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The impact of domestication on the genetic variability in the orange carrot, cultivated *Daucus carota* ssp. *sativus* and the genetic homogeneity of various cultivars

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Summary. Isozyme analysis of wild and domesticated accessions indicated that domestication of the cultivated carrot *Daucus carota* ssp. *sativus* resulted in an insignificant reduction of all genetic variability and genetic distance estimates. Although they are less variable genetically, cultivated forms maintain a high proportion of observed heterozygosity. Relative to the overall genetic variability of the species, samples from four common cultivars 'Red Cored Chantenay', 'Scarlet Nantes', 'Danvers Half Long' and 'A Plus' demonstrated a high degree of genetic similarity. This is attributed to the recent development of orange cultivars and the limited gene pool utilized in their development.

Key words: Carrot – *Daucus* – Isozyme electrophoresis – Cultivar – Genetic bottleneck

Introduction

Cultivated crops are usually excluded from variability estimate studies since they are likely to be biased by the manner of their maintenance and cultivation (Gottlieb 1981). Hamrick et al. (1979) concluded that cultivated plants had greater expected heterozygosity values (H_{exp}) than wild species due to artificial genetic manipulation. In wild populations most of the allozyme variation is found within rather than among populations (Brown 1979). Allozyme surveys of cultivated plants generally report little within cultivar variation, but considerable among cultivar variation (Tanksley and Orton 1983; Hamrick et al. 1979). The degree of genetic variability found within a crop species is a function of the method of its domestication, its breeding system and the method by which it is maintained (Hamrick et al. 1979). Agricultural crops are subjected to numerous artificial selection and breeding techniques. The genetic variability of a cultivated crop may vary considerably from its progenitor depending on the length and degree of the bottleneck they were subjected to as well as the degree of introgression with wild populations. *Daucus carota* ssp. *carota* is a cosmopolitan anthropochorous, weedy subspecies, and great care is therefore necessary to ensure that commercial seed plots are properly isolated from any wild populations (Wijnheijmer et al. 1989).

Affirming trueness to type in cultivars is becoming increasingly difficult today with the release of many new lines and the convergence of many of these lines on a few of the most desirable characters. Isozyme data quickly determine a particular genotype, and electrophoresis is now being utilized as a means of determining the genotypes of various crop species. It is also of use to ensure F_1 hybrid purity (Arus 1983; Surrs 1986). Mass populations of breeder lines represent a wide variety of genotypes that demonstrate uniform phenotypes under agronomic conditions. Isozyme polymorphisms act as useful markers to estimate the genetic diversity found in these populations.

The objectives of this paper are two fold. First, to determine the degree of genetic reorganization in domesticated carrot cultivars. Secondly, to determine the genetic integrity of commercial cultivars by assessing the genetic variability within and among 13 lines of four commercial carrot cultivars.

Materials and methods

A. Plant material

Genetic variability estimates for 168 accessions of *D. carota* were utilized (St. Pierre 1989). Values for the proportion of

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polymorphic loci (P), the mean number of alleles per locus (A), the observed mean heterozygosity (H_{obs}) as well as some of Nei's measures of intraspecific genetic diversity (Nei 1972, 1973) including total gene diversity within a taxon (H_t), gene diversity within populations of a taxon (H_s), gene diversity between populations within a taxon (D_{st}) and the coefficient of gene differentiation (G_{st}) were obtained from 123 cultivated and 45 wild accessions of *D. carota*. Mean values for cultivated and wild accessions were calculated and compared statistically to determine whether any significant changes had occurred in the genetic makeup as a result of domestication.

Thirteen of the 123 seed samples from domesticated carrots represented four commonly grown bunching and processing carrot cultivars. 'Red Cored Chantenay', 'Scarlet Nantes', and 'Danvers Half Long' are varieties maintained by open pollination, while 'A-Plus' is a hybrid variety. Seeds were sown in the University of Windsor greenhouses and allowed to grow to maturity.

