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Recurring Origins of Allopolyploid Species in *Asplenium*

Abstract. *A large proportion of plant species has originated through allopolyploidy: interspecific hybridization followed by chromosome doubling. Heterozygosity remains fixed in allopolyploids because of nonsegregation of parental chromosomes. Two allotetraploid species of the fern genus* Asplenium *show allozyme polymorphisms at loci that are polymorphic in their diploid progenitors, indicating that each has originated more than once and implicating continued gene flow from diploids to tetraploids.*

Allopolyploidization is a prevalent mode of speciation in plants (*1*). In contrast to speciation through divergence at a diploid level, which requires numerous generations (*2*), allopolyploids form abruptly through interspecific hybridizations and subsequent doubling of chromosomes (*3*). Reproductive isolation from progenitors is effected instantaneously through differences in ploidy. If well-differentiated species hybridize, resulting allopolyploids have fixed heterozygous genotypes at many loci because of nonsegregation of nonhomologous chromosomes (*4, 5*). In this respect the population genetic structure of allopolyploids is similar to that of apomictic species, which are often of hybrid origin as well. Apomixis is an alternative reproductive strategy for a genetically favorable but meiotically impaired hybrid (*6*). The retention of sexuality in an allopolyploid, however, is important in that it favors increased diversity in the gene pool of the allopolyploid species. Our alloyzme data on the Appalachian *Asplenium* fern complex show that a major source of genetic diversity in allopolyploids can be the recurrence of allopolyploidization events.

The hybridization that launches an allopolyploid species is often recognizable many generations later by the fixed genotype that results. Parentages of numerous allopolyploids have been described throughout the world's floras. Perhaps there are no firmer data than those on the Appalachian-Ozarkian species of *Asplenium* in which the presumed parentages of three allotetraploid species, as originally proposed on morphological grounds (*7*), have been corroborated by data from flavonoids (*8*) and allozymes (*5*). One of the allotetraploids, *A. ebenoides* (*platyneuron* × *rhizophyllum*) is known from a single locality in Alabama, although its analogous sterile F_1 allodiploid is found at numerous localities where it occurs with its relatively common parents. In contrast, the other two allotetraploids, *A. bradleyi* (*montanum* × *platyneuron*) and *A. pinnatifidum* (*montanum* × *rhizophyllum*), are wide ranging and roughly coterminous although somewhat local. Their ranges extend considerably westward of that of their mutual diploid progenitor *A. montanum*, which is nearly restricted to the Appalachian provinces. Both of these allotetraploids occur in habitats quite similar to that of *A. montanum*—that is, in siliceous, partially shaded rock outcrops. Only a single sterile allodiploid *A. bradleyi* plant has been reported (*9*), and *A. pinnatifidum* is known only as an allotetraploid. Thus it would appear that these hybrids are less readily formed than *A. ebenoides*. However, our allozyme data indicate that both *A. bradleyi* and *A. pinnatifidum* have originated repeatedly.

Electrophoretic data phenotypes for nine enzyme loci from several populations of all six species of the complex (Tables 1, 2, and 3) (*10*) show that two loci (*GDH* and *6-PGDH*) are invariable for all species; two others (*ACPH* and *LAP*) vary among, but not within, diploid species; and the other five (*GOT-1, IDH, PGI-2, PGM-2*, and *SKDH*) show intraspecific variability in the diploids (Table 1) (*11*). *Asplenium montanum* has a duplicated *PGM-2* locus, as judged from populations in which all individuals have two bands for *PGM-2* (*12*). Other populations contain individuals with single bands, indicating either a lack of this duplication or, more probably, possession of electrophoretically identical alleles at both loci.

The allotetraploids *A. bradleyi* and *A. pinnatifidum* are generally heterozygous for alleles present in their respective diploid progenitors (Tables 2 and 3) (*13*). Two types of variation in genotype are evident. Some individuals, or entire populations, express only one parental allele at one or two loci (for example, *LAP* in *A. pinnatifidum* from Chester County, Pennsylvania), whereas the rest of the loci conform to the expected hybrid genotype. This loss of gene expression may be a derived condition resulting from mutation (*14*), rare recombinational events (*15*), or regulational silencing (*16*).

