An isozyme-based assessment of the genetic variability within the *Daucus carota* complex (Apiaceae: Caucalideae)

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Received March 6, 1990

ST. PIERRE, M. D., BAYER, R. J., and WEIS, I. M. 1990. An isozyme-based assessment of the genetic variability within the *Daucus carota* complex (Apiaceae: Caucalideae). Can. J. Bot. **68**: 2449–2457.

Daucus carota sensu lato is a phenotypically variable Eurasian species complex that has been naturalized and cultivated worldwide. One hundred and sixty-eight accessions from 32 countries were surveyed by enzyme electrophoresis to determine the extent of genetic variation present in the species. Eight enzyme systems coded by 16 putative loci were surveyed. They indicate that the recognized subspecies have diverged only slightly from one another with respect to the enzymes surveyed. The samples of wild taxa are more genetically diverse than those of the cultivated taxa, but not significantly different with respect to the mean number of alleles per locus, the proportion of polymorphic loci, the observed and expected heterozygosities, or Nei's genetic diversity statistics ($P > 0.05$). Members of the aggregate group *gingidium* have significantly lower total gene diversity within a taxon ($P < .05$) than do members of the aggregate group *carota*, as well as lower gene diversity within populations of the taxa ($P < 0.01$). Thus allozyme divergence is concordant with morphological divergence. Cultivars with carotene-pigmented roots show negligible amounts of genetic divergence. They appear to have evolved in a simple pattern after an initial genetic bottleneck.

Key words: *Daucus carota*, isozyme, electrophoresis, genetic variability.

ST. PIERRE, M. D., BAYER, R. J., et WEIS, I. M. 1990. An isozyme-based assessment of the genetic variability within the *Daucus carota* complex (Apiaceae: Caucalideae). Can. J. Bot. **68** : 2449–2457.

Daucus carota sensu lato est un complexe d'espèces phénotypiquement variables d'origine eurasiatique qui ont été naturalisées et cultivées partout au monde. Les patrons enzymatiques de 168 taxons originaires de 32 pays ont été étudiés par électrophorèse, dans le but d'établir l'amplitude de la variation génétique présente chez cette espèce. Huit systèmes enzymatiques codés par 16 loci putatifs ont été examinés. Les résultats montrent que les sous-espèces reonnues n'ont que peu divergé les unes des autres en ce qui a trait aux enzymes étudiées. Les échantillons de taxons sauvages diffèrent plus fortement que ceux des taxons cultivés, mais non significativement en termes de nombres moyens d'allèles par locus, de la proportion de loci polymorphiques, de l'hétérozygocité observée et attendue ou des données statistiques de diversité génétique de Nei ($P < 0,05$). Les membres du groupe agrégé *gingidium* montrent une diversité génétique totale plus faible à l'intérieur d'un taxon ($P < 0,05$) que les membres du groupe agrégé *carota*, de même qu'une diversité génétique plus faible àl'intérieur des populations d'un taxon ($P < 0,01$). Ainsi, la divergence allozymique correspond à la divergence morphologique. Les cultivars ayant des racines pigmentées de carotène montrent des divergences génétiques négligeables. Ils semblent avoir évolué selon un patron simple à la suite d'un goulot d'étranglement génétique initial.

Mots clés : *Daucus carota*, isozyme, électrophorèse, variabilité génétique.

[Traduit par la revue]

Introduction

Daucus carota L. sensu lato is a wild-cultigen species complex of north temperate regions. It contains several weedy, semidomesticated, and domesticated forms. The cultivated carrot *Daucus carota* ssp. *sativus* (Hoffm.) Arcangeli is a cool-season crop grown throughout much of the world. One of the wild taxa, *D. carota* ssp. *carota*, is an anthropochorous, early successional inhabitant, establishing itself in cleared areas and disturbed sites (Vavilov 1951). It is widely distributed and is morphologically diverse (Small 1978). The species' distribution ranges from the Canary Islands and British Isles eastward through Siberia to the Kamchatka peninsula (Nehou 1961; Shishkin 1974) and from central Sweden and Norway south to North Africa and India (Dale 1974). *Daucus carota* was introduced into North America and has since spread throughout most of eastern North America, Mexico, Central America and the West Indies (Mathias and Constance 1944–1945). It is a highly outcrossing, protandrous annual-biennial with an unspecialized, entomophilous breeding system (Thompson 1962; Bell 1971).

The *Daucus carota* L. complex poses one of the most difficult classification problems in the Apiaceae (Sean de Rivas and Heywood 1974; Small 1978) owing to the variability of morphological characters and the occurrence of frequent hybridization within the complex. Various subgroups within the species may have evolved because of environmental pressures, for example environmental conditions along the seashore (Onno 1936; Nehou 1961); they are nevertheless readily

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Printed in Canada / Imprimé au Canada

TABLE 1. Various taxonomic treatments of *Daucus carota* L.

Thellung (1926)	Onno (1936)	Heywood (1968*a*)
D. carota ssp. *Gummiferi* (Drude) Thellung	*D. gingidium* L.	*D. carota* ssp. *gummifer* Hooker fil. ssp. *commutatus* (Paol.) Thell. ssp. *hispanicus* (Gouan) Thell. ssp. *hispidus* (Arcangeli) Heywood ssp. *gadecaei* (Rouy & Camus) Heywood ssp. *drepanensis* (Arcangeli) Heywood ssp. *rupestris* (Guss.) Heywood
D. carota ssp. *Eucarota* (Battand et Trabut) Thellung	*D. carota* L.	*D. carota* ssp. *carota* L. ssp. *maritimus* (Lam.) Batt. ssp. *major* (Vis.) Arcangeli ssp. *maximus* (Desf.) Ball ssp. *sativus* (Hoffm.) Arcangeli

interfertile and probably interbreed on a regular basis (Thellung 1927; McCollum 1977; Owens 1974).

