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Allozyme variation and evolutionary relationships of grain amaranths (*Amaranthus* spp.)

H. Hauptli and S. Jain

Department of Agronomy and Range Science, University of California, Davis, CA 96616, USA

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Summary. Allozyme studies in amaranth provided useful assays of genetic variation in order to verify the patterns inferred from morphological traits, for elucidating the genetic structure of landraces, and for the studies of evolutionary relationships among wild, weedy and crop species. Thirty-four populations of cultivated New World amaranths were surveyed along with 21 weedy New World populations for allozyme variation at nine electrophoretic enzyme loci. Eleven populations of cultivated amaranths from the Indian State of Uttar Pradesh and six from Nepal were also surveyed for a comparison. In the New World populations, heterozygosity was low, and different populations ranged from 0 to 44% polymorphic loci. Adjacent populations were often fixed for different alleles or had very different allele frequencies at certain loci, with no apparent geographical patterns. Diversity index H′ was partitioned into the intra- and interpopulation as well as the interspecific components of variability. The crop versus weed genetic distances were the largest, whereas the intra- and interpopulation components of H′ were about equal. Genetic structure of all three species of the New World amaranths together can be described as a collection of distinct populations, each more or less a heterogeneous collection of highly homozygous individuals. The North Indian populations showed relatively less allozyme variability with the most common alleles same as those of Mexican landraces. Alleles at several loci proved to be diagnostic of the crop and weed groups, and of the three individual crop species. Genetic distances based on pooled gene frequencies showed the three crop species to be generally more closely related inter se than they were to their putative weedy progenitor species, respectively (with the exception of the weed-crop pair *A. quitensis* and *A. caudatus*). This implies a single domestication event involving *A. hybridus* as the common ancestor rather than three separate domestication events. Close similarity between *A. caudatus* and *A. quitensis* might have resulted from transdomestication based on a weedy or semi-domesticated species having migrated from Meso-America to South America. This preliminary report must now be expanded by further ecogeographical, cytogenetic and population studies on new extensive collections from the areas of early domestication. Some evidence of recent introgression and/or segregation of crop-weed hybrids between *A. caudatus* and *A. retroflexus* is available in the form of rare individuals in crop populations with crop allozyme genotypes except for a single homozygous weedy allele.

Key words: Amaranth – Crop evolution – Crop-weed relations – Allozyme variation

Introduction

Measures of genetic variation at allozyme loci have often proven useful in the study of biosystematics and evolutionary relationships of numerous plant taxa.

Moreover, an efficient and complete germplasm collection would be guided by a knowledge of the geographic patterns of variability and the structure of landrace populations (Brown 1978; Senadhira 1976). Levels of heterozygosity and similarity between populations may provide evidence for the importance of heterosis, interpopulation migration, or introgression in crop's development, when this is coupled with the information about the breeding systems and morphology of germplasm accessions. Genetic similarity between species and their putative ancestral progenitors may help elucidate domestication patterns in cultivated plants and provide information on their dispersal routes (Rick and Fobes 1975; Second 1982). Allozyme studies are utilized here to help answer questions on the relationships and amounts of genetic variation in populations of cultivated amaranths, especially those sampled from their centers of origin, as compared with the secondary centers of culti-

vation after dispersal on one hand, and populations of weedy relatives on the other.

Grain amaranth as a crop includes three ancient New World domesticates: *Amaranthus caudatus, A. hypochondriacus,* and *A. cruentus,* which have spread worldwide since the European colonization of the Americas. In an elegant and thorough study of the history of grain amaranths, Sauer (1950) showed that indigenous populations of cultivated *A. cruentus* and *A. hypochondriacus* occurred only in Guatemala and Mexico, while cultivation of *A. caudatus* is restricted to the Andean South America. Early references to the occurrence of related weedy species *A. quitensis, A. hybridus,* and *A. powellii,* place their pre-Columbian distributions in highland Ecuador, Northern Central America, and western North America respectively (Sauer 1950). A polyphyletic origin of the three crops from these three weeds has long been assumed whereas Sauer (1967) proposed an alternative monophyletic explanation involving a *cruentus*-like progenitor in Guatemala which subsequently migrated north and west to Mexico where it involved introgression from *A. powellii* to yield *A. hypochondriacus,* and south to the Andean region, where it underwent introgression from *A. quitensis* and gave rise to *A. caudatus.* Study of the genetic similarities between these species (along with historical data) would allow a test of these alternative hypotheses, and provide evidence for the potential role of introgression between the crop and weedy species. An understanding of the patterns of genetic variation among and within populations could further describe the relative roles of various factors of evolution (selection, migration and drift).

The status of grain amaranth in the New World has declined from an important grain to a relic in South America and to a minor grain in Mexico. Sauer (1950, 1967) postulated from the available historical records that grain species were introduced to many areas of the Old World in post-Columbian times; specifically, their dispersal to Ceylon through Portuguese trade occurred during the 18th Century and subsequent spread to the Indian subcontinent reaching as far north as the Himalayan foothills in northern India. The existing variation in the American centers of origin and the areas of introduction in the Old World have not been described. This study reports the first survey of allozyme variation in populations from the centers of origin of the crop in the New World and from areas of introduced cultivation in India, as well as in populations of the related weedy species occurring in North America.

Materials and methods

Bulk population samples of the three domesticates were collected in Latin America (Hauptli et al. 1979), and India (Jain et al. 1979). Weedy populations were collected and kindly contributed by several colleagues across the USA. These accessions are listed in Appendix 1. Approximately 50 seeds of each accession were planted in each of two 10×10 cm^2 plastic pots using an equal mixture of soil, sand, and peat as the planting medium. Seedlings were grown in a greenhouse for three weeks, or until the first two true leaves had fully expanded. The crude extracts from seedlings in approximately 50 ml of 0.1 M Tris-HCl buffer with 0.014 M mercaptoethanol were run on a starch gel electrophoretic system following Scandalios (1969). Duplicate wicks of crude extract were made for each plant, and were run on different discontinuous buffer systems, as all enzymes did not exhibit adequate activity, and/or proper allozyme separation did not occur with the same gel pH. Both gel buffers were 0.1 M Tris-Citrate buffers, pH 7.6 and 8.3, with the same bridge buffer 0.1 M Na Borate, pH 8.7 and starch content was 12.8% for both. Each gel was sliced horizontally and slices were stained for the following enzymes: xanthine dehedrogenase (XDH), glutamate dehydrogenase (GDH), glutamine oxaloacetic transaminase (GOT), leucine amino peptidase (LAP), glutamine oxaloacetic transaminase (GOT), leucine amino peptidase (LAP), acid phosphatase (ACPH), malate dehydrogenase (MDH), shikimic acid hydrogenase (SDH), phosphoglucoisomerase (PGI), and alcohol dehydrogenase (ADH). Thirty samples were run on each gel along with three interspersed samples from a "control" population known to be electrophoretically uniform (accession PI 337611). Each gel had five samples each of six populations.

In this preliminary study, inheritance of the enzyme phenotypes was inferred from the kinds and numbers of different phenotypes found for each enzyme in families of known mothers, or from the patterns of band frequencies within populations. Three measures of variation, namely, percent polymorphic loci (PLP), heterozygosity (Het), and fixation indices (F), were calculated for each population. Gene frequencies for each species were calculated as an arithmatic mean of population freuencies which were then used to calculate the genetic distances between species as described by Nei (1972). To describe the distribution of variability in the whole New World collection, the Shannon-Weaver information index, $H' = -\Sigma p_i \ln p_i$ was used, where p_i is frequency of the i^{th} allele at the j^{th} locus in a population. The components of H' can be appropriately estimated to compare the amounts of genetic variability among and within populations (Lewontin 1972 for method and rationale).