Alleles for which the allopolyploids are heterozygous show another type of variation. For loci that are invariant within the diploid progenitors, the allotetraploids uniformly express the same alleles as are fixed in the diploids (*17*). However, four of the five loci that are variable in the diploids are also variable in both allotetraploids (Tables 2 and 3). Genotypic differences exist within some and between most allotetraploid populations. The alleles that show this variation are electrophoretically identical to those detected in the diploids with the follow-

ing exceptions: *PGI-2* (allele 83) (found in *A. bradleyi* from Warren County, Virginia, and *A. pinnatifidum* from Powell and Rowan Counties, Kentucky); *PGI-2* (85) (found in *A. bradleyi* from Gaston County, North Carolina); and *SKDH* (140) (found in *A. pinnatifidum* from Powell County, Kentucky, and Union County, Illinois). These three "orphan" alleles represent the *A. montanum* contribution, for their respective loci, to the allotetraploid genotype (*18*). A further pattern of variation related to the *A. montanum* contribution is the expression of both or only one of the two alleles (140 and 115) of the duplicated *PGM-2* locus. Whether expression of only one allele represents a loss of expression or an original incorporation of only one allele is uncertain.

This pattern of variation in the allotetraploids for loci that are also variable in their diploid progenitors is almost certainly a result of repeated allopolyploidizations involving diploid pairs of different genotypes (*19*). Each different alloploid genotype now detected in both *A. bradleyi* and *A. pinnatifidum* might be directly derived from an ancestral genotype and may have had a separate origin. However, the role of recombination between genotypes is uncertain. The polymorphisms in some allotetraploid populations indicate that recombination between the original hybrid genotypes might have occurred. Populations that are fixed for alternative genotypes may result from founder effect and drift (population sizes are often quite small) rather than antiquity of the genotypes. Data on the geographic distribution of genotypes may provide evidence on the precise evolutionary history of the complex. Each original allotetraploid genotype might spread from its point of origin to form recombinants with other similarly

Table 1. Alleles detected at nine enzyme loci of diploid *Asplenium* species (listed for each species in order of decreasing frequency). Alleles are named relative to mobility of most frequent allele of *A. platyneuron* (designated 100). Loci: *ACPH*, acid phosphatase; *GOT-1*, glutamate oxaloacetate transaminase; *GDH*, glutamate dehydrogenase; *IDH*, isocitrate dehydrogenase; *LAP*, leucine amino-peptidase; *PGI-2*, phosphoglucose isomerase; *PGM-2*, phosphoglucomutase; *6-PGDH*, 6-phosphogluconate dehydrogenase; and *SKDH*, shikimate dehydrogenase.

Locus	*A. platyneuron*	*A. montanum*	*A. rhizophyllum*
ACPH	100	119	90
GOT-1	100, 87	130	130
GDH	100	100	100
IDH	100	78, 63	100
LAP	100	118	110
PGI-2	100, 112, 125	71, 80, 63	100
PGM-2	100, 42, 75	140, 115*	165, 100
6-PGDH	100	100	100
SKDH	100	135, 120	160

*The two alleles listed for *PGM-2* are present in most individuals of this species, probably because of a duplication of this locus.

Table 2. Inferred genotypes of *Asplenium bradleyi*. Numbers in parentheses represent dosage of each allele as inferred from relative banding intensities on gels.

Locus	Warren Co., Virginia (n = 30)	Gaston Co., North Carolina (n = 28)	Garland Co., Arkansas (n = 5)	Yell Co., Arkansas (n = 25)	Callaway Co., Missouri (n = 27)
ACPH	100 (2), 119 (2)	100 (2), 119 (2) 100 (4)	100 (2), 119 (2)	100 (2), 119 (2)	100 (2), 119 (2)
GOT-1		100 (2), 130 (2)	100 (2), 130 (2)	100 (2), 130 (2)	100 (2), 130 (2)
GDH	100 (4)	100 (4)	100 (4)	100 (4)	100 (4)
IDH	100 (4)	100 (2), 78 (2)	100 (2), 63 (2)	100 (2), 63 (2) 100 (1), 63 (3) 63 (4)	100 (2), 63 (2)
LAP	100 (2), 118 (2)	100 (2), 118 (2)	100 (2), 118 (2)	100 (2), 118 (2)	100 (2), 118 (2)
PGI-2	125 (2), 83 (2)	125 (2), 71 (2) 125 (2), 85 (2)	112 (2), 80 (2)	112 (2), 80 (2)	112 (2), 80 (2)
PGM-2	42 (2), 115 (2)	100 (2), 140 (2)	42 (2), 115 (2), 140 (2)	42 (2), 115 (2), 140 (2)	42 (2), 115 (2), 140 (2)
6-PGDH	100 (4)	100 (4)	100 (4)	100 (4)	100 (4)
SKDH	100 (2), 135 (2)	100 (2), 135 (2)	100 (2), 135 (2)	100 (2), 135 (2)	100 (2), 135 (2)

Table 3. Inferred genotypes of *Asplenium pinnatifidum*. Numbers in parentheses represent dosage of each allele as inferred from relative banding intensities on gels.

