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Evolution

Allopolyploidy in bryophytes: Multiple origins of *Plagiomnium medium*

(Bryophyta/polyploidy/electrophoresis/isozymes/chloroplast DNA)

ROBERT WYATT, IRENEUSZ J. ODRZYKOSKI*, ANN STONEBURNER, HENRY W. BASS†, AND GLENN A. GALAU

Department of Botany, University of Georgia, Athens, GA 30602

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ABSTRACT **Bryophytes are thought to be unique among land plants in lacking the important evolutionary process of allopolyploidy, which involves interspecific hybridization and chromosome doubling. Electrophoretic data show, however, that the polyploid moss *Plagiomnium medium* is an allopolyploid derivative of *Plagiomnium ellipticum* and *Plagiomnium insigne*, that *P. medium* has originated more than once from these progenitors, and that cross-fertilization results in interlocus genetic recombination. Evidence from restriction fragment length polymorphisms in chloroplast DNA implicates *P. insigne* as the female parent in interspecific hybridizations with *P. ellipticum*. Contrary to prevailing views, it appears that those evolutionary processes responsible for genetic differentiation and speciation in other land plants occur in the bryophytes as well.**

It is estimated that 47% of all flowering plants and 95% of all pteridophytes are polyploids and that the majority of these are allopolyploids, which have arisen through doubling of chromosomes in interspecific hybrids (ref. 1, but see ref. 2). Among the bryophytes 79% of mosses, 11% of liverworts, and 2% of hornworts are believed to be polyploids (3, 4). In contrast to other land plants, however, all of these have been assumed on the basis of morphology to be autopolyploids (5, 6). Furthermore, hybridization has been documented in only a few isolated cases involving sterile sporophytes from intergeneric crosses (3–6). The absence of hybrid speciation, an important mode of evolution in other land plants, has contributed to the characterization of bryophytes as a group with severely limited evolutionary potential (7, 8).

Within the moss genus *Plagiomnium*, section *Rosulata* includes one polyploid species (*Plagiomnium medium*; n = 12) and six haploid species (9). On the basis of morphology, Lowry (10) concluded that *Plagiomnium ellipticum* (n = 6) is the progenitor of autopolyploid *P. medium*. Koponen (9), however, stressed different characters and argued that *Plagiomnium insigne* (n = 6) is the only possible progenitor. Because the morphological evidence is ambiguous, we elected to use biochemical evidence to resolve the origin of *P. medium*.

MATERIALS AND METHODS

Four gametophytic populations each of *P. medium* and *P. ellipticum* were sampled in northern Michigan from many of the same localities sampled in Lowry's (9) original cytological study. Because Lowry's (9) chromosome counts were done on plants collected in Michigan [all vouchers verified by Wyatt (11)] and his and all other counts [reviewed by Koponen (12)] for these species are in agreement, we did not deem it necessary to examine our material cytologically. We also obtained population samples of *P. insigne* from northern California. From each population, we collected 36 moss clumps (approximately 5 × 5 cm). These were maintained in a growth chamber on a light/dark cycle of 16:8 hr at 15°C and 10°C, respectively. Single healthy shoots were subjected to horizontal starch gel electrophoresis after 1–2 months under these conditions. Electrophoretic procedures were identical to those used in our earlier studies of *Plagiomnium ciliare* (13). Because populations differed only slightly in allele frequencies, we pooled all samples for making interspecific comparisons. A more detailed analysis of these data will appear elsewhere.

We also prepared DNA from crude chloroplast fractions of each of the three species by conventional methods. It was digested with *Eco*RI, electrophoresed in agarose gels, and blotted (14) and cross-linked (15) onto nylon. Hybridization with nick-translated maize *psbA* and subsequent washing of the nylon was done in aqueous buffer at 23°C (16). The *psbA* probe was an 800-nucleotide-long *Eco*RI/*Pvu* I subfragment of the insert in *pzmC426* (17). It probably consists exclusively of amino acid coding sequence.