Thellung (1926) observed two phyletic lineages within the *D. carota* complex. The lineages were described as two subspecies groups: ssp. *Eucarota* (Battand et Trabut) Thellung, having thin, dull, highly dissected leaves, and ssp. *Gummiferi* (Drude) Thellung, having thick, shiny foliage. The latter group is primarily indigenous to Mediterranean and Atlantic beaches of Europe. Thellung's subspecies were later elevated to the species level by Onno (1936), naming them *D. carota* L. and *D. gingidium* L., respectively. Heywood (1968*a*, 1968*b*) further separated the two divisions into 11 subspecies largely based on fruit morphology (Heywood and Dakshini 1971; Heywood 1968*c*; Sean de Rivas and Heywood 1974) to explain the wide morphological diversity observed (Table 1). Using additional morphological traits and multivariate analysis, Small (1978) attempted to define the species as groups but found a continuum throughout the complex. He avoided the assignment of strict subspecies designations to his groupings, using instead broad, generalized assemblies consisting of three cultivated and three wild groups. These include ssp. aggregate *gingidium*, ssp. aggregate *carota*, and inland plants of Asia as the wild groups, and "eastern carrot" variety *atrorubens*, "western carrot" variety *sativus*, and eastern × western hybrids (*atrosavitus*) for the cultivated groups. Two groups of cultigens were distinguishable, but a clear morphological gap was not evident (Small 1978).

Early morphological studies (Rubashevskaya 1931; Matzkevitzh 1929) suggest that a comprehensive genetic investigation into the nature of this plant species would aid in the delineation of the complex. Enzyme electrophoresis is a useful technique for describing the amount and distribution of genetic variability within populations, the extent of divergence between them (Gottlieb 1981), and is, therefore, a valuable tool in the study of origin, evolutionary relationships, and distribution of crop species (Kiang and Gorman 1983). The purpose of this study was to undertake an extensive, isozyme-based assessment of the *D. carota* complex and then put it in clearer taxonomic and evolutionary perspective.

Materials and methods

Seed samples, from 168 accessions representing domestic, semi-domestic and nine putative wild subspecies of *Daucus carota* sensu lato were sown and grown to maturity in the greenhouses at the University of Windsor. Identification of all accessions was determined from flowering specimens. Each accession was keyed twice, using both Heywood's (1968*a*) and Small's (1978) taxonomic schemes. Most cultivar and landrace samples were obtained from collections of genetic stocks maintained by the United States Department of Agriculture (USDA) in Ames, Iowa. Other cultivated varieties were obtained from commercial North American seed companies. Wild and weedy accessions were obtained from North American and Eurasian botanical gardens, universities, and research stations. One hundred and twenty-three cultivated and 45 wild accessions, representing 32 countries, were used in the analysis (St. Pierre 1989). Voucher specimens were deposited at DAO and WOCB.

Fresh leaf samples were ground with a mortar and pestle in a cold extracting buffer (Gottlieb 1981) with approximately 20 mg of polyvinylpolypyrrolidone (Sigma P6755). Extracts were decanted into microcentrifuge tubes and centrifuged at 15 000 rpm for 2 min. The supernatant was then 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; Bayer and Crawford 1986). Some results for TPI, PGI, and LAP were reexamined on a cellulose acetate system (Hebert and Payne 1985). The methods of Soltis et al. (1983) were used for staining gels and visualizing the enzymes. The most rapidly migrating locus (most anodal) form of each enzyme was designated 1, and slower migrating loci were progressively labelled with higher values. This labelling system was also followed for allelic variants, the most anodal allele being designated *a* and slower alleles in progressive alphabetical order.

Intact chloroplasts were isolated from leaf tissue, osmotically ruptured, and run next to whole leaf extracts for comparison, using the techniques of Gastony and Darrow (1983). To complement these results, a similar comparison using pollen leachates was examined to determine the cytosolic forms of the isozymes (Weeden and Gottlieb 1980).

Controlled selfings were undertaken by covering umbels from prior to anthesis until seeds were mature (3–4 weeks). Selfed seed were harvested, vernalized for 3 months at 0°C, and sown for later electrophoretic analysis of segregation patterns at particular loci.

The proportion of polymorphic loci (P; using the 0.99 criterion for polymorphism), the mean number of alleles per locus (A; including monomorphic loci), and the observed (H_{obs}) and expected (H_{exp}) mean heterozygosities were calculated by hand. χ^2 tests determined whether

TABLE 2. Genetic variation in 12 subgroups (Heywood 1968*a*) of *Daucus carota* L.

Subgroup	*A*	*P*	H_{obs}	H_{exp}	*N*
atrorubens	1.499	0.434	0.166	0.150	19
atrosativus	1.492	0.434	0.161	0.143	51
carota	1.544	0.471	0.165	0.161	20
commutatus	1.539	0.468	0.104	0.137	3
drepanensis	1.337	0.310	0.148	0.103	2
gadecaei	1.598	0.533	0.190	0.130	1
gingidium	1.485	0.392	0.140	0.135	3
gummifer	1.529	0.548	0.166	0.156	4
major	1.647	0.483	0.139	0.178	2
maritimus	1.597	0.503	0.179	0.178	5
maximus	1.631	0.497	0.178	0.180	5
sativus	1.515	0.474	0.160	0.153	53
Species	1.516 (±0.003)	0.457 (±0.016)	0.161 (±0.009)	0.151 (±0.001)	168

NOTE: *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; *N*, number of populations. Pairwise values of heterozygosity are not significantly different. Values for the species are a weighted average (± SEM) of those for the subgroups.