Results

Allozyme loci

Zymograms of all crop phentoypes and their inferred inheritance are shown in Fig. 1. GDH was monomorphic over all species with a single banded phenotype. XDH phenotypes always appeared fixed in a population, and the very rare two single-banded phenotypes suggested a diallelic system. LAP, MDH, and SDH behaved as monomeric enzymes controlled by genes with 3, 3, and 4 alleles, respectively. ADH and ACPH were assumed to be dimeric enzymes each controlled by single genes. Inheritance of GOT phenotypes could not be ascertained, as all crop populations were fixed for phenotype A except one fixed for phenotype B. As they were fixed within each of the populations, treating the

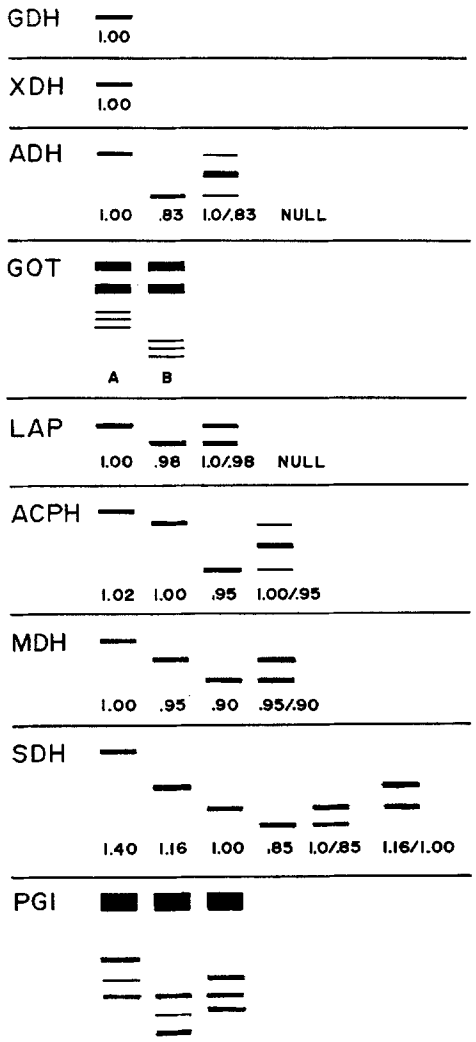

Fig. 1. All allozyme phenotypes found in the three crop species. The phenotypes of the monomorphic control population is designated 1.00 or A. Numerical designations for other alleles are migration distances proportional to the 1.00 alleles

two phenotypes as two alleles would not bias the calculation of H', but genetic distance may be slightly underestimated if these phenotypes actually differ by more than one allele. Inheritance of PGI phenotypes was inferred mainly on the basis of their frequency in populations. The band patterns A and B are fixed in some populations, but pattern C occurs in low frequencies similar to those of heterozygotes at other loci in the population (C was assumed to be the heterozygote carrying alleles for A and B).

For ADH and LAP, an individual was considered to be homozygous for a "null" allele if there was no activity for one of these enzymes but normal activity for all other enzymes. On the other hand, an individual showing a given band was considered as homozygous for the allele controlling this band. The existence of null alleles, undetected in the heterozygotes, might bias the allele frequency estimates. However, amaranths are highly self-pollinated species so that this bias would be small. The rare instances of populations with null homozygotes were excluded in the estimation of heterozygosity level (Het) and values of F. Weedy phenotypes not shared by the crop species group are not shown here because of their multiplicity and complexity; for instance, six additional PGI phenotypes and four additional GOT phenotypes were found. Data on weedy species involved numerous prevalent null alleles, allozyme phenotypes which require further Mendelian analysis.

Interpopulation diversity and the presence of diagnostic alleles

Table 1 shows the mean frequencies of designated alleles at all loci for the crop versus weedy species. Allelic frequency data for all individual populations are too bulky to present here, but Tables 2 and 3 give a few selected examples in *A. caudatus* and *A. hybridus*. A surprisingly high number of alleles are fixed or nearly so over all species (e.g. GDH, XDH, GOT, LAP, and ADH). Due to this relatively low level of diversity in the group of species sampled, few species-specific diagnostic alleles were found. These include MDH 1.00 (*A. hypochondriacus, A. hybridus* and *A. retroflexus,* ACPH 1.02 (*A. hypochondriacus*), ACPH 0.95 (*A. caudatus*), GOT B (*A. cruentus*), SDH 1.40 (*A. cruentus*), ADH 0.83 for weeds versus crops, LAP 0.98 in some weeds, and XDH 1.03 (*A. hybridus*). No crop species is fixed for any allele not found in another crop species. Comparisons of the New World *A. hypochondriacus* and *A. cruentus* with the Indian accessions show several common as well as rare alleles to have been lost during migration to the Indian subcontinent. Presence of different ADH allele suggests *A. hybridus,* not *A. powellii,* to be closely related to *A. hypochondriacus.*

Genetic distances among species

Table 4 shows genetic distances between species based upon these specific frequencies; crop-crop distances are found to be relatively lower than the weed-weed distances. Of the three pairwise crop-progenitor weed comparisons, only the *A. caudatus–A.quitensis* comparison matches the level of similarity found in the crop-crop comparisons. *A. quitensis* demonstrates relative affinity with the other two crop species as well.

Evidence of introgression

Low frequencies of characteristic "weedy" alleles (e.g. ADH 0.83 and null; LAP 0.98 and null) are found in several populations of *A. caudatus.* These are most

Table 1. Frequencies of designated alleles pooled over species for each enzyme locus

	Crop species					Weedy species			
Locus	Allele	*caudatus*	*cruentus*	*hypochondriacus*	Indian populations	*quitensis*	*hybridus*	*powellii*	*retroflexus*
GDH	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
XDH	1.03	0.00	0.00	0.00	0.00	0.00	0.45[b]	0.00	0.00
	1.00	1.00	1.00	1.00	1.00	1.00	0.55	1.00	1.00
ADH	1.00	0.99	0.97	1.00	1.00	1.00	0.39	0.00	0.00
	0.83	0.00	0.01	0.00	0.00	0.00	0.61	0.70	0.78
	null	0.01[a]	0.02	0.00	0.00	0.00	0.00	0.30[a]	0.22
LAP	1.00	0.98	1.00	1.00	1.00	1.00	1.00	0.00	0.00
	0.98	0.00	0.00	0.00	0.00	0.00	0.00	0.65	0.92
	null	0.02[a]	0.00	0.00	0.00	0.00	0.00	0.35[a]	0.08
ACPH	1.02	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00
	1.00	0.62	1.00	0.98	1.00	0.88	1.00	1.00	1.00
	0.95	0.38	0.00	0.00	0.00	0.12	0.00	0.00	0.00
MDH	1.00	0.00	0.00	0.64	0.50	0.00	0.69	0.00	0.75
	0.95	0.94	0.93	0.26	0.50	0.23	0.31	1.00	0.25
	0.90	0.06	0.07	0.10	0.00	0.77	0.00	0.00	0.00
SDH	1.40	0.00	0.09	0.00	0.00	0.00			
	1.16	0.00	0.09	0.43	0.00	0.00			
	1.00	0.31	0.79	0.52	1.00	0.25			
	0.85	0.69	0.03	0.05	0.00	0.75			
PGI	A	0.33	1.00	1.00	1.00	0.27	0.25	0.00	0.00
	B	0.67	0.00	0.00	0.00	0.73	0.00	0.00	0.00
Others		0.00	0.00	0.00	0.00	0.00	0.75	1.00	1.00
GOT	A	1.00	0.88	1.00	1.00	1.00	0.58	1.00	1.00
	B	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00
Others		0.00	0.00	0.00	0.00	0.00	0.42	0.00	0.00
No. populations		23	8	3	17	6	10	1	4
No. individuals		1,118	281	102	337	300	408	20	93

[a] These are frequencies of homozygotes, as heterozygotes are not discernible without progeny testing
[b] Only 4 populations assayed

probably the result of recent hybridization with weedy *A. retroflexus*, as F_1 hybrids and their advanced generations were apparently seen in the field. These plants with intermediate crop-weed morphology had more branches than the crop species, and intermediate flower morphology with long bracts, although they were not as branched as the weedy species, and had intermediate-sized leaves. The presence of long (3 mm) recurving tepals with spatulate to emarginate tips is suggestive of *A. retroflexus*.