RESULTS AND DISCUSSION

Using horizontal starch gel electrophoresis, we scored genetic variation at 18 enzyme loci. Of these, 9 contributed no useful information regarding interspecific relationships because they were monomorphic or nearly so (Table 1). The other 9 included alleles present in only one of the two haploids, *P. ellipticum* or *P. insigne*, which therefore could be used as genetic markers to establish unequivocally the source of the alleles expressed by *P. medium*. When species are as well-differentiated as the haploid mosses considered here, their allopolyploid derivatives display fixed heterozygosity because of nonsegregation of nonhomologous chromosomes (18–20). Furthermore, when one or both of the progenitors is polymorphic, different fixed heterozygous genotypes of the allopolyploid may be produced. The existence of such variability constitutes a strong case for multiple origins of the derivative species.

Our data show that *P. medium* displays a number of different combinations of alleles, one in each case originating from *P. insigne* and one from *P. ellipticum* (Table 1). The existence of fixed heterozygosity at every locus where it could reasonably be expected constitutes strong evidence that *P. medium* is indeed an allopolyploid. This is shown clearly by loci such as *Pgm-2*, in which the haploids *P. ellipticum* and *P. insigne* possess different alleles (Fig. 1*A*). The allopolyploid *P. medium* expresses both alleles, producing a fixed heterozygous pattern (Fig. 1*A*). *Hk-1* shows a

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*Present address: Department of Genetics, Institute of Biology, Adam Mickiewicz University, Dabrowskiego Street 165, Poznan 60-594, Poland.
†Present address: Department of Botany, North Carolina State University, Raleigh, NC 27695-7612.

Table 1. Allele frequencies for 18 enzyme loci of the allopolyploid moss *P. medium* and its two haploid progenitors, *P. ellipticum* and *P. insigne*

Locus	Allele	*P. ellipticum* ($\bar{n}$ = 112)		*P. medium* ($\bar{n}$ = 292)		*P. insigne* ($\bar{n}$ = 90)
Aco-1	*a*	0.991	→	0.483		—
	b	—		0.500	←	1.000
	c	0.009	→	0.010		—
	d	—	→	0.007*		—
Aco-2	*a*	—		0.500	←	1.000
	b	0.111	→	0.146		—
	c	0.889	→	0.354		—
Ald-1	*a*	1.000		1.000		1.000
Dia-1	*a*	0.714		1.000		1.000
	b	0.286		—		—
Dia-2	*a*	1.000		1.000		1.000
G3p-1	*a*	1.000		1.000		1.000
G3p-2	*a*	1.000		0.983		1.000
	b	—		0.017*		—
Hk-1	*a*	1.000	→	0.500		—
	b	—		0.500	←	1.000
Idh-1	*a*	0.974		1.000		1.000
	b	0.026		—		—
Mdh-1	*a*	1.000	→	0.500		—
	b	—		0.500	←	1.000
Mdh-2	*a*	0.966		0.910		0.372
	b	0.034		—		—
	c	—		0.090	←	0.628
Mdh-3	*a*	1.000		1.000		1.000
Per-1	*a*	0.530	→	0.102		—
	b	0.385	→	0.398		—
	c	—		0.500	←	1.000
	d	0.085		—		—
Pgi-1	*a*	0.991		1.000		1.000
	b	0.009		—		—
Pgm-1	*a*	0.983	→	0.500		—
	b	0.017		0.242	←	0.149
	c	—		0.236	←	0.851
	d	—		0.222*	←	—
Pgm-2	*a*	0.846	→	0.261		0.340
	b	0.136		—		—
	c	—		0.500	←	0.660
	d	0.009	→	0.189		—
	e	0.009	→	0.050		—
Tpi-1	*a*	0.103	→	0.040		—
	b	0.897		0.960		1.000
Tpi-2	*a*	0.205		—		—
	b	0.761		1.000		1.000
	c	0.034		—		—

Arrows designate the presumed source of particular alleles. Note that allele frequencies for *P. medium* do not represent segregating polymorphisms, but rather alleles present in fixed heterozygous genotypes. Loci: *Aco*, aconitase; *Ald*, aldolase; *Dia*, diapharase; *G3p*, glucose-3-phosphate dehydrogenase; *Hk*, hexokinase; *Idh*, isocitrate dehydrogenase; *Mdh*, malate dehydrogenase; *Per*, peroxidase; *Pgi*, phosphoglucose isomerase; *Pgm*, phosphoglucomutase; *Tpi*, triosephosphate isomerase. $\bar{n}$, Mean number of haploid genomes screened per locus.
*Origins of these "orphan alleles" are discussed in the text.

similar pattern of variation (Fig. 1*B*). At loci such as *Per-1*, a single species-specific allele is contributed by *P. insigne* to the allopolyploid *P. medium*. The contribution of the polymorphic *P. ellipticum*, however, is variable (Fig. 1*C*). The existence of different *Per-1* genotypes of *P. medium* is strong evidence for multiple origins of this allopolyploid. Other loci, including *Aco-2* (Fig. 1*D*) and *Tpi-1* (Fig. 1*E*), further illustrate this pattern.

