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Evolution

Gene number in species of Astereae that have different chromosome numbers

(plant evolution/isozymes/aneuploidy/Compositae)

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ABSTRACT **Differences in the gametic chromosome numbers ($n = 4, 5, 9$) of species in the Astereae tribe of the Compositae have been variously interpreted. One hypothesis proposes that $n = 9$ was the original base number of the group and that the lower numbers resulted from aneuploid reduction. The alternative hypothesis asserts that the ancestral base number was $n = 4$ or $n = 5$ and that species in which $n = 9$ are allotetraploids derived by hybridization between taxa with the lower numbers. Electrophoretic analysis of 17 enzyme systems in five species of *Machaeranthera*, in which $n = 4$, 5, and 9, and two species of *Aster* in which $n = 5$ and 9, demonstrates that all of these species have the same number of gene loci specifying the tested enzymes. The absence of isozyme multiplicity in the species in which $n = 9$ suggests that they did not arise by polyploidy.**

Ploidy level in plants has usually been determined primarily on the basis of chromosome number. Although satisfactory in most cases, sharp differences characterize the interpretation of chromosome numbers in the Astereae tribe of the Compositae. The most common chromosome number in the tribe is $n = 9$, but the numbers vary between $n = 2$ and $n = 9$, with many species having $n = 4$ or $n = 5$. One hypothesis proposes that $n = 9$ was the original base number of the group and that lower numbers resulted from aneuploid reduction (1). Evidence claimed to support this view is the association of $n = 9$ with the primitive woody habit, the high symmetry of the $n = 9$ karyotypes, and the widespread occurrence of this chromosome number in divergent lineages within the tribe. The alternative hypothesis calls attention to the rarity of species with the intermediate chromosome numbers $n = 6$ and $n = 7$ and asserts that the ancestral base number was $n = 4$ or $n = 5$, so that species in which $n = 9$ are allotetraploids derived by hybridization between taxa with the lower numbers (2, 3).

The essential attribute of polyploidy, however, is not relative chromosome number but genome multiplication and its attendant increases in number of gene loci. Consequently, I have used gel electrophoresis to determine whether species in which $n = 9$ have more gene loci coding particular isozymes than those in which $n = 4$ or 5. The approach builds on two recent findings: (*i*) allopolyploid species display isozyme multiplicity relative to diploids because they inherit from their diploid parents homoeologous loci that are frequently fixed for different alleles (4–6) and (*ii*) decrease in chromosome number from the ancestral diploid level as a result of aneuploidy does not change the number of structural genes specifying isozymes (7). Thus, if species in which $n = 9$ have many more isozyme loci than those in which $n = 4$ or $n = 5$, they are likely to be polyploid. Polyploidy is rejected if they have the same number of loci.

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MATERIALS AND METHODS

Seven species were examined: *Machaeranthera tenuis*, $n = 4$ (Jackson 7607, Ft. Davis, TX); *M. mexicana*, $n = 4$ (Jackson 7547, 57 miles west of Durango, Mexico); *M. boltoniae*, $n = 4$ (Jackson 7551, 27 miles north of Durango, Mexico); *M. turneri* $n = 5$ (Jackson 7564, Meoqui, Chihauhua, Mexico); *M. brevilingulata*, $n = 9$ (Jackson 7526, Aguascalientes, Mexico); *Aster riparius*, $n = 5$ (Jackson Lordsburg, NM, and Jackson 7550, 27 miles north of Durango, Mexico); *Aster hydrophilus*, $n = 9$ (Jackson 7640, Beatty NV). The seeds were generously provided by R. C. Jackson. Several of the collections had been previously grown for electrophoretic studies (8).

The seeds were germinated by nicking their radicle end after they had imbibed water for 24 hr. After radicle emergence, which usually occurred within the following 24–48 hr, the seedlings were placed in cups of moistened vermiculite and grown for 2–3 weeks. They were fertilized with one-half strength Hoagland's solution at weekly intervals.

