Selbyana 10: 36–55

ELECTROPHORETIC EVIDENCE FOR THE EVOLUTIONARY RELATIONSHIP OF THE TETRAPLOID CHENOPODIUM BERLANDIERI TO ITS PUTATIVE DIPLOID PROGENITORS

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ABSTRACT. An electrophoretic analysis of the western North American alveolate-fruited *Chenopodium* (subsect. *Cellulata*) was undertaken to examine the evolutionary relationships among the three diploid species *C. neomexicanum, C. palmeri, C. watsonii,* and the allotetraploid *C. berlandieri.* Data suggest that eight and 16 isozyme loci code for the five enzyme systems GOT, IDH, LAP, MDH, and PGI in the diploids and tetraploid, respectively. Results confirm that *C. berlandieri* is an allotetraploid, originating by hybridization between at least two different diploid genomes. On the basis of the electrophoretic phenotypes, geographical ranges, and past morphological studies, *C. neomexicanum* and *C. watsonii* are suggested as ancestors to the tetraploid. Enzyme multiplicity in *C. berlandieri* may account for the tetraploid's widespread distribution throughout western North America.

Isozyme analysis has been extremely useful for probing the identity of diploid progenitors to polyploid taxa (e.g., Cherry et al., 1970; Roose & Gottlieb, 1976; Hancock & Bringhurst, 1981; Crawford & Smith, 1984; Murdy & Carter, 1985; Werth et al., 1985; Bayer & Crawford, 1986). In general, an allopolyploid possesses a subset of the allozyme alleles from each of the parental diploid species. The polyploid and/or diploids may also possess unique alleles depending on the age of the polyploid and subsequent evolutionary events (Walters, 1985; Werth et al., 1985). This paper focuses on the phylogenetic relationship between the tetraploid *Chenopodium berlandieri* Moq. ($2n = 4x = 36$; Cole, 1962; Keener, 1970, 1974) and its putative diploid progenitors ($2n = 2x = 18$), *C. neomexicanum* Standley, *C. palmeri* Standley, and *C. watsonii* A. Nelson. The morphological and isozyme variation among the three diploid species is examined elsewhere (Walters, 1988).

Chenopodium section *Chenopodium* subsection *Cellulata* appears to be of New World origin with radiation from relatively arid, montane areas of Central America and western North and South America (Wahl, 1952–1953). Six tetraploids and three diploids are included in this morphologically complex and taxonomically difficult group (Aellen, 1929; Aellen & Just, 1943; Wahl, 1952–1953; Crawford, 1973, 1974; Wilson & Heiser, 1979). Western North American elements include the tetraploid *C. berlandieri* and three diploids: *C. neomexicanum, C. palmeri,* and *C. watsonii.* Species of the subsection have an inflorescence composed of many flowers clustered in compact glomerules, a perianth consisting of five sepals, an ovary with one basal ovule, and a fruit that is an utricle. The plants are self-pollinating, anemophilous inbreeders (Crawford & Wilson, 1977; Wilson, 1981); however, cross-pollination does occur (Walters, unpubl.).

Chenopodium berlandieri is a weedy annual that occupies disturbed open ground from Alaska south to Guatemala with an eastern extension along the Gulf and Atlantic Coasts. It is one of the most abundant and widespread species of the genus in North America. Past research has led to the hypothesis that *C. berlandieri* originated in the arid southwestern United States or northern Mexico (Wahl, 1952–1953). From this hypothesized center of origin, it supposedly radiated most extensively northward into the Great Plains and southward into Mexico (Wahl, 1952–1953). Based on a preliminary electrophoretic study, Wilson (1976b) suggests that *C. berlandieri* is an allotetraploid originating by hybridization of at least two different diploid genomes.

Chenopodium neomexicanum is found along disturbed, weedy roadsides in mountains above 1,500 m from west Texas to Arizona. The species is uncommon and localized; populations are often small and difficult to find. This diploid is easily mistaken in the field for *C. berlandieri* when mature fruits are not available. Crawford (1973) suggested that "plants similar to *C. neomexicanum* may be ancestral to *C. berlandieri.*"

Chenopodium palmeri also occurs along roadsides, as well as in other semidisturbed habitats from west Texas through northern Mexico (Walters, 1985). Although results of one morphological study indicated that this species was syn-

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onymous with *C. neomexicanum* (Crawford, 1973), recent analyses suggest that these species are morphologically distinct but isozymically similar (Walters, 1985, 1988).

Chenopodium watsonii is unique among North American chenopods in that it has relatively large seeds with white, attached pericarps, sepals that enclose the fruits completely at maturity, and an ill scent (Crawford, 1974). It occurs at higher elevations in disturbed soil in central and southern Colorado, New Mexico, Arizona, and Utah; a few populations have been located as far north and east as Kansas, South Dakota, Montana, and Alberta (Crawford, 1974).

The geographic distributions of the diploids and *Chenopodium berlandieri* correspond to general distributional patterns of other diploid/polyploid complexes. As is often the case (Stebbins, 1940, 1971; Jackson, 1976), the diploid taxa, *C. neomexicanum, C. palmeri,* and *C. watsonii,* are relatively restricted in distribution while the tetraploid is widespread. The generalization that tetraploids are found in labile or successional habitats and are dominant in regions that only recently have been opened to occupation, while the diploids occupy the older more stable habitats (Löve & Löve, 1943; Ehrendorfer, 1980), seems to hold true for this diploid/tetraploid complex. *Chenopodium berlandieri* is typically found as a pioneer species in crop fields, gardens, and areas under construction, while the diploids are found primarily along maintained roadsides. The tetraploid also occurs along roadsides, supporting the hypothesis that successful polyploids often compete with their diploid parents for the same habitat as well as colonize new habitats (DeWet, 1980).

The diploid elements of subsect. *Cellulata* have been implicated as contributors to the origin of *Chenopodium berlandieri* (Crawford, 1973; Wilson & Heiser, 1979). *Chenopodium neomexicanum, C. palmeri,* and *C. watsonii* are the only known extant diploid members of the subsection and they are sympatric with *C. berlandieri* (Wilson & Heiser, 1979). The primary centers of distribution for the diploids are the southwestern United States and northern Mexico, the suggested region of origin for the tetraploid (Wahl, 1952–1953; Crawford, 1973; Wilson & Heiser, 1979). Finally, a number of morphological and chemical characters found in *C. neomexicanum* (e.g., leaf shape, attached pericarp, fruits exposed at maturity, flavonoid chemistry, leaf epidermal patterns) and *C. watsonii* (e.g., ill scent, fruit enclosed within the sepals, opaque pericarp) are also found in *C. berlandieri* (Crawford, 1973; Walters, 1985), suggesting possible phylogenetic relationships between these two diploids and the tetraploid (Walters, 1985).

Materials and Methods

Fruits from plants of the three diploid species and the tetraploid species were collected from a sampling region ranging from Montana south to the Mexican state of Zacatecas and from Kansas west to the California coast. Southwestern United States and northern Mexico were intensively sampled because Wahl (1952–1953) hypothesized that this area was the center of origin and diversity for the tetraploid. A total of 188 populations of the four species was subjected to electrophoresis (see Walters, 1985, for complete locality information). All voucher specimens, including progeny from artificial pollinations, and seed packets are maintained in the Biology Department Herbarium, Texas A&M University (TAMU).

Population samples for starch-gel electrophoresis consisted of two types: 1) a mixed population seed sample ("mixed packet") and 2) an individual plant seed packet ("plant packet"). The mixed packet contained ten fruits from each of ten plants in a population. The plant packet, of which there were at least ten per population, contained the fruits from an individual plant.

Of the 188 populations, 99 were assayed using plants grown from mixed packets. All statistical analyses were based on the results from these populations. Nineteen of these 99 populations represented the three diploid species. All information and isozyme data relating to the diploid populations are presented in Walters (1988). Eighty of these populations represented the tetraploid (Appendix 1). Each of the 99 population samples consisted of at least eight plants grown from fruits of a mixed packet. These plants were used for electrophoresis. If variation in banding patterns was detected for a mixed packet, then the population was examined in more depth by sampling the associated plant packets. Ninety-five of the 188 populations were assayed in this manner. A total of 300 plant packets were sampled using at least eight fruits per plant packet. This sampling method assisted in determining the genetic basis for observed variants.