Such hybrids and weedy populations of *A. retroflexus* were seen in crop fields in the Callejon de Huaylas area, and may account for the ADH and LAP variation seen in populations UCA037, UCA039, and UCA040 (Table 2). *A. hybridus* was rarely seen in the area, and often only along the roadsides rather than in cultivated fields, but it cannot be ruled out as a possible introgressant. The fields of populations UCA031 and UCA032 were not visited but the white-seeded samples from this area are found mixed with some black seed which suggests some hybridization with the weedy species.

Hybrids between *A. quitensis* and *A. caudatus* were distinctly seen in the crop field of population UCE004, by their striking deep purple pigmentation, long bracts, and black seed (all characteristics of *A. quitensis*). Individuals of advanced generations with only one or two of these traits were also present. These plants were seen in the field, even though the farmers indicated that black seeds are systematically culled from the seed before sowing.

Crop diversity analysis

Table 5 provides a summary of the levels of variation at 5 out of a total 9 electrophoretic loci scored in 41 crop populations grouped by countries, Even this limited survey suggests a large region of diversity centered in Peru, decreasing in the north and south directions. Considering just the crop species and *A. quitensis*, the es-

Table 2. Allele frequencies in 6 populations located in the Callejon de Huaylas area of central Peru, a narrow valley 75 kilometers long from Huaraz in the south to Caras in the north

Locus and allele	Huaraz UC031	Huaraz UC032	Carhuaz UC037	Yungay UC040	Caras UC039	Caras UC042
GDH 1.00	1.00	1.00	1.00	1.00	1.00	1.00
XDH 1.00	1.00	1.00	1.00	1.00	1.00	1.00
ADH 1.00	1.00	1.00	0.96	1.00	0.99	1.00
0.83			0.00		0.01	
Null			0.04		0.00	
Net.[a]			?		0.02	
LAP 1.00	1.00	1.00	1.00	0.96	1.00	1.00
0.98				0.04		
Het.[a]				0.00		
ACPH 1.00		1.00		0.84	0.56	0.16
0.95	1.00		1.00	0.16	0.44	0.84
Het.[a]				0.12	0.09	0.04
MDH 0.95	1.00	0.24	1.00	0.45	1.00	1.00
0.90		0.76		0.55		
Het.[a]		0.04		0.12		
SDH 1.00	0.15	0.23	0.41	0.49	0.05	1.00
0.85	0.85	0.77	0.59	0.51	0.95	
Het.[a]	0.04	0.08	0.07	0.03	0.00	
PG1 A		0.99		1.00	0.18	0.07
B	1.00	0.01	1.00		0.75	0.86
A/B					0.07	0.07
GOT A	1.00	1.00	1.00	1.00	1.00	1.00
No. of individuals	38	76	34	42	79	57

[a] Het = proportion of heterozygotes

Table 3. Allele frequencies in four populations of *A. hybridus* from the USA (UCY273 and UCY267), Peru (UCY043), and Guatemala (UCY082).

Locus and allele	UCY273	UCY267	UCY043	UCY082	Non-crop alleles
GDH 1.00	1.00	1.00	1.00	1.00	
XDH 1.03	[a]	[a]	1.00	–	[a]
1.00			–	1.00	
ADH 1.00	–	1.00	0.125	1.00	
0.83	1.00	–	0.750	–	[a]
Null	–	–	0.125	–	[a]
LAP 1.00	–	1.00	1.00	1.00	
0.98	1.00	–	–	–	[a]
ACPH 1.00	1.00	1.00	1.00	1.00	
MDH 1.00	0.50	1.00	0.07	–	
0.95	0.50	–	0.93	1.00	
0.90	–	–	–	–	
PG1 A	–	1.00	–	0.97	
B	–	–	0.07	0.03	
Others	–	–	–	–	
GOT A	1.00	1.00	1.00	1.00	
B	–	–	–	–	
Others	–	–	–	–	[a]

[a] Alleles common in the weedy amaranths

Table 4. Genetic distances based on specific gene frequencies

		Crops		Weeds			
		cruentus	*hypochondriacus*	*quitensis*	*hybridus*	*powellii*	*retroflexus*
Crops	*caudatus*	0.129	0.155	0.082	0.341	0.431	0.602
	cruentus		0.070	0.181	0.282	0.422	0.579
	hypochondriacus			0.190	0.215	0.541	0.477
Weeds	*quitensis*				0.337	0.544	0.588
	hybridus					0.326	0.257
	powellii						0.262

Table 5. Allozyme variation at five representative loci and three measures of average intra-population variation in grain amaranth collections from Central and South America (including *A. quintensis*)

	Argentina	Bolivia	South Peru	North Peru	Ecuador	Guatemala	Mexico
No. of populations	1	8	7	10	4	5	6
GOT-1	M	M	M	M	M	M	P
Adh-1	M	M	M	P	M	M	P
Sdh-1	M	P	P	P	P	P	P
Pgi-1	M	P	P	P	P	P	P
Lap	M	M	P	P	M	M	M
$\bar{k}$	1.00	1.44	1.44	1.77	1.25	1.33	1.55
PLP	0	24	20	26	22	9	16
H′	0	0.265	0.133	0.274	0.310	0.067	0.209

Nine allozyme loci were scored. M = monomorphic, P = polymorphic. Nearly 50 plants were assayed for each accession. $\bar{k}$ = number of alleles per locus; PLP = average % polymorphic loci per population; H′ = Shannon-Weaver diversity index

timates of percent polymorphic loci (PLP) for each population, plotted in Figs. 2 and 3, also show such a geographic pattern of larger diversity in Peru. In contrast, all the Indian populations were almost monomorphic, and there was a restriction in the number of alleles from on overall 22 in the New World populations to 10 in the Indian collection. Of course, this is a limited survey of Indian germplasm and many more collections will have to be examined before genetic diversity in Indian amaranths can be properly compared with the patterns observed in South America.

For the entire New World collection, no clines or gradual geographic shifts in allele frequency could be detected (Figs. 4–6). Significant geographic differences are evident between the Central and South American centers. Marked geographic shifts in allele frequency seem to be of the type described above in which nearby populations of the same species are very different in allele frequency, or are in fact fixed for different alleles. Table 6 gives the means of H′ components estimated by pooling all polymorphic loci in the New World crop collection. *A. quitensis* is included here because of its close affinity to *A. caudatus* and its semi-domesticated habit. Pooling all species showed that about half of the total H′ values was due to species differences. The remainder was about equally divided into the within- and between-population components. Considering each species separately, the same kind of pattern is evident, where the within-population heterogeneity approximately equals the between-population heterogeneity. GOT in *A. cruentus* is an exception, due to one population fixed for the B phentoype, while all others were fixed for A. The other two loci, MDH and SDH, show *A. cruentus* with the same pattern as the other species.