Locus	Chester Co., Pennsylvania (n = 9)	Fluvanna Co., Virginia (n = 6)	Powell Co., Kentucky (n = 13)	Rowan Co., Kentucky (n = 15)	Union Co., Illinois (n = 30)	Garland Co., Arkansas (n = 14)	Benton Co., Arkansas (n = 25)
ACPH	119 (2), 90 (2)	119 (2), 90 (2)	119 (2), 90 (2)	119 (2), 90 (2)	119 (2), 90 (2)	119 (2), 90 (2)	119 (2), 90 (2)
GOT-1	130 (4)	130 (4)	130 (4)	130 (4)	130 (4)	130 (4)	130 (4)
GDH	100 (4)	100 (4)	100 (4)	100 (4)	100 (4)	100 (4)	100 (4)
IDH	100 (2), 63 (2)	100 (2), 63 (2)	100 (2), 78 (2)	100 (2), 63 (2)	100 (2), 63 (2)	100 (4)	100 (4)
LAP	118 (4)	118 (2), 110 (2)	118 (2), 110 (2)	118 (2), 110 (2)	118 (2), 110 (2)	118 (2), 110 (2)	118 (2), 110 (2)
PGI-2	100 (2), 80 (2)	100 (2), 80 (2)	100 (2), 83 (2) 100 (4)	100 (2), 80 (2) 100 (2), 83 (2)	100 (2), 80 (2)	100 (2), 83 (2)	100 (2), 80 (2)
PGM-2	165 (2), 115 (2)	165 (2), 140 (2), 115 (2)	165 (2), 140 (2), 115 (2) 165 (2), 115 (2) 165 (4)	165 (2), 140 (2)	165 (2), 140 (2)	165 (2), 115 (2)	165 (4)
6-PGDH	100 (4)	100 (4)	100 (4)	100 (4)	100 (4)	100 (4)	100 (4)
SKDH	160 (2), 135 (2)	160 (2), 135 (2)	160 (2), 140 (2) 160 (2), 135 (2)	160 (2), 135 (2)	160 (2), 135 (2)	160 (2), 140 (2)	160 (2), 135 (2)

spreading genotypes. Thus, extensive areas now occupied by single genotypes may circumscribe centers of allopolyploid origin. This may well apply to *A. bradleyi* in the Ozark-Ouachita region. Three widely separated populations from this region (Garland and Yell counties, Arkansas and Callaway County, Missouri) shared essentially identical genotypes; the only exception is that *A. bradleyi* plants from Yell County are variable for *IDH*, being either heterozygous (100^2, 63^2), homozygous (63^4), or asymmetrically heterozygous (100^1, 63^3). The latter pattern probably resulted from crosses between the former two genotypes.

How general is the phenomenon of recurrent origins of allopolyploid species? Studies of allozyme variation in polyploid complexes are few, and most consider crops or anthropogenic weeds (*20*). There are indications from these and from morphological and cytological data on other taxa that the phenomenon of multiple allopolyploidizations may be much more general than has been thought. Studies of wheat (*21*), *Phlox* (*22*), and European *Asplenium lepidum* (*23*) have shown that differently named species may represent analogous combinations of different morphotypes of the same diploid progenitor species. Variation in esterase genotypes indicates that *Tragopogon mirus*, a recent allotetraploid derivative of two species introduced into North America, originated independently at widely separated localities (*4*). Allozyme data also suggest multiple origins for the fern allotetraploid *Pellaea wrightiana* (*24*). These studies suggest an important aspect of the dynamics of reticulate evolution: species genetically isolated at the diploid level may repeatedly contribute to a common gene pool at the polyploid level. The continued acquisition of genetic diversity by allopolyploids, such as those in *Asplenium*, may substantially augment their potential to evolve and speciate.

