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AN ELECTROPHORETIC SURVEY OF THE GENETIC VARIABILITY  
WITHIN THE DAUCUS CAROTA L. COMPLEX (APIACEAE: CAUCALIDEAE)

by

© Michel Denis St. Pierre

A Thesis  
submitted to the Faculty of Graduate Studies  
and Research through the Department of Biological Sciences  
in Partial Fulfillment of the requirements  
for the Degree of Master of Science at  
The University of Windsor

Windsor, Ontario, Canada

1989



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## ABSTRACT

Daucus carota sensu lato is a phenotypically variable Eurasian species complex which has been naturalized and cultivated worldwide. 168 accessions from 32 countries were surveyed by enzyme electrophoresis to determine the extent of genetic variation present in the species. Eight enzymes, coded by 16 putative loci were utilized to indicate that the subspecies have diverged only slightly from one another with respect to biochemical genetics. Wild taxa are more genetically diverse than cultivated taxa, but not significantly different with respect to genetic variability or Nei's genetic diversity statistics ( $P > 0.05$ ). Members of the aggregate group gingidium have significantly lower  $H_t$  ( $P < 0.05$ ) and  $H_s$  ( $P < 0.01$ ) values when compared to members of the aggregate group carota, thus demonstrating allozyme divergence is concordant with morphological divergence. Cultivars with carotene pigmented roots show negligible amounts of genetic divergence as a result of their recent development. They appear to have co-evolved relative to one another in a simple pattern after the initial genetic bottleneck. Seedsmen successfully maintain genetic integrity in modern cultivars. Maps of the spatial distribution of gene frequencies support the notion that the regions surrounding Afghanistan and Turkey act as regions of maximum genetic diversity, hence it is probable that early landraces originated in these areas and were later dispersed throughout Europe and the Orient. After a series of hybridizations,

selections and possible mutations, carotene cultivars were developed in western Europe, and these have formed the base of all modern carrot varieties. Wild populations are a good source of genetic variability for the improvement of modern cultivars.

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I. AN ELECTROPHORETIC ASSESSMENT OF THE GENETIC VARIABILITY  
S WITHIN DAUCUS CAROTA L. SENSU LATO.



## INTRODUCTION

The cultivated carrot Daucus carota L., is a cool season crop grown throughout the world. It is a staple food crop in many Asian countries, and is of increasing importance in our health conscious society. Carrots were initially a fodder crop for farm animals. Later, carotene, a vitamin A precursor known to reduce night blindness in aviators, was extracted from carrots prior to its artificial synthesis in 1947. Today, with the advent of higher quality varieties, it is a key ingredient in many soups and dishes or eaten alone as a vegetable.

The cultivated carrot is a member of a morphologically diverse species complex. The species has been described as the most difficult taxonomic problem within the Apiaceae (carrot family) due to the lack of morphologically stable characters and the occurrence of frequent hybridizations within the complex (Sean de Rivas and Heywood, 1974). One of the wild taxa, subspecies 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 morphological diversity is continuous because of a high level of gene flow and a wide geographic distribution. Because of this, anatomic characters are of little value in distinguishing the various subspecies (Sean de Rivas and Heywood, 1974). Early morphological studies (Rubashevskaya, 1931; Matzkevitch, 1929) reflected this sense of frustration and suggested that a comprehensive genetic investigation into the nature of this plant species would aid in the proper delineation of the species.

Although enzyme electrophoretic methods only determine a portion of a plant species genetic variation (Hamrick et al., 1979; Gottlieb, 1981), it is an improvement over other techniques. It is a useful technique for describing the amount and distribution of genetic variability within populations, as well as the extent of divergence between them (Gottlieb, 1981). Enzyme electrophoresis data is fundamentally different from morphology and secondary chemistry (Crawford, 1983). Electrophoretic variants are the result of variation in structural genes coding for polypeptides, therefore electrophoretic variation reflects genetic differences. It is important in phylogenetic inferences to utilize markers based on genetic variability. Morphological and secondary chemical qualities are usually the result of complex multi-locus interactions, and are not always present in all populations studied. Therefore, morphology and secondary chemistry do not always provide clear and consistent genetic markers. Electrophoresis provides an unbiased technique as neither banding patterns of particular populations nor the properties of gene loci scored are known prior to the investigation. All loci are given equal weight, and innate conclusions based on morphological structure are of no power (Crawford, 1983). Isozymes therefore are an extremely valuable tool in the study of origin, evolutionary relationships and distribution of crop species (Kiang and Gorman, 1983).

Crops which have been cultivated since antiquity no longer resemble their wild progenitors. Rather, under the careful selection and manipulation of man they have become increasingly

specialized. Important factors in a species' survival are no longer significant in a controlled, fertilized environment thereby allowing for aesthetic, nutritional and yield improvements. Determining the place of origin, the phylogeny and the sequences of development of these man-made plants is a challenge. Ancestral origin is of vital importance for the accession of diverse germplasm. Quite often, disease and pest resistance, colour morphs, precocity and other physiological and morphological genes which may be of significance in a sound breeding program are available in the more diversified wild ancestral populations. Determining areas of highest genetic diversity therefore may have strong economical implications by maximizing the gene pool available for these species.

Through this study, I undertook an extensive, isozyme based, taxonomic assessment of the D. carota sensu lato complex and put it in clearer phylogenetic perspective. I also assessed the degree of divergence and genetic reorganization in cultivated taxa, and the geographical distribution of genetic diversity thereby developing a factual and sensible proposal of historical gene flow and possible domestication routes.

## DISTRIBUTION

Daucus carota L. sensu lato is a wild-cultigen species of north temperate regions. It is best known to North Americans and Europeans as the bright orange carrot commonly grown in gardens. The most common weedy variant, known as Queen Anne's lace, bird's nest, or devil's plague is cosmopolitan. Its geographic distribution ranges from the Canary and British Isles eastward through Siberia as far east as the Kamchatka peninsula (Nehou, 1961; Shishkin, 1974) and from central Sweden and Norway south to North Africa and India (Dale, 1974). The species was introduced by North American settlers, probably as a seed contaminant and has since spread throughout most of eastern North America, Mexico, Central America and the West Indies (Mathias and Constance, 1944-45; Crompton et al., 1988). It has also been introduced into Australia, New Zealand, Hawaii, Patagonia, Chile and South Africa (Hegi, 1956; Shishkin, 1974; Drude, 1898; Fiori et al., 1900; Helweg, 1908).

It is commonly found in dry woodland or grassland, common near banks, roads, dikes, and wastelands (Krause, 1904). Its habitat ranges from grassy disturbed sites and sparse meadows (Hegi, 1956; Fiori et al., 1900), to rocky calcareous seashores (Nehou, 1961), and to 3000m in elevation in the Himalayas (Hegi, 1956). Figures 1.1 and 1.2 illustrate the natural distribution of the various subspecies of D. carota L.