Discussion

Introgression and potential hybridization barriers

Some recent introgression is indicated here by the rare occurrence of weedy alleles in crop populations. Crop-weed hybrids involving *A. retroflexus* and *A. quitensis*

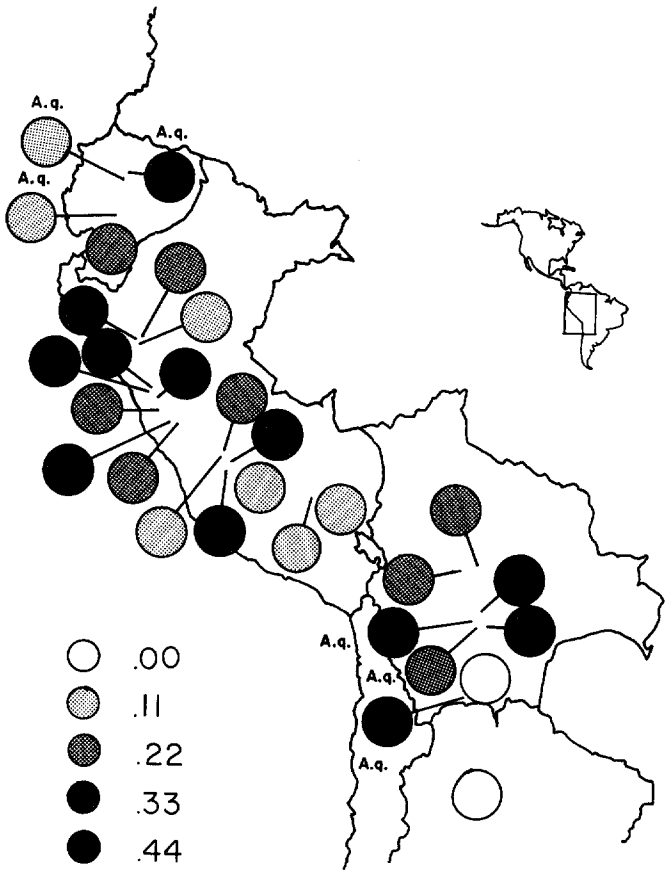

Fig. 2

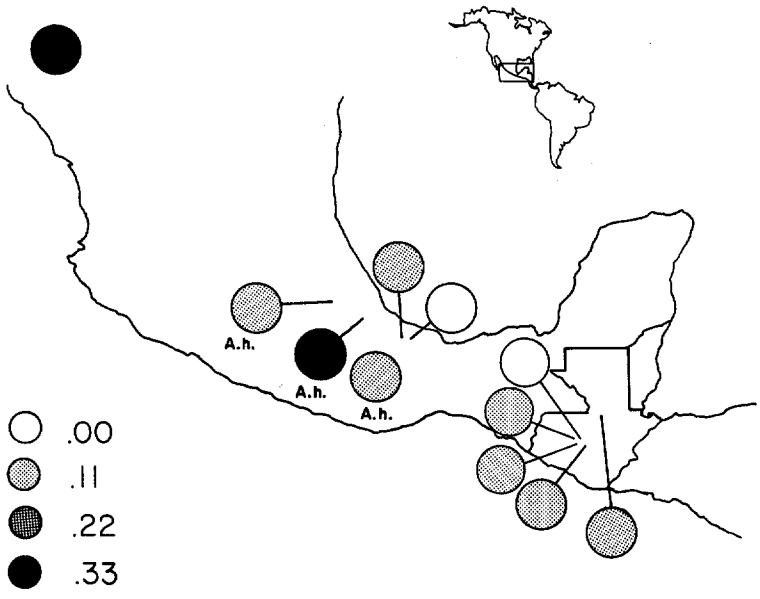

Fig. 3

Figs. 2 and 3. Percent polymorphic loci, with each circle denoting a population. **2** South American populations; All are *A. caudatus* except those designated A.q. for *A. quitensis.* **3** Central American populations; all are *A. cruentus* except those designated A.h. for *A. hypochondriacus*

and their advanced generation derivatives occur in these populations. A few individuals, for example, were homozygous for weedy allele ADH 0.83 or null in the background of other loci homozygous for the crop-specific alleles. Whether these were the result of selection for crop genes among the products of segregation or backcrossing is not known.

There is no weedy species that encompasses all of the allozyme variation range present in the crop species although we fully recognize that this study involved rather small numbers of weedy accessions. Estimates of genetic distances (Table 4) indicate that the weedy species are less closely related to each other than the crop species are related inter se, indicating a common ancestor. However, the weedy species are relatively more diverse, and certain populations of *A. hybridus,* for example, show closer affinity to the crop species as a group. The same may be true for *A. powellii* of which, however, many more populations need to be studied. That *A. quitensis* is the sole progenitor of *A. caudatus* seems unlikely because of the former's similarity to the other crop species as well. Coons (1975) described *A. quitensis* morphologically as a subspecies of *A. hybridus,* mainly on the basis of flower morphology; allozyme comparisons, however, showed them to be quite distinct, *A. hybridus* not having the MDH 0.90 allele, or the PGI B phenotype, and reciprocally, *A. quitensis* not having the common XDH 1.03, ADH 0.83, or MDH 1.00 alleles of *A. hybridus.*

It is likely that *A. quitensis* arose from hybridization between *A. hybridus* and *A. caudatus,* judging from the conflicting morphological and allozyme data. Hauptli and Jain (1978) reported the fixation of ADH 1.00 and the slow EST allele (both exclusively crop alleles) in the California populations of crop-weed hybrid origin that retained their intermediate morphology. The same events may have occurred in the hybrid derivatives of *A. hybridus*×*A. caudatus. A. quitensis* has intermediate morphology for branching and flowering time, and all *A. quitensis* individuals showed a deep purple color found in *A. caudatus* but not in *A. hybridus* populations. Moreover, *A. quitensis* is not as weedy as *A. hybridus,* occurring rather infrequently along the roadsides and waste places, but found more as a tolerated or encouraged semidomesticate in crop fields. In fact, although *A. quitensis* was collected as a semidomesticate in Guaranda, Ecuador, in 1948, it could not be found there in 1979 (Hauptli et al. 1979), whereas the weedy *A. hybridus* was abundant in the adjacent abandoned fields.

More cytogenetic and hybridization work is needed to indicate whether or not *A. quitensis* is the progenitor of *A. caudatus.* There is no obvious divergence in their allele frequencies corresponding to their geographical divergence patterns, as seen in several species of tomato occurring in the same region, albeit in different habitats (Rick et al. 1977, 1979). Nor do pat-

