy and subsequent gene silencing. However, other explanations for these defective CAB genes are possible: (1) several copies of a diploid complement of CAB genes have mutated to a nonfunctional state; and (2) some defective CAB genes have been specifically amplified in the genome of *P. munitum* during the course of evolution. These latter two explanations seem less likely, however, given that neither of these processes has been observed in CAB genes from angiosperms.

Preliminary data for the fern *Ceratopteris richardii* also support an ancient polyploid origin for ferns (McGrath and Pichersky, unpublished). These workers attempted to estimate copy number for several genes in *Ceratopteris* by gel blot hybridization with cloned cDNAs. With each cDNA probe, a strong band of hybridization was apparent, as were intense smears in the background. Although these data are preliminary, McGrath and Pichersky suggest that these smears may represent related sequences resulting from ancient polyploidy.

Because mechanisms of gene silencing may involve DNA methylation, McGrath and Pichersky[93] examined the level and distribution of 5-methylcytosine nucleotides in three homosporous pteridophytes: *C. richardii* ($n = 39$), *P. munitum* ($n = 41$), and *Psilotum nudum* ($n = 52$). Each species shows an overall level of 5-methylcytosine similar to angiosperms. Thus, these data are inconclusive with regard to massive gene silencing due to the fact that genes can be silenced through a variety of mechanisms. Significantly, however, genomic blots indicate that the majority of signals detected from CAB gene sequences appears in methylated regions in all three of these species, thus suggesting widespread silencing of CAB genes in ferns.

At this point, therefore, some molecular data strongly support the hypothesis that ferns are ancient polyploids and that massive gene silencing has occurred in these plants. Additional data for other genes and other species of pteridophytes must be obtained, however, in order to evaluate unambiguously the possibility of massive gene silencing in pteridophytes.

*Z. mays* and *Sorghum bicolor* represent two additional possibilities of extensive genome diploidization. However, the controversy regarding the evolutionary history of maize and sorghum is similar to that reviewed earlier for homosporous pteridophytes. Based on cytogenetic data,

Rhoades[129] first suggested that the genome of maize ($n = 10$) may be the result of ancient polyploidy from an ancestor having $n = 5$. This argument was later extended to sorghum and other Andropogoneae.[17,44] Duplicate genes for isozymes have been reported for several isozyme systems in maize, including HK, MDH, TPI, PGM, and 6PGD (reviewed by Wendel et al.[185]). In addition, linkage analyses have shown that most duplicate loci occur in parallel linkage relationships with members of other gene pairs, supporting an ancient polyploid origin. Duplicate isozyme loci also are present in sorghum.[98] However, many maize and sorghum enzyme systems show the number of isozymes typical of diploid seed plants.[98,185] Two hypotheses can be advanced to explain these data:[185] (1) the genomes of maize and sorghum are of polyploid origin, but diploidization and gene silencing have occurred extensively in many genomic regions; and (2) the present level of gene duplication and parallel linkages reflect extensive duplication of chromosomal segments in diploid plants.[47,184]

Analyses of the maize and sorghum genomes using a large number of cloned maize sequences and RFLP analysis revealed extensive duplication of RFLP loci.[60,67] Helentjaris et al.[60] concluded that maize may be of ancient polyploid origin, but because duplicate loci do not primarily involve five pairs of chromosomes, the genome of maize is not now structured as an allopolyploid (this is discussed in more detail later).

Comparative genome mapping of sorghum and maize also provided evidence to support an ancient polyploid origin for both genera.[191] However, the chromosomal patterns of both maize and sorghum also could be derived via segmental duplication without polyploidy. Whitkus et al.[191] favor polyploidy as the more parsimonious explanation.

Thus, at this point, the question of ancient polyploidy vs. segmental duplication is not completely resolved in sorghum and maize, although the data seem to support ancient polyploidy.[60,191] If maize and sorghum truly are ancient polyploids, then considerable gene silencing has occurred in both genera.[185,191] Furthermore, because a larger proportion of duplicated loci is present in maize than in sorghum, the loss of duplicated segments has occurred more rapidly in the lineage leading to sorghum than in that leading to maize.[191]