The seedlings were assayed when they were 3–4 weeks old. Enzyme extraction was accomplished by crushing whole seedling shoots in a small plastic weighing boat in ≈0.2 ml of cold extraction buffer [0.1 M Tris·HCl, pH 7.5/14 mM 2-mercaptoethanol/1 mM EDTA(Na_4)/10 mM KCl/10 mM $MgCl_2$] and 5–10 mg of solid polyvinylpolypyrrolidone. Extracts were maintained on ice. Electrophoresis was conducted in 12.8% starch gels made with different combinations of gel and electrode buffers according to the particular enzymes assayed. System I was buffer A (0.038 M lithium hydroxide/0.188 M boric acid, pH 8.3) and buffer B (0.05 M Tris/0.007 M citric acid, pH 8.3); gel buffer was B/A = 9:1, and electrode buffer was A only. This system was used for phosphoglucomutase (EC 2.7.5.1); phosphoglucose isomerase (EC 5.3.1.9); glucose-6-phosphate dehydrogenase (EC 1.1.1.49); triose phosphate isomerase (EC 5.3.1.1); aldolase (EC 4.1.2.7); and aspartate aminotransferase (EC 2.6.1.1). System II was stock buffer [0.9 M Tris/0.02 M EDTA (Na_4)/0.5 M boric acid] diluted 1:20 for use as gel buffer and 1:5 for use as electrode buffer. This system was used for 6-phosphogluconate dehydrogenase (EC 1.1.1.44); fructose-1,6-bisphosphatase (EC 3.1.3.11); and catalase (EC 1.11.1.6). System III was gel buffer (0.076 M Tris/0.005 M citric acid, pH 8.7) and electrode buffer (0.3 M boric acid/0.06 M NaOH). This system was used for glutamate dehydrogenase (EC 1.4.1.2) and leucine aminopeptidase (EC 3.4.1.--). System IV was gel buffer (0.02 M histidine·HCl titrated to pH 7.0 with 4 M NaOH) and electrode buffer (0.4 M sodium citrate titrated to pH 7.0 with HCl). This system was used for shikimate dehydrogenase (EC 1.1.1.25), malic enzyme (EC 1.1.1.40), adenylate kinase (EC 2.7.4.3), aconitase (EC 4.2.1.3), and malate dehydrogenase (EC 1.1.1.37). System V was gel buffer (0.005 M histidine·HCl titrated to pH 7.0 with 4 M NaOH) and electrode buffer (0.4 M sodium citrate titrated to pH 7.0 with HCl). This system was

