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The impact of domestication on distribution of allozyme variation within and among cultivars of radish, *Raphanus sativus* L.

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Summary. Allozyme surveys of cultivated plant species generally report little within-cultivar variation, but considerable among-cultivar variation. This trend contrasts with natural plant populations in which most allozyme variation resides within, rather than among, populations. The difference may be an artifact of the extreme inbreeding techniques used to develop and propagate these crops, rather than a consequence of domestication per se. To test this hypothesis, we compared the population genetic structure of 24 lines of radish cultivars – a domesticated species developed and maintained as open-pollinated, outcrossed populations – with four wild radish populations in California. Although the wild populations displayed more overall allozyme variation than the cultivars, most of the allozyme variation in the cultivars remains partitioned within, rather than among, lines. Apparently, how a crop is developed and maintained can have a profound influence on the organization of genetic variation of that species.

Key words: Breeding methods – Isozymes – Polymorphism – Radish – *Raphanus sativus*

Introduction

Allozyme variation in crop plants generally occurs among cultivars with little or no within-cultivar polymorphism (Tanksley and Orton 1983). In contrast, allozyme variation in natural plant populations occurs primarily within, rather than among, populations (Brown 1979). Even those species with high selfing rates typically are organized such that 50% or more of their total allozyme variation resides within populations (Brown 1979). The different population genetic structure of crop species may be an artifact of the methods of cultivar development and maintenance. In the case of clonally-maintained cultivars (especially woody species, cf. Torres 1983), it is obvious that each cultivar should represent a single genotype. For sexually-maintained inbred lines, Levin (1976) proposed that the bottlenecks and isolation utilized to develop and maintain such lines should redistribute existing variation among those lines. If these techniques, rather than some other aspect of domestication, are responsible for the typical patterns of allozyme variation observed in cultivated plants, then a very different genetic structure should occur in those crops developed and maintained as open-pollinated populations, rather than as inbred lines. Under such conditions, some redistribution of variation might still occur from isolation and hitchhiking, but it would not be expected to occur to the extent found among inbred cultivars. However, whether an outcrossing crop retains a population genetic structure relatively unchanged from is wild relatives remains to be addressed directly.

Raphanus sativus L., radish, is an ideal species to test this hypothesis because cultivars of this species have been developed and maintained in open-pollinated, outcrossing populations (Crisp 1976; Simmonds 1979). Furthermore, since this introduced species also occurs in natural, weedy populations in California (Panetsos and Baker 1967), a control is available to compare the impact of domestication on the population genetic structure of this species. Specifically, we asked the following questions: (1) how does the population genetic structure of the cultivars contrast with that of wild populations; and (2) how do these differences compare with those for cultivated plants maintained as inbred lines?

Table 1. Allozyme variation in cultivars[a]

Class[b]		Black Round	Black Long	White Spherical		White Long			Red and White Long		
Cultivar		'Round Black Spanish'	'Long Black Spanish'	'Grant White Globe'	'Hailstone White Globe'	'Short Top Icicle'	'White Icicle'		'French Breakfast'		
Source[c]		FB	FR	N	FB	FM	B	N	B	G	N
Locus	Allele										
LDH	1	0.60	0.23	0.67	0.57	0.80	0.84	0.90	0.50	0.60	0.63
	2	0.40	0.77	0.33	0.43	0.20	0.16	0.10	0.50	0.40	0.37
PGM-1	1	0.30	0.33	0.73	0.30	0.18	0.16	0.33	0.07	0.10	0.10
	2	0.57	0.60	0.10	0.70	0.75	0.84	0.67	0.60	0.87	–
	3	0.13	0.07	0.17	–	0.07	–	–	0.33	0.03	0.90
PGM-2	1	0.20	0.60	1.00	0.77	0.18	–	0.13	–	0.07	–
	2	0.70	0.37	–	0.17	0.79	0.91	0.77	0.70	0.93	0.20
	3	0.10	0.03	–	0.06	0.03	0.09	0.10	0.30	–	0.80
PGD	1	0.27	–	0.07	0.50	0.37	0.16	0.27	–	0.03	0.17
	2	–	0.03	–	0.10	–	0.03	–	–	–	–
	3	0.73	0.97	0.93	0.40	0.63	0.81	0.73	1.00	0.97	0.83
EST	1	0.03	0.10	0.60	0.33	0.07	0.16	0.13	0.03	–	–
	2	0.60	0.90	0.40	0.43	0.93	0.84	0.87	0.47	0.37	1.00
	4	0.03	0.03	–	–	–	–	–	–	–	–
	null	0.34	–	–	0.24	–	–	–	0.50	0.63	–
LAP	1	0.20	0.07	–	0.20	–	–	0.03	–	–	–
	2	0.43	0.23	–	0.43	0.39	0.19	0.57	0.80	0.70	0.67
	3	0.04	0.03	–	–	–	–	–	0.07	–	0.07
	4	0.33	0.67	1.00	0.37	0.61	0.81	0.40	0.13	0.30	0.26