TABLE 3. Mean genetic identities and (ranges) for comparisons of populations within 11 subgroups (Heywood 1968*a*) of *Daucus carota*

Subgroup	Mean genetic identity (*I*)	*N*
atrorubens	0.902 (0.697–0.987)	19
atrosativus	0.884 (0.628–0.997)	51
carota	0.803 (0.482–0.994)	20
commutatus	0.903 (0.837–0.966)	3
drepanensis	0.778 (0.778–0.778)	2
gingidium	0.868 (0.819–0.929)	3
gummifer	0.809 (0.709–0.891)	4
major	0.881 (0.881–0.881)	2
maritimus	0.758 (0.565–0.906)	5
maximus	0.829 (0.774–0.911)	5
sativus	0.932 (0.777–0.996)	53
Weighted mean	0.884 (0.482–0.997)	168

NOTE: *N*, the number of populations within each group.

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 by the GENESTAT program (Whitkus 1985) and include Nei's measures of intraspecific mean genetic identity (*I*) and distance (*D*), 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}). A distance phenogram was constructed, based on the genetic distance matrix by the unweighted pair group method using arithmetic averages (UPGMA; Sneath and Sokal 1973) with the TAXON subroutine of the NT-SYS program (Rohlf et al. 1974). The accessions were identified, compared on a one-to-one basis, and grouped according to the taxonomic schemes of Heywood (1968*a*), and Small (1978). Genetic distance matrices, as well as distance phenograms, were analyzed with these identifications in mind. Centroid, average, median, single (nearest neighbor), and complete (farthest neighbor) linkage method clustering strategies were computed using SYSTAT (Wilkinson 1988). The accessions were also grouped and compared based on their cultivated status, and distance phenograms were constructed for the groups.

Results

Genetic interpretation of variable banding patterns

Daucus carota is a diploid species ($2n = 18$; Moore 1971; Owens 1974), and genetic interpretation of isozyme banding patterns was based on this premise. A total of 16 presumed enzyme loci coding for 34 alleles were identified including *Adh-1a* to *Adh-1c, Adh-2a, Gdh-1a, Gdh-1b, Idh-1a, Lap-1a, Lap-1b, Lap-2a, Lap-2b, Mdh-4a, Mdh-4b, Mdh-5a, Mdh-5b, Pgi-1a, Pgi-1b, Pgi-2a* to *Pgi-2d, Pgm-1a* to *Pgm-1c, Pgm-2a, Pgm-2b, Pgm-3a, Pgm-3b, Tpi-1a* to *Tpi-1d, Tpi-2a,* and *Tpi-3a*. The genetic basis of the banding patterns was initially inferred by the segregating patterns scored on gels, but was confirmed through the analysis of segregation patterns produced by F_1 progeny of controlled selfings and by the exclusion of subcellular material or the leaching of cytosolic enzymes.

Putative duplications of structural loci were observed in MDH, TPI, and PGM. MDH-1, MDH-2, and MDH-3 were scored throughout the study but could not be included in the analysis because it was impossible to adequately interpret some banding patterns owing to isozyme overlap. *Mdh-4* was the only locus that possessed a null allozyme. Two ADH isozymes were observed in *D. carota*. The more anodal form, ADH-1, is polymorphic, typically observed with a fast and a slow allelic form. One accession, a North American cultivar (Chantenay Long, Dominion Seeds), possessed a third, intermediate allozyme. A single polymorphic GDH isozyme was resolved, displaying one or three bands. The two outer allozymes, which should have resolved from this tetrameric system, were usually not observed, probably owing to their reduced activity within the system used. Three banded patterns were scored as heterozygotes.

Statistical analysis

Daucus carota maintains a high degree of genetic variability (Table 2). Randomly sampled populations were found to be in Hardy–Weinberg equilibrium for the majority of the polymorphic loci investigated. Genetic identity values for within group comparisons (Tables 3 and 4) were highest among cultivated groups and lowest in the wild *maritimus* ($I = 0.758$) and *drepanensis* ($I = 0.778$) subspecies. Expected average heterozygosity is highest in members of the *carota* group, especially sspp. *maximus, maritimus,* and *major,* and lowest in ssp. *drepanensis* (Table 2).

Total genetic diversity (H_t) varies considerably among taxa (Table 5) and loci (Table 6). The highest value was observed

TABLE 4. Mean genetic identities and (ranges) for comparisons of populations within six subgroups (Small 1978) of *Daucus carota*

Subgroup	Mean genetic identity (*I*)
atrorubens	0.902 (0.697–0.987)
atrosativus	0.884 (0.628–0.997)
carota	0.794 (0.482–1.000)
gingidium	0.854 (0.702–0.997)
Inland	0.868 (0.819–0.929)
sativus	0.932 (0.777–0.996)

TABLE 5. Nei's genetic diversity statistics for 12 subgroups (Heywood 1968*a*) of the *D. carota* complex

Taxa	H_t	H_s	D_{st}	G_{st}
atrorubens	0.230	0.150	0.080	0.254
atrosativus	0.242	0.146	0.095	0.257
carota	0.383	0.227	0.155	0.358
commutatus	0.200	0.134	0.068	0.169
drepanensis	0.153	0.097	0.056	0.167
gadecaei	0.087	0.087	—	—
gingidium	0.150	0.117	0.042	0.103
gummifer	0.299	0.155	0.144	0.346
major	0.158	0.137	0.021	0.054
maritimus	0.353	0.210	0.143	0.311
maximus	0.285	0.193	0.092	0.220
sativus	0.218	0.159	0.059	0.213
Species	0.271	0.164	0.107	0.403