## TAXONOMY

The Daucus carota L. complex poses one of the most difficult classification problems in the Apiaceae (Thellung, 1926a, 1926b;

Figure 1.1. Natural distribution of *D. carota* L. subspecies within Heywood's (1968) gingidium group.

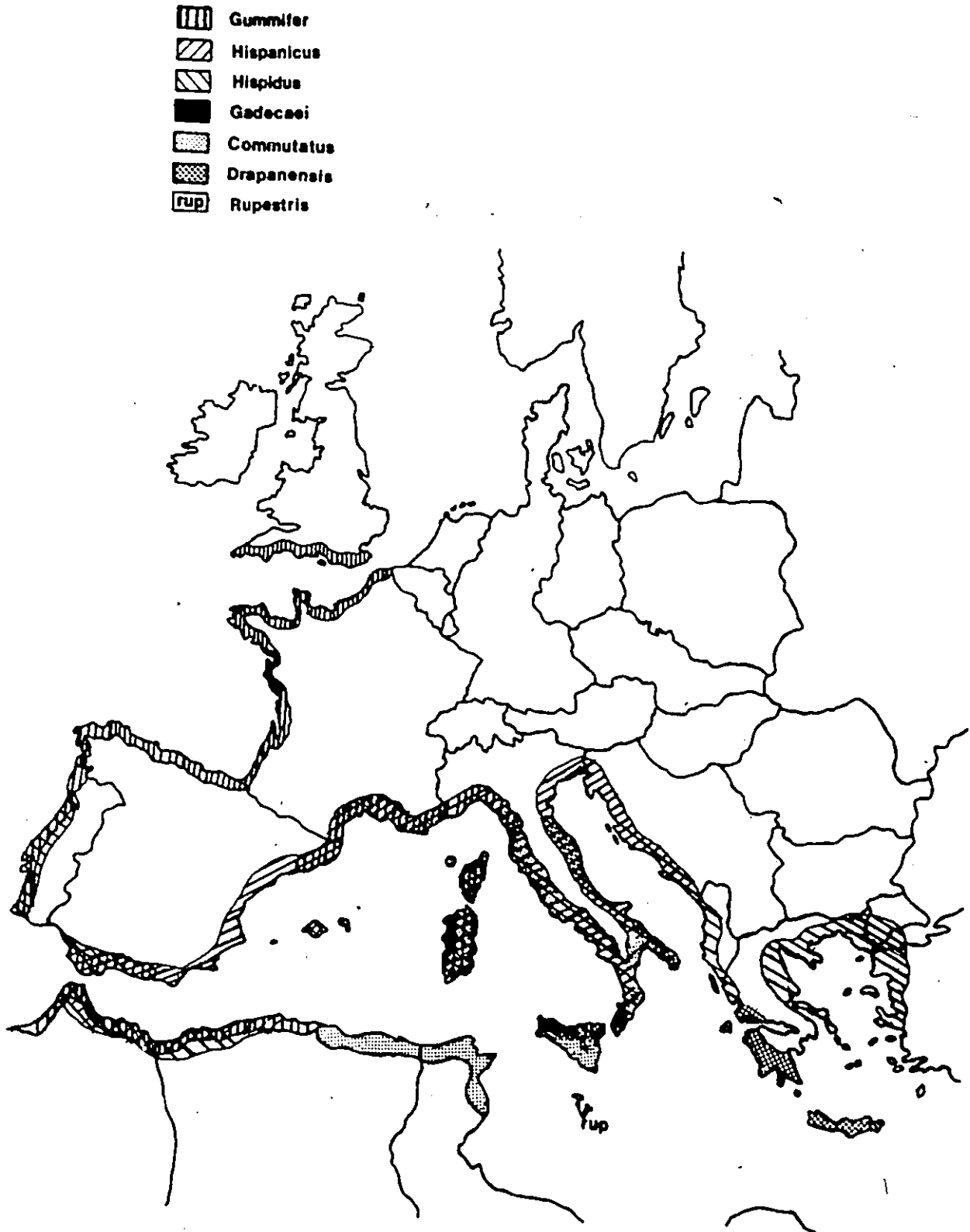
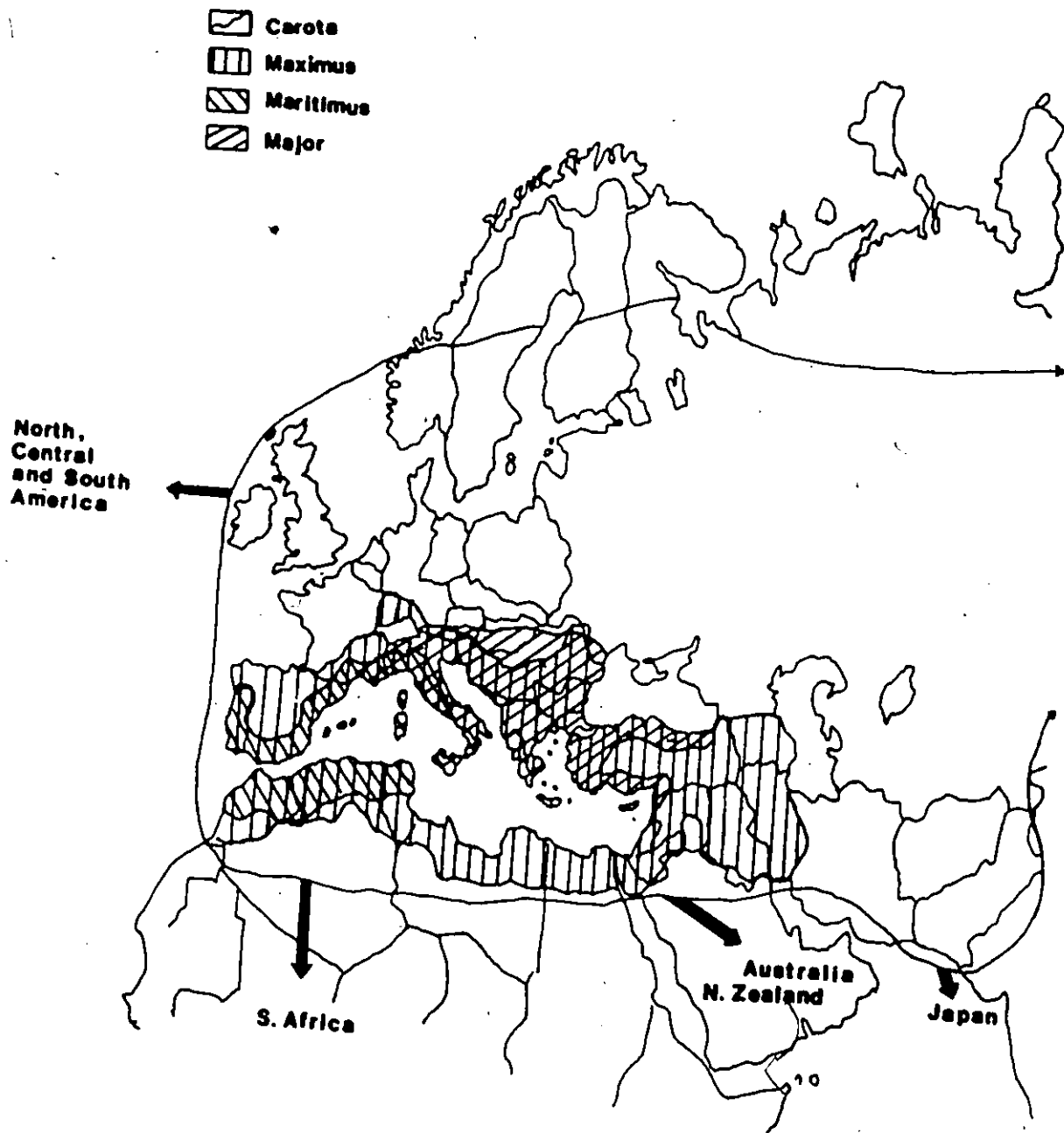