B. Electrophoresis

Twenty-five individuals were examined from each accession. Fresh leaf samples were ground in a cold 0.1 *M* TRIS-HCl extracting buffer, pH 7.5 (Gottlieb 1981) with approximately 20 mg polyvinylpolypyrrolidone (Sigma P6755). The extracts were centrifuged at 15,000 rpm for 2 min. The supernatant was absorbed onto double-thickness filter paper wicks and immediately subjected to horizontal starch gel electrophoresis for approximately 4 h. The enzyme systems were resolved utilizing 12.5% starch gels and two buffer systems. Phosphoglucose isomerase (PGI), triose phosphate isomerase (TPI), leucine amino peptidase (LAP), alcohol dehydrogenase (ADH) and glutamate dehydrogenase (GDH) were resolved using a TRIS-citrate and lithium borate system (Bayer and Crawford 1986). Malate dehydrogenase ((NAD)MDH), phosphoglucomutase (PGM), isocitrate dehydrogenase (IDH) and 6-phosphogluconate dehydrogenase (6-PGD) were resolved using a citric acid and *L*-histidine (free base) system (Cardy et al. 1981). The staining of gels and visualization of enzymes were done employing the methods of Soltis et al. (1983).

C. Statistical analysis

Values for P, A, H_{obs} and H_{exp} were calculated by hand. Chi-square tests determined whether observed and expected mean heterozygosities from each population deviated from Hardy-Weinberg expectations. Nei's genetic identity and genetic distance values (Nei 1972, 1973) were calculated utilizing the GENESTAT program (Whitkus 1985) and include Nei's measures of intraspecific mean genetic identity (I) and distance (D), H_t, H_s, D_{st} and G_{st}. The accessions were compared on a one to one basis and also grouped within cultivars. A distance phenogram was constructed based on the genetic distance matrix by the unweighted pair-group method using arithmetic averages (Sneath and Sokal 1973) within the TAXON subroutine of the NT-SYS program (Rohlf et al. 1974). Centroid, average, median, single (nearest neighbor) and complete (farthest neighbor) linkage method clustering strategies were computed with SYSTAT (Wilkinson 1988).

Results

A. General

Both cultivated and wild forms demonstrated a relatively large genetic variability. Details of the genetic interpretation of enzyme phenotypes can be found in St. Pierre et al. (1990). Wild forms demonstrated greater variability though not significantly so (Wilcoxon two-sample test, Table 1). The value of P obtained for cultivated forms (0.451) is less than that obtained for wild accessions (0.474, Table 1). This apparent reduction in variability was also observed in A, the mean number of alleles per locus, estimated at 1.503 for cultivars and 1.551 for wild forms. Total genetic diversity, H_t, in wild subspecies (0.307) is not significantly larger than that observed in cultivated accessions (0.230, Table 1).

A great deal of genetic variability is maintained in all accessions of the four cultivars (Table 2). Values range from 0.378 to 0.636 for P, 0.121 to 0.244 for H_{obs} and 0.149 to 0.265 for H_{exp}. This variability is probably due to the method in which the cultivars are maintained. The proportion of polymorphic loci in the four cultivars ranges from 0.457 in 'A-Plus' to 0.564 in 'Red Cored Chantenay' (Table 3). The mean number of alleles per locus ranges from 1.468 in 'A-Plus' to 1.659 in 'Danvers Half Long'. This trend is not clearly reflected in H_t values, as they range from 0.175 in 'Scarlet Nantes' to 0.238 in 'Danvers Half Long'. Expected and observed mean heterozygosities (Table 2) are not significantly different and conform well with those of the species in general (St. Pierre et al. 1990).

B. Specific distribution of variation

Genetic variability in wild subspecies, as compared to domesticated forms, is almost equally shared between the portion of gene diversity occurring within populations (H_s) and the portion of gene diversity occurring between populations (D_{st}, Table 1). Twenty-eight percent of the allozyme variation at polymorphic loci is due to interpopulation variation in wild taxa, as opposed to 24% for cultigens (Table 1) as determined by the relative magnitude of gene differentiation among populations (G_{st}). A greater proportion of genetic variation is found within both wild and cultivar accessions rather than among them.

Three of the four cultivars demonstrated higher than average H_s values (Table 3) for cultivated forms, owing probably to their wide distribution and popularity. 'Scarlet Nantes' is an exception. The smaller H_s value for 'Scarlet Nantes' is indicative of lower genetic diversity, and thus perhaps produces a more uniform crop. The four varieties demonstrated fairly low D_{st} values (Table 3), therefore only a small fraction of their genetic variability occurs between accessions. This is reflected in their small G_{st} values (Table 3), which indicate that a range of 7.5% ('Scarlet Nantes') to 15.5% ('Danvers Half Long') of the allozyme variability at polymorphic loci resides between accessions. The genome of these accessions is uniform, as is indicated by relatively low H_s and D_{st} values relative to those found in wild taxa. Clus-

Table 1. Genetic variation and Nei's genetic diversity statistic averages for 45 wild and 123 cultivated *Daucus carota* L. accessions (St. Pierre 1989)