## C. Regulatory or Functional Divergence of Duplicate Genes

Regulatory or functional divergence of duplicate genes has been studied more extensively in animals than in plants. Perhaps the best example of differential expression of duplicate genes is provided by creatine kinase B loci in the polyploid fish *Carpiodes cyprinus*,[41] where the B21 form predominates in eye tissue and the B22 form of the enzyme is expressed only in heart tissue. Functional divergence of loci also was demonstrated for salmonid fishes, which represent ancient tetraploids. H4 and H41 lactate dehydrogenases in salmonid fishes differ in thermal stability, as well as in all catalytic properties examined, including substrate optima, Michaelis constants, and susceptibiltiy to inhibition by high levels of substrate.[5,82] Ferris and Whitt[41] maintain that in catostomid fishes of ancient polyploid origin, divergence in regulation or function appears to have occurred at most of the duplicate loci that are still retained. Similarly, Allendorf[1] has emphasized the importance of these phenomena in tetraploid fishes.

Convincing examples of divergence in regulation or function of duplicate genes in polyploid plants are few. One possible example of functional divergence involves duplicated Lb genes in soybean.[76] Only one major Lb is produced in *P. vulgaris*, which is diploid. In contrast, in *G. max*, an ancient tetraploid, Lee and Verma[76] note that the multiplicity, variability, and temporal expression of Lb genes indicate a possible distinct role for each during nodule development.

Another possible example of divergence in gene function and regulation involves actin genes in soybean.[61] DNA sequence analysis and genomic blotting experiments indicated that five of six soybean actin gene family members studied were significantly more diverged from one another than are members of other known actin gene families. This heterogeneity suggests that the members of this gene family also may have diverged in function and/or regulation. More recently, tissue-specific expression of three diver-

gent actins was demonstrated in soybean roots.[92] One hypothesis, therefore, is that divergence and differential expression of actin genes in soybeans followed an ancient polyploidy event. Another possibility, however, for the existence of three pairs of actin genes in soybean is recent gene duplication.

## D. Chromosomal Repatterning

RFLP data and RAPD markers have provided unprecedented insights into the evolution of nuclear genomes. Comparative genome mapping is a powerful approach for investigating chromosomal evolution. Using molecular markers such as RFLPs, RAPDs, and isozyme loci, comparative genome mapping facilitates the analysis of the extent of chromosomal rearrangements between species.[169]

These approaches also have been applied to the study of several polyploid genomes and have yielded important new information regarding genomic changes following polyploidization. Perhaps the best studied system to date is represented by some of the economically important *Brassica* species. Based on interspecific hybridization and cytogenetic data, relationships were proposed among three diploid species:[172] *B. campestris* (= *B. rapa*) (*n* = 10), *B. nigra* (*n* = 8), and *B. oleracea* (*n* = 9); and three allotetraploids: *B. napus* (*n* = 19), *B. juncea* (*n* = 18), and *B. carinata* (*n* = 17). The parentage of the three allopolyploids in the "triangle of U" are as follows (Figure 5): *B. napus* = *B. campestris* × *B. oleracea; B. juncea* = *B. campestris* × *B. nigra; B. carinata* = *B. nigra* × *B. oleracea.* Numerous lines of evidence subsequently confirmed U's hypothesis, including data from enzyme electrophoresis[21] and nuclear RFLPs[28,65,160] (reviewed in Song et al.[160]). In addition, restriction site analysis of cpDNA provided information on the maternal parents of each allotetraploid (Figure 5).[39,109,161] Thus, this well-studied complex provides an important model for the study of polyploid genomes.

It has been suggested that the three diploid *Brassica* species (*B. nigra, B. campestris,* and *B. oleracea*) are actually balanced ancient polyploids derived from a lower original base chromosome number,[118] and isozyme data support this hypothesis.[120,122] Recently, the genome organization of *B. oleracea* has been studied in detail based on the inheritance of RFLPs using random genomic fragments and cDNA clones.[69,94,140] Both studies revealed extensive duplication of genetic markers, in support of the hypothesis that *B. oleracea* is actually an ancient polyploid. Using the cDNA RFLPs located to chromosomes of *B. oleracea,*[94] a comparative map of *B. campestris* was obtained.[95] As with *B. oleracea,* the genome of *B. campestris* also showed extensive gene duplication, and the majority of duplicated loci was again unlinked. Comparison of the *B. oleracea* and *B. campestris* genomes suggests that these secondary, or ancient, polyploids share a common diploid ancestor. Significantly, these molecular data also suggest that some linkage blocks have been conserved between *B. oleracea* and *B. campestris.* In addition, chromosome repatterning also has occurred because some loci have altered syntenic associations.[95] A similar conclusion was reached by Slocum[139] in a comparative analysis of RFLP maps for these two *Brassica* species based on random genomic clones.