Figure 1.2. Natural distribution of *D. carota* L. subspecies within Heywood's (1968) *carota* group. The entire range of subspecies *carota* is not shown, but extends north of Mongolia and China and east through Siberia, as far as the Kamchatka peninsula (Shishkin, 1974). Arrows indicate areas in which subspecies *carota* has been introduced.



Sean de Rivas and Heywood, 1974; Small, 1978). Taxonomists have generally avoided this species complex, probably because of the limited morphological variability and large environmental plasticity found within the group. It contains several weedy, semi-cultivated and cultivated forms. Nearly 60 putative species of Daucus have been described through time, almost half of these being variations of the polymorphic D. carota (Whitaker et al., 1970).

Daucus carota belongs to the section of the Apiaceae with primary and secondary ridges on the fruit, which Bentham and Hooker (1867) and Boissier (1872) regarded as comprising the tribe Caucalideae. Drude (1898), recognized two tribes within Bentham and Hooker's (1867) Caucalideae. He suggested separating the existing tribe into the tribes Dauceae with spines on the primary and secondary ridges of the fruit, and Laserpiteae whose fruit does not have spines, but possesses prominent primary and secondary ridges. Therefore, there are conflicting classifications even at the tribal and sub-tribal levels, as the line of demarcation in fruit morphology is not clear (McNeil et al., 1969). The tribe Caucalideae contains 18 genera with approximately 75 species (McNeil et al., 1969). The genus Daucus which contains approximately 25 species, is centered in central Europe, North Africa, and south-west Asia. Linnaeus described D. carota in 1753.

Thellung (1926b), observed two phyletic lineages within the species. He divided them into two subspecies groups: ssp. Eucarota (Battand et Trabut)Thellung, having thin, dull, highly dissected leaves, and ssp. Gummiferi (Drude)Thellung, possessing

thick shiny foliage and 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. The most recent taxonomic revision was by Heywood (1968a, 1968b). Characters clearly distinguishing either group were not found and therefore Heywood (1968a, 1968b) further separated the two divisions into 11 subspecies to explain the wide morphological diversity observed. These various subspecies are largely based on fruit morphology (Heywood and Dakshini, 1971; Heywood, 1968c; Sean de Rivas and Heywood, 1974) (figure 1.3). They can be broadly divided into two groups as Thellung and Onno suggested (table 1.1), however, overlapping characters exist. A noteworthy example is the small coastal variant, ssp. gadecaei. It is clearly a member of the gingidium group, yet possesses contracting or birds-nest like umbels, characteristic of the carota group.

Utilizing additional morphological traits and multivariate analysis, Small (1978) attempted to define the species as groups but found a continuum throughout the complex. Two groups of cultigens were distinguishable, however, a clear morphological gap was not evident (Small, 1978). Various subgroups within the species have evolved largely due to environmental constraints, particularly seashore habitat (Onno, 1936; Nehou, 1961) yet are nevertheless readily interfertile and probably interbreed on a regular basis (Thellung, 1927; McCollum, 1977). Gene flow among subgroups would maintain species integrity and prevent genetic segregation. Small (1978) therefore relaxed from assignment of



Figure 1.3

Various taxonomic treatments of Daucus carota L.

Thellung (1926)

Onno (1936)

Heywood (1968)

D. carota -----> D. gingidium----->  
subsp. Gummiferi

---D.carota subsp. gummifer  
commutatus  
hispanicus  
hispidus  
gadecaei  
drepanensis  
rupestris

D. carota-----> D. carota----->  
subsp. Eucarota

---D. carota subsp. carota  
maritimus  
major  
maximus  
sativus

Table 1.1

Characters distinguishing the 2 major intraspecific taxa within the Daucus carota L. complex.

Character	Taxa	
	ssp. agg. <u>carota</u>	ssp. agg. <u>gingidium</u>
foliage	dull, highly dissected	thick, shiny, less dissected
involucral bracts	linear/linear-lanceolate	lanceolate/ovate
stem	straight or flexuous	usually flexuous
fruiting umbel	nest-like, strongly contracting	convex or slightly contracted (except ssp. <u>gadecae</u> )
gum exudation from wounds	no	yes
native distribution	inland plants of Eurasia	Mediterranean and Atlantic coasts

from: Heywood (1968), Small (1978)

strict subspecies designations to his groupings, using instead broad, generalized assemblies consisting of 3 cultivated and 3 wild groups. These include ssp. aggregate gingidum, ssp. aggregate carota and inland plants of Asia as the wild group and "eastern carrot" variety atrorubens, "western carrot" variety sativus, and "eastern" x "western" hybrids for the cultivated group (figure 1.4). Both Heywood's (1968b) and Small's (1978) taxonomic schemes were utilized during the course of this project.

The cultivated carrot has been recognized taxonomically for a long time. It is most easily distinguished by its swollen tap root. Hoffman (1791), first separated it as variety sativus of D. carota L.. Arcangeli (1882, in Small, 1978) later raised it to the subspecies level D. carota subsp. sativus (Hofm.) Arcangeli. Recent taxonomic studies on the cultivated carrot have largely been conducted by Russian investigators. Rubashevskaya (1931), inspired by the work of Vavilov and Bukinich (1929) and Matzkevitch (1929), defined two groups of cultivated carrots based on their geographic distribution, D. carota ssp. orientalis M. Rubashevskaya and D. carota ssp. occidentalis M. Rubashevskaya. D. carota ssp. occidentalis, usually has orange, sometimes yellow or white, unbranched roots, yellowish green, highly dissected, glabrous foliage, green petioles, white petals, absence of a central anthocyan flower, and a biennial habit. D. carota ssp. orientalis usually has yellow or purple (anthocyan) branched roots, grayish-green pubescence, less dissected, darker green leaves, purplish petioles, pinkish flower buds, a purplish central umbel flower,

Figure 1.4

Key to the 6 Groups of Daucus carota L. (Small, 1978).