There are only three alleles present in *P. medium* that were not detected also in *P. ellipticum* or *P. insigne*. These

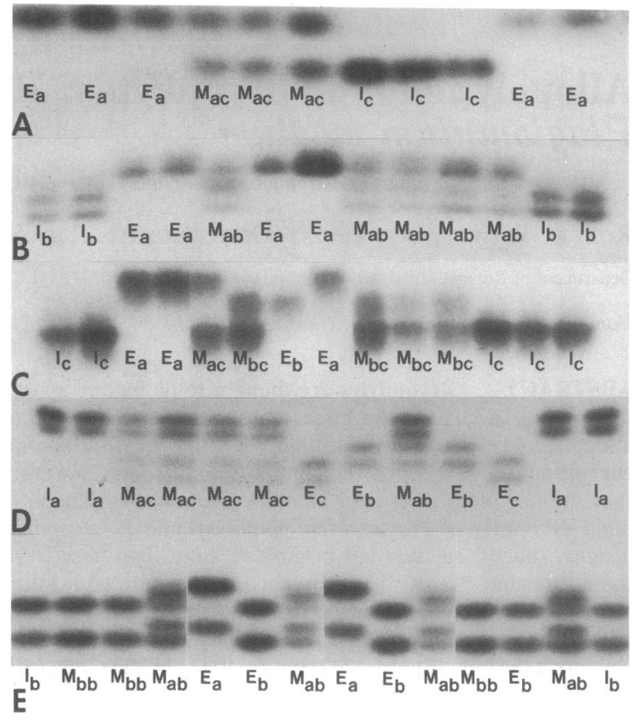

FIG. 1. Starch gels illustrating electrophoretic banding patterns of individual gametophytic plants of the allopolyploid moss *P. medium* (M) and its two haploid progenitors, *P. ellipticum* (E) and *P. insigne* (I), for five enzyme loci. Allelic designations match those in Table 1, but note that not all allelic variants appear on these gels. Subscripts to species designations indicate allelic combinations. Loci: *A, Pgm-2; B, Hk-1; C, Per-1; D, Aco-2; E, Tpi-1.*

"orphan alleles," whose frequency was never higher than 2.2%, may represent mutations that have occurred in the gene pool of *P. medium* since its origin. On the other hand, they may represent low-frequency variants in populations of the haploids that our population samples failed to include. Alternatively, it has been suggested that orphan alleles may serve as a "genetic memory" of variants that were once present in the progenitors but that have been lost from present-day populations (20). If the three orphan alleles in *P. medium* are derived from its haploid progenitors, *Aco-1d* must have come from *P. ellipticum*, since the species-specific allele (*Aco-1b*) contributed by *P. insigne* is present in every plant. By similar reasoning, *Pgm-1d* must have come from *P. insigne*. At the third locus (*G3p-2*) both progenitors are monomorphic, so that no definite statement can be made about the likely origin of allele *G3p-2b*.

The extremely low number of apparently unique alleles in allopolyploid *P. medium* suggests that this species originated very recently from ancestors whose gene pools were virtually identical to extant *P. ellipticum* and *P. insigne*. A very recent origin is suggested further by the absence of gene silencing of duplicated loci (2). Present-day distributions of the progenitor taxa, however, show little overlap. *P. insigne* is endemic to the Pacific Northwest of the United States and Canada, whereas *P. ellipticum*, like *P. medium*, is circumboreal (9). It is possible that *P. medium* originated during the Pleistocene, when southward migration of *P. ellipticum* would have brought that species into contact with *P. insigne* in the western United States.