In a recent review of DNA-based genome maps for *Brassica* species, Quiros et al.[121] provide evidence for considerable plasticity of *Brassica* genomes and suggest the possibility of both intra- and intergenomic recombination. Evidence

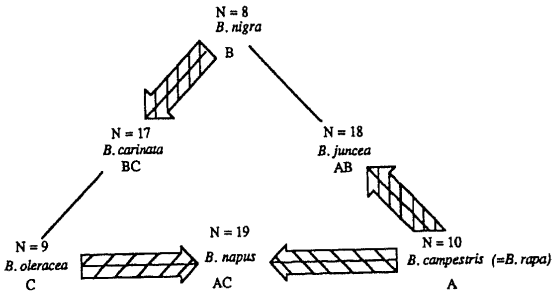


**FIGURE 5.** "Triangle of U" ($U^{172}$), depicting relationships among cultivated diploid and allotetraploid *Brassica* species. The triangle has been updated to show the maternal parents of each allotetraploid (indicated by arrows) based on Erickson et al.,[39] Palmer et al.,[109] and Song and Osborn (in preparation). The origin of *B. napus* apparently is more complex than indicated here.[161]

for intragenomic recombination is provided by a study of the C genome (contributed by *B. oleracea*) in the allotetraploid *B. napus* (genomes AC). In a comparison of natural and synthetic *B. napus*, syntenic differences were observed in the C genome.[94] Although chromosomes 3, 4, 5, and 6 align well in both natural and synthetic *B. napus*, chromosomes 1, 2, and 7 display rearrangements. These and other data (reviewed in Quiros et al.[121]) suggest intragenomic rearrangements following allopolyploidy in *B. napus*. Similarly, Hoenecke and Chyi[62] (reviewed in Quiros et al.[121]) identified major conserved linkage groups in the A genome of *B. napus*, as well as significant linkage rearrangement between the A genomes from the diploid and allotetraploid. Some evidence for intergenomic recombination also has been obtained for *B. napus*[90] (reviewed in Quiros et al.[121]).

Based on nuclear RFLP data, Song et al.[160] provide intriguing information regarding genome evolution in *Brassica*. Their data not only support the proposed ancestries of the three allopolyploids (Figure 5), but also suggest that the cytoplasmic genomes in the alloploids have played important roles in the subsequent evolution of the nuclear genomes of these polyploids. Their analysis reveals that the nuclear DNA composition of each allotetraploid is more closely related to the diploid parent that contributed the cytoplasm to that tetraploid. This result was most pronounced in the tetraploids *B. juncea* (AB genomes) and *B. carinata* (BC genomes) (Figure 5). Rather than showing a complete combination of nuclear RFLP patterns of the parental diploid species in each case, the allotetraploid RFLP patterns were actually more similar to those of the cytoplasmic donor. That is, the nuclear RFLP pattern of the allotetraploid *B. juncea* (AB) was very similar to *B. campestris* (A genome) and less similar to the other parental genome (B genome) contributed by *B. nigra*. Similarly, the RFLP pattern of *B. carinata* (BC) was more similar to *B. nigra* (B genome) than to the other parental genome (C genome) contributed by *B. oleracea*.

Song et al.[160] propose that the cytoplasm has a profound influence on the evolution of the nuclear genome in these allotetraploid *Brassica* species. When the parental diploid species of an allotetraploid exhibit highly differentiated cytoplasms (as measured by cpDNA restriction site data), the nuclear genome of the male donor has been altered much more than the nuclear genome from the female parent. Thus, in the example of the tetraploids *B. carinata* (BC) and *B. juncea* (AB), the B cytoplasm is distinct from the A and C cytoplasms, and for each of these two tetraploids, the nuclear genome of the allotetraploid is more similar to that of the parental diploid that contributed the cytoplasm. In contrast, the A and C cytoplasms are very similar as determined by cpDNA restriction site analyses.[39,109] Significantly, in the allotetraploid *B. napus*, which has parental nuclear genomes A and C (Figure 5), both the A and C nuclear genomes have evolved with similar rates of change based on nuclear RFLP data.[160]

These data suggest a coevolution of the nuclear and cytoplasmic genomes,[160] and suggest that the native cytoplasm may provide selection pressure on portions of the foreign nuclear genome, stabilizing the newly produced allotetraploid by establishing an "harmonious relationship between cytoplasmic and nuclear genomes." In contrast, if there are no significant differences between the cytoplasms of the parental diploids, as in the allotetraploid *B. napus*, this selection pressure will be minimized and the two nuclear genomes in the allotetraploid will change with similar frequency during the course of evolution.