- 1- Storage organs brittle and usually heavily pigmented, rarely all white.....cultivars A, B, and C
- 2- Storage organs exclusively orange.....group A ("Western" cultivars)  
SATIVUS
- 2- Storage organs yellow, red, and/or purple.....groups B and C
  - 3- Leaf pubescence limited.....group B (East-west intermediates)  
ATROSATIVUS
  - 3- Leaf pubescence abundant.....group C ("Eastern" cultivars)  
ATRORUBENS
- 1- Storage organ pliable, lacking pigment, usually entirely white .....wild groups D, E, and F
  - 4- Basal foliage strongly dissected.....group D (ssp. agg. carota)  
CAROTA
  - 4- Basal foliage weakly dissected.....groups E and F
    - 5- Inland plants of Asia.....group E (wild Asian ssp.)  
INLAND
    - 5- Plants of the Mediterranean and adjacent Atlantic, mostly coastal.....group F (ssp. agg. gingidium)  
GINGIDIUM

and usually bolts in the first year of growth (Small, 1978; Matzkevitzh, 1929; Rubashevskaya, 1931). Additional information on the taxonomy and classification of cultivars are found in papers by Alefeld (1866), Thellung (1926a), Matzkevitzh (1929), Vavilov and Bukinich (1929), Rubashevskaya (1931), Zagorodskikh (1939), and Small (1978).

The need for a classification scheme is obvious to professionals studying particular crop species. In several crops, hundreds of cultivars have been named for few distinct varieties (Babb et al., 1950). It is therefore imperative to produce an understandable classification scheme as well as to provide a list of synonymous varieties.

#### TAXONOMIC TREATMENTS

##### A) MORPHOMETRIC

Numerical taxonomic studies have been quite limited, due to the great phenotypic plasticity found throughout the species. McNeil et al., (1969) utilized a taximetric approach for the classification of the tribe Caucalideae, utilizing both qualitative and quantitative measurements. Factors delimiting the tribe include fruit and inflorescence characters, while foliar characters were of little importance. The study included five D. carota accessions which clustered out well, but did little to further quantify the species.

Spine and ridge formation are widely used as taxonomic traits in the Apiaceae, however, many taxonomists object to the use of these controversially plastic traits. With electron microscopy, researchers have developed microcharacters which may

further clarify the taxa (Heywood, 1968a; Heywood and Dakshini, 1971; Seanz de Rivas and Heywood, 1974). Seanz de Rivas (1973), looked at mature fruit of D. carota ssps. carota, gummifer, and gadecaei and found little difference between subspecies, but large differences among individuals within subspecies.

Pollen exine morphology has been found to be an excellent morphological character for generic distinction (Cerceanu Larrival, 1971), but is of little use at the species level. Wild Daucus exhibiting the most advanced pollen type has a short vegetative cycle, and is distributed in the Northern Hemisphere to the Mediterranean basin (Cerceanu Larrival, 1971).

In a detailed taxonomic analysis of the D. carota sensu lato complex, Small (1978) was able to separate wild and cultivated forms, although a continuum occurred within each group, and to a lesser extent extended between the two groups. It was clear that members of the subspecies aggregate gingidium did not influence the development or evolution of the cultivated carrot, as a result of their unique ecological and morphological traits.

#### PIGMENTATION AS A TAXONOMIC CHARACTERISTIC

Pigmentation has been utilized as a racial diagnostic character solely in cultivated and semi-cultivated forms, as wild forms have very little or no pigmentation. The overall colour of a root may be affected by chlorophyll, anthochlors, anthocyanins and carotenoids of the xanthophyll, carotene and lycopene types (Lyubimenko et al, 1936; Banga and DeBruyn, 1964). From these, four major colourmorphs are distinguished: (1) the purple or anthocyan Asian semi-cultivated carrot, (2) the yellow Asian

semi-cultivated carrot, (3) the European white forage type carrot, and (4) the European orange or carotene carrot.

Multiple pigments can be found within a single root due to the varying rate of pigment synthesis cessation in various tissues (Lyubimenko et al, 1936). Also, pigment synthesis and accumulation may occur in various tissue layers. Violet colour in Asiatic accessions is mainly concentrated in the cortical layers (Vavilov and Bukinich, 1929; Lyubimenko et al, 1936; Banga and DeBruyn, 1964). In the white variant, the yellow pigment is either concentrated in the xylem and the secondary cortex, or is totally absent.

The two major Asiatic colourmorph groups have distinct geographic ranges (Vavilov and Bukinich, 1929). The anthocyan type is more prevalent in the Eastern provinces of Afghanistan, southward to India, while the anthochlor type prevails north of the Hindu Kush mountain range, into the Russian provinces, northward and eastward through Iran and Turkey. The common orange carrot, containing plastid carotene, is not known in this area (Vavilov and Bukinich, 1929), but transitional forms with pinkish, slightly yellowish and white roots do occur.

Biochemical data support the absence of carotene in Asian material (Harbourne, 1975).

Harbourne (1975), concluded that the Apiaceae as a whole demonstrate a relatively uniform anthocyan pattern. Anthocyan may be found in the root, petioles, and inflorescence (notably in the central group of florets). Anthocyanin carrots contain 3 cyanadin glucosides: 3 lathyroside, 3-xylosylglucosylgalactoside

and its ferulyl derivative (Harbourne, 1975). Subspecies maritimus possesses a unique, taxonomically significant anthocyanin pattern through the loss of xylose, and instead produces a disaccharide pigment (Harbourne, 1975).

One form of yellow pigmentation, anthochlors, belongs to the same group of flavones and flavanols as the anthocyanins. Lyubimenko et al. (1936), postulated that it is quite probable that these yellow water soluble pigments are the first stage in the synthesis of anthocyanins since there are always anthochlors in roots containing anthocyanins.