[a] The cultivars are monomorphic for TPI[1] and FOR[2]; [b] George and Evans (1981); [c] B = Burpee, E = Excell, FB = Fort Berthold, FM = Ferry Morse, FR = Fredonia, G = Germaine, L = Los Angeles, N = Northrup-King

Materials and methods

Twenty-four samples of radish cultivars were obtained from commercial outlets in Riverside, California, and Pullman, Washington. These samples represent 16 named varieties from eight different seed companies (detailed in Table 1). They include 7 of the 11 common root morphology categories described for winter radish cultivars (George and Evans 1981). In addition, three collections were made from southern California populations of wild radish and one from a northern California population (Table 2). Sample sizes were 15 to 45 individuals per cultivar or collection.

Mature seeds or buds served as enzyme sources. Techniques for enzyme electrophoresis of *Raphanus sativus* material have been described elsewhere (Ellstrand 1984) except for esterase (EST), triosephosphate isomerase (TPI), and formate dehydrogenase (FOR). EST and TPI were run in the discontinuous Tris-citrate, lithium-borate system of Heywood (1980) at 75 milliamps until the front had moved 5 cm from the origin. FOR was run on the Tris-EDTA-borate system described by Ellstrand (1984). FOR staining technique followed that of Wendel and Parks (1982); EST, Shaw and Prasad (1970); and TPI, Pichersky and Gottlieb (1983). Seeds from plants of known genotype served as internal standards on each gel.

The genetic basis for the gel banding patterns has been determined by comparisons of half-sib isozyme phenotypes to that of their maternal parent (Ellstrand 1984). The allozyme polymorphisms reported below are governed by single loci; alleles are (with one exception) co-dominant. A total of eight loci coding for seven enzymes were scored (Tables 1 and 2). A null allele was detected for esterase in some cultivars. In those cases, which were polymorphic for the null, we calculated the allele frequency of the null by assuming its genotypes to be in Hardy-Weinberg equilibrium.

Data were analyzed with the aid of the BIOSYS computer package (Swofford and Selander 1981). A dendrogram based on Nei's (1978) unbiased genetic distances was constructed using the unweighted pair group method (Sneath and Sokal 1973).

Results

Overall variation

Both cultivars and natural populations of *Raphanus sativus* show considerable allozyme variation. The cultivars were polymorphic at six of the eight loci assayed (Table 1) with an average of 2.6 alleles per locus and 3.2 alleles per polymorphic locus. The total gene diversity (Nei 1973), H_T, was 0.37 ± 0.24 per locus. The natural populations were somewhat more variable with all eight loci polymorphic (Table 2) and

Table 1 (continued)