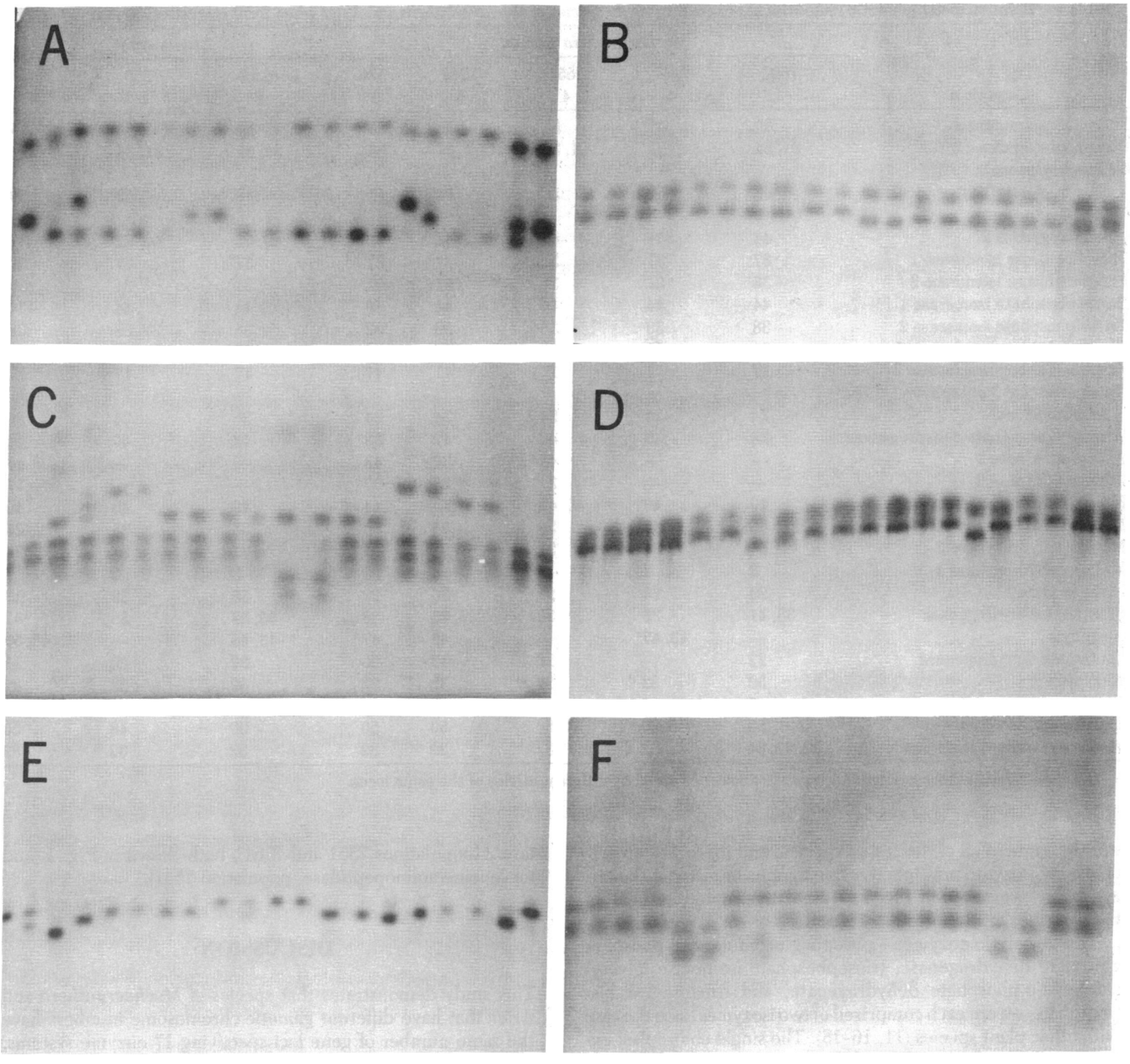

FIG. 1. Zymograms showing electrophoretic patterns of six enzyme systems in seven species of *Machaeranthera* and *Aster*. (*A*) Phosphoglucomutase. (*B*) Phosphoglucose isomerase. (*C*) Malate dehydrogenase. (*D*) 6-Phosphogluconate dehydrogenase. (*E*) Shikimate dehydrogenase. (*F*) Triose phosphate isomerase. The linear order of the sampled individuals of each species is the same on each zymogram; reading from left to right, each pair of tracks contains extracts from different individuals of each of the species: 1 and 2, 7564; 3 and 4, 7551; 5 and 6, 7526; 7 and 8, 7550; 9 and 10, 7607; 11 and 12, 7640; 13 and 14, 7547; 15 and 16, Lordsburg; 17 and 18, 7526; 19 and 20, 7564. The anode is toward the top of each zymogram.

used for glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12).

In all of these systems, all of the isozymes migrated toward the anode. Sample sizes were four individuals of *M. brevilingulata* and *A. riparius* and two individuals of each of the other five species for each enzyme system. Small sample size per species was justified because our concern is not with allelic variability but only with number of gene loci, an attribute expected not to vary among conspecific individuals. Standard assay methods were used (9–11).

The enzymes used in this study were selected because they could be reliably separated by electrophoresis into cleanly resolved bands of activity, the number of isozymes for each system in diploid plants was generally understood (12, 13), formal genetic studies for many of them had been carried out in *Stephanomeria* (14, 15), and in the Compositae and, except for leucine aminopeptidase, they were assayed with natural substrates. Nonspecific esterases, phosphatases, and peroxidases were deliberately not assayed because plants possess many isozymes in these systems, which would make it difficult to know whether differences in their number from species to species reflected developmental changes, changes in substrate specificities, or the addition of gene loci.