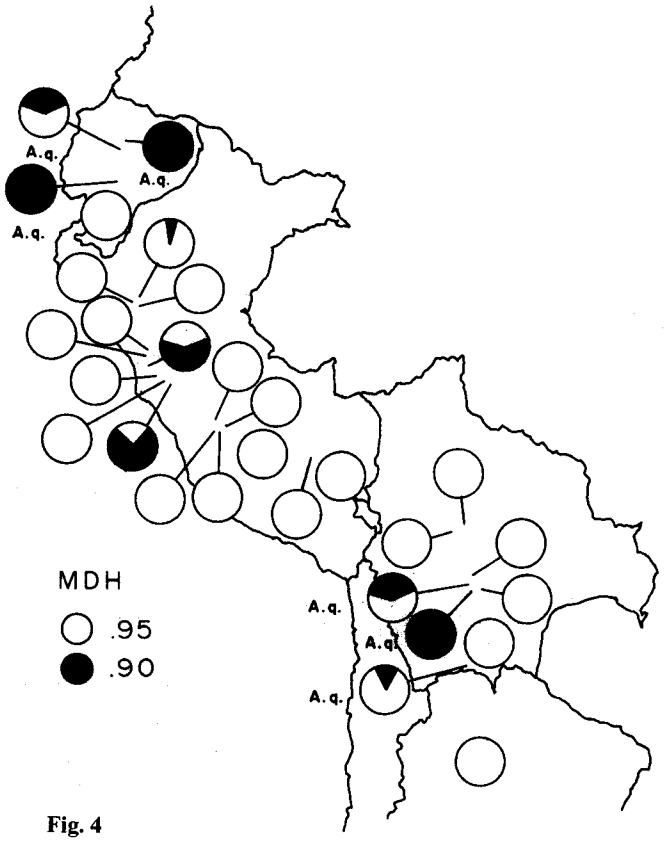

Fig. 4

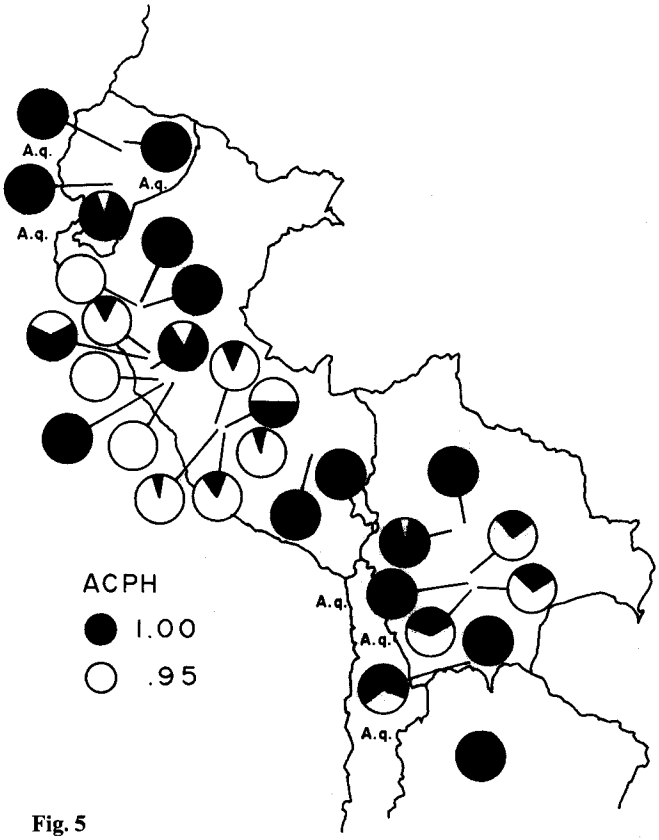

Fig. 5

Figs. 4–6. Allelic frequencies at MDH, ACPH and PGI loci in Central and South America

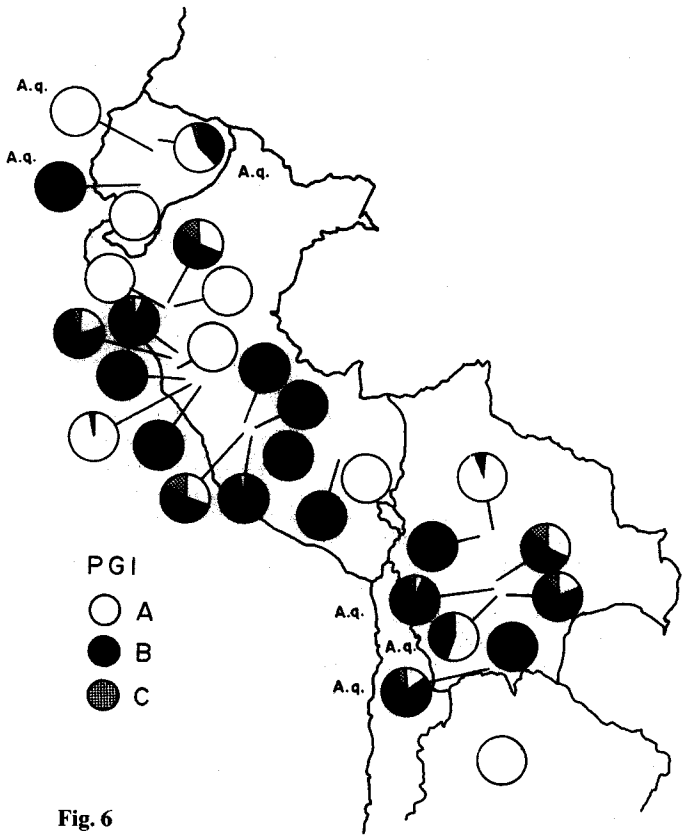

Fig. 6

terns in amaranth variation seem to correspond to any obvious environmental differences as was reported in wild barleys by Nevo et al. (1979). The distribution of amaranth variability is more likely the result of human movements and local agricultural preferences than a response to an environmental or eco-geographical gradient.

For example, *A. quitensis* was found in southern Bolivia (UC052 and UC053 from the Sucre area, a previously unreported site for this species), and nowhere between Sucre and southern Ecuador. The Spanish immigrants, and Inca Indians before them, were known to have transported groups of people to distant locations of their empires whenever employment demanded so, or to control noncooperative communities. Similarities in the dress of some indigenous peoples of the Bolivian state of Chuquisaca is reminiscent of the Canari of southern Ecuador. Thus, it seems conceivable that *A. quitensis* was a relatively recent introduction to southern Bolivia brought in by the Ecuadorian slaves. *A. quitensis* is sold as medicinal and magical plants in southern Bolivia on to the Argentine border by the migratory Callahuayan herbalists who are native of the Lake Titicaca region. These travelling merchants may be spreading *A. quitensis* to even newer areas in the Andes, as evidenced by the presence of *A. quitensis* in population UCE004 of Argentina. In any case, market samples of white-seeded *A. cruentus* from this region included some black seed, and reciprocally, rare white seed can be found in the batches of *A. quitensis* seed from the area. Unfortunately, there are no allozyme differences that separate the two species, and no evidence of hybridization or introgression is available in our data.

Table 6. Within and between populations, and between species components of H' considering all species pooled (total) and each species separately

Locus	Total			*caudatus*		*quitensis*		*cruentus*		*hypochondriacus*	
	W/IN POPN	BTWN POPN	BTWN SPEC	W/IN POPN	BTWN POPN	W/IN POPN	BTWN POPN	W/IN POPN	BTWN POPN	W/IN POPN	BTWN POPN
ADH	0.24	0.00	0.76	0.67	0.33	0.00	0.00	0.00	0.00	0.00	0.00
GOT	0.00	0.78	0.22	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
LAP	0.43	0.00	0.57	0.67	0.33	0.00	0.00	0.00	0.00	0.00	0.00
ACPH	0.21	0.29	0.50	0.36	0.64	0.39	0.61	0.00	0.00	0.80	0.20
MDH	0.29	0.48	0.23	0.38	0.62	0.50	0.50	0.31	0.69	0.34	0.66
SDH	0.40	0.20	0.40	0.52	0.48	0.91	0.09	0.51	0.49	0.34	0.66
PGI	0.15	0.29	0.56	0.20	0.80	0.50	0.50	0.00	0.00	0.00	0.00
Mean	0.25	0.29	0.46	0.47	0.53	0.58	0.42	0.27	0.73	0.49	0.51

Further variation assays using additional allozyme loci as well as morphological marker loci and quantitatively inherited traits in these collections could ascertain the importance of introgression and extent of hybridization between these two species. The occurrence of rare alleles at high frequencies in a few populations may have been aided by recent human migration.

Stuber et al. (1982) have shown that selection solely on the basis of allozyme genotype was successful in increasing means for yield component characteristics in a maize population within one generation. Whether the allozymes themselves were responsible for yield differences, or whether linked loci were responsible, this example indicates that quantitative changes in morphological and/or physiological characters under selection may be reflected in changes in allozyme frequencies, and vice versa (also Tanksley and Rick 1980). Accordingly, *A. hybridus–A. caudatus* hybrids may have been poorly adapted to weedy environments, but may have undergone selection for the alleles characteristic of *A. caudatus* (or alleles at linked loci) resulting in adaptation to cultivation, and the semi-domesticate habit of *A. quitensis.*

A similar situation was discussed by Stephens (1949) for cotton, in which the apparent breakup of coadapted clusters of polygenes arranged in cryptic structural differences between the two species resulted in the inviability of advanced generations of an interspecific cross. Selective elimination of genes from the donor species occurred in interspecific backcrosses. A similar event was experimentally shown in the backcrosses involving two *Phaseolus* species (Wall 1968). This would suggest pollen competition rather than structural differentiation and/or coadapted multilocus genotypes as the mode of elimination (cf. Kulakow and Jain 1983). Although the mechanisms differ, these two examples show experimentally how one parent of an interspecific cross can be largely eliminated, and yet bring about the transfer of a few genes. Such a phenomenon may have been involved in the origin of *A. quitensis.*

Interpopulation variation and germplasm resources

The distinctness of amaranth populations is exemplified in the Andean *A. caudatus* populations that may differ radically even over a few kilometers. Coons (1982) reports some surprisingly strong reproductive barriers between the Peruvian populations of *A. caudatus.* As each plant can produce hundreds of thousands of small seeds a single plant progeny could conceivably be saved to plant the entire next year's background garden crop, and provide a possible explanation for the fixation of different alleles in adjacent populations. This kind of drastic selection and drift could also lead to rapid divergence between populations and the development of reproductive barriers.