Taxa	H_t	H_s	D_{st}	G_{st}	P	A	H_{obs}
Wild	0.307	0.189	0.118	0.277	0.474	1.551	0.161
Cultivated	0.230	0.152	0.078	0.238	0.451	1.503	0.161

Presented are:
H_t, Total gene diversity within a taxon; H_s, gene diversity within populations of a taxon; D_{st}, gene diversity between populations within a taxon; G_{st}, coefficient of gene differentiation; P, proportion of polymorphic loci, where the frequency of the most common allele is less than 0.99; A, mean number of alleles per locus; H_{obs}, observed average heterozygosity
Paired values are not significantly different ($P>0.05$) utilizing the Wilcoxon two-sample test

Table 2. Genetic variation in 13 accessions of four orange rooted carrot cultivars

Cultivar	Source[a]	A	P	H_{obs}	H_{exp}
Scarlet Nantes	Jung	1.565	0.560	0.186	0.255
	Dominion	1.510	0.500	0.169	0.164
	Harris-Moran	1.454	0.378	0.116	0.151
Red Cored Chantenay	Burpee	1.622	0.560	0.128	0.162
	Jung	1.565	0.500	0.121	0.149
	Seedway	1.622	0.560	0.154	0.198
	USDA-225862	1.699	0.636	0.244	0.265
A-Plus	Jung	1.454	0.439	0.211	0.155
	Seedway	1.510	0.500	0.157	0.178
	Dominion	1.446	0.433	0.145	0.174
Danvers Half Long	Burpee	1.733	0.621	0.198	0.237
	Dominion	1.733	0.621	0.154	0.227
	Dam	1.510	0.439	0.162	0.182

A, Mean number of alleles per locus; P, proportion of polymorphic loci, where the frequency of the most common allele is less than 0.99; H_{obs}, observed average heterozygosity; H_{exp}, expected average heterozygosity
[a] Commercial sources of the seed
Pairwise values of heterozygosity are not significantly different ($P>0.05$)

Table 3. Genetic variation and Nei's genetic diversity statistics within and among four cultivars of *Daucus carota* L

Cultivar	H_t[a]	H_s	D_{st}	G_{st}	P	A
A-Plus	0.232	0.182	0.050	0.121	0.457	1.468
Danvers Half Long	0.238	0.189	0.049	0.155	0.560	1.659
Red Cored Chantenay	0.218	0.185	0.033	0.100	0.564	1.627
Scarlet Nantes	0.175	0.148	0.027	0.075	0.479	1.510
Mean	0.238	0.191	0.047	0.161	0.515	1.566

[a] Definition of symbols is presented in footnote of Table 1

Table 4. Nei's genetic distances (upper triangle) and genetic identities (lower triangle) for all pairwise comparisons of accessions among four cultivars of *D. carota*

	A-Plus	Danvers Half Long	Red Cored Chantenay	Scarlet Nantes
APL	–	0.037	0.029	0.023
DAN	0.963	–	0.018	0.027
RCC	0.971	0.982	–	0.014
SNA	0.977	0.973	0.986	–

Table 5. Mean genetic identities and (ranges) for pairwise comparisons of accessions within four cultivars of *D. carota* L

Cultivar	Genetic identity	
	Mean (I)	Range
A-Plus	0.934	(0.925–0.952)
Danvers Half Long	0.925	(0.909–0.955)
Red Cored Chantenay	0.940	(0.885–0.992)
Scarlet Nantes	0.957	(0.934–0.980)

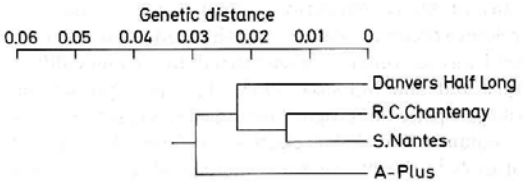

Fig. 1. Distance phenogram (UPGMA) derived from a matrix of genetic distances for 13 accessions of four orange rooted *D. carota* cultivars

ter analysis of values of D suggested that wild forms cluster fairly well from cultivated forms, demonstrating some degree of divergence (St. Pierre et al. 1990). Because relatively high H_t levels were obtained, and more variability occurred within taxa than among them, the division of clusters were almost arbitrary (St. Pierre et al. 1990).