Pigmentation in European or western type carrots is mainly the result of carotenoids (orange) and oxygenated xanthophylls (yellow) (Umiel and Gabelman, 1972; Banga and DeBruyn, 1964). Commercial orange carrots are largely (95%) pigmented with alpha and beta carotenes (Harper and Zscheil, 1944; Banga and DeBruyn, 1964; Umiel and Gabelman, 1972). Orange carrots have a higher beta:alpha carotene ratio, while yellow carrots have the opposite ratio (Ben Schaul and Klein, 1965). Other reported carotenes in carrots include phytoene, phytofluene, lycopene, gamma, zeta, and delta carotene (Banga and DeBruyn, 1964; Umiel and Gabelman, 1972). Red-rooted Japanese varieties are almost exclusively pigmented with lycopene, the same pigment found in tomatoes (Umiel and Gabelman, 1972), while white and yellow rooted types are 75 to 90 percent pigmented with xanthophylls (Umiel and Gabelman, 1972). Anthochlors and carotenoids are therefore of primary importance in pigmentation.

Inheritance of the purple petiole in carrots was found to be controlled by a single dominant gene (Angell and Gabelman, 1970).



In root pigmentation, at least three major genes determine the dominance of white over orange, and at least two genes determine the dominance of yellow over orange (Laferriere and Gabelman, 1968). Independent inheritance was found for pigmentation of the root phloem and xylem (Laferriere and Gabelman, 1968). Imam and Gabelman (1968) further demonstrated the single gene dominance of lemon over light orange, and light orange over orange. Umiel and Gabelman (1972) postulated the existence of a dominant red and a dominant orange allele with the locus coding for the orange allele epistatic to the locus coding for the red allele, even when the orange allele is homozygous recessive. In other words, red carrots are the result of an orange pigment locus deletion. These authors stress the genetic complexity of root colour phenotypes. Several factors contribute to the production of a specific root colour and the nature and number of genes responsible is still unknown (Umiel and Gabelman, 1972). Lyubimenko et al., (1936) therefore conclude that the final colouration of a particular root depends on the stage at which the successive conversion of carotenoids ceases. They also concluded that the complexity of the carotenoid system appears to be closely linked with the geographic origin of the accession. This would suggest an important environmental impact on phenotypic expression.

Utilizing pigmentation of carrot roots as a racial diagnostic tool, the most similar group to wild types are Asiatic varieties with primary carotenoids combined with anthochlor and anthocyanin. Next most similar are the Asiatic varieties

containing xanthophylloids and anthocyanins, then Asiatic varieties with beta xanthophyll and more complex carotenoids such as lycopeneoids, and finally European and American cultivars containing beta xanthophyll and carotenes (Lyubimenko et al., 1936). Wild Asiatic carrots differ in the nature of their primary carotenoids from European wild types and therefore it is assumed that these groups represent two different phylogenetic branches (Lyubimenko et al., 1936).

#### B) KARYOTYPE

Cytotaxonomy of the Apiaceae is still in a juvenile stage, therefore there are limitations in chromosomal data currently available (Moore, 1971). The species is unquestionably diploid, containing nine pairs of chromosomes i.e.,  $2n = 2x = 18$  (Whitaker, 1949; Hiroe, 1962; Zenkteler, 1962; Moore, 1971, 1973; Turkov et al., 1974; Owens, 1974; Banga, 1976; Dudits et al., 1977).

Karyotypes of individual subspecies have also been reported  $2n = 18$  for ssps. carota, drapanensis, gummifer, sativus, and gadecaei (Moore, 1973; Owens, 1974). The size of the chromosomes range from 1.8 to 2.5 microns (Turkov et al., 1974; Owens, 1974). The total length of the karyotype is 39.0 microns (Turkov et al., 1974). Karyotype morphology of various Daucus species is very different (Owens, 1974), and therefore may explain the lack of genetic exchange between species (Moore, 1971; Owens, 1974). The chromosomes of all Daucus species are small and therefore detailed karyotype analysis is liable to error (Owens, 1974). Karyotype data are inconclusive at the subspecies level.

### C) CHEMOTAXONOMY

Essential oils were of limited usefulness, capable only of separating tribes into genera (Williams and Harbourne, 1972). It was thought that flavanoids (Crowden et al, 1969; Harbourne, 1971; Harbourne and Williams, 1972), essential oils (Williams and Harbourne, 1972), and general proteins (Crowden et al, 1969), would be of systematic value, but their results were not conclusive enough to form a rational classification scheme (Heywood, 1971). Simon (1982a, 1982b) and Senalik and Simon (1987), have shown that a 5 to 10 fold variation in the amount of terpenes and total volatile terpenoids may exist within one cultivar alone.

### D) ELECTROPHORESIS

A detailed electrophoretic survey of the D. carota complex has not been previously undertaken. Due to the ease of in vitro culture, and the prolific production of somatic cells (Matthews and Widholm, 1985), D. carota L. is a convenient species to maintain under laboratory conditions and thus has been utilized in a few electrophoretic surveys of plant structural proteins and enzymes (Matthews and Widholm, 1985; Schiavo et al, 1980; Matthews et al, 1984). Enzyme systems observed include glucose phosphate isomerase (GPI), alcohol dehydrogenase (ADH), malate dehydrogenase (MDH-NAD), and lactate dehydrogenase (LDH), (Schiavo et al, 1980) on cellogel sheets; peroxidase (PER), esterase (EST), and general protein (GP) by acrylamide gel electrophoresis (Crowden et al, 1969); homoserine dehydrogenase (HSDS) and ADH on 5% polyacrylamide gels (Matthews et al, 1984);

aspartokinase, HSDH, dihydrodipicolinic acid synthase on 0.7% horizontal agarose gels (Matthews and Widholm, 1985); PER on 7% (w/v) polyacrylamide gels (Dudits et al, 1977); glutamate dehydrogenase (GDH), MDH, EST, PER, aspartate aminotransferase (AAT), gamma glutamyl transferase (gamma GT) and acid phosphatase (ACP) (Lee and Dougall, 1973) on an acrylamide system and two major groups of EST utilizing a polyacrylamide system (Chibbar et al, 1988). Multiple bands were resolved by Crowden et al, (1969) on the general substrate enzymes studied, and slight variation occurred between ssp. sativus, carota, and godecaei.

The work of Schiavo et al, (1980) is of little taxonomic interest as a single somatic cell line was utilized. A zymogram comparison of wild carrot lines in the presence and absence of 2,4-dichlorophenoxyacetic acid (2,4-D) was undertaken by Lee and Dougall (1973). Variation in the banding patterns of MDH, ACP, AAT, and gamma GT were noted. The changes are probably due to metabolic and developmental changes in the affected lines. Matthews et al, (1984) were able to separate ssp. sativus cv. "Danvers" from ssp. gummifer, D. capillifolius and D. pusillus through the activity of HSDH. Matthews and Widholm (1985) utilized HSDH to identify intraspecific hybrids of D. carota x capillifolius. The hybrid possesses a unique band not found in either parent- a hybrid band. Dudits et al, (1977) were able to separate D. carota ssp sativus from D. capillifolius and identify their hybrids via a cathodal peroxidase band. These are largely pilot projects, utilizing electrophoretic variants to demonstrate the genetic variation found between somatic cell lines, or

hybrids; As has been noted, sufficient variation exists to utilize this technique as a valuable taxonomic tool in the systematic appraisal of the species complex.