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1. W. H. Lewis, Ed., 1980, *Polyploidy: Biological Relevance* (Plenum, New York, 1980).
2. The average rate of speciation through divergence is controversial. Nonetheless, even models of extremely rapid divergent speciation [for example, H. L. Carson, *Science* **168**, 1414 (1970)] require numerous generations before speciation is complete.
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10. Starch gel electrophoresis of foliar extracts was used [see C. R. Werth *et al.* (*5*) on methodology and collection localities for diploids]. More recent extracts have used polyvinylpyrrolidone in place of caffeine [as described in Werth *et al.*, *Isozyme Bull.* **15**, 139 (1982)].
11. Putative heterozygotes have been found to segregate electromorphs into gametophyte progeny, supporting the interpretation of the electromorphs as alleles at genetic loci (C. R. Werth, unpublished observations).
12. The *PGM-2* homozygotes in the other two diploid species have a single band of corresponding intensity and general position.
13. The heterozygosity referred to here is for equivalent genes coded in the nonhomologous genomes of the two parental species. Genotypes are only inferred since segregation does not ordinarily occur in allotetraploids.
14. Compare loss of parental gene expression in *Chenopodium* [H. Wilson, S. C. Barber, T. Walters, *Biochem. Syst. Ecol.* **11**, 7 (1983)] attributed to nonfunctional (null) alleles.
15. Recombination could be accomplished through chromosome breakages or through rare pairing of nonhomologous chromosomes, either in multivalents as suggested by Roose and Gottlieb (*4*) or in bivalents, as suggested by E. J. Klekowski, [*Am. J. Bot.* **60**, 535 (1973)].
16. S. D. Ferris and G. S. Whitt, [*J. Mol. Evol.* **12**, 267 (1979)] discuss regulational silencing of duplicated loci.
17. An exception is the previously mentioned instances of nonexpression of parental alleles.
18. *Asplenium montanum* occurs in small isolated populations that show considerable interpopulational differentiation resulting from founder effect [C. R. Werth, thesis, Miami University, Oxford, Ohio (1983); T. Reeves, *Am. Fern J.* **64**, 105 (1974)]. A more extensive survey of populations in this species may well turn up these "orphan" alleles [see B. J. Turner, B-L. H. Brett, E. M. Rasch, J. S. Balsano, *Evolution* **34**, 246 (1980)].
19. Although alleles may, of course, arise through mutation, it is unlikely that most or all the variation in the heterozygous genotypes of the allotetraploid genotypes resulted from mutations following a special hybridization. Most mutations would give rise to nonfunctional alleles that would be sheltered from selection by the fixed heterozygosity of the allopolyploids. However, for those loci that are variable in the diploids, most of the variation in the allotetraploids involves functional alleles present in the diploids. In contrast, for loci that are invariant in the diploids, the only type of variation in the allotetraploids involves nonexpression of parental alleles. Moreover, the occurrence of a single *A. bradleyi* genotype (with the exception of *IDH*) occupying the entire range of this species west of the Mississippi is not consistent with a supposed origin of all or most variability in the allotetraploids from posthybridization mutations.
20. Other reports of geographic variation for isozyme loci in polyploids are those of Roose and Gottlieb (*4*) and Wilson *et al.*, in (*14*).
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25. We are indebted to A. A. Karlin for advice on techniques, to S. Taylor and S. Swartz for technical assistance, and the following who assisted us in locating populations: R. Cranfil, J. Lankalis, J. Matthews, L. Mellichamp and A. Mellichamp, R. Moran, P. Nelson, C. Stevens, and C. Taylor. Supported in part by grants from the Willard Sherman Turrell Herbarium Fund (grants 11, 20, 23, 36) and Sigma Xi.

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Human Immunoglobulin D: Genomic Sequence of the Delta Heavy Chain

Abstract. *The DNA coding for the human immunoglobulin D (IgD) heavy chain (δ, delta) has been sequenced including the membrane and secreted termini. Human δ, like that of the mouse, has a separate exon for the carboxyl terminus of the secreted form. This feature of human and mouse IgD distinguishes it from all other immunoglobulins regardless of species or class. The human gene is different from that of the mouse; it has three, rather than two, constant region domains; and its lengthy hinge is encoded by two exons rather than one. Except for the third constant region, the human and mouse genes are only distantly related.*

Immunoglobulin D (IgD) was discovered in human serum in 1965 (*1*), and the corresponding mouse Ig was identified subsequently (*2, 3*). In both the mouse and the human, IgD is a predominant surface component of B cells and is only a minor component of serum Ig (*1, 4*). The function of IgD has been a subject of considerable speculation, but despite a wealth of structural information, the function is basically unknown (*5, 6*).

IgD has been studied in great detail at physiological and molecular levels in the mouse. The majority of antigen-reactive splenic B cells co-express surface IgM and IgD of shared light chain type, idiotype, and antigen specificity (*7, 8*). The ratio of IgM to IgD varies with the state of B-cell differentiation (*9*), with early B cells expressing IgM only. As cell differentiation progresses, IgD is turned on; this leads to "double-producers" expressing both IgM and IgD on the cell surface with identical variable regions. Most mature B cells have ten times as much IgD as IgM on the surface (*10*). As the B cell is activated, IgD is shut off rather than being secreted copiously as with IgM and other immunoglobulin classes.

The murine δ chain has several properties that set it apart from all other immunoglobulin heavy (H) chains: (i) Murine IgD has only two H-chain constant re-