Seedlings for electrophoresis were grown in growth chambers with light provided by a mixture of incandescent and fluorescent bulbs. After leaf samples were taken for electrophoresis, plants with new phenotypes, plants with possible heterozygous phenotypes, and/or plants to be used as pollen parents for crosses were transferred to a greenhouse. Selfing of plants entailed covering the inflorescence primordia with a plastic bag and tying the base of the bag around the stem. Hand pollination and/or inflorescence to inflorescence contact followed by subsequent bagging of the female parent were the general procedures

for artificial hybridization. Since flowers of *Chenopodium* species are small and tightly compacted in the inflorescence, identifying the fruit(s) produced from the cross is nearly impossible. Isolation of the hybrids requires testing numerous progeny from the inflorescence of the female plant. Since *Chenopodium* species are inbreeders, progeny from a single inflorescence will reflect selfing as well as crossing events. Further details on these methods are described in Wilson (1980).

All electrophoretic runs contained two standards for consistency in the scoring of electrophoretic phenotypes. The two standards, *Chenopodium berlandieri* ssp. *nuttalliae* (Safford) Wilson & Heiser and *C. quinoa* Willd., are two cultivated members of subsection *Cellulata* that have been standards in the laboratory since 1978 (Wilson, unpubl.). Plants of the two standards were grown from fruits obtained from self-pollinated individuals. No variation was detected within either of the two standards. Banding pattern phenotypes were scored based on their relationship to these standards. Phenotypes for each population sample were later transformed into genotypes once isozyme loci and allelic variants were identified.

Past electrophoretic studies on species in the genera *Chenopodium* and *Cucurbita* provided the basis for buffer systems used and enzymes assayed for this study (Crawford, 1976, 1979; Wilson, 1976a, 1976b, 1981; Crawford & Wilson, 1977, 1979; Wilson & Heiser, 1979; Wilson et al., 1983; Kirkpatrick, 1984). Sample preparation followed Wilson (1981); buffer and gel recipes followed Wilson (1981) and Kirkpatrick (1984). In accordance with these established methods, two buffer systems (one continuous and one discontinuous) were used for assaying five enzyme systems: 1) glutamate oxaloacetate transaminase (GOT), 2) isocitrate dehydrogenase (IDH), 3) leucine aminopeptidase (LAP), 4) malate dehydrogenase (MDH), and 5) phosphoglucose isomerase (PGI). The Tris-citrate gel buffer (pH 8.7), sodium hydroxide-boric acid electrode buffer (pH 8.1), and an 8.8 percent starch (Connaught) concentration were used for assaying GOT and LAP (APPENDIX 2). The gel and electrode buffers (pH 6.5) of L-histidine-citric acid and a 9.5 percent starch concentration (Connaught) were used to assay IDH, MDH, and PGI (APPENDIX 2).

Samples for electrophoresis consisted of two 9 mm discs of leaf tissue (30 mg) from the first two primary leaves. The leaf sample, along with one drop of extract buffer (0.02 M Tris-HCl, 0.03 M mercaptoethanol, pH 7.0), was machine ground and the resulting crude homogenate was absorbed onto a 2 × 12 mm filter paper wick (Whatman #3). The wick was then inserted in a slit cut across the width of a 20.5 × 14.5 × 0.6 cm horizontal starch slab 7.5 cm from the cathodal end of the gel; each gel held 30 wick samples. Electrophoresis was conducted at 4°C. Gels were run at 30 mA and 250 V for the L-histidine buffer system. For the Tris-citrate buffer system, gels were run at 30 mA and 100 V rising to 250 V. Wicks were removed after 15 min. Electrophoresis continued for seven hours on the Tris-citrate system and eight hours for the L-histidine system. Stain assay recipes are presented in APPENDIX 2.

Disc electrophoresis was performed in eight percent acrylamide gels using procedures adapted from those described by Davis (1964). Leaf tissue was machine ground in 0.3 ml of a pH 7.5 buffer (Carlson, 1972). A pH 8.57 Tris-glycine buffer (5.0 g Tris and 28.8 g glycine/liter) was used in the reservoirs. Detailed description of the procedures and recipes for disc gel electrophoresis are described in Hart (1975) and Hart and Langston (1977). Disc electrophoresis was only performed to resolve banding patterns in GOT and LAP. Gels were stained following the procedures described in APPENDIX 2.

Genetic interpretation of electrophoretic phenotypes was based on several lines of evidence. The first was the frequency and common occurrence of suites of phenotypes in plant and mixed packets. The second was based on the results of self-pollination and crossing experiments where the banding phenotypes of the parents were known. Heterozygous progeny obtained from a cross were scored and often selfed to produce an

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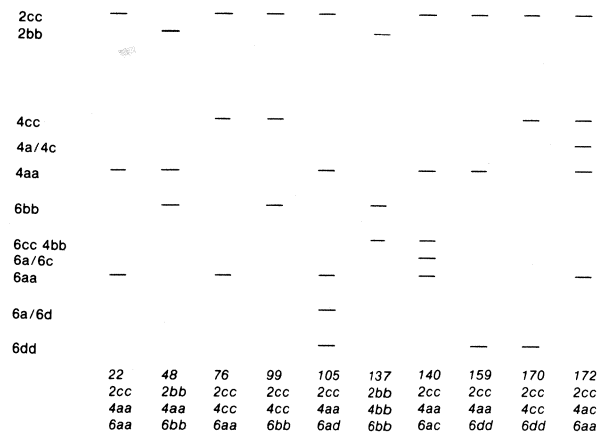
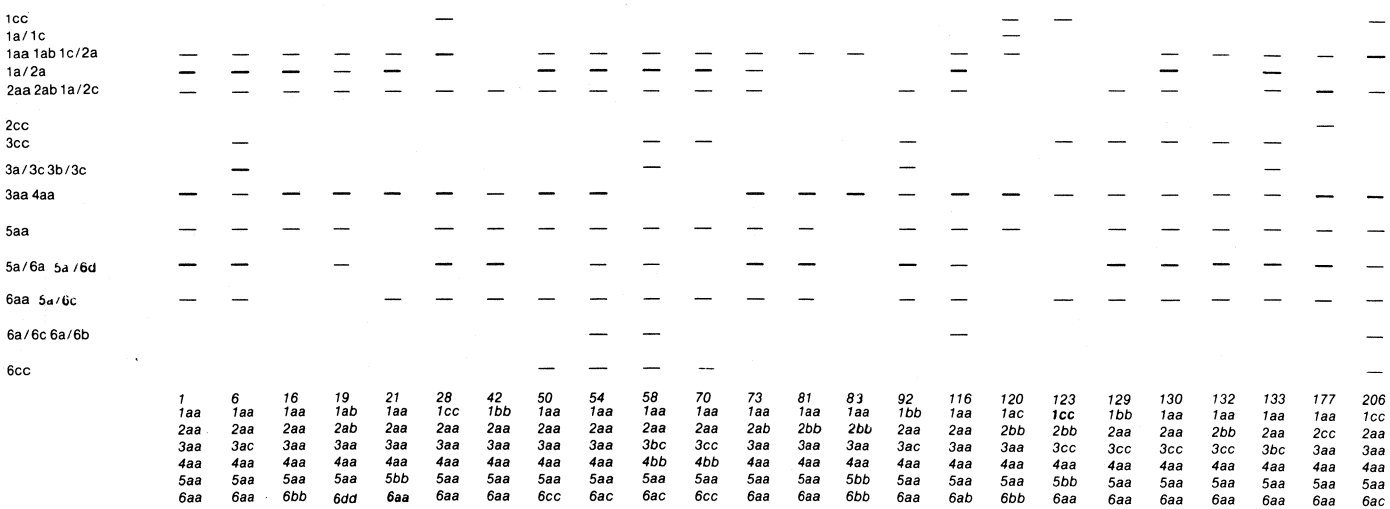
FIGURE 1. Genetic interpretation of electrophoretic banding patterns for GOT in the tetraploid *Chenopodium berlandieri,* and the diploids, *C. neomexicanum, C. palmeri,* and *C. watsonii.* The phenotype designation is given directly below each pattern. Alleles present at all loci contributing to a banding pattern are given below each phenotype. To the left of the phenotypes are the enzyme subunit combinations that form the bands. Allelic products forming intralocus and interlocus heterodimers are indicated by a slash between subunits of the dimer (e.g., 1a/2a). In the tetraploid, *Got-6d* and all "b" alleles represent null (inactive) alleles. In cases where a locus was heterozygous for an active and null variant, and the heterodimer comigrated with the active homodimer, the subunits are presented without a slash (e.g., 2ab). Since the position of an inactive enzyme product associated with a locus homozygous for a null allele (e.g., 2bb in the tetraploid) is not known, these are not given on the left side of the diagram.