#### E) DNA CONTENT AND RESTRICTION FRAGMENT PATTERNS

Mean DNA values of Owens (1974), vary from 2.01 to 3.35 picograms within subspecies of D. carota, a considerable variation. The variation is even greater between species which indicates that the species are genetically isolated and may explain the limited amount of gene exchange observed.

Endonuclease restriction fragment length patterns from plastid DNA digests were compared by Matthews et al., (1984). No differences were observed between ssp. sativus cv. "Danvers" and D. capillifolius when examined using 8 different restriction enzymes. Small differences were observed between ssp. sativus cv. "Danvers" and ssp. gummifer and D. capillifolius, while large differences were observed between the cultivar and D. pusillus plastid DNA patterns. Further examination of plastid and mitochondrial DNA by DeBonte et al., (1984) also confirm the closer homology of ssp. sativus to D. capillifolius than to ssp. gummifer or D. pusillus. The degree of divergence was found to be more pronounced in the mitochondrial genome (DeBonte et al., 1984; Matthews and Widholm, 1985). The restriction patterns of the mitochondrial DNA (mtDNA) were different for each cell line (Matthews and Widholm, 1985). Ichikawa et al., (1989) report a closer homology between 13 cultivars and ssp. carota than that between ssp. carota and ssp. gummifer, and D. capillifolius when observing restriction fragment patterns of mtDNA.

## BREEDING SYSTEM

The floral uniformity found throughout the Apiaceae may represent an ancient adaptive peak (Bell, 1971). The inflorescence structure is virtually unchanged across several genera (Bell, 1971; Owens, 1974). Daucus carota flowers are borne in a large, flat umbels bearing over 100 small, white flowers (Owens, 1974; Paci, 1956). The central floret may or may not be deep purple in colour. Floral symmetry is slightly zygomorphic in wild populations and actinomorphic in cultivars. Gynodioecy is well documented within umbels of a plant (Brandenburg, 1981; Owens, 1974). The ratio of hermaphroditic to male sterile flowers varies among ecotypes (Rubashevskaya, 1931).

Umbels bloom for several days, in an indeterminate pattern of development. Blooming of a plant can continue for months, moving from the primary umbel, downwards. Two or three petals may expand, allowing a few stamens to exert. The dehiscence of the anthers begins as soon as the flower begins to open, therefore, when the flower is completely opened, the anthers are almost emptied (Paci, 1956), i.e., it is protandrous in nature. Protandry is common in several plant families as a mechanism to encourage cross pollination eg., Beta vulgaris (Chenopodiaceae), Allium cepa (Liliaceae), Zea mays (Poaceae), Rubus idaeus (Rosaceae) and Trifolium pratense (Fabaceae) (Owens, 1974). It occurs in both wild and domesticated D. carota subspecies (Owens, 1974) and encourages genetic exchange, maintaining variability within the species.

Wild variants are annual under favourable conditions, while

domestic cultivars are biennial. A physiological change in photoperiod sensitivity has been bred in cultivated forms through methodological screening of unwanted early bolters (Banga, 1976). F1 hybrids between wild and domestic forms are mostly annuals (Brandenburg, 1981).

Bell (1971) summarized the breeding system of D. carota: "Thus the term 'breeding system' does not quite seem to fit when applied to the Umbelliferae which have 'unspecialized' flowers that are pollinated by unspecialized pollinators". Most members of the tribe Caucalideae are facultative outbreeders and are protandrous (Owens, 1974; Thompson, 1962). Daucus carota is an outcrosser, though not self incompatible (Bell, 1971; Owens, 1974) and natural cross pollination has been estimated at 95-99% (Thompson, 1962) when utilizing root colour of seedlings as a marker. Factors promoting outcrossing include: protandry, attractive inflorescence (especially zygomorphic flowers), the spatial separation of gynoecious and androecious parts in the hermaphroditic flower, long filaments, large number of flowers per inflorescence and the occurrence of staminate flowers (Owens, 1974). Daucus carota has long enough filaments to promote geitonogamy. This mechanism ensures pollination after a brief time in which cross pollination could have occurred (Bell, 1971).

Outcrossing success may vary due to environmental and spatial factors as well as the availability of pollinators as D. carota is entomophilous (Bell, 1971). Two to three hundred types of pollinators, representing 5 major orders, but largely Dipterans, have been observed (Bell, 1971; Judd, 1969).

Promiscuous pollination by several species of unspecialized insects lowers isolation barriers and promotes panmixis.

Seeds produced through self fertilization are smaller and less viable than those resulting from cross pollination (Paci, 1956). Selfed plants produce 1-10% of a normal seed set (Paci, 1956; Owens, 1974). Self fertilization is usually not realized in nature. Plants produced through selfing exhibit inbreeding depression and are believed to be eliminated through competition in natural environments (Owens, 1974).

#### HYBRIDIZATION

Hybridization is almost unknown in the family. This may appear striking as ethological barriers are unlikely due to the species' promiscuous pollinating mechanism. There are no references to mechanical, seasonal or temporal isolating barriers, however genetic isolating barriers have been documented (Owens, 1974).

Owens (1974) attempted to hybridize seven species in the genus Daucus: D. aureus Desf., blanchei Reut., carota L., crinitus Desf., montanus Humb. & Bonpl. ex Sprengel, muricatus L., and syrticus Murb.. In all instances, crosses failed to set seed due to unilateral incompatibility. Only D. carota accepted foreign pollen tubes, yet no seeds resulted, probably due to the relatively large genetic differences separating the species (Owens, 1974). These differences are indicated by the variation in chromosome numbers and amount of DNA. Successful interspecific hybridization has only been documented once; D. carota x capillifolius hybrids were successfully produced under



artificial conditions (McCollum, 1975). Lack of hybridization in the field may be due to geographic and ecological isolating barriers. There appear to be no barriers to inter subspecific crosses in D. carota (Owens, 1974; McCollum, 1977; Banga, 1976). Hybrids between ssps. carota, gadenaie, gummifer, gingidium and sativus have all been successful (Owens, 1974; McCollum, 1977). Hybrids are intermediate in all morphological traits (McCollum, 1977). Inter-subspecific crosses in D. carota are successful and also occur in natural populations (Nehou, 1961) where both subspecies co-exist. Reproductive isolation is accomplished through geographic and ecological isolating barriers (Nehou, 1961; Owens, 1974).