The distinctive nature of populations is also obvious from the high between-population component of the total diversity index H′ (Table 6). Some of the loci contributing the most to the within-species values of H′ were those in which one population was fixed for an allele not found in any other population (i.e. GOT B in *A. cruentus*), or populations which were fixed for different alleles. Concurrently, many populations were polymorphic for at least one locus with gene frequencies in the intermediate range (0.25–0.75). The distribution of the total variability in amaranth germplasm appears much different than that found in some inbreeding species, for example, wild barleys (Nevo et al. 1979). Here, most of the variation in a species as a whole could be included in an extensive collection from a single population. For grain amaranth, many populations would have to be collected to achieve the same probability of conserving an allele as its frequency in the species as a whole. Also, highly homozygous structure of the landraces requires that more individuals are collected per population than for a randomly mating species. Since populations are well-differentiated from each other and no geographic patterns could be found, as many landrace populations as possible must be sampled.

Similar to the occurrence of rare alleles or allozyme phenotypes in a single population (e.g. GOT B in one *A. cruentus* population), the distribution of heterozygotes is also highly variable across populations. Several populations have much lower values of fixation index (F) indicating heterozygosity levels closer to those expected under random mating than the ones approaching 1.0, characteristic of the majority of collections. We can only speculate from our observations on the male : female flower ratios and environmental or cultural conditions that may increase outcrossing rates in certain populations; however, no obvious differences in sex expres-

sion characteristics were found between the highly heterozygous populations and the others that had variation for the outcrossing rates (Hauptli and Jain 1984). The Callejon de Huaylas area of northern Peru (starred populations, Figs. 4–6) is a good example of a region of extensive amaranth cultivation but with only slightly higher levels of heterozygosity than others.

Breeding system

If the high levels of homozygosity and high F values are primarily the result of deviations from random mating, breeding system may be characterized by predominant selfing. The estimated outcrossing values (Figs. 7 and 8) are lower than the estimates of 10 to 30% reported in a population grown at Davis (Hauptli and Jain 1984). The Latin American populations are almost ubiquitously interplanted with maize at lower population densities with restricted pollen dispersion in the field. Also, plants from different regions may have undergone inadvertent human selection for decreased outcrossing, perhaps via such interplanting and selection against the black seeded crop-weed hybrids.

Evolutionary trends towards increased inbreeding in the course of domestication have been discussed for tomato (Rick 1950), rice (Oka and Morishima 1967) and other crop species. Amaranth may be an example of this generality, although outcrossing rates of weedy species are not available for comparison. All the known related species are monoecious, with male and female flowers intermixed within glomerules along the panicle. Other more distantly related species have a wider spatial separation of male and female flowers. *A. spinosus* (an Old World member of the *Amaranthus* section is also monoecious, as are the grain domesticates, but male and female flowers are separated in clusters on each flowering branch. Many species of Section *Blitopsis* in the genus *Amaranthus* are dioecious. It is not too surprising, then, to find variation in the cultivated materials for sex expression characteristics leading to highly variable outcrossing rates. There is some evidence for opposing selective pressures toward higher inbreeding under domestication as well as toward retention of varying outcrossing rates, primarily due to heterosis in crop-weed hybrids (unpublished data).

Crop origins

Neither of the two proposed hypotheses (monophyletic vs polyphyletic) for the origin of the three domesticated species is unequivocally supported by this study. Genetic distances between crop versus crop pairs are lower than between the weed-weed pairs, thus implying a common monophyletic origin of the three domesticates with *A. hybridus* as the most probable common ancestor (Sauer 1967). However, information on most weedy re-

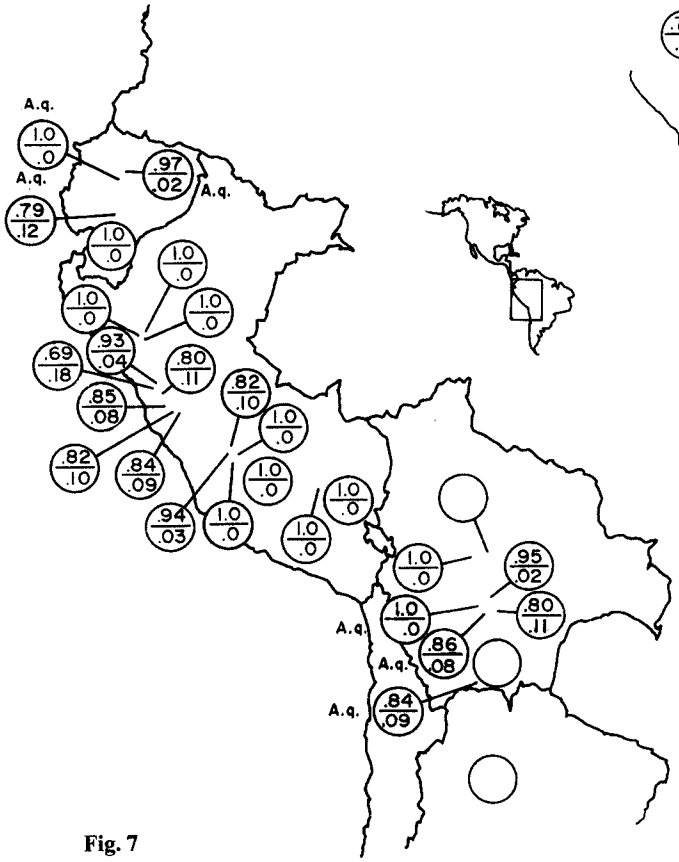

Fig. 7

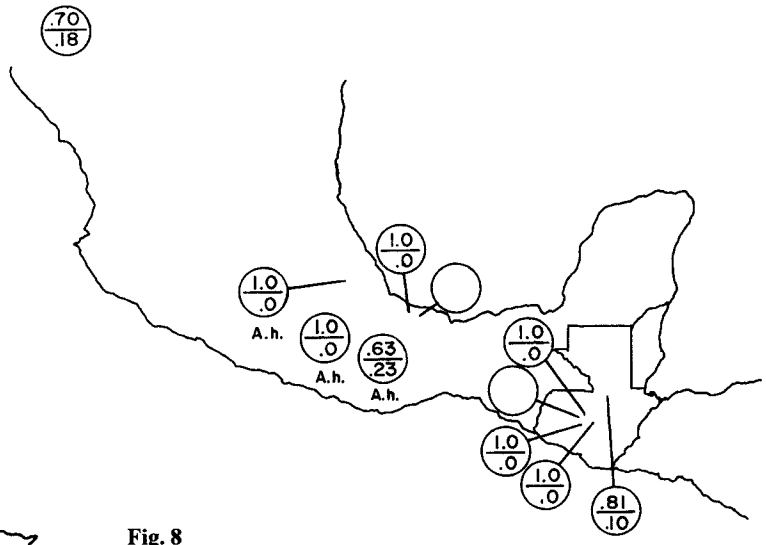

Fig. 8

Figs. 7 and 8. Graphical representation of the estimates of fixation index F (upper number), and outcrossing rate t (lower number) in each population. Blank circles are monomorphic populations

latives, e.g. *A. powellii,* must be gathered on a larger number of field collections. The very low genetic distance between *A. quitensis* and *A. caudatus* (Table 4) points to a separate domestication of *A. caudatus* from *A. quitensis.* One cannot exclude the idea that *A. quitensis* is derived from *A. caudatus* (via hybridization with *A. hybridus* or escape from cultivation). As noted earlier, Hauptli et al. (1979) found *A. quitensis* to be ecologically a semi-domesticate in Ecuador, occurring only in cultivated fields, and not other disturbed environments inhabited by *A. hybridus* or other truly weedy *Amaranthus.* It has not persisted in some sites where it was previously recorded, whereas other weedy *Amaranthus* spp. are present. Thus, currently the main distribution of *A. quitensis* in Ecuador does not overlap with that of the white seeded *A. caudatus* to the south (Heiser 1962; Coons 1975; Hauptli et al. 1979).