GOT - Diploid



GOT - Tetraploid



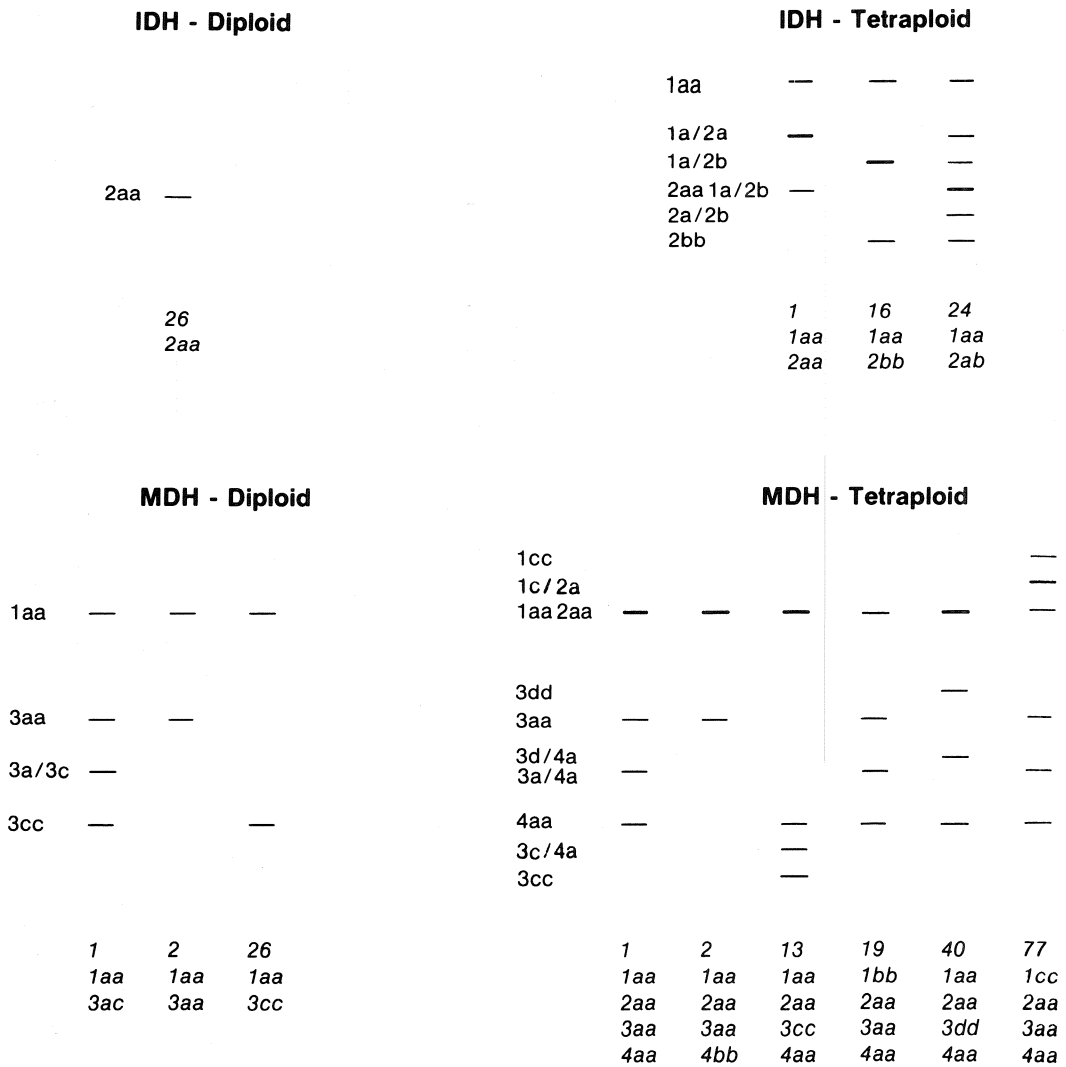


FIGURE 2. Genetic interpretation of electrophoretic banding patterns observed for IDH and MDH in the tetraploid *Chenopodium berlandieri,* and the diploids, *C. neomexicanum, C. palmeri,* and *C. watsonii.* Null variants in the tetraploid are *Mdh-1b* and *Mdh-4b.* Loci and allele designations as for GOT (FIGURE 1).

F_2 generation. The third line of evidence was the relative banding intensities of phenotypes. Fourth was the interpretation of the electrophoretic phenotypes in the diploid species. Results from previous electrophoretic studies within the genus were also considered (Wilson, 1976a, 1976b, 1981; Crawford & Wilson, 1977, 1979; Crawford, 1979; Wilson et al., 1983). Finally, putative genetic loci and genotypes were inferred from the known substructures of enzymes (Gottlieb, 1982).

Determination of paralogous loci (defined as homologous loci within a species that are the result of gene duplication; Wiley, 1981) was based on three lines of evidence. First, there appears to be a characteristic minimum number of isozymes in diploid plants for several enzyme systems (Gottlieb, 1982). Second, for any particular enzyme, there appears to be a consistent pattern of subcellular compartmentalization in higher plants which affects patterns of heterodimer formation (Gottlieb, 1981). Finally, if the most frequent products of the allelic variants of two loci comigrated to identical positions on the gel, the loci were considered to be paralogous.

The manner in which loci and alleles were designated is described in FIGURE 1. Note that locus

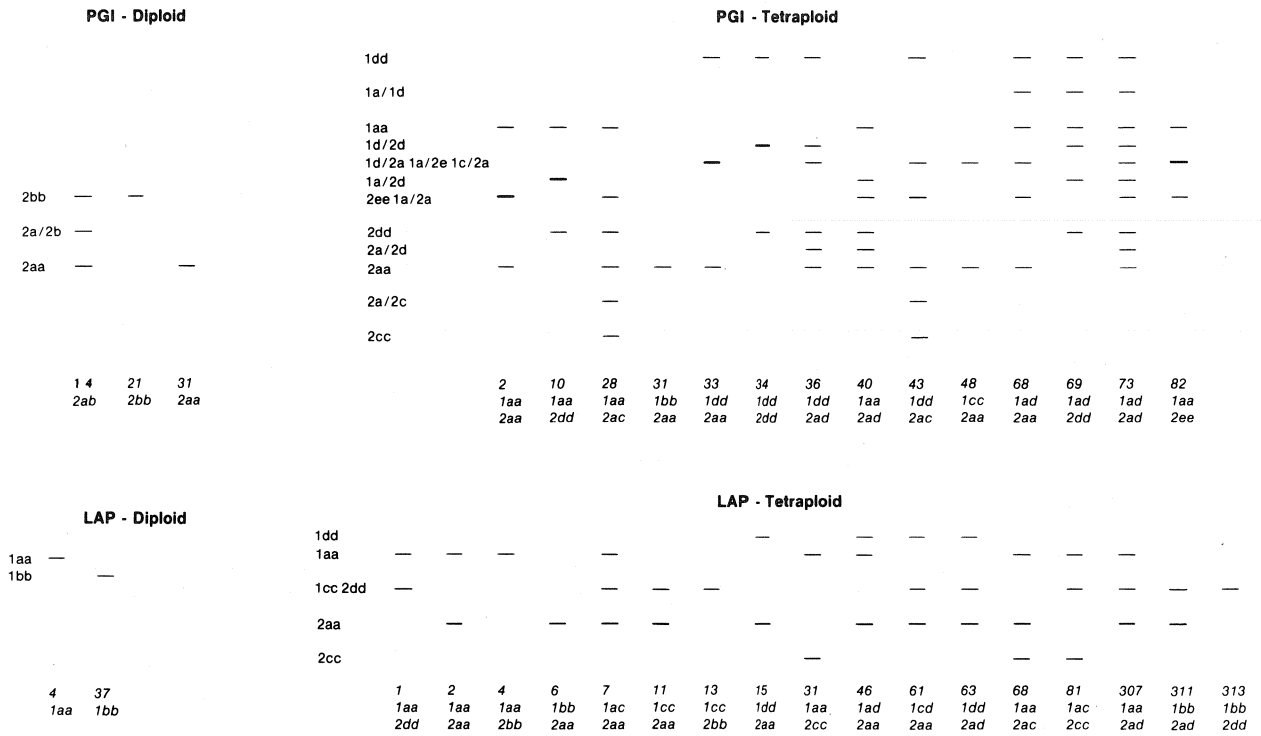


FIGURE 3. Genetic interpretation of electrophoretic banding patterns for LAP and PGI observed in the tetraploid *Chenopodium berlandieri,* and the diploids, *C. neomexicanum, C. palmeri,* and *C. watsonii.* Null variants are *Pgi-1b, Pgi-1c, Lap-1b* and *Lap-2b.* Uppermost bands in PGI are not illustrated because of poor resolution. Loci and allele designations as for GOT (FIGURE 1).