NOTE: 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. All values are not significantly different when taxa are compared as wild versus cultivated groups (Wilcoxon's two-sample test), while H_t ($P<0.05$) and H_s ($P<0.01$) are significantly different when the taxa are compared as *carota* versus *gingidium* groups (Wilcoxon's two-sample test).

in the most widely distributed subspecies, and the lowest value was in an endemic subspecies. The species demonstrates an overall H_t of 0.271 (Table 5). Among the loci surveyed, the highest total genetic diversity value was observed in *Lap-2* (H_t = 0.527), closely followed by *Pgi-2* (H_t = 0.507, Table 6).

A greater proportion of the total variability was observed within populations (H_s = 0.164) than between populations (D_{st} = 0.107; Table 5). Therefore, the species as a whole shows great cohesion. Subspecies *major* shows the lowest value for the proportion of gene diversity occurring between populations (D_{st} = 0.021). The proportion of allozyme diversity owing to interpopulation differences (G_{st}) is useful in estimating the relative magnitude of gene differentiation (Table 5). Subspecies *carota*, perhaps owing to its wide spatial distribution and genetic variability, had the greatest relative differentiation, as 36% of the allozyme variation at polymorphic loci resides between accessions. Excluding ssp. *gadecaei*, ssp. *major* demonstrated by far the lowest relative differentiation value (G_{st} = 0.054; Table 5).

A phenogram of genetic distances was constructed (Fig. 1) illustrating the homogeneity of the species as well as the lack of clearly definable subgroups. Advanced carotene type carrots (group A; Small 1978) cluster tightly as noted by the number of A-designated lines at the top of the figure, yet several other accessions assigned to this group are scattered throughout the phenogram. Landraces, denoted by B and C lines, are more widely dispersed among wild taxa. The most genetically diversified groups are members of wild taxa, especially those in

TABLE 6. Nei's gene diversity statistics for 12 polymorphic isozymes scored in 168 populations of *D. carota* sensu lato

Isozyme	H_t	H_s	D_{st}	G_{st}	D_m
ADH-2	0.015	0.007	0.008	0.527	0.008
GDH-1	0.097	0.076	0.021	0.214	0.021
LAP-1	0.459	0.278	0.181	0.394	0.182
LAP-2	0.527	0.311	0.216	0.410	0.217
MDH-4	0.431	0.196	0.235	0.545	0.236
MDH-5	0.078	0.034	0.044	0.562	0.044
PGI-1	0.490	0.373	0.117	0.238	0.118
PGI-2	0.507	0.344	0.163	0.321	0.164
PGM-1	0.243	0.110	0.133	0.547	0.134
PGM-2	0.328	0.222	0.106	0.323	0.107
PGM-3	0.456	0.267	0.189	0.414	0.190
TPI-1	0.406	0.257	0.149	0.367	0.150
Mean	0.271	0.164	0.107	0.403	0.108

NOTE: 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; D_m, absolute degree of gene differentiation.

group D. Wild taxa are found almost exclusively in the lower half of the phenogram. The high degree of genetic relatedness and the difficulty in adequately separating wild and cultivated taxa for classification purposes is apparent in the interdispersal of various groups throughout the figure. Genetic distance matrices (Tables 7 and 8) as well as distance phenograms (Figs. 2, 3, 4, and 5) of the clustered groups showed a relatively close relationship between the designated subspecies. All but two interspecific comparisons had values of D less than 0.10 (Fig. 2). There appears to be no clear demarcation between the two major groups, *carota* and *gingidium*, in Fig. 2, and in fact, ssp. *sativus* (*carota* group) appears to be more similar to ssp. *gummifer* (*gingidium* group) than any other subspecies (D = 0.011). Among cultivated groups, ssp. *sativus* was closely associated with the other two cultivar groups (Figs. 2 and 4), separated by D = 0.016. Another cluster includes sspp. *maritimus*, *commutatus*, and *maximum*, among which maximum D = 0.029. The two major groups were more clearly divided in Fig. 5, in which the cultivated forms were excluded. Because wild taxa did not retain a similar clustering pattern when cultivated taxa were excluded (Fig. 2 vs. Fig. 5), other clustering algorithms were examined to see if any apparent pattern arose. All five methods consistently demonstrated two major clusters: the *atrorubens*, *atrosativus*, *sativus*, and *drepanensis* cluster and the *maximus*, *gummifer*, and *maritimus* cluster. The latter cluster is apparent in Fig. 5. The most divergent taxon by far in all phenograms was ssp. *major*. With its extremely low D_{st} and G_{st} values, this subspecies clearly differs from the rest of the *D. carota* complex.

Variability and genetic distance estimates were more conservative when the taxa were classified into Small's (1978) six general groups (Fig. 3). The eastern and east–west hybrid groups appeared to be more genetically similar to the *gingidium* taxa than to the *sativus* taxa. However, all taxa were within the D = 0.06 range, demonstrating a high degree of homogeneity (Tables 3 and 4).