Electrophoretic data for three of the other four haploid species in *Plagiomnium* section *Rosulata* indicate that *Plagiomnium affine, Plagiomnium ciliare*, and *Plagiomnium elatum* cannot be involved in the parentage of *P. medium*. Each of these species is fixed at several loci for alleles that do not occur in *P. medium*. Furthermore, many of the alleles

that occur in different genotypes of *P. medium* have not been detected in these three potential progenitors (data not shown). We have been unable to secure material of the remaining species, *Plagiomnium tezukae*, a rare moss endemic to the high mountains of Japan. Based on its morphology and distribution, however, it would appear unlikely to have been involved in the parentage of *P. medium*.

We have detected more than 30 multilocus genotypes of allopolyploid *P. medium*. This discovery implies that extensive genetic recombination has taken place among the original genotypes produced through allopolyploidy. An alternative hypothesis, that each of the multilocus genotypes represents a separate polyploid origin, seems less likely. Such an explanation would require a very large number of presumably rare events involving interspecific hybridization and polyploidization. The fact that at some loci *P. medium* is fixed for low frequency alleles of the progenitors also suggests that the number of hybridization events was probably limited. Most workers have assumed that synoecious polyploid mosses (those with the sex organs mixed together on the same plant), like *P. medium*, typically self-fertilize (3–6). Our discovery of apparent extensive interlocus recombination suggests, on the contrary, that cross-fertilization is possible, if not common. Electrophoretic evidence of cross-fertilization has been presented previously for the bisexual liverwort *Pellia epiphylla* (21).

Recent research on these moss species with restriction fragment length polymorphisms has enabled us to identify the likely female parent in the original crosses that gave rise to allopolyploid *P. medium*. We hybridized a portion of the maize chloroplast *psbA* DNA, which encodes the photosystem II 32-kDa polypeptide, to *Eco*RI-digested chloroplast DNA from *P. medium*, *P. ellipticum*, and *P. insigne*. Assuming maternal inheritance of the chloroplast genome, the restriction fragment pattern of the polyploid should be identical to the pattern of its maternal parent (22, 23). The pattern for *P. medium* matched that of *P. insigne*, rather than that of *P. ellipticum* (Fig. 2). Therefore, it appears that *P. insigne* is the sole or most common maternal parent of *P. medium*. Maternal inheritance of the chloroplast genome has yet to be demonstrated in bryophytes, but circumstantial evidence from ultrastructural studies of spermatogenesis (e.g., see ref. 24) suggests that it is likely.

Our results have wide-ranging implications for bryophyte phylogeny and evolutionary biology. We have demonstrated that allopolyploidy occurs in bryophytes. This important mode of speciation in other land plants has only rarely been mentioned (25, 26) and never documented with evidence in mosses or liverworts (3–5). Because allopolyploidy necessarily involves interspecific hybridization, we have also demonstrated that crossing between natural populations belonging to two different species of bryophytes is possible. Our data regarding multiple origins of *P. medium* join a growing body of literature that suggests that allopolyploids may originate independently numerous times and in different places (18–20). This implies that gene flow from progenitor species into derivative allopolyploid gene pools is possible over long time spans. Our observation of interlocus genetic recombination in synoecious *P. medium* demonstrates that cross-fertilization can occur in mosses with mixed sex organs. Finally, we have shown that plants of *P. insigne* probably served as the female parent in the interspecific hybridizations that gave rise to *P. medium*. Identification of the maternal parent of a natural allopolyploid plant has been done previously only for a few flowering plants (27).

In total, our research suggests that, contrary to prevailing views (7, 28), bryophytes are not organisms with severely limited evolutionary possibilities. Rather, it appears that most, if not all, of the evolutionary processes responsible for genetic differentiation and speciation in other land plants occur in the mosses as well. Careful study undoubtedly will reveal additional examples of allopolyploidy, interspecific hybridization, and other evolutionary processes in bryophytes.

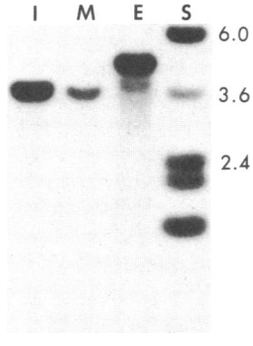

FIG. 2. Agarose gel illustrating restriction fragment length polymorphisms in chloroplast DNA from the allopolyploid moss *P. medium* (lane M) and its two haploid progenitors, *P. ellipticum* (lane E) and *P. insigne* (lane I). The sizes of fragments of restriction enzyme-digested pBR325 (lane S) are given in kilobase pairs.

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