TABLE 1. Results from selfing experiments. See text for enzyme system abbreviations. B = *Chenopodium berlandieri*, N = *C. neomexicanum*, P = *C. palmeri*, W = *C. watsonii*.

Enzyme	Parent species	Phenotype (genotype)		Number of F_1 progeny
		Parent	F_1 progeny	
GOT	N	22 (2cc/4aa/6aa)	22 (2cc/4aa/6aa)	38
	N	140 (2cc/4aa/6ac)	14:140:22 (2cc/4aa/6cc 2cc/4aa/6ac 2cc/4aa/6aa)	14:21:11
	P	172 (2cc/4ac/6aa)	22:172:76 (2cc/4aa/6aa 2cc/4ac/6aa 2cc/4cc/6aa)	2:5:8
	W	48 (2bb/4aa/6bb)	48 (2bb/4aa/6bb)	16
	B	50 (1aa/2aa/3aa) (4aa/5aa/6cc)	50 (1aa/2aa/3aa) (4aa/5aa/6cc)	10
	B	1 (1aa/2aa/3aa) (4aa/5aa/6aa)	1 (1aa/2aa/3aa) (4aa/5aa/6aa)	18
	B	54 (1aa/2aa/3aa) (4aa/5aa/6ac)	1:54:50 (1aa/2aa/3aa 1aa/2aa/3aa 1aa/2aa/3aa) (4aa/5aa/6aa 4aa/5aa/6ac 4aa/5aa/6cc)	3:19:5
	B	73 (1aa/2ab/3aa) (4aa/5aa/6aa)	1:73:81 (1aa/2aa/3aa 1aa/2ab/3aa 1aa/2bb/3aa) (4aa/5aa/6aa 4aa/5aa/6aa 4aa/5aa/6aa)	5:3:7
	B	116 (1aa/2aa/3aa) (4aa/5aa/6ab)	1:116:16 (1aa/2aa/3aa 1aa/2aa/3aa 1aa/2aa/3aa) (4aa/5aa/6aa 4aa/5aa/6ab 4aa/5aa/6bb)	2:5:6
IDH	P	26 (2aa)	26 (2aa)	27
	B	16 (1aa/2bb)	16 (1aa/2bb)	11
	B	1 (1aa/2aa)	1 (1aa/2aa)	57
	B	24 (1aa/2ab)	1:24:16 (1aa/2aa 1aa/2ab 1aa/2bb)	6:5:5
LAP	N	4 (1aa)	4 (1aa)	38
	W	37 (1bb)	37 (1bb)	18
	B	1 (1aa/2dd)	1 (1aa/2dd)	23
	B	2 (1aa/2aa)	2 (2aa/2aa)	57
	B	15 (1dd/2aa)	15 (1dd/2aa)	12
	B	7 (1ac/2aa)	2:7:11 (1aa/2aa 1ac/2aa 1cc/2aa)	8:9:5
	B	62 (1cd/2aa)	11:62:15 (1cc/2aa 1cd/2aa 1dd/2aa)	13:9:3
	B	307 (1aa/2ad)	1:307:2 (1aa/2dd 1aa/2ad 1aa/2aa)	4:11:5
MDH	P	26 (1aa/3cc)	26 (1aa/3cc)	15
	P	1 (1aa/3ac)	2:1:26 (1aa/3aa 1aa/3ac 1aa/3cc)	3:3:3
	B	1 (1aa/2aa/3aa/4aa)	1 (1aa/2aa/3aa/4aa)	25
	B	40 (1aa/2aa/3dd/4aa)	40 (1aa/2aa/3dd/4aa)	17
	B	77 (1cc/2aa/3aa/4aa)	77 (1cc/2aa/3aa/4aa)	57

TABLE 1. Continued.

Enzyme	Parent species	Phenotype (genotype)		Number of F_1 progeny
		Parent	F_1 progeny	
PGI	N	21 (2bb)	21 (2bb)	38
	W	14 (2ab)	21:14:31 (2bb 2ab 2aa)	5:8:3
	B	2 (1aa/2aa)	2 (1aa/2aa)	28
	B	31 (1bb/2aa)	31 (1bb/2aa)	13
	B	48 (1cc/2aa)	48 (1cc/2aa)	25
	B	69 (1ad/2dd)	10:69:34 (1aa/2dd 1ad/2dd 1dd/2dd)	2:4:2

and allele designations between levels of ploidy do not necessarily reflect homology.

The BIOSYS-1 package of Swofford and Selander (1981) was used to determine 1) percentage of loci polymorphic (PLP) using the 0.99 criterion; 2) average heterozygosity based on "direct count" (H_{obs}) and an unbiased estimate (Nei, 1978) based on Hardy-Weinberg expectations (H_{exp}); 3) Nei's (1972) identity value (I); and 4) mean number of alleles per locus. Only data obtained from the mixed packets were included in the statistical analyses.

RESULTS

The most common electrophoretic phenotypes observed from plants grown from mixed and plant packets are diagrammed in FIGURES 1–3. All bands migrated anodally from the origin. For the tetraploid, those loci producing the most anodal variants received the lowest numerical designation. In the diploids, a locus that had allelic variants migrating to positions similar to the allelic variants at a tetraploid locus was given the same locus designation as the tetraploid. The most common allelic variant at each locus was designated with an "a" for both the diploid species and the tetraploid species.

In the three diploids, a total of eight loci were determined for the five enzyme systems: *Got-2, Got-4, Got-6, Idh-2, Lap-1, Mdh-1, Mdh-3,* and *Pgi-2* (FIGURES 1–3). Sixteen enzyme loci were identified in the tetraploid; paralogous loci were given separate loci designations [1) *Got-1* and *Got-2,* 2) *Got-3* and *Got-4,* 3) *Got-5* and *Got-6,* 4) *Idh-1* and *Idh-2,* 5) *Lap-1* and *Lap-2,* 6) *Mdh-1* and *Mdh-2,* 7) *Mdh-3* and *Mdh-4,* and 8) *Pgi-1* and *Pgi-2*]. The allelic frequencies observed at these loci for the mixed packets varied among many of the 80 populations of *Chenopodium berlandieri* (APPENDIX 3).

Results from selfing and artificial hybridization experiments in some cases showed at most three phenotypes (TABLE 1). Results indicating heterozygosity at more than one locus within an enzyme system are not presented here. Although in some experiments the low number of progeny tested makes the data statistically inconclusive, these data do indicate the nature of the progeny produced. Almost all crossing experiments resulted in two phenotypes in the F_1 progeny, one phenotype representing self-pollination of the homozygous parent and the second phenotype representing hybridization with a genotypically distinct pollen parent (TABLE 2).

GOT. The presence of three loci, as well as the absence of interlocus heterodimer formation, in the *Chenopodium* diploids appears consistent with other studies of diploid plants for dimeric GOT (Gottlieb, 1981). In the diploids, two alleles were identified for *Got-2,* three alleles for *Got-4,* and four alleles for *Got-6* (FIGURE 1). Alleles *Got-2b* and *Got-4b* are exclusive to populations of *C. watsonii, Got-4c* and *Got-6a* are exclusive to *C. neomexicanum* and *C. palmeri* populations, *Got-6d* is found only in *C. palmeri,* while the remaining alleles are shared by all three diploid species (Walters, 1988). Allele *Got-6b* is common in *C. watsonii* and rare in the other diploids.