Discussion

Phenotypic plasticity and genetic variability are the major strategies by which plant species adapt to environmental change. (Schlichting 1986). Phenotypic plasticity allows the formation of locally adapted ecotypes in corresponding micro-

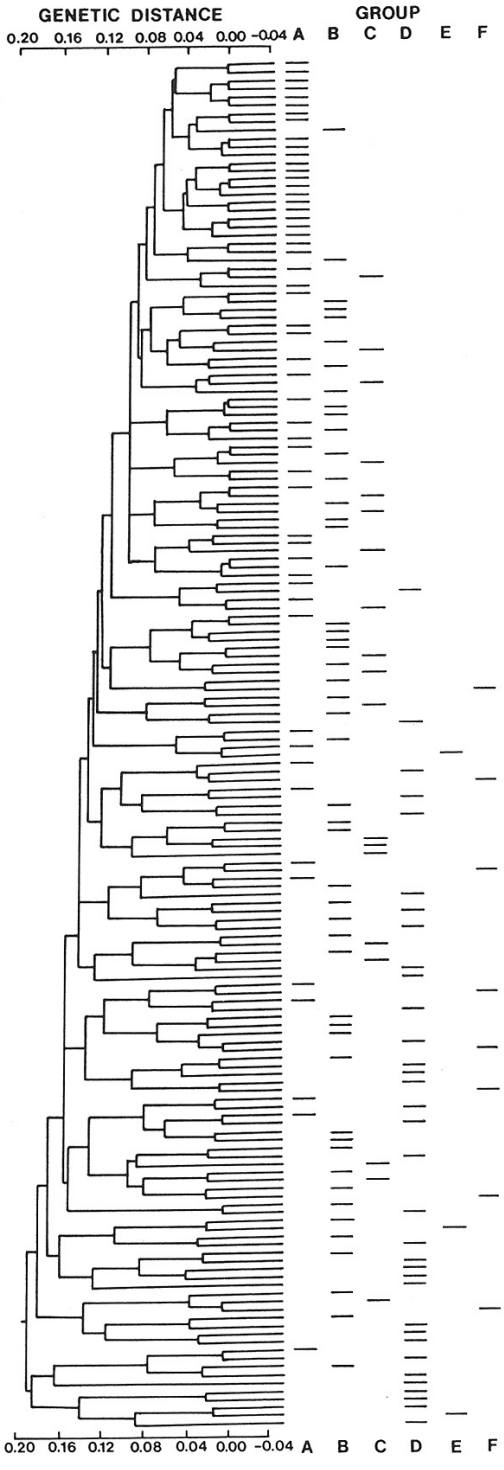

FIG. 1. Distance phenogram (UPGMA) derived from a matrix of genetic distances for 168 accessions of *C. carota* L. Lines to the right indicate membership in Small's (1978) taxonomic groups. A, *sativus*; B, *atrosativus*; C, *atrorubens*; D, *carota*; E, inland; and F, *gingidium*.

habitats and allows the preservation of high degrees of genetic variability within species. *Daucus carota* was a paleotemperate species that has now been widely distributed. Therefore, it is no surprise that with such wide ecological conditions, so many phenotypes have been described (Nehou 1961). In addition to plasticity, breeding system and resulting gene flow influence the genetic structure of populations and species (Brown 1979). Geographically widespread subspecies of plants typically demonstrate moderate rates of gene flow and low interpopulational divergence, while endemic subspecies should be genetically depauperate (Loveless and Hamrick 1984; Hamrick et al. 1979).

The techniques used in developing and maintaining a cultivar can have important influences on the genetic variation found within the taxon (Brown 1979). The differences observed among cultivated accessions may be due to the extreme inbreeding techniques used in selecting and breeding cultivars. Cultivars resulting from highly inbred lines may demonstrate a reorganization of isozyme distributions once bottleneck periods have been overcome (Levin 1976). Those derived from open-pollinated populations, as is *D. carota*, should have more evenly distributed genetic variation than if inbred lines were used. As gene flow occurs easily between populations, intrapopulation variability should contribute a large proportion of total genetic diversity, and interpopulation homogeneity should restrict the contribution from divergence between populations (Table 5).

Domestic orange carrot cultivars, which have undergone both strict selection for a brief period and large scale open pollination to propagate and maintain the lineage, show some restrictions of gene flow, yet still maintain an amount of genetic variability comparable to wild taxa (H_{obs} = 0.160; Table 2). The uniform distribution of genetic diversity is apparent, as semicultivars are intermingled among both cultivars and wild accessions in Fig. 1. The species' relatively low D_{st} value of 0.107 supports this trend (Table 5). The species' strong outcrossing tendencies (approximately 95%; Thompson 1962) and ability to hybridize readily wherever two subspecies coexist reduce the chance of genetic differentiation, thus uniting the species into a more panmictic unit. Outcrossing species show much less geographic differentiation and fewer multilocus associations than predominantly inbreeding species (Brown 1979; Loveless and Hamrick 1984). It has been shown (Owens 1974) that *D. carota* quickly declines in seed viability and vigour after a few generations of selfing. This strong selective force encourages outcrossing and maintains genetic diversity throughout the species (G_{st} = 0.402 and H_t = 0.271; Table 5). The H_t value for *D. carota* sensu lato is comparable to that found in other predominantly outcrossing species (Loveless and Hamrick 1984) and can be attributed to both the wide diversity of taxa studied and the fact that both cultivated and wild forms were compared. The G_{st} values in Table 5 are comparable to those for inbreeders. Loveless and Hamrick (1984) reported an inconsistent decline in G_{st} values with increasing outcrossing rate, demonstrating that other ecological factors affect this gene statistic.