The importance of successful inter-subspecific crosses is that it may significantly increase the germplasm available for carrot breeding. The D. carota-gingidium complex, with all its morphological and biochemical ecotypes and geographical adaptations, encompasses a vast source of germplasm. Traits peculiar to members of the gingidium group and D. capillifolius are therefore available to carrot breeders. Genes for resistance to disease, insects, nematodes, and environmental stresses found in wild populations may be incorporated into cultivated carrots. Very few disease resistant cultivars exist, although some such as "Spartan Delight" and "Spartan Fancy" demonstrate some tolerance for rusty root (Crete, 1980).

## MATERIALS AND METHODS

Seed samples, from 168 accessions representing domestic, landrace and 9 putative wild subspecies of Daucus carota L. sensu lato were sown and allowed to grow to maturity in the greenhouses at the University of Windsor. Most cultivar and landrace samples were obtained from collections of genetic stocks maintained by the United States Department of Agriculture (USDA) in Ames, Iowa and Fort Collins, Colorado. 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. 123 cultivated and 45 wild accessions, representing 32 countries were utilized in the analysis (appendix A and figures 1.5 and 1.6). Voucher specimens were deposited in the Agriculture Canada (Ottawa) and University of Windsor herbariums.

Fresh leaf samples were ground utilizing a mortar and pestle in cold extracting buffer consisting of 0.1 M tris-HCl, pH 7.5, 4mM 2-mercaptoethanol, 1mM EDTA (tetrasodium salt), 10mM KCl and 10mM MgCl<sub>2</sub> (Gottlieb, 1981). Approximately 20 mg of polyvinylpolypyrrolidone (Sigma P6755) was added to the crude extract at the time of grinding. Leaf bracts, stems, roots, seeds and entire seedlings were employed successfully. However, seedlings less than 10 days old produced less satisfactory results. The extracts were decanted into microcentrifuge tubes and centrifuged at 15,000 r.p.m. for 2 minutes. The supernatant was then absorbed onto double thickness filter paper wicks and immediately subjected to horizontal starch gel electrophoresis for approximately 4 hours. The enzyme systems were resolved

Figure 1.5. Origin of Eurasian domestic and landrace accessions.

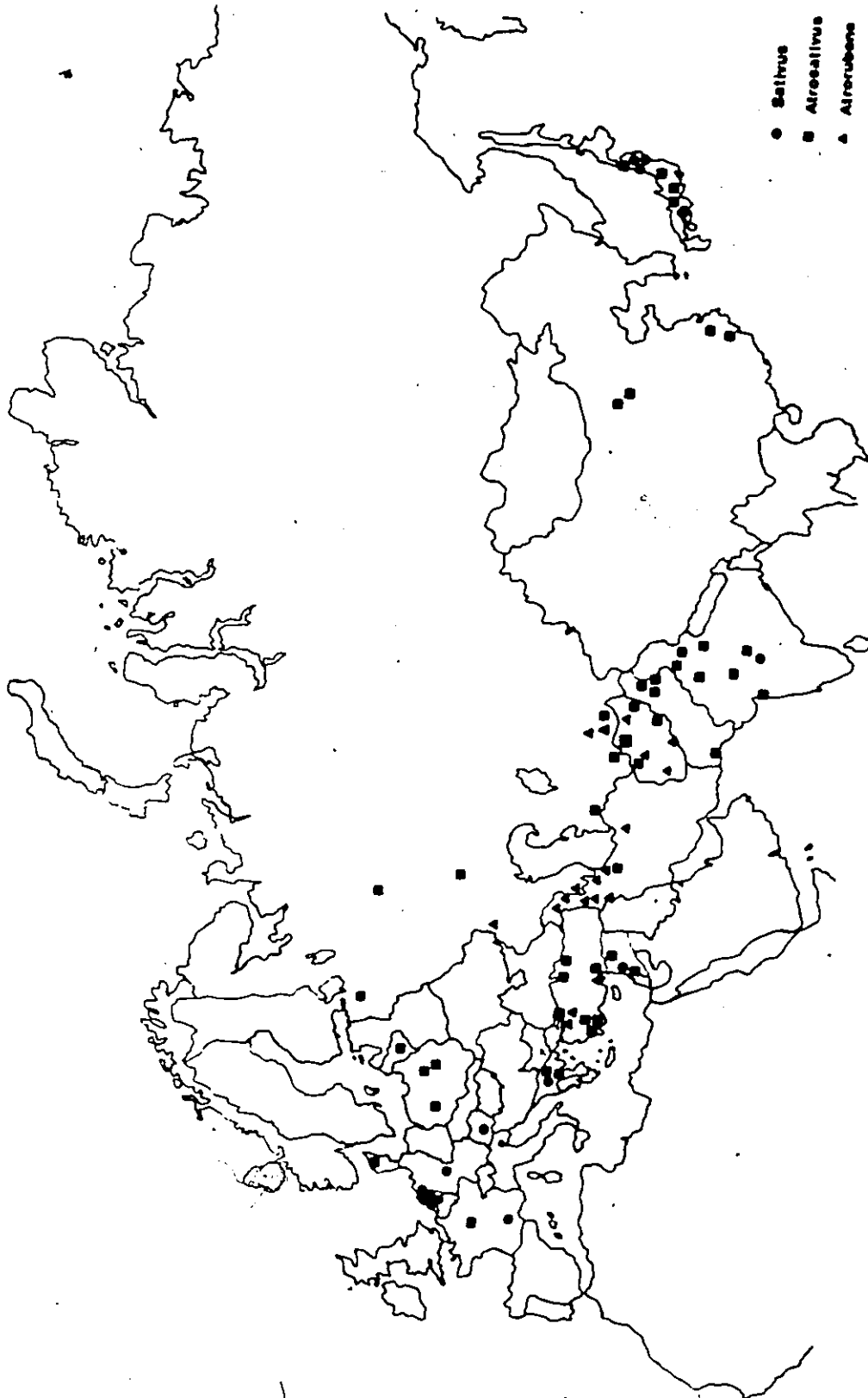
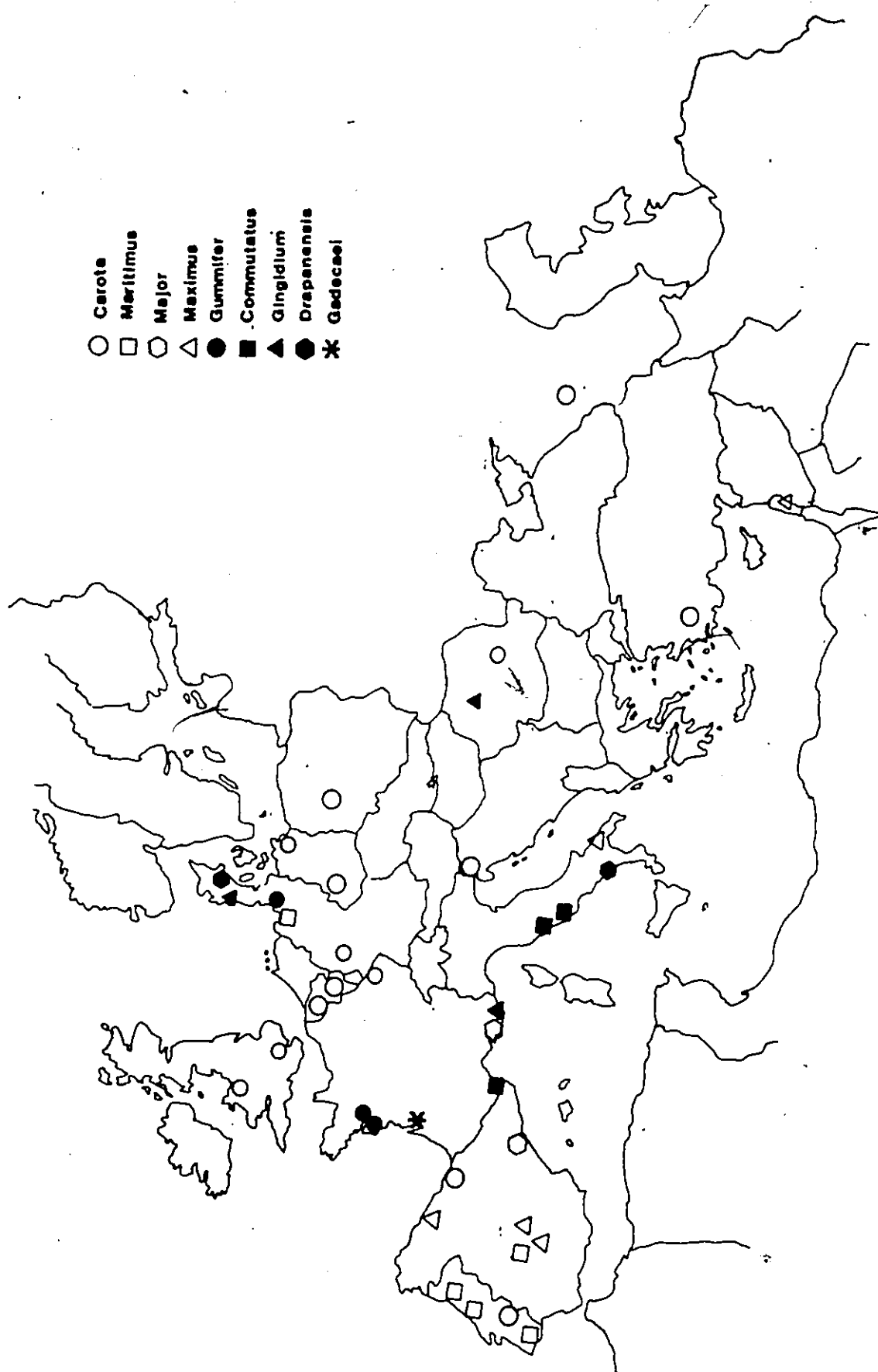