More recently, Song et al.[162] produced a series of synthetic allotetraploids corresponding to *B. napus*, *B. juncea*, and *B. carinata* to test further their hypothesis that mutual interactions between the maternal cytoplasmic genomes (chloroplastic and mitochondrial) and the paternal nuclear genome may result in changes in the structure of the nuclear genome in a newly produced alloploid. Comparison of nuclear RFLP patterns of these synthetic allotetraploids to the natural allotetraploids demonstrated that the nuclear genomes of the natural alloploids are considerably more distant from the progenitor diploid species than are those of the synthetic allotetraploids. These data suggest that the nuclear genomes of allotetraploid Brassicas may have changed considerably since the origin of these alloploids, and the results parallel those presented above and reviewed by Quiros et al.[121]

The hypothesis of Song et al.[160] that the cytoplasm can influence and provide selection pressure on the nuclear genome has potentially major implications regarding reciprocal origins of polyploids (discussed earlier). That is, if those polyploids having multiple origins due to reciprocal maternal parents have differentially altered nuclear genomes, then the genetic/evolutionary consequences of this type of multiple origin could be considerable.

Another possible example of genome evolution and chromosomal repatterning in polyploids involves comparative genome mapping of sorghum (*S. bicolor*) and maize (*Z. mays*). As previously reviewed, because sorghum and maize both have $n = 10$, they have been considered by some to be ancient polyploids (reviewed in Whitkus et al.[191]). The maize genome was recently characterized in great detail using RFLP analysis and cloned maize sequences.[60] Mapping of the many duplicate RFLP loci revealed a complex pattern of organization. That is, the duplicate loci do not primarily involve five pairs of chromosomes; thus five pairs of homologous chromosomes are not currently present in maize. Although RFLP data support an ancient polyploid origin for maize, the genome of maize is not now structured as an allopolyploid, implying the possibility of extensive chromosomal repatterning following polyploidization.

More recently, Whitkus et al.[191] used maize probes to produce a sorghum linkage map, thus facilitating an evaluation of the processes of genome evolution involved in the divergence of maize and sorghum. These authors determined that most sorghum linkage groups are composed of RFLP loci that predominanatly represent only two maize chromosomes. This pattern is consistent with the hypothesized ancient polyploid origin of maize and sorghum.[60,191] Significantly, nine cases were found in which the locus order within linkage groups shared by sorghum and maize was inverted in sorghum relative to maize. Whitkus et al.[191] propose that this is due either to inversions or to intrachromosomal translocations, finding no evidence of large interchromosomal translocations.

Although the data for maize and sorghum genomes are consistent with chromosomal rearrangement following ancient polyploidy, the occurrence of duplicated chromosome segments in maize may result from duplicated chromosomal segments rather than polyploidy.[60,129,184,191] Whitkus et al.[191] point out that an argument against the chromosomal segment duplication hypothesis is that the extensive duplications in the maize genome would necessitate the occurrence of numerous independent segmental duplications. Thus, they conclude that ancient polyploidy is the more parsimonious explanation, especially when combined with the cytotaxonomic evidence for ancient polyploidy. Finally, these molecular data for maize and sorghum genomes exemplify well the paradox of highly diploidized polyploid genomes. That is, if maize and sorghum truly are ancient polyploids, their genomes have been so extensively reorganized that it is now difficult to discern unambiguously whether these genomes are polyploid or diploid.

A new approach that holds great promise for the study of genome evolution in polyploids is the use of genomic *in situ* hybridization (GISH), which involves the use of total genomic DNA probes.[3,77,136] Molecular approaches such as GISH, which essentially "paint" chromosomes, offer tremendous potential for testing genome relationships and also for studying genome evolution in polyploids. Bennett et al.[10] used GISH to test the hypothesis that *Milium montianum* ($2n = 22$) is an alloploid with diploid *M. vernale* as one parent. Biotinylated total genomic DNA of diploid *M. vernale* was hybridized *in situ* to root tip chromosomes of *M. montianum*. The *M. vernale* probe hybridized preferentially to the eight large, or L, chromosomes of *M. montianum* that some had hypothesized had been donated by the diploid *M. vernale*. These results not only confirmed the alloploid origin of *M. montianum* but, more pertinent to this portion of our review, also show that subsequent to the formation of the allopoloid *M. montianum* the L chromosomes donated by *M. vernale* have largely retained their genomic integrity. Significantly, however, a few regions along the L chromosomes clearly lacked the hybridization signal of the *M. vernale* genomic probe, suggesting that either (1) these sequences arose after the formation of the alloploid, or (2) a limited intergenomic transfer of DNA from chromosomes representing the second genome present in this alloploid (unidentified) to