Red and White Spherical				Red Spherical									
'Scarlet Turnip White-Tipped'		'Sparkler'		'Champion'	'Cherry Bell'	'Comet'	'Crimson Giant'		'Early Scarlet Globe'			'Red Devil	Scarlet Knight'
E	FM	B	NK	FB	B	N	B	FM	FM	L	N	FM	N
0.63	0.47	0.23	0.57	0.90	0.89	0.72	0.57	0.77	0.80	0.87	0.87	0.74	0.80
0.37	0.53	0.77	0.43	0.10	0.11	0.28	0.43	0.23	0.20	0.13	0.13	0.26	0.20
0.07	0.43	–	0.33	0.50	0.53	0.28	0.07	0.10	0.03	0.10	–	0.80	0.30
0.73	0.57	0.60	0.60	0.10	0.47	0.35	0.63	0.50	0.87	0.80	0.80	0.10	0.70
0.20	–	0.40	0.07	0.40	–	0.37	0.30	0.40	0.10	0.10	0.20	0.10	–
–	–	–	–	0.30	–	0.02	0.77	0.07	–	–	–	0.03	0.02
0.57	0.13	0.80	0.10	0.63	0.68	0.33	0.67	0.87	0.90	0.63	33	0.43	0.68
0.43	0.87	0.20	0.90	0.07	0.32	0.65	0.16	0.06	0.10	0.37	0.67	0.53	0.30
0.70	0.87	0.93	0.80	0.70	–	0.17	0.53	0.33	0.20	0.43	0.37	0.03	0.03
–	–	–	–	–	–	–	0.03	–	–	–	0.03	–	–
0.30	0.13	0.07	0.20	0.30	1.00	0.83	0.43	0.67	0.80	0.57	0.60	0.97	0.97
0.03	0.20	0.37	–	0.07	–	–	0.13	0.10	–	–	–	–	0.02
0.40	0.80	0.63	1.00	0.93	1.00	1.00	0.87	0.90	1.00	1.00	1.00	1.00	0.98
–	–	–	–	–	–	–	–	–	–	–	–	–	–
0.57	–	–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	0.27	–	–	0.07	0.11	0.13	0.26	0.17	–	0.13
0.30	0.60	0.30	0.47	0.70	0.93	0.90	0.90	0.79	0.80	0.57	0.67	1.00	0.87
0.07	0.07	0.10	0.07	0.03	0.07	–	–	0.07	0.07	0.07	0.03	–	–
0.63	0.33	0.60	0.46	–	–	0.10	0.03	0.04	–	0.10	0.13	–	–

3.1 alleles per polymorphic locus. For these populations, gene diversity per locus was 0.54 ± 0.09, not significantly different from the cultivars.

The natural populations also show more overall variation when compared with the cultivars locus-by-locus. In six of eight comparisons, the total gene diversity (H_T) of each locus is greater for the natural populations (Table 3, $P = 0.039$; one-tailed Wilcoxon's signed-ranks test).

Distribution of variation

How genetic variation is organized can be described with both absolute and relative measures. In terms of absolute variation, the total gene diversity at a locus can be expressed as the sum of H_S, the portion of gene diversity that occurs within subpopulations, plus D_{ST}, the portion of gene diversity occurring among subpopulations (Nei 1973). These values are reported for both the natural populations and cultivars in Table 3. H_S values are uniformly greater in the natural populations ($P = 0.004$; one-tailed Wilcoxon's signed-ranks test). On the other hand, when all loci are compared, D_{ST} differences are not significantly different ($P \gg 0.05$; one-tailed Wilcoxon's signed-ranks test). Thus, differences in the distribution of total allozyme variation between radish cultivars and wild populations are accounted for primarily by a relative deficiency of within-population variation in the cultivars.

The parameter for the measurement of relative interpopulation differentiation, G_{ST} is obtained from D_{ST}/H_{ST} (Nei 1973), giving the population of allozyme diversity arising from interpopulation differences. These values, which can be computed only for polymorphic loci, are given in Table 3. When these G_{ST} values for shared polymorphic loci are compared, it is clear that the cultivars display the greater relative differentiation ($P = 0.031$; Wilcoxon's signed-ranks test, one-tailed). However, at every locus more of the allozyme variation resides within wild populations and within cultivars than among those groups.