RESULTS

The isozyme patterns of the 17 enzyme systems examined in the seven species of *Machaeranthera* and *Aster* were closely similar. The number of isozymes for each of the enzyme systems

Table 1. Relative electrophoretic mobilities of enzymes in seven species of *Machaeranthera* and *Aster* when front migrates 100 mm

	Machaeranthera species					*Aster* species		
	7607	7547	7551	7564	7526	Lordsburg	7550	7640
Gametic *n*	4	4	4	5	9	5	5	9
Phosphoglucomutase 1	55	55	55	55	55	54	55	55
Phosphoglucomutase 2	31, 34	31	31, 39	31, 34	31	34, 39	31, 34	31
6-Phosphogluconate dehydrogenase 1	50	50	50	50	51	48	50	50
6-Phosphogluconate dehydrogenase 2	44	44	44	44	46	40, 42	40, 42	44
Phosphoglucose isomerase 1	37	37	37	37	37	37	37	37
Phosphoglucose isomerase 2	33	31	31	31	31	31	31	30
Triose phosphate isomerase 1	44	44	44	44	38	44	44	44
Triose phosphate isomerase 2	38	38	38	38	32	38	32, 38	38
Fructose-1,6-bisphosphatase 1	28	28	28	28	28	28	28	28
Fructose-1,6-bisphosphatase 2	17	17	17	17	17	17	17	17
Glyceraldehyde-3-phosphate dehydrogenase	24	24	24	24	24	24	24	24
Glucose-6-phosphate dehydrogenase	38	40	40	40	38	42	40	40
Adenylate kinase 1	—	—	43	39	43	—	—	43
Adenylate kinase 2	—	—	37	38	37	—	—	37
Malate dehydrogenase 1	40	40	40, 44	—	44	49	40	40
Malate dehydrogenase 2	35	35	35	35	35	35	35	27
Malate dehydrogenase 3	31	31	31	31	31	31	31	24
Malate dehydrogenase 4	3	3	3	3	3	3	3	3
Malic enzyme	29	25	25	36	22	36	39	18
Shikimate dehydrogenase	38, 41	36	34, 36	36, 38	38	36, 38	38	41
Aconitase	—	43, 47	—	45	47	43, 45	—	45, 50
Glutamate dehydrogenase	22	—	20	13	20	20	20	16
Aldolase	32	32	32	32	32	32	32	32
Catalase	5	5	5	5	5	5	5	5
Leucine aminopeptidase	50	48, 50	50	50	50	50	48	50
Aspartate aminotransferase	30, 32, 34	32	32	32	32	32	32	32

Two mobilities in the same column indicate allozymes coded by different alleles of the same locus.

was the same in all of the tested species, and there was no evidence for isozyme multiplicity. Zymograms of many of the enzymes are presented in Fig. 1. The relative mobilities of all of the isozymes are reported in Table 1.

The glycolytic enzymes—phosphoglucomutase, 6-phosphogluconate dehydrogenase, triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, and fructose-1,6,-bisphosphate—were each comprised of two isozymes, as is the case with other plant species (11, 16–18). The single chloroplast enzyme, glucose-3-phosphate dehydrogenase, one glucose-6-phosphate dehydrogenase, and two adenylate kinase isozymes were detected. Malic enzyme, shikimate dehydrogenase, aconitase, aldolase, glutamate dehydrogenase, catalase, and leucine aminopeptidase each showed a single enzyme band. Only one aspartate amino transferase isozyme was detected although many plants have three or four (19, 20). Four malate dehydrogenase isozymes were observed. These apparently included both cytoplasmic and mitochondrial forms, on the basis of differential resistance to inactivation when extracted with 6% ascorbic acid/16% sucrose (21), as well as a microbody isozyme, by analogy with the similarly slow mobility of the microbody isozyme in spinach (22, 23).