Measures of genetic variability in *D. carota* can be compared with averages of data for outcrossers and inbreeders compiled by Gottlieb (1981). The proportion of polymorphic loci in *D. carota* (Table 2, *P*) is similar to that for other outcrossers (0.370 ± 0.05) and differs significantly (Tukey's test; Sokal and Rohlf 1981) from that for selfers (0.044 ± 0.014). However, the H_{obs} is particularly high in *D. carota* (Table 2)

TABLE 7. Nei's genetic distances (upper triangle) and genetic identities (lower triangle) for all pairwise comparisons of accessions within 12 groups (Heywood 1968*a*) of *D. carota*

	atrorubens	*atrosativus*	*carota*	*commutatus*	*drepanensis*	*gadecaei*	*gingidium*	*gummifer*	*major*	*maritimus*	*maximus*	*sativus*
atrorubens	—	0.008	0.053	0.025	0.019	0.092	0.040	0.039	0.256	0.071	0.037	0.021
atrosativus	0.992	—	0.052	0.031	0.012	0.104	0.049	0.035	0.263	0.066	0.030	0.011
carota	0.947	0.948	—	0.075	0.086	0.118	0.108	0.074	0.170	0.083	0.058	0.075
commutatus	0.975	0.969	0.925	—	0.039	0.108	0.059	0.057	0.254	0.074	0.048	0.044
drepanensis	0.981	0.988	0.914	0.961	—	0.140	0.051	0.053	0.320	0.102	0.054	0.011
gadecaei	0.908	0.896	0.882	0.892	0.860	—	0.153	0.076	0.409	0.088	0.098	0.165
gingidium	0.960	0.951	0.892	0.941	0.949	0.847	—	0.077	0.364	0.113	0.074	0.060
gummifer	0.961	0.965	0.926	0.943	0.947	0.924	0.923	—	0.316	0.026	0.027	0.044
major	0.744	0.737	0.830	0.746	0.680	0.591	0.636	0.684	—	0.313	0.284	0.263
maritimus	0.929	0.934	0.917	0.926	0.898	0.912	0.887	0.974	0.687	—	0.030	0.083
maximus	0.963	0.970	0.942	0.952	0.946	0.902	0.926	0.973	0.716	0.970	—	0.044
sativus	0.979	0.989	0.925	0.956	0.989	0.835	0.940	0.956	0.737	0.917	0.956	—

TABLE 8. Nei's genetic distances (upper triangle) and genetic identities (lower triangle) for all pairwise comparisons of accessions within six groups (Small 1978) of *D. carota* L.

	atrorubens	*atrosativus*	*carota*	Inland	*gingidium*	*sativus*
atrorubens	—	0.008	0.038	0.040	0.016	0.021
atrosativus	0.992	—	0.035	0.049	0.014	0.011
carota	0.962	0.965	—	0.091	0.034	0.053
Inland	0.960	0.951	0.909	—	0.059	0.060
gingidium	0.984	0.986	0.966	0.941	—	0.028
sativus	0.979	0.989	0.947	0.940	0.972	—

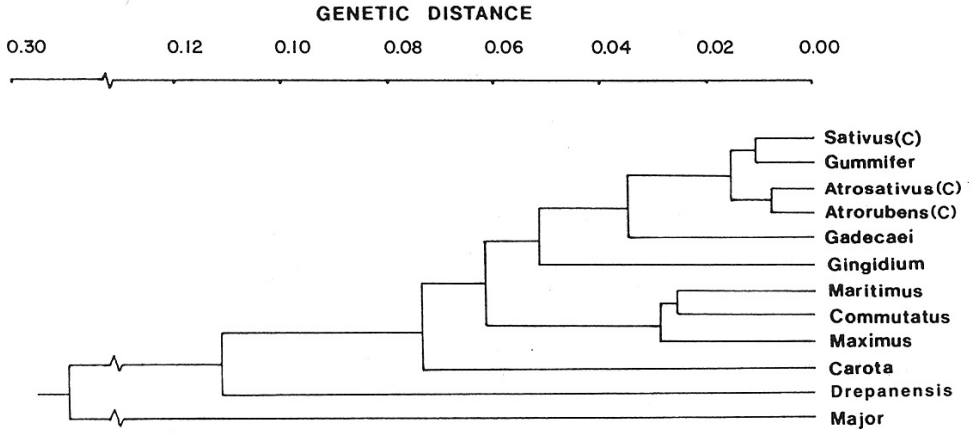

FIG. 2. Distance phenogram (UPGMA) derived from a matrix of genetic distances (Table 7) for 12 groups (Heywood 1968*a*) of *D. carota* L. Groups labelled (C) are cultivated.

compared with that for either selfers (0.001 ± 0.0004) or outcrossers (0.086 ± 0.017). The low levels of *I* in sspp. *maritimus* (0.758) and *drepanensis* (0.778) (Table 3) are similar to mean genetic identity values for different species (Crawford 1983; Loveless and Hamrick 1984), and the species as a whole has a lower genetic identity than the values for either selfers (0.975 ± 0.01) or outcrossers (0.956 ± 0.01). The ecological and life history traits that may contribute to the maintenance of the high observed levels of genetic variability in the species include its high outcrossing rates, its abundance, its high reproductive capacity, and its promiscuous and generalized entomophilous pollinators (Hamrick et al. 1979). Selfing, inbreeding, and associated population substructuring forces appear highly unlikely. Therefore a high degree of recombination and heterozygosity is expected within populations.