Discussion

While cultivars of *Raphanus sativus* show some reorganization of allozyme variation relative to natural

Table 2. Allozyme variation in wild populations

Locus	Allele	Population			
		UC Moreno field station	Riverside CRC-East	Riverside CRC-West	5 S Carmel
IDH	1	0.73	0.52	0.61	0.18
	2	0.26	0.58	0.38	0.82
	3	0.01	–	0.01	–
PGM-1	1	0.04	0.32	0.26	0.16
	2	0.70	0.55	0.63	0.82
	3	0.26	0.13	0.11	0.02
PGM-2	1	0.60	0.24	0.07	0.02
	2	0.17	0.40	0.59	0.50
	3	0.23	0.36	0.34	0.48
PGD	1	0.11	0.17	0.26	0.18
	2	0.17	0.17	0.06	0.05
	3	0.72	0.66	0.68	0.77
EST	1	0.29	0.13	0.11	–
	2	0.62	0.52	0.56	0.74
	3	0.05	0.04	0.19	–
	4	0.04	0.31	0.14	0.26
LAP	1	0.10	0.16	0.23	0.25
	2	0.51	0.24	0.56	0.27
	3	0.17	0.10	0.05	0.02
	4	0.22	0.50	0.11	0.39
	5	–	–	0.05	0.07
TPI	1	0.56	1.00	0.91	–
	2	0.44	–	0.09	1.00
FOR	1	0.23	0.04	0.14	–
	2	0.35	0.70	0.58	0.88
	3	0.42	0.26	0.28	0.12

Table 3. Distribution of allozyme variation in *Raphanus sativus*

Locus	Cultivars				Wild populations			
	H_T	H_S	D_{ST}	G_{ST}	H_T	H_S	D_{ST}	G_{ST}
IDH	0.44	0.36	0.08	0.17	0.51	0.42	0.09	0.18
PGM-1	0.61	0.42	0.19	0.31	0.48	0.46	0.02	0.04
PGM-2	0.58	0.34	0.24	0.41	0.65	0.57	0.09	0.13
PGD	0.46	0.27	0.19	0.41	0.45	0.44	0.01	0.02
EST	0.33	0.22	0.11	0.33	0.57	0.54	0.07	0.06
LAP	0.54	0.40	0.14	0.26	0.71	0.66	0.05	0.07
TPI	0.00	0.00	0.00	–	0.47	0.16	0.31	0.65
FOR	0.00	0.00	0.00	–	0.52	0.47	0.05	0.09

populations, the magnitude of such changes is considerably smaller than the typical redistribution of isozyme variation after domestication (Brown 1978). The differences are best illustrated with an example. *Phlox drummondii* contrasts with *R. sativus* in its history of domestication because the former is a naturally self-incompatible species developed and maintained as self-compatible, inbred cultivars (Levin 1975), while the latter, also a naturally self-incompatible species, was developed and maintained in self-incompatible, open-pollinated populations (Crisp 1976; Simmonds 1979). The genetic correlates of domestication are presented for each species in Table 4. In the case of phlox, we compare the allozyme diversity uncovered at the same loci assayed by a study of 16 cultivars (Levin 1976) and a study of 73 natural populations (Levin 1978). The most important parameter to compare is the G_{ST} values, the proportion of allozyme variation distributed among populations. For wild phlox populations 29% of the allozyme variation at polymorphic loci resides among populations (G_{ST}), while for the phlox cultivars 78% resides among populations. The situation for radishes is very different; 15% of the allozyme variation at polymorphic loci is partitioned among natural populations, and the value increases to only 31% in the cultivars. Clearly, the conditions for the domestication of radish cultivars are not accompanied by the severe reorganization of genetic variation which goes along with the establishment of inbred lines. In fact, the data presented here suggests that maintenance of cultivars through outcrossing, open-pollinated populations may be a good way to conserve genetic variation within cultivars (see also Brown 1978 and references therein).