The occurrence of the ACPH 0.95 allele and PGI B phenotype only in *A. caudatus* and *A. quitensis* indicates a close relationship between them. If multiple origins are to be assumed it seems more likely on the basis of distances and alleles unique to the two groups of species that two events took place; one in Mesoamerica and the other in the Andean area. An alternative synthesis following Sauer's (1967) logic may be put forth involving a single domestication event of *A. hybridus* in Mesoamerica leading to the *A. cruentus–A. hypochondriacus* complex there, and ancient migration of this domesticate to South America, where it underwent radical divergence in the very different Andean environment. *A. caudatus* could then be closely related to this complex and still be unique. It could then have hybridized with *A. hybridus* or escaped to yield *A. quitensis.* If the latter scenario is valid, a kind of "transdomestication" event, similar to that described by Hymowitz (1970) for the origin of guar, may have taken place in the origin of *A. caudatus.* Accordingly, the weed or semi-domesticate hypothetically introduced to south America from Mesoamerica was probably not *A. quitensis,* as its distribution is comparatively limited.

This picture of divergent, yet relatively close groups of domesticates in Meso- and South America is in dramatic contrast to the two very different *Chenopodium* domesticates described by Wilson and Heiser (1979). The Mexican and Andean domesticates (*C. rutalliae* and *C. quinoa,* respectively) are less closely related to each other than to their postulated weedy antecedents (*C. berlandieri* and *C. hircinum,* respectively) on the basis of morphology, an allozyme locus, and cross-compatibility. It is curious that the chenopods, with such an overall morphology similar to the amaranths (even some indigenous people confuse them at times), and similar distribution and uses as pseudocereal crops, might differ in their histories of domestication. Perhaps the South American farmers chose amaranth after the successful domestication of quinoa, as the former contains no toxic substances that must be leached from the seed; indeed, amaranth is called "quinoa dulce" in some parts of its distribution. A careful philological study of names given to amaranths and quinoas in South America and Mexico might elucidate some of the questions as to their relative importance as crops, and their mutual interaction in early domestication.

It may be difficult to compare species crossability in *Amaranthus,* as has been done for the *Chenopodium* species, as there appear to be varying cross-incompatibility barriers between populations of *A. caudatus* (Coons 1982; also Kulakow and Jain 1983), and different chromosome numbers have been reported within *A. cruentus* and *A. powellii* (Pal et al. 1982). Further examination of genetic relation between domesticates should include tests of complementation of allelism of various segregating marker loci controlling plant pigmentation, seed color, and seed translucency.

In his elegant review of New World domestication, Heiser (1979) noted numerous difficulties in testing phylogenetic hypotheses. Both ancestry and places of origin often remain speculative in most examples since the descriptive genetic variation studies often fail to establish clear-cut patterns of species relationships. Clearly, this first study of variation in amaranths posed even more uncertainties. The Meso-American and South American amaranth groups might be more closely related to one another than to their weedy companions, unlike the *Chenopodium.* No suitable unique progenitor for the *A. caudatus–A. quitensis* complex was guessed as we suggest that an earlier semi-domesticate or weed, now extinct, was involved in a transdomestication type event. Multiple domestication events have been proposed for a number of crops of New World Origin, for instance, beans (Gentry 1969) and avocado (Heiser 1979). Different wild or weedy species of the same genus seem to have been repeatedly domesticated independently in the case of peppers (Heiser 1979; MecLeod et al., in press), manioc (Renvoise 1970), and *Solanum* species cultivated for their fruits rather than tubers (*S. quitoense, S. topiro,* and *S. muricatum*) (Heiser 1979). Maize domestication remains enigmatic, but most agree on a single event, probably occurring in Mexico. The potato and tomato also show relatively uncomplicated monophyletic origins (Heiser 1979; Rick and Fobes 1975). The amaranth scenario proposed here of concurrent migration and domestication of a semi-domesticate between the two New World centers appears to be a new mode of origin among the New World crops.

References

Brown AHD (1978) Isozymes, plant population genetic structure, and genetic conservation. Theor Appl Genet 52:145–157

Brown AHD, Munday J (1982) Population genetic structure of landraces of barley from Iran. Genetica

Coons MP (1975) The Genus *Amaranthus* in Ecuador. PhD Dissertation, Indiana University

Coons MP (1982) Relationships of *Amaranthus caudatus.* Econ Bot 36:129–146

Gentry HS (1968) Origin of the common bean, *Phaseolus vulgaris.* Econ Bot 20:55–69

Goodman MM, Bird RM (1977) The races of maize. 4. Tentative grouping of 219 Latin American races. Econ Bot 31:204–221

Hauptli H, Jain SK (1978) Biosystematics and agronomic potential of some weedy and cultivated amaranths. Theor Appl Genet 52:177–185

Hauptli H, Lutz RL, Jain SK (1979) Germplasm exploration in Central and South America. In: Proc 2nd Amaranth Conf, Rodale Press, pp 117–122

Hauptli H, Jain SK (1984) Genetic variation on outcrossing rate and correlated morphological traits in a population of grain amaranth (*Amaranthus cruentus* L.). Genetica (in press)

Heiser CB Jr (1962) Sangorache, an amaranth used ceremonially in Ecuador. Am Anthropol 66:136–139

Heiser CB (1979) Origins of some cultivated New World plants. Annu Rev Ecol Syst 10:309–326

Hymowitz T (1970) The trans-domestication concept as applied to guar. Econ Bot 22:49–60

Jain SK, Vaidya KR, Joshi BD (1979) Collection and evaluation of Indian grain amaranths. In: Proc 2nd Amaranth Conf, Rodale Press, pp 123–128

Jain SK, Kulakow P, Bryant D, Hauptli H (in preparation) A germplasm catalog for the grain amaranths

Kulakow P, Jain SK (1983) Crop-weed relationships in the genus *Amaranthus:* genetic analysis of multilocus systems and of reproductive isolation. In: Proc 15th Int Genet Congr (Abstr) (in press)

Lewontin RC (1972) The apportionment of human diversity. Evol Biol 6:381–398

McLeod MJ, Guttman SI, Eshbaugh WH, Rayle RE (in press) An electrophoretic study of evolution in *Capsicum* (Solanaceae)

Nei M (1972) Genetic distance between populations. Am Nat 106:283–292

Nevo E, Brown AHD, Zohary D (1979) Genetic diversity in the wild progenitor of barley in Israel. Experientia 35:1027–1029

Oka HI, Morishima H (1967) Variation in the breeding systems of a wild rice, *Oryza perennis.* Evolution 21:249–258

Pal M, Pandey RM, Khoshoo TN (1982) Evolution and improvement of cultivated amaranths. 9. Cytogenetic relationship between the two basic chromosome numbers. J Hered 73:353–356

Renvoise BS (1970) The area of origin of *Manihot esculenta* as a crop plant – a review of the evidence. Econ Bot 22:352–359

Rick CM (1950) Pollination relations of *Lycopersicon esculentum* in native and foreign regions. Evolution 4:110–122

Rick CM, Fobes, JG (1975) Allozyme variation in the cultivated tomato and closely related species. Bull Torrey Bot Club 101:376–384

Rick CM, Fobes JF, Holle M (1977) Genetic variation in *Lycopersicon pimpinellifolium:* evidence of evolutionary change in mating systems. Plant Syst Evol 127:139–179