Morphological studies have shown the variation to be continuous from wild subspecies in the aggregate group *gingidium* to modern cultivars (Small 1978). The greatest variation in vegetative morphology occurs in wild forms (Small 1978). Cultivated morphotypes show less vegetative variability, yet demonstrate a diversity of root shapes and colours due to selection by humans (Matzkevitzh 1929). The division of landraces and cultivars into several taxa (Zagorodskikh 1939;

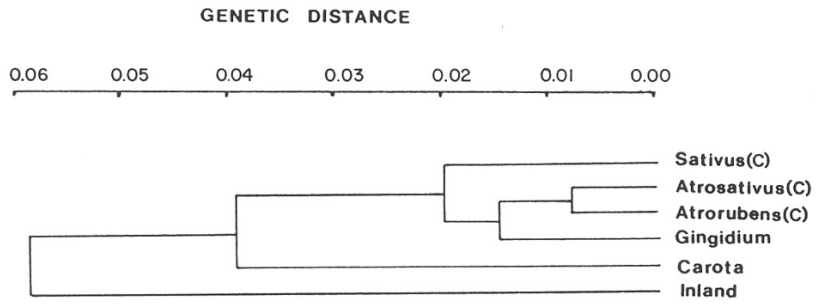

FIG. 3. Distance phenogram (UPGMA) derived from a matrix of genetic distances (Table 8) for six groups (Small 1978) of *D. carota* L. Groups labelled (C) are cultivated.

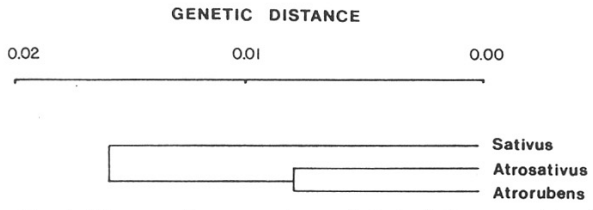

FIG. 4. Distance phenogram (UPGMA) derived from a matrix of genetic distances for 123 cultivated accessions of *D. carota* L.

Rubashevskaya 1931; Alefeld 1866; Matzkevitzh 1929) is based on a few highly variable traits. Variability in semicultivars has been noted in other crop species (Ford-Lloyd and Williams 1975; Kiang and Gorman 1983). Root shape and pigmentation distributions are considered to be artificial agronomic traits and therefore should be used with caution in the systematic evaluation of a species complex. Cultivated taxa are highly uniform genetically, evident in low D and D_{st} values (Fig. 4; Table 5). Because the western group is a recent agronomic development presumably originating from few individuals (Banga 1957), genetic and morphological variability are not correlated. The taxon has only recently been of major agricultural importance, and the various morphotypes have not yet had time to diverge. Therefore, from a genetic perspective, the eastern and western divisions are largely artificial.

The general taxonomic scheme proposed by Thellung (1926) for *D. carota* is a plausible one, based on morphological data, as two major phyletic groups are distinguishable. They are also genetically divisible, as significantly different H_t ($P < 0.05$) and H_s ($P < 0.01$) values divide them (Table 5). However, no clear pattern of division is consistent among phenograms (Figs. 2 and 5). Small (1978) also divided the taxon into two groups by morphological traits; however, appropriate character weighting was necessary. The small genetic distance between groups does not warrant raising them to the rank of species as suggested by Onno (1936). Based on electrophoretic data, division of the species into 11 subspecies (Heywood 1968*a*) also seems unwarranted. The pattern of genetic variation evident in Table 4, as well as the inconsistent morphological diversity within groups, suggest combining many of Heywood's subspecies (1968*a*) into fewer subgroups.

Inconsistent clustering patterns also suggest that the species cannot be clearly divided based on electrophoretic data. Two trends are apparent in all phenograms. The cultivated taxa form a united group and sspp. *maritimus* and *maximus* are consistently clustered. The large genetic distance isolating ssp. *major* is not explicable in view of its morphology, breeding system, or ecological habits. In fact, it has been suggested as one of the original parents in the hybridization of ssp. *sativus* (Krause 1904). The relatively large value of D for ssp. *major* may be partially attributed to the small sample size (two accessions).

The taxonomic divisions of Small (1978), although not intended to formally delineate taxa, do not adequately encompass the genetic variability observed. When clustered into these six groups (Fig. 3), very little genetic differentiation of groups was observed. We attribute this to the larger proportion of genetic variation within as opposed to among groups. Electrophoretic data do very little in further classifying cultigens. These demonstrate a closer homology of the *atrosativus* group

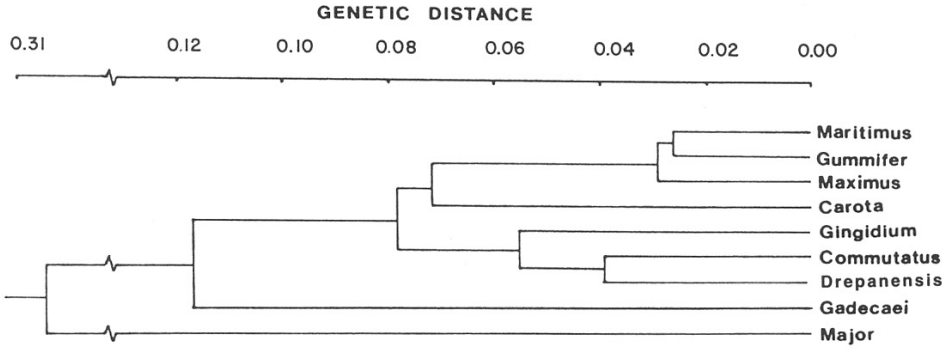

FIG. 5. Distance phenogram (UPGMA) derived from a matrix of genetic distances for 45 wild populations of *D. carota* L.

to the *atrorubens* group than to the *sativus* group (Figs. 2, 3, and 4), and all could be grouped as a single taxon.