Table 4. Genetic correlates of domestication in *phlox* radish population genetic parameters

	% Polymorphic loci	No. alleles/ polymorphic locus	H_T[a]	H_S[a]	D_{ST}[a]	G_{ST}[a]
Phlox drummondii						
Wild[b]	0.31	2.0	0.24	0.17	0.07	0.29
Cultivated[c]	0.38	2.0	0.33	0.07	0.26	0.78
Raphanus sativus[d]						
Wild	1.00	3.1	0.54	0.45	0.08	0.15
Cultivated	0.75	2.6	0.49	0.34	0.15	0.31

[a] Mean over all polymorphic loci; [b] from Levin 1976; [c] from Levin 1978; [d] from this study

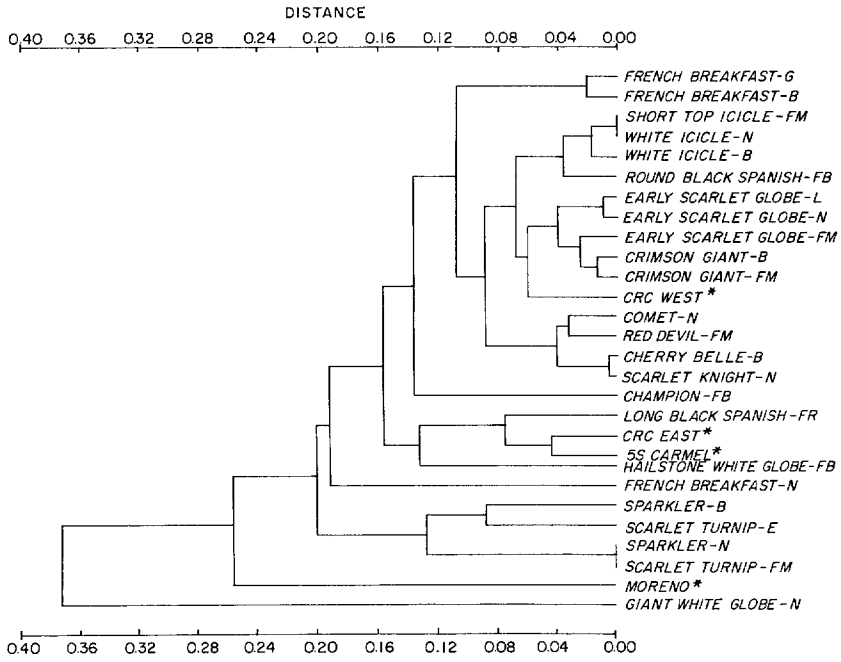

Fig. 1. Phenogram comparing affinities of radish cultivars and wild (*) populations. See text for details

Another way to ask whether domestication has dramatically altered the genetic composition of cultivars is to measure degree of divergence of cultivars from the wild populations. For example, a numerical taxonomic analysis of the phlox data described above (Levin 1976) yielded a dendrogram which clearly removed the native populations from the cultivars. We have performed a similar comparison with the radish data presented in Fig. 1. All but one of the wild populations cluster well within the cultivars, and even that population (Moreno) shows greater affinities to the other cultivars than does Giant White Globe. The phenogram based on allozyme data confirms, for the most part, the taxonomic organization of radish cultivars into root morphology categories (George and Evans 1981). However, the fact that cultivars of different color and similar root shape or those of the same color and different root shape do not cluster in patterns suggests a simple history of the overall evolution of these categories relative to each other. This analysis confirms our assertion that the domestication and maintenance of radish cultivars has not only retained a population structure similar to that of wild populations, but has not resulted in any dramatic divergence in overall composition of allozyme alleles.

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