Of the six loci in the tetraploid, only the products of *Got-1* and *Got-2,* as well as *Got-5* and *Got-6,* appeared to form interlocus heterodimers (FIGURE 1). The most common allelic variants, "a", at the paralogous loci *Got-3* and *Got-4* comigrated to identical positions on the gel (FIGURE 1). Four alleles at *Got-6,* three alleles each at *Got-1, Got-2,* and *Got-3,* and two at *Got-4* and *Got-5* were identified in the tetraploid population system (FIGURE 1). Alleles *Got-3b, Got-6b,* and *Got-6d* are "activity nulls" (see Goodman & Stuber, 1983), meaning that each forms an active subunit combination which produces a

TABLE 2. Results from artificial hybridization experiments. See text for enzyme system abbreviations. B = *Chenopodium berlandieri,* N = *C. neomexicanum,* Q = *C. quinoa.*

Enzyme	Species crossed	Phenotype (genotype) Parent ♀	Parent ♂	F_1 progeny	Number of F_1 progeny
GOT	N × N	22 (2cc/4aa/6aa)	14 (2cc/4aa/6cc)	22:140 (2cc/4aa/6aa 2aa/4aa/6ac)	7:5
	B × B	1 (1aa/2aa/3aa) (4aa/5aa/6aa)	50 (1aa/2aa/3aa) (4aa/5aa/6cc)	1:54 (1aa/2aa/3aa 1aa/2aa/3aa) (4aa/5aa/6aa 4aa/5aa/6ac)	44:15
	B × Q	1 (1aa/2aa/3aa) (4aa/5aa/6aa)	123 (1cc/2bb/3cc) (4aa/5bb/6aa)	1:99 (1aa/2aa/3aa 1ac/2ab/3ac) (4aa/5aa/6aa 4aa/5ab/6aa)	3:3
	B × B	42 (1bb/2aa/3aa) (4aa/5aa/6aa)	82 (1aa/2bb/3aa) (4aa/5aa/6aa)	42:194 (1bb/2aa/3aa 1ab/2ab/3aa) (4aa/5aa/6aa 4aa/5aa/6aa)	5:1
IDH	B × B	1 (1aa/2aa)	16 (1aa/2bb)	1:24 (1aa/2aa 1aa/2ab)	12:19
LAP	B × B	2 (1aa/2aa)	1 (1aa/2dd)	2:307 (1aa/2aa 1aa/2ad)	28:23
	B × B	1 (1aa/2dd)	11 (1cc/2aa)	1:315 (1aa/2dd 1ac/2ad)	4:4
MDH	B × Q	1 (1aa/2aa) (3aa/4aa)	77 (1cc/2aa) (3aa/4aa)	1:105 (1aa/2aa 1ac/2aa) (3aa/4aa 3aa/4aa)	32:12
PGI	B × B	2 (1aa/2aa)	33 (1dd/2aa)	2:68 (1aa/2aa 1ad/2aa)	23:115
	B × B	2 (1aa/2aa)	48 (1cc/2aa)	2:103 (1aa/2aa 1ac/2aa)	4:4

band on the gel. Bands representing intralocus heterodimers between the alleles *Got-3b* and *Got-3c* and between the alleles *Got-6b* and *Got-6a* are shown in phenotypes 58 and 116 (FIGURE 2). However, allele *Got-6b* appears not to form interlocus heterodimers with alleles at *Got-5* (FIGURE 1: phenotype 16; TABLE 1: see phenotype 116 selfing data). In contrast, *Got-6d* forms an active subunit combination with *Got-5a* (FIGURE 1: phenotype 19).

IDH. All three diploids were monomorphic for the same variant at *Idh-2* for the dimeric enzyme system IDH (FIGURE 2: phenotype 26). The nonsegregating interlocus heterodimer in the tetraploid suggests that the two loci are paralogous. Locus *Idh-1* was monomorphic for the same allele in all populations of the tetraploid; two allelic variants were identified at *Idh-2* (FIGURE 2).

MDH. No interlocus heterodimer occurred between *Mdh-1* and *Mdh-3* in the diploids for this dimeric enzyme (FIGURE 2). Two allelic variants at *Mdh-3* and one at *Mdh-1* were shared by the three diploid species (FIGURE 2). Interlocus heterodimers were detected between the products of the paralogous loci 1 and 2, and loci 3 and 4 in the tetraploid. This is illustrated in phenotype 77 which was always true breeding (TABLE 1). Three variants at *Mdh-1,* three at *Mdh-3,* and two at *Mdh-4* were observed in the tetraploid populations (FIGURE 2). All tetraploid populations were monomorphic for *Mdh-2aa,* which produced an enzyme that comigrated with that of *Mdh-1aa* (FIGURE 2). The null allele *Mdh-1b* was only detected in plants grown from plant packets.

PGI. The most anodal band(s) for PGI in the diploids and tetraploid could not be scored due to resolution problems. On the basis of the lower bands, a one-locus model for the diploids and a two-locus model for the tetraploid is indicated for this dimeric enzyme (FIGURE 3). Two allelic variants at the PGI locus in the *Cellulata* diploids and four variants at each of the tetraploid loci were observed (FIGURE 3). Among the diploids, allele *Pgi-2a* was found only in *Chenopodium watsonii,* while *Pgi-2b* was common to all three species (Walters, 1988).

Interlocus heterodimers formed in the tetraploid (FIGURE 3). Two phenotypes, 31 and 48, were found to be homozygous for different null variants at locus *Pgi-1* and homozygous for an identical allele at *Pgi-2* (*Pgi-2a*). Phenotype 31 is a one-banded pattern, while phenotype 48 is a two-banded pattern (FIGURE 3). Based on selfing and artificial hybridization experiments, the

TABLE 3. Genetic statistics within each species. N = *Chenopodium neomexicanum*, P = *C. palmeri*, W = *C. watsonii*, B = *C. berlandieri*. Standard deviations are in parentheses.

Species	Number of populations sampled	Mean sample size/population	Number of loci	Mean no. of alleles per locus	Total loci polymorphic	Mean percentage loci polymorphic	Mean observed heterozygosity	Mean expected heterozygosity
N	9	27.41	8	1.01 (0.03)	12	1.39 (4.17)	0.000 (0.00)	0.007 (0.02)
P	6	22.02	8	1.13 (0.18)	37	10.42 (14.61)	0.001 (0.00)	0.037 (0.06)
W	4	20.85	8	1.30 (0.16)	50	25.00 (17.68)	0.016 (0.01)	0.076 (0.06)
B	80	16.10	16	1.13 (0.21)	80	10.02 (10.60)	0.008 (0.02)	0.038 (0.04)

additional band in phenotype 48 appears to represent an active interlocus heterodimer (TABLES 1, 2). When a plant with phenotype 48 was crossed with a plant with phenotype 2, the hybrid progeny showed a five-banded phenotype representing the following subunit combinations: 1a/1c, 1aa, 2aa, 1a/2a, and 1c/2a. Here again is the unusual condition in which an "activity null" encoded for by one locus combines with an active variant from the other locus to form a functional heterodimer. In contrast, the null allele *Pgi-1b* appears not to recognize the enzyme subunits produced by the paralogous locus. This may represent two successive stages toward diploidization of a polyploid.

LAP. *Chenopodium neomexicanum* and *C. palmeri* populations were monomorphic for the same allele (*Lap-1a*) at the single monomeric LAP locus (FIGURE 3: phenotype 4). *Chenopodium watsonii* populations were monomorphic for *Lap-1b* (FIGURE 3: phenotype 37). Four allelic variants were identified at each of the two loci in the tetraploid (FIGURE 3).

During the course of the study tetraploid phenotype 7 occurred in two different suites of phenotypes: 1, 7, and 2, as well as 2, 7, and 11 (FIGURE 3). Based on the segregation data and the genetic interpretation of the phenotypes, phenotype 7 may in fact represent two different genotypes (*1ac 2aa* and *1aa 2ad*). Therefore, plants, which when selfed produced progeny with phenotypes 11 and 2, were scored as phenotype 7; plants producing progeny with phenotypes 1 and 2 were rescored as phenotype 307 (TABLE 1; FIGURE 3). When segregation data were not available, other phenotypes scored in the population from which a plant was obtained were used to determine whether the phenotype of that plant should be designated as 7 or 307. In a similar fashion, phenotypes 11 and 13 were determined to represent two genotypes each. Phenotype 11 was rescored as 11 or 311 and phenotype 13 as 13 or 313 as appropriate (FIGURE 3).

Values for observed mean heterozygosity were consistently lower than the expected mean heterozygosity values for the tetraploid and the diploids (TABLE 3). The three diploid species and the tetraploid generally exhibited PLP and mean H_{exp} (TABLE 3) higher than those expected for selfing species (PLP = 4.4, H_{exp} = 0.001) but lower than those of the average outcrossing species (PLP = 37.0, H_{exp} = 0.086; Gottlieb, 1981). The identity value (Nei, 1972) of 0.930 (0.665–1.000) for the tetraploid populations is comparable to those presented for the diploid species (Walters, 1988) and other *Chenopodium* species (Crawford, 1983).