Karyotype (Moore 1971; Owens 1974), chemotaxonomic (Williams and Harbourne 1972; Heywood 1971), quantitative DNA analysis (Owens 1974), and morphological studies (Cerceau Larrival 1971; Small 1978) have proven to be of limited use in further classifying the species complex. Similarly, electrophoretic data do not provide strong evidence for separating the taxa into distinct groups. The complex is a young one in terms of evolutionary history, as marginal groups have not yet differentiated into sharply distinguishable subgroups either morphologically or genetically. Results from electrophoretic data tend to unite the morphological and ecological groupings into a more cohesive, unified system. The occurrence of hybridizations between wild and cultivated taxa, as well as within wild taxa, the degree of microevolution among wild forms and among cultivated forms, and the narrow genetic distances observed all suggest that the various taxa cannot be clearly defined into separate subgroups.

Acknowledgments

We wish to thank Dr. Mark Widrlechner (USDA, Ames, IA) for the supply of valuable germ plasm, and Dr. Ernest Small (Agriculture Canada, Ottawa) for useful literature, herbarium specimens, and translated material. Dr. Paul Hebert, Dr. John Jacobs, Dr. Ernest Small, and two anonymous reviewers are gratefully acknowledged for constructive criticism on earlier versions of this manuscript. This research was supported by a Natural Sciences and Engineering Research Council grant to R.J.B. (No. 3797) and a University of Windsor Research Board grant. This study was done in partial fulfillment of the M.Sc. degree for M.D.S.

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Appendix: Mean allelic frequencies of 12 taxa in the *D. carota* complex (Heywood 1968*a*)

	atrorubens	*atrosativus*	*carota*	*commutatus*	*drepanensis*	*gadecaei*	*gingidium*	*gummifer*	*major*	*maritimus*	*maximus*	*sativus*
Adh-1a	0.927	0.968	0.754	1.00	1.00	1.00	1.00	0.905	0.444	1.00	0.966	0.798
Adh-1ab	0.073	0.032	0.246	—	—	—	—	0.095	0.555	—	0.034	0.199
Adh-1c	—	—	—	—	—	—	—	—	—	—	—	0.003
Adh-2a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Gdh-1a	0.042	0.026	0.060	0.031	—	0.143	0.013	0.179	0.130	0.200	0.083	0.049
Gdh-1b	0.958	0.963	0.940	0.969	1.00	0.857	0.987	0.821	0.870	0.800	0.917	0.951
Idh-1a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lap-1a	0.223	0.295	0.153	0.238	0.067	0.391	0.200	0.221	—	0.302	0.142	0.171
Lap-1b	0.777	0.705	0.846	0.762	0.933	0.609	0.800	0.779	—	0.698	0.858	0.829
Lap-2a	0.617	0.427	0.544	0.785	0.392	0.864	0.990	0.413	—	0.454	0.427	0.280
Lap-2b	0.383	0.573	0.456	0.215	0.608	0.136	0.010	0.587	—	0.546	0.573	0.720
Mdh-4a	0.740	0.655	0.562	0.867	0.833	—	0.745	0.415	0.680	0.341	0.528	0.799
Mdh-4b	0.260	0.345	0.438	0.133	0.167	1.00	0.255	0.585	0.320	0.659	0.472	0.194
Mdh-5a	0.027	0.019	0.093	—	—	0.040	0.165	0.359	—	0.278	0.042	0.011
Mdh-5b	0.973	0.981	0.907	1.00	1.00	0.960	0.835	0.641	1.00	0.722	0.958	0.989
Pgi-1a	0.462	0.575	0.502	0.768	0.683	—	0.290	0.450	0.686	0.378	0.331	0.602
Pgi-1b	0.538	0.425	0.498	0.232	0.317	—	0.710	0.550	0.314	0.622	0.669	0.398
Pgi-2a	—	0.006	0.004	0.055	—	—	—	0.210	0.020	—	0.016	—
Pgi-2b	0.648	0.546	0.428	0.746	0.600	—	0.585	0.726	0.670	0.870	0.532	0.734
Pgi-2c	0.038	0.096	0.078	—	—	—	—	—	0.030	0.026	0.295	0.011
Pgi-2d	0.313	0.352	0.491	0.199	0.400	—	0.415	0.064	0.280	0.104	0.157	0.256
Pgm-1a	0.897	0.926	0.457	0.991	1.00	0.900	1.00	0.786	—	0.625	0.787	0.908
Pgm-1b	0.040	0.061	0.360	—	—	0.100	—	0.107	—	0.340	0.213	0.080
Pgm-1c	0.063	0.013	0.184	0.009	—	—	—	0.107	—	0.035	—	0.011
Pgm-2a	0.737	0.770	0.543	0.675	1.00	0.950	1.00	0.855	—	0.649	0.703	0.843
Pgm-2b	0.262	0.230	0.457	0.325	—	0.050	—	0.145	1.00	0.351	0.297	0.157
Pgm-3a	0.398	0.308	0.512	0.553	0.250	1.00	—	0.526	—	0.632	0.541	0.178
Pgm-3b	0.602	0.692	0.487	0.447	0.750	—	1.00	0.474	—	0.368	0.459	0.822
Tpi-1a	—	—	—	—	—	—	—	—	—	0.072	0.058	—
Tpi-1b	0.101	0.221	0.316	0.450	0.167	0.040	0.396	0.381	0.380	0.577	0.512	0.286
Tpi-1c	—	0.001	0.013	—	—	—	—	0.034	—	—	0.060	0.003
Tpi-1d	0.899	0.778	0.672	0.550	0.833	0.960	0.604	0.585	0.620	0.351	0.369	0.710
Tpi-2a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Tpi-3a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00