The four cultivar groups are genetically similar, as all pairwise comparisons have genetic distances of less than 0.04 (Fig. 1, Table 4). The high degree of genetic similarity (Table 5) suggests there is uniformity within and among cultivars. Utilizing centroid, average, median, single (nearest neighbor) and complete (farthest neighbor) linkage method clustering strategies, we found that some accessions consistently cluster well ('Red Cored Chantenay') while others do not. Some accessions of the same variety ('A-Plus' and 'Danvers Half Long') do cluster together across many clustering algorithms.

Discussion

In 1629, short and long root types of carrots were known, and within these, red and yellow pith types were noted (Helweg 1908). Through time the list of available cultivars has risen from a few unspecialied varieties (Villmorin Andrieux 1885; Goff 1880) to hundreds of specialized varieties (Magruder et al. 1940; Babb et al. 1950 and others). Recently, carrot breeding has drastically changed. Early methods included mass selection or combined mass pedigree selection, recent methods involve inbred lines and hybridizations. Resulting cultivars from highly inbred lines may demonstrate a reorganization of gene frequencies once the genetic bottleneck has been overcome (Levin 1976). The genetic variation of cultivars developed and maintained as open-pollinated populations is usually distributed more evenly: there is less intra-populational and more inter-populational homogeneity among this group, as gene flow occurs more easily. Domestic orange cultivars, which have undergone both strict selection for a brief period and large scale open pollination to propagate and maintain the line, show a reduction in genetic variation. Yet modern cultivars demonstrate the same amount of heterozygosity in the more limited gene pool as the wild subspecies (H_{obs} = 0.161) (Table 1). Obviously, maintenance of strongly outcrossing species through open-pollinated populations is a good way to conserve genetic variability within cultivars (Ellstrand and Marshall 1985). This presupposes that wild subspecies and rogues are removed to prevent genetic contamination. While electrophoretic markers are useful tools in the systematic evaluation of a species, their ability to separate carrot cultivars is limited. This is due to the much greater H_s than D_{st} values observed.

Although some genetic re-organization within carrot cultivars is observed, it is considerably less than that typically found after domestication (Brown 1978). Compared to wild populations, domestic carrots have a 4.9% decrease in P, a 3.1% decrease in A, a 19.6% decrease in H_s, a 33.9% decrease in D_{st}, resulting in a 25.1% decrease in H_t as well as a 14.3% decrease in G_{st} (Table 1). Owing to the large genetic variability in wild subspecies, it is not surprising to observe a restriction in genetic variability in cultivated forms. The differences observed may be due to the inbreeding techniques utilized in selective cultivar breeding. Also, the genetic base of modern carotene-pigmented cultivars is limited, being based on a few eighteenth century selections (Banga 1976; Helweg 1908).

The introduction of male-sterile lines has enabled carrot breeders to maintain F_1 hybrid lines (Banga 1976). 'A Plus' is a three-way hybrid between three inbred lines $(A \times B) \times C$. That origin may explain its lower A and P values and high genetic uniformity. Any variation observed is a function of the variability found in the female lines and the pollen donor male lines used to produce it. The heterozygosity is not fixed in a diploid three-way hybrid in that certain plants are $A \times C$ hybrids while others are $B \times C$ hybrids for any given locus.

Cultivars with a high H_t value may be mor universally acceptable. They have been bred to be uniform in a variety of situations. The low D_{st} and G_{st} values observed in cultivars are a good reflection on the companies producing these seeds. Phenograms produced by various clustering strategies suggest that some cultivars are genetically homogeneous across seed companies while others are slightly more divergent. This divergence is minimal when compared to within accession genetic variability. The European accession of 'Red Cored Chantenay' is consistently the most divergent. There is very little genetic variability due to variation between populations tested, demonstrating a high degree of genetic purity in these lines. This is remarkable, considering the breeding system and levels of heterozygosity within these cultivars. It should be noted that many vegetable seed re-sellers may be buying seed from the same seed producers. Therefore, the origin of the seed may be more similar than expected.

In conclusion, no real pattern of genetic differentiation is observed among cultivars. Therefore, the maintenance and domestication of cultivated carrots has resulted in only minor divergences; basically a reduction in overall genetic variability. The population structure of these cultivars is similar, yet less variable than that of wild populations. In both groups a greater proportion of the population variability is maintained within rather than between populations. The distribution of genetic variability in carrot is not consistent with most wild-cultivar genetic studies (Hamrick et al. 1979) because more variability is observed in wild accessions. However, most wild progenitor species do not demonstrate the morphological, geographic, and genetic variability observed in *D. carota* L. The cultivars appear to have evolved in a simple pattern following the initial genetic bottleneck of domestication.

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