The most common phenotypes observed in the tetraploid population for each of the five enzyme systems are GOT 1, IDH 1, LAP 2, MDH 1, and PGI 2 (FIGURE 4). The overall common phenotype for the tetraploid (the composite of common phenotypes over all enzyme systems) was found in at least one plant in 54 of the 80 populations, especially in populations from southwestern United States.

DISCUSSION

The low expected mean heterozygosity values for the diploids and tetraploid are probably due to the consequences of frequent self-fertilization and the difficulty in identifying heterozygous phenotypes for some of the enzyme systems. This conclusion is supported by greenhouse studies and other electrophoretic results which also suggest that *Chenopodium* species are facultative outcrossers (Walters, 1985).

To test if the overall common phenotype may represent the primitive phenotype for the tetraploid, diploid phenotypes that, when combined, might produce the overall common phenotype

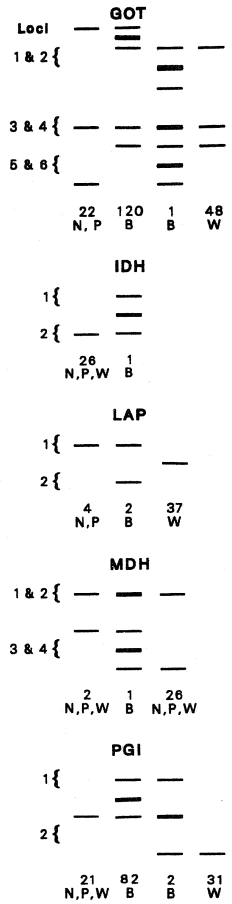


FIGURE 4. Electrophoretic phenotypes of the three diploid species (N = *Chenopodium neomexicanum*, P = *C. palmeri*, W = *C. watsonii*) and the tetraploid (B = *C. berlandieri*) which expressed the highest degree of similarity in band migration. The phenotype designation and the species in which the phenotype occurred are given directly below each banding pattern. The diagram incorporates those tetraploid phenotyes (GOT 1, IDH 1, LAP 2, MDH 1, PGI 2) which together form the most common and widespread composite tetraploid phenotype. To the left of each enzyme system are locus designations (see APPENDIX 3). The darker bands represent either the products of two homozygous loci that comigrate to the same position or a heterodimer.

were identified (FIGURE 4). In those cases where all the bands of the common tetraploid phenotype could not account for or be accounted for by known diploid phenotypes (e.g., GOT, IDH, LAP, PGI), additional tetraploid phenotypes were surveyed to look for match-ups (FIGURE 4).

Combining any two of the observed diploid phenotypes does not produce the tetraploid GOT phenotype 1 (FIGURE 4). However, combining the diploid phenotype 48 found in *Chenopodium watsonii* with phenotype 22 found in *C. neomexicanum* and *C. palmeri* would account for the four slowest bands of the common tetraploid phenotype. The additional band in the tetraploid represents an interlocus heterodimer. Tetraploid phenotype 120, on the other hand, possesses the most anodal three bands that would be expected between a cross of these diploid phenotypes. Note that the diploid phenotype 48 (FIGURE 1) represents one allele (*Got-2b*) that is exclusive to *C. watsonii* and another (*Got-6b*) which is rare in the other diploids. Also, diploid phenotype 22 represents allele *Got-6a,* which is only found in *C. neomexicanum* and *C. palmeri.*

A band corresponding to the most anodal band of the tetraploid IDH phenotype was not detected in any of the diploid populations (FIGURE 4). However, all taxa exhibited the lowermost band, which presumably represents the same allelic variant. Similarly, one LAP band in the tetraploid was shared by *Chenopodium neomexicanum* and *C. palmeri,* while the other band was not detected in any of the diploids (FIGURE 4). A similar situation exists with respect to PGI, although this time it is *C. watsonii* that shares a band, and possibly the same allele, with the common tetraploid phenotype 2. Tetraploid phenotype 82, however, does contain the identical band (and presumably the same allele) found in phenotype 21 of all three diploids (FIGURE 4). Finally, if one combines the MDH phenotypes 2 and 26 found in the diploid species, the common tetraploid phenotype 1 can be formed. A similar phenotype (1) was found in *C. watsonii* (FIGURE 3), although, in the diploid, the central band of the lower trio of bands represents an intralocus rather than an interlocus heterodimer.

Paralogous loci designation in the tetraploid, concordance of electrophoretic phenotypes between the diploids and tetraploid, and morphological studies (Walters, 1985) suggest a number of possibilities concerning the parentage of the tetraploid. The first possibility is that the tetraploid may have been formed by the hybridization of *Chenopodium neomexicanum* or *C. palmeri* and *C. watsonii.* The lack of more correspondence between the diploid and tetraploid phenotypes may be due to the small number of diploid populations sampled or to the loss of these allozyme alleles in the diploids through time. A second possibility is that one of the two species involved in the origin of the tetraploid is extinct. The third possibility is that the ancestral form of the tetraploid is no longer present. If this is true, then the common tetraploid phenotypes presented here represent divergent phenotypes rather than the primitive phenotypes. This most

likely explains the lack of total correspondence between diploid and the common tetraploid phenotypes.

The following is suggested as a working hypothesis for the primitive tetraploid phenotype. For GOT, the primitive phenotype was a combination of the upper three bands of tetraploid phenotype 120 and the slower four bands of phenotype 1 (FIGURE 4). For IDH, the primitive phenotype was a single band similar to the diploid phenotype 26. Mutation at one of the paralogous IDH loci early in the evolution of the tetraploid produced the common three-banded pattern. Similarly, the original LAP tetraploid phenotype was like the diploid phenotype 4, followed by divergence at one of the paralogous loci. The primitive phenotype for PGI was a three-banded pattern containing the bands of diploid phenotypes 21, 31, and the interlocus heterodimer (FIGURE 4). As with IDH and LAP, mutation early in the evolution of the tetraploid occurred at one of the paralogous loci to produce a faster variant which is now common in tetraploid populations.

The high degree of morphological similarity between *Chenopodium neomexicanum* and *C. berlandieri* (Crawford, 1973; Walters, 1985) and the concordance of their electrophoretic phenotypes (FIGURE 4) support the hypothesis that *C. neomexicanum* was involved in the origin and/or evolution of *C. berlandieri* as suggested by Crawford (1973). Although *C. palmeri* exhibits the same degree of isozyme concordance with the tetraploid as *C. neomexicanum,* morphological studies suggest that *C. palmeri* is not as similar to the tetraploid as are *C. neomexicanum* and *C. watsonii* (Walters, 1985). Isozyme and morphological evidence (Walters, 1985) indicate that *C. watsonii* is probably the other parent in the polyploid event. *Chenopodium berlandieri* and *C. watsonii* exhibit a high affinity with respect to banding phenotypes, most significantly in GOT and PGI.

Enzyme multiplicity and novel enzyme heterodimers, as a direct consequence of possessing two divergent genomes, may extend the range of environments in which an allotetraploid can exist, thereby accounting for the relatively wide distribution of many polyploids (Roose & Gottlieb, 1976; Murdy & Carter, 1985; Soltis & Rieseberg, 1986). Roose and Gottlieb (1976) suggested that the ecological success of the tetraploids of *Tragopogon* reflects, in part, their enzyme multiplicity. The extensive distribution of *Chenopodium berlandieri* throughout North America, in contrast to the restricted range of its putative diploid progenitors, further supports the notion that enzyme multiplicity in polyploids may contribute to the ability of the polyploid to adapt to varying environmental conditions, thereby increasing its geographic range.

ACKNOWLEDGMENTS

This paper is based in part on a Ph.D. dissertation submitted to the Biology Department at Texas A&M University. The research was supported in part by NSF Grant DEB-8212148 and the Department of Biology at Texas A&M University. I am most grateful to Deena Decker Walters for her comments on a preliminary draft of the manuscript. I thank J. Kress and two anonymous reviewers for their critiques.

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APPENDIX 1. Locality information of the 80 populations of *Chenopodium berlandieri*.