Rick CM, Fobes JF, Tanksley SD (1979) Evolution of mating systems in *Lycopersicon hirsutum* as deduced from genetic variation in electrophoretic and morphological characters. Plant Syst Evol 132:279–298

Sauer JD (1950) The grain amaranths: a survey of their history and classification. Ann Mo Bot Gard 37:561–632

Sauer JD (1967) The grain amaranths and their relatives: a revised taxonomic and geographic survey. Ann Mo Bot Gard 54:103–137

Scandalios JG (1969) Genetic control of multiple molecular forms of enzymes in plants: a review. Biochem Genet 3:37–79

Second G (1982) Origin of the genic diversity of cultivated rice (*Oryza,* spp.): study of the polymorphism scored at 40 isozyme loci. Jpn J Genet 57:25–57

Senadhira D (1976) Genetic variation in corn and its relatives. PhD Thesis, University of California, Davis

Stephens SG (1949) The cytogenetics of speciation in *Gossypium.* 1. Selective elimination of the donor parent genotype in interspecific backcrosses. Genetics 34:627–637

Stuber CW, Goodman MM, Moll RH (1982) Improvement of yield and ear number resulting from selection at allozyme loci in a maize population. Crop Sci 22:737–740

Tanksley SD, Rick CM (1980) Isozymic gene linkage map of the tomato; Applications in genetics and breeding. Theor Appl Genet 57:161–170

Wall JR (1968) Leucine aminopeptidase polymorphism in *Phaseolus* and differential elimination of the donor parent genotype in interspecific backcrosses.

Wilson HD, Heiser CB Jr (1979) The origin and evolutionary relationships of 'Huauzontle' (*Chenopodium nuttalliae* Safford), domesticated chenopod of Mexico. Am J Bot 66:198–206

Appendix 1. Site location, and species of population

Accession	Species	Location
UC004	*A. edulis*	Family farm, 2 km east of corralitos, Tucuman, Argentina
UC008	*A. caudatus*	Family farm, 1 km south of San Lorenzo, Tarija, Bolivia.
UC0010	*A. caudatus*	Family farm, 5 km east of Cochabamba, Cochabamba
UC011	*A. caudatus*	Market sample, Sacbab, Cochabamba, Bolivia
UC015	*A. caudatus*	Family farm just northwest of San Jeronimo, Cuezeco, Peru
UC018	*A. caudatus*	Family farm just northwest of rail station, Pactar, Urubamba, Peru
UC019	*A. caudatus*	Market sample, Ayacucho, Ayacucho, Peru
UC021	*A. caudatus*	Market sample, Huancayo, Huancayo, Peru
UC022	*A. caudatus*	Market sample, Huancayo, Huancayo, Peru
UC023	*A. caudatus*	Family farm outside La Majorada, Huancavelica, Peru
UC025	*A. caudatus*	Family farm outside Manuel Tellenia, Huancavelica, Peru
UC031	*A. caudatus*	Market sample, Huarez, Ancash, Peru
UC032	*A. caudatus*	Family farm, 5 km south of Huaraz, Ancash, Peru
UC037	*A. caudatus*	Family farm, Carhuaz, Ancash, Peru
UC040	*A. caudatus*	Market sample, Yungay, Ancash, Peru
UC039	*A. caudatus*	Market sample, Caras, Ancash, Peru

Appendix 1 (continued)

Accession	Species	Location
UC042	*A. caudatus*	Family farm, southern edge of Caras, Ancash, Peru
UC044	*A. caudatus*	Market sample, Cajamarca, Cajamarca, Peru
UC045	*A. caudatus*	Family farm, southernedge of Cajamarca, Cajamarca, Peru
UC047	*A. caudatus*	Family farm, southernedge of Cajamarca, Cajamarca, Peru
UC050	*A. caudatus*	Market sample, Sucre, Chuquisaca, Bolivia
UC054	*A. caudatus*	Market sample, Tarabuco, Chuquisaca, Bolivia
UC066	*A. caudatus*	Family farm, Caruncay, Azuay, Ecuador
UC007	*A. quitensis*	Market sample, Villazon, Tarija, Bolivia
UC052	*A. quitensis*	Market sample, Tarabuco, Chuquisaca, Bolivia
UC053	*A. quitensis*	Family farm 5 km east of Gualaceo, Azogues, Ecuador
UC073	*A. quitensis*	Family farm, San Andres, (7 km south of Riobamba, Ecuador
UC075	*A. quitensis*	Family farm east of Tabacundo, Quito, Ecuador
UC085	*A. quitensis*	Family farm, Aldea San Miguel, Chimattenango, Guatemala
UC086	*A. cruentus*	Family farm above Aldea Choatalum, Chimaltenango, Guatemala
UC088	*A. cruentus*	Family farm just below Aldea Choatalum, Chimaltenango, Guatemala
UC096	*A. cruentus*	Family farm, Ojittan, Veracruz, Mexico
UC097	*A. cruentus*	Family farm, Jilapa de Diaz, Veracruz, Mexico
UC106	*A. cruentus*	Market sample, Coban, Alto Verapaz, Guatemala
UC107	*A. cruentus*	Family farm near San Bernardo, Sonora, Mexico
UC093	*A. hypochondriacus*	Market sample, Oaxaca, Oaxaca, Mexico
UC099	*A. hypochondriacus*	Market sample, Cordoba, Veracruz, Mexico
UC104	*A. hypochondriacus*	Market sample, San Martin Texmelucan, Puebla, Mexico
UC154	*A. cruentus*	Family farm at Rancho Terrero near San Bernardo, Sonora, Mexico
UC155	Unknown crop	Village Joshimath, Chamolli District, Uttar Pradesh, India
UC13k	Unknown crop	Village Vinyak Chatti Channolli District, Uttar Pradesh, India
UC157	Unknown crop	Village Vinyak Chatti Channolli District, Uttar Pradesh, India
UC157	Unknown crop	Village Joshi Matta, Chamolli District, Uttar Pradesh, India
UC160	Unknown crop	Village Joshi Matta, Chamolli District, Uttar Pradesh, India
UC15k	Unknown crop	Village Pando Keshawar Bamani, Chamolli District, Uttar Pradesh, India
UC169	Unknown crop	Gauri kund above San Prayag, Channolli District, Uttar Pradesh, India
UC172	Unknown crop	Village pando Keshawar Bamani, Chamolli District, Uttar Pradesh, India
UC175	Unknown crop	Village Pando Kesawar Bamani, Chamolli District, Uttar Pradesh, India
UC184	Unknown crop	Village Subash Nagar, Chamolli District Uttar Pradesh, India
UC186	Unknown crop	Village Rampur, Chamolli District, Uttar Pradesh, India
UC260	Unknown crop	Nepalese collection, FAO # 101
UC261	Unknown crop	Nepalese collection, FAO # 102
UC262	Unknown crop	Nepalese collection, FAO # 103
UC263	Unknown crop	Nepalese collection, FAO # 104
UC264	Unknown crop	Nepalese collection, FAO # 105
UC265	Unknown crop	Nepalese collection, FAO # 106
UC266	*A. hybridus*	Fayette Co., Pennsylvania, USA
UC267	*A. hybridus*	Pickens Co., South Carolina, USA
UC268	*A. hybridus*	Ingham Co., Michigan, USA
UC269	*A. hybridus*	Fayette Co., Kentucky, USA
UC270	*A. hybridus*	Douglas Co., Kansas, USA
UC271	*A. hybridus*	Tippecanoe Co., Indiana, USA
UC272	*A. hybridus*	Richland Co., South Carolina, USA
UC043	*A. hybridus*	Riverbank, west site of the Plaza de Armas, Yungay, Ancash, Peru
UC082	*A. hybridus*	Weed population along roadsides, Antigua, Guatemala
UC273	*A. hybridus*	University of Texas, Austin, Texas, USA
UC274	*A. hybridus*	Tulelake Agricultural Experimental Station, Modoc Co., California, USA