On the basis of the electrophoretic patterns, the 17 enzyme systems appear to be coded by a minimum of 26 gene loci. Heterozygous loci were recognized in a number of plants. Thus, three-banded heterozygous patterns were observed for the dimeric triose phosphate isomerase 2 (population 7550) and malate dehydrogenase 1 (population 7551) (Fig. 1). Double-banded heterozygotes were found for the monomeric shikimate dehydrogenase (populations 7564 and 7607) and phosphoglucomutase 2 (populations 7551 and 7564), both shown in Fig. 1, and for leucine aminopeptidase (population 7547).

DISCUSSION

This study demonstrates that species of *Machaeranthera* and *Aster* that have different gametic chromosome numbers have the same number of gene loci specifying 17 enzyme systems. A previous electrophoretic study of six enzyme systems in the *Machaeranthera* species also did not find differences in isozyme number (8). The complete absence of enzyme multiplicity makes it most unlikely that *M. brevilingulata* and *Aster hydrophilus*, the two species in which $n = 9$, originated by polyploidy, as previously proposed on indirect grounds (2, 3). The electrophoretic results are also consistent with the finding that both species have half or less than half the amount of total DNA per nucleus compared with the related species in which $n = 4$ or 5 (24).

The use of the number of structural genes coding enzymes rather than the number of chromosomes to identify ploidy level follows from the widespread observation that allopolyploid plants possess more electrophoretically detectable isozymes than diploids (5, 6, 14, 25, 26). The additional isozymes result from the inheritance of genes coding different enzyme forms from the diploid parents and from evolution subsequent to the origin of the polyploid during which new mutations become established at homoeologous gene loci. In many cases, polyploids express fixed or true-breeding multiple-banded patterns whereas these segregate at the diploid level.

Recognition of increased number of isozymes requires differences in their electrophoretic mobilities. As the mobilities of the enzymes coded by 19 of the 26 putative loci assayed have diverged among the examined species, even in the limited sample of individuals (see Table 1), it is reasonable to propose that, if the $n = 9$ genomes were polyploid, then at least some of these systems would exhibit multiplicity on electrophoresis, but none do.

It is also possible that the $n = 9$ species are polyploid but the additional loci were silenced by mutations so that their electrophoretic patterns now resemble those of diploid species. It seems unlikely, however, that all of the tested loci in two $n = 9$ species would have been turned off. Indeed, insofar as this problem has been studied in plant polyploids, genes inherited from different diploid species appear to maintain their function (5, 6). Also, to silence so many loci by gradual processes would mean that the $n = 9$ species were ancient. This seems improbable because of the relatively small morphological divergence between them and related species.

The observed constancy of structural gene number in the species of *Machaeranthera* and *Aster* is not unexpected if the species with lower numbers represent lineages that originally arose by aneuploid reduction. This process is generally conceived to involve translocation of essential euchromatin and loss of only heterochromatin and centromeres. The genetic evidence in this paper is supported by a cytological analysis of meiosis in a hybrid between *Aster hydrophilus* ($n = 9$) and *M. parviflora* ($n = 5$), a species very closely related to *M. turneri* examined in the present study, which showed cells with two or more small chromosomes of *A. hydrophilus* paired with single large chromosomes of *M. parviflora* (24). Constancy of genes coding enzymes detected by electrophoresis was also observed in seven species of *Crepis* that comprise a classical aneuploid series from $n = 6$ to $n = 3$ (7). A similar result has been found in *Coreopsis* (Compositae), a genus that includes a nearly complete aneuploid series from $n = 13$ to $n = 6$, for genes coding six enzyme systems (D. J. Crawford, personal communication).

The use of electrophoretic evidence of gene number to assign ploidy level takes advantage of an inherent genetic attribute of polyploids that can be analyzed simply and directly. It is now appropriate to examine other groups in the *Astereae* and elsewhere.

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