Population number	Latitude (N)	Longitude (W)	Altitude (m)	Locality	Collector**	Collector number
10	19.17	099.40	2,134	Mexico, D.F.*	HDW	02943
11	18.48	097.11	1,829	Veracruz*	HDW	02953
66	44.06	103.14	990	South Dakota	HDW	03042
88	19.07	099.33	2,624	Mexico, D.F.*	HDW	03172
94	14.45	091.12	9	Guatemala	HDW	03626
98	34.30	112.30	1,636	Arizona	ELE	20797
103	30.30	096.40	0	Texas	HDW	03737
107	30.50	103.80	1,631	Texas	TWW	01685
108	30.50	103.80	1,372	Texas	TWW	01682
109	30.50	103.80	1,585	Texas	TWW	01684
111	30.19	104.01	1,433	Texas	TWW	01687
113	36.75	106.50	1,875	New Mexico	TWW	01656
114	36.75	106.50	2,164	New Mexico	TWW	01660
115	33.20	110.50	1,524	Arizona	TWW	01641
116	33.20	110.50	1,829	Arizona	TWW	01643
117	33.20	110.50	1,113	Arizona	TWW	01640
118	36.90	106.60	2,347	New Mexico	TWW	01661
120	35.15	111.40	2,118	Arizona	TWW	01644
121	34.55	110.15	2,134	Arizona	TWW	01645
123	30.21	103.41	1,722	Texas	TWW	01689
124	36.25	105.35	2,118	New Mexico	TWW	01668
127	35.36	105.13	2,057	New Mexico	TWW	01676
128	34.60	106.25	2,042	New Mexico	TWW	01678
129	35.10	106.00	2,210	New Mexico	TWW	01677
131	30.21	103.41	1,280	Texas	TWW	01690
132	27.32	097.52	0	Texas	TWW	01708
133	32.45	108.20	1,768	New Mexico	TWW	01609
134	33.10	107.20	2,423	New Mexico	TWW	01611
137	32.15	111.00	1,570	Arizona	TWW	01636
141	33.75	108.70	1,920	New Mexico	TWW	01620
142	34.30	109.25	1,733	Arizona	TWW	01624
143	33.75	108.70	1,875	New Mexico	TWW	01621
145	35.15	111.40	2,393	Arizona	TWW	01693
147	35.15	111.40	2,225	Arizona	TWW	01650
149	36.25	105.35	2,728	New Mexico	TWW	01663
150	26.18	098.08	0	Texas	TWW	01702
155	28.03	097.03	0	Texas	TWW	01696
157	26.12	098.14	0	Texas	TWW	01705
158	35.58	105.17	2,255	New Mexico	TWW	01674
159	35.58	105.17	2,652	New Mexico	TWW	01672
161	30.50	103.80	1,722	Texas	TWW	01686
165	28.03	097.03	0	Texas	TWW	01699
167	38.49	104.48	1,900	Colorado	TWW	01709
168	38.49	104.48	1,900	Colorado	TWW	01710
174	30.15	097.42	0	Texas	TWW	01730
179	28.03	097.03	0	Texas	TWW	01737
180	28.03	097.03	0	Texas	TWW	01738
183	31.35	097.06	0	Texas	HDW	03895
188	25.42	100.59	1,509	Coahuila*	TWW	01804
189	28.25	106.52	2,012	Chihuahua*	TWW	01799
191	30.40	096.22	0	Texas	HDW	03899
192	30.40	096.22	0	Texas	HDW	03898
193	23.25	103.13	2,164	Zacatecas*	TWW	01787
194	25.30	104.44	1,859	Durango*	TWW	01793
196	22.04	100.28	2,057	San Luis Potosí*	TWW	01776
197	25.24	100.59	2,073	Coahuila*	TWW	01758
198	25.24	100.59	2,042	Coahuila*	TWW	01754
200	23.53	104.15	1,951	Nuevo León*	TWW	01752
206	22.44	102.32	1,981	Zacatecas*	TWW	01767

APPENDIX 1. Continued.

Population number	Latitude (N)	Longitude (W)	Altitude (m)	Locality	Collector**	Collector number
209	22.08	100.58	2,057	San Luis Potosí*	TWW	01774
210	21.48	100.56	2,200	San Luis Potosí*	TWW	01777
213	22.17	102.13	1,951	Aguascalientes*	TWW	01781
214	22.14	102.15	2,073	Aguascalientes*	TWW	01782
216	25.24	100.59	1,631	Coahuila*	TWW	01805
218	22.22	102.32	2,316	Zacatecas*	TWW	01786
219	28.49	100.30	0	Texas	TWW	01807
249	43.40	103.36	1,097	South Dakota	TWW	01846
250	43.46	103.36	1,097	South Dakota	TWW	01847
251	43.50	103.36	1,646	South Dakota	TWW	01848
252	44.06	103.41	1,542	South Dakota	TWW	01849
253	44.06	103.14	1,463	South Dakota	TWW	01850
255	46.21	104.12	1,000	Montana	TWW	01852
258	46.15	106.41	869	Montana	TWW	01856
282	41.35	109.13	2,012	Wyoming	TWW	01895
284	41.46	107.15	1,920	Wyoming	TWW	01899
286	42.15	106.18	1,567	Wyoming	TWW	01902
290	42.04	104.52	1,372	Wyoming	TWW	01908
324	29.20	099.08	0	Texas	HDW	05003
327	30.75	103.60	671	Texas	HDW	05026
331	29.14	099.47	0	Texas	TMK	03304

* Mexico.
** ELE = Emilo Leht, HDW = Hugh Wilson, TMK = Tony Keeney, TWW = Terrence Walters.

APPENDIX 2. Electrode and gel buffer systems, stain buffer, and stain assay procedures used in the electrophoretic analysis.

Electrode and gel buffer systems

1. Discontinuous system modified from Yang (1971).
   a. Electrode buffer (pH = 8.1)
      37.1 g H_3BO_3 (0.3 M)
      ca. 4.8 g NaOH (0.056 M)
      1.5 liters H_2O
      pH to 8.1 with NaOH. Fill for total volume of 2.0 liters with H_2O.
   b. Gel buffer (pH = 8.7)
      20 g Tris (0.083 M)
      ca. 2.2 g citric acid (0.005 M)
      1.5 liters H_2O
      pH to 8.7 with citric acid. Fill for total volume of 2.0 liters with H_2O.
2. Continuous system modified from Cardy et al. (1980).
   a. Stock solution (pH = 6.5; must be refrigerated)
      40.32 g histidine
      ca. 6.0 g citric acid
      3.5 liters H_2O
      pH to 6.5 with citric acid. Fill to 4.0 liters with H_2O.
   b. Electrode buffer (pH = 6.5)
      3 parts stock solution to 4.5 parts H_2O (0.0064 M histidine; 0.0008 M citric acid)
   c. Gel buffer (pH = 6.5)
      1 part stock solution to 3 parts H_2O (0.004 M histidine; 0.0005 M citric acid)

Stain buffer

0.1 M Tris base (12.1 g/liter)
ca. 3 ml HCl conc. (pH to 7.0 with HCl)

Stain assay procedures

1. Glutamate oxaloacetate transaminase (GOT)
   Modified from Cardy et al. (1980).
   a. GOT substrate solution (pH = 7.4)
      200 ml H_2O
      532.4 mg L-aspartic acid
      2.0 g PVP-40
      200.0 mg EDTA
      5.68 g Na_2HPO_4
   b. GOT stain solution
      25 ml H_2O (warm)
      100 mg Fast Blue BB Salt (BB)
      146.1 mg alpha-ketoglutaric acid

   After slicing, combine 25.0 ml of the substrate solution, H_2O, and alpha-ketoglutaric acid. Pour over gel and incubate at 37°C for 10 min. Then add the BB salt to solution in the staining tray; mix and incubate for an additional 15 min. Rinse and fix in 50% glycerol.

APPENDIX 2. Continued.

2. Isocitrate dehydrogenase (IDH)
   Modified from Guries and Ledig (1978).
      50 ml stain buffer (0.1 M Tris-HCl; warm)
      1 ml MTT (10 mg/ml conc.)
      1 ml 10% $MgCl_2$
      1 ml NADP (5 mg/ml conc.)
      20 mg Na_3 DL-isocitric acid
      0.4 ml PMS (5 mg/ml conc.)

   After slicing, combine all above components and pour over gel. Incubate at 37°C for 45 min. Rinse and fix in 50% EtOH.
3. Leucine aminopeptidase (LAP)
   Modified from Gottlieb (1973).
   a. Stock substrate
      0.5 g L-leucyl-beta-naphthylamide HCl
      100 ml N,N dimethylformamide
   b. Stock buffer
      0.01 M phosphate buffer (pH = 6.0)
   c. Stain
      50 mg Black K Salt

   After slicing, combine 1 ml stock substrate, 10 ml stock buffer, and H_2O to 100 ml total volume. Pour over gel and incubate at 37°C for 20 min. Pour off solution, combine stain with 75 ml of warm stock buffer and pour over gel. Incubate again until bands appear (ca. 10 min). Fix in 50% EtOH.
4. Malate dehydrogenase (MDH)
   Modified from Cardy et al. (1980).
   a. Malic acid substrate solution
      Malic acid (50 mg/ml conc.) neutralized to pH 8.0 with 2 M NaOH
   b. Stain solution
      50 ml stain buffer (0.1 M Tris-HCl; warm)
      0.4 ml PMS (5 mg/ml conc.)
      1.0 ml MTT (10 mg/ml conc.)
      1.0 ml NAD (25 mg/ml conc.)