the L chromosomes donated by *M. vernale*. These results not only demonstrate further the dynamic nature of polyploid genomes, but also indicate the great potential of GISH in studies of polyploid genome evolution.

## VI. FUTURE DIRECTIONS

Molecular data have increased greatly our understanding of polyploid evolution during the past decade. Although these data are highly significant in that they reveal a much more dynamic nature of polyploid taxa and their genomes than perhaps has been historically envisioned, in many ways these studies are only a prelude to future research. Clearly, molecular studies have set the stage for what could be considered the next generation of investigations into the evolution of polyploids and polyploid genomes. Although we have learned a great deal about polyploids and polyploid genomes indirectly through studies aimed at elucidating the parentage of polyploids, it seems clear that simple documentation of polyploid ancestors will provide few new data of importance to the students of polyploid evolution. In the following paragraphs, we provide some suggestions for future studies of polyploids.

Multiple origins of both allo- and autoploids clearly appear to be the rule, rather than the exception, in natural populations of plants. However, the compelling evidence for recurrent polyploidization within species has in virtually all cases been a byproduct of other investigations. Few detailed studies have been conducted using a suite of molecular markers with the specific objective of determining the frequencies of recurrent polyploid events for a given polyploid. Such analyses are needed to determined more accurately just how many times a given polyploid may have been formed. Those few comprehensive studies that have been conducted (e.g., polyploid Nordic *Draba* species and Ownbey's tetraploid Tragopogons) suggest that at least some polyploids may have originated numerous times. The use of RAPD markers, together with allozyme and cpDNA data, may be of particular value in estimating the number of independent polyploidizations.

Numerous questions remain to be addressed regarding the genetic consequences of recurrent polyploidization events within a species. For example, do multiple origins lead to different, locally adapted genotypes? Do plants of different polyploid origin have different evolutionary potentials? These types of questions are best addressed via basic research involving ecological genetics, quantitative genetics, and ecological physiology. Polyploid plants of reciprocal maternal parentage are of particular interest because the two maternal lineages may display profoundly different evolutionary potentials. That most polyploids appear to be of multiple origin also raises the following questions: how likely is gene exchange among allopatric genotypes produced via multiple origins in a given polyploid species and how frequently are new genotypes produced via recombination? In addition, studies are needed to ascertain the extent of gene flow between diploid progenitors and their polyploid derivative; this source of genetic variation in polyploids needs to be critically compared to that resulting from recurrent polyploidization.

Although molecular data show unequivocally that autopolyploidy is much more common than is traditionally maintained, and in fact is widespread in natural populations, we know very little about the underlying reasons for the success of these plants in nature. Diploid-autotetraploid pairs are particularly intriguing because morphological differences between ploidal levels may be cryptic or nonexistent. Basic research involving detailed comparisons of autotetraploids and their diploid progenitors are needed in the areas of ecological genetics, quantitative genetics, and ecological physiology.

In recent years, molecular data have yielded unprecedented insights into genome evolution in polyploid plants. The pioneering work on polyploid *Brassica* genomes exemplifies well the type of molecular genetic work that should be extended to naturally occurring polyploids. What are the extent and frequency of genome reorganization in naturally occurring auto- and allopolyploids? Technological advances such as GISH may offer a relatively rapid means of addressing this question in a wide array of polyploids.

With regard to the fate of duplicated genes and genomes, many basic questions remain. For example, how quickly do gene silencing and genome reorganization occur? Can massive diploidization of a polyploid genome occur? Recently

formed alloploids, such as the Tragopogons and *Senecio cambrensis*, may offer particularly important models for studying the rate of genomic change in polyploids, and molecular studies of putative ancient polyploids could reveal multiple copies of silenced genes. Furthermore, what are the effects of these types of genomic changes on sterility barriers? Addressing these and other related questions will provide a better understanding of the evolutionary significance of auto- and allopolyploidy in plants.

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