   After slicing, combine all components of the stain solution with 10 ml of the substrate solution. Pour over gel and incubate for 30 min at 37°C. Rinse and store in H_2O.
5. Phosphoglucose isomerase (PGI)
   Modified from Gottlieb (1973).
      50 ml stain buffer (0.1 M Tris-HCl; warm)
      1.0 ml PMS (5 mg/ml conc.)
      1.0 ml MTT (10 mg/ml conc.)
      1.0 ml fructose-6-phosphate (50 mg/ml conc.)
      1.0 ml NAPD (5 mg/ml conc.)
      2 drops G-6-PDH (100 units/ml conc.)

   After slicing, combine all components and pour over gel. Incubate for 30 min at 37°C. Rinse and fix in 50% EtOH.

APPENDIX 3. Allelic frequencies (percent) for populations polymorphic for at least one locus. All other populations (see APPENDIX 1) were monomorphic for the "a" allele at each of the 16 loci except for the following: 216 was monomorphic for *Lap-1c,* 94 and 103 were monomorphic for *Lap-2b,* 10, 88, 193, 194, 196, and 214 were monomorphic for *Lap-2d,* 249 was monomorphic for *Idh-2b,* 180 was monomorphic for *Got-1b,* 189 was monomorphic for *Got-1c,* and 94 was monomorphic for *Pgi-1d.* Some alleles presented in FIGURES 1–3 are not given here since this listing only contains population samples in which at least eight progeny were examined from a mixed packet. Loci *Idh-1* and *Mdh-2* are not listed since all 80 populations were monomorphic for allele "a."

Population	*Got-1*			*Got-2*			*Got-3*			*Got-4*		*Got-5*		*Got-6*				*Idh-2*	
	a	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*a*	*b*	*a*	*b*	*c*	*d*	*a*	*b*
11	100			100			100			100		100		100				100	
66	93	7		93	7		100			100		60	40	81		5	14	100	
98	100			100			100			100		90	10	86	14			100	
107	100			100			100			100		100		100				100	
108	100			100			100			100		100		100				100	
109	100			100			100			100		100		100				100	
111	100			100			100			100		100		100				100	
113	100			100			100			100		100		100				100	
114	89	11		100			67	6	27	89	11	78	23	94		6		100	
117	100			100			100			100		100		100				100	
118	100			100			100			100		100		29		71		100	
120	100			100			100			100		100		100				100	
123	89	11		100			100			100		100		100				100	
127	100			100			88		12	100		100		100				100	
128	100			100			100			100		100		100				100	
129	100			100			100			100		100		55		45		100	
131	100			100			100			100		100		100				100	
132	100			100			33	67		33	67	100		100				100	
133	100			77		23	100			100		100		100				98	2
141	100			100			100			100		100		87		13		95	5
143	100			100			100			100		100		100				24	76
145	100			100			100			100		20	80	90		10		100	
147	100			100			100			100		100		100				100	
150	100			100			100			100		100		100				100	
155	93	7		100			100			100		100		100				100	
157	100			100			100			100		100		100				100	
158	100			100			50		50	58	42	100		58		42		100	
159	100			100				50	50		100	100		50		50		100	
161	100			100			100			100		100		100				100	
165	84	16		100			97		3	100		100		100				100	
167	100			100			100			100		100		91		9		100	
168	100			100			100			100		100		71		29		100	
174	100			50	50		100			100		100		100				100	
188	91	9		59	41		59		41	100		82	18	100				100	

APPENDIX 3. Continued.

Population	*Got-1*			*Got-2*			*Got-3*			*Got-4*		*Got-5*		*Got-6*				*Idh-2*	
	a	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*a*	*b*	*a*	*b*	*c*	*d*	*a*	*b*
192	100			100			100			100		100		94	6			100	
197	100			100			100			100		100		100				100	
200	100			100			100			100		100		100				89	11
206	100			56	44		100			100		100		100				100	
209	100			100			100			100		100		100				100	
210	100			100			100			100		100		100				100	
213	100			100			100			100		100		100				100	
218	100			33	67		100			100		100		100				83	17
219	100			100			100			100		100		100				100	
250	100			54	46		100			100		100		100				100	
252	93		7	85	15		100			100		100		85	15			100	
253	100			100			100			100		100		88		12		100	
255	100			100			100			100		100		63	37			100	
258	100			69	31		100			100		100		77	23			100	
282	29		71	100			100			100		100		97		3		100	
284	100			88		12	100			100		100		88	6	6		100	
286	100			100			100			100		100		100				100	
290	94	6		100			78		22	100		100		100				100	
324	83	17		100			100			100		100		100				100	
327	100			58		42	70		30	100		100		100				100	
331	100			100			100			100		100		100				100	

APPENDIX 3. Continued.

Population	*Lap-1*				*Lap-2*				*Mdh-1*		*Mdh-3*			*Mdh-4*		*Pgi-1*				*Pgi-2*	
	a	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*a*	*c*	*a*	*c*	*d*	*a*	*b*	*a*	*b*	*c*	*d*	*a*	*d*
11	100					40		60	100		100			100		100				100	
66			46	54	100				100		100			100		100				100	
98	100				100				100		100			100		41			59	100	
107	86		14		100				100		100			100		100				100	
108	32	47	21		100				100		100			100		100				100	
109	88		12		71		29		100		100			100		100				100	
111		78	22		100				100		100			100		100				100	
113	67		33		100				100		100			100		100				100	
114	56		44		100				78	22	78	22		100		100				100	
117	88	12			100				100		100			100		100				100	
118	77	6	17		100				100		100			100		100				100	
120	40	60			100				100		100			100		100				100	
123	100				100				100		100			100		100				100	
127	50	25	25		63	37			100		100			100		100				100	
128	88	12			100				100		100			100		100				100	
129	85		15		100				100		100			100		100				100	
131	75		25		100				100		100			100		100				100	
132	100							100	100		100			100		100				100	
133	100				100				100		100			100		100				100	
141	100				100				100		100			100		100				100	
143	100				100				100		100			100		100				100	
145	90		10		100				90	10	90	10		100		75	25			100	
147	69	31			100				100		100			100		100				100	
150	99		1		39			61	100		100			100		43			57	36	64
155	100				100				100		100			100		100				100	
157	30	11	59		59	37		4	100		100			100		22		78		100	
158	83	17			100				100		100			100		100				100	
159	100				100				100		100			100		100				100	
161	71		29		100				100		100			100		100				100	
165	100				100				100		100			100		100				100	
167	9	18	73		100				100		100			100		100				100	
168	52	42	6		100				100		100			100		66			34	100	
174	100				100				100		100			100		100				100	
188	91		9		100				100		100			100		100				100	
192	100					100			100		100			100		100				100	
197	60		40		100				100		100			100		100				100	
200	56	11	33		100				100		100			100		100				100	
206	100				33			67	100		100			100		100				100	

APPENDIX 3. Continued.

Population	*Lap-1*				*Lap-2*				*Mdh-1*		*Mdh-3*			*Mdh-4*		*Pgi-1*				*Pgi-2*	
	a	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*a*	*c*	*a*	*c*	*d*	*a*	*b*	*a*	*b*	*c*	*d*	*a*	*d*
209	100							100	100		100			100		65			35	100	
210	100							100	100		100			100		38			62	100	
213	100							100	100		53		47	100		81			19	100	
218	100				100				100		100			100		100				100	
219	96			4	100				100		27	9	64	100		100				100	
250			25	75	100				100		100			100		100				100	
252	43		15	42	77		23		100		100			100		93			7	100	
253			38	62	100				100		100			100		88			12	100	
255			38	62	77		23		100		100			100		78			22	100	
258	31			69	100				100		100			100		73			27	100	
282	41		47	12	100				100		100			100		91			9	100	
284	15	6	29	50	100				100		100			100		88			12	100	
286	60		40		100				100		30	70		30	70	55			45	100	
290			100		100				100		100			100		100				100	
324	42			58	100				100		83		17	100		100				75	25
327	100				100				100		100			100		100				52	48
331	36		64		100				100		100			100		100				64	36