Figure 1:6. Origin of Eurasian wild accessions.



utilizing 12.5% starch gels and two buffer systems. The electrical conditions consisted of 100 mA (constant current) and 200 V for the lithium borate system and 35 mA and 200 V (constant voltage) for the L-histidine system. Phosphoglucose mutase (PGI), triose phosphate isomerase (TPI), leucine amino peptidase (LAP), alcohol dehydrogenase (ADH) and glutamate dehydrogenase (GDH) were resolved using a gel buffer of nine parts tris-citrate (0.05 M tris, 0.007 M citric acid  $H_2O$ , pH 8.3) and one part lithium borate (0.038 M lithium hydroxide, 0.188 M boric acid, pH 8.3). The lithium borate solution was also employed as electrode buffer (Bayer and Crawford, 1986). Malate dehydrogenase ((NAD)MDH), phosphoglucomutase (PGM), isocitrate dehydrogenase (IDH) and 6-phosphogluconate dehydrogenase (6-PGD) were resolved using a gel buffer of 0.016 M L-histidine (free base) and 0.002 M citric acid  $H_2O$ , pH 6.5, and an electrode buffer of 0.065 M L-histidine (free base), 0.007 M citric acid  $H_2O$ , pH 6.5 (Cardy, Stuber and Goodman, 1981; Bayer and Crawford, 1986). The methods of Soltis et al., (1983), were employed for the staining of gels and visualization of 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 nomenclature was also followed for allelic variants, the most anodal allele being designated as A and slower alleles in progressive alphabetical order.

Results for TPI, PGI, 6-PGD and LAP were re-examined on a cellulose acetate system (Hebert and Payne, 1985) using a buffer of 0.024 M tris and 0.19 M glycine, pH 8.4. Again the methods of Soltis et al., (1983) were used in staining, however, with

aliquots of one half volume due to the smaller surface area to stain.

Additional enzyme systems were resolved with infrequent and/or unreliable results. These include superoxide dismutase (SOD), malic enzyme (ME), acid phosphatase (ACP) and shikimate dehydrogenase (SKDH).

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

Controlled crosses were performed to interpret enzyme systems. However, due to the miniscule size and frailty of unopened florets, and the unavailability of male sterile lines, these crosses were not successful. Instead, 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 degrees celsius, and sown for electrophoretic observation of the segregation of particular banding patterns.

Some genetic variation data were calculated by hand. These include:

a) proportion of loci polymorphic in the species, using the 0.99 criterion for polymorphism.

b) the mean number of alleles at the average polymorphic locus.

The maximum number of alleles at such a locus in a diploid plant

is two.

c) the mean number of alleles per locus (including monomorphic loci).

d) proportion of loci polymorphic per population. This value will be equal to a) if a locus is polymorphic in every population studied within the species.

e) observed mean heterozygosity. This is the proportion of all loci in the average individual which is heterozygous. It is defined as :

$$h = 1 - \sum x_i^2$$

where  $x_i$  is the frequency of the  $i^{\text{th}}$  allele. The average heterozygosity, HET is the mean of  $h$  over all loci (Gottlieb, 1981). HET is equal to Nei's  $D_{x(m)}$  which he calls average heterozygosity or gene diversity (Nei, 1972). Allelic frequencies were tabulated and are summarized in table 1.2.

Nei's genetic identity and genetic distance values (Nei, 1972, 1973) were calculated from the allelic frequencies utilizing the GENESTAT program (Whitkus, 1985). The genetic identity statistic is commonly used to measure the similarity of all pairs of populations within a taxon.

1) Genetic identity of the total population,  $J_t$ . For a single locus, with  $k$  alleles and  $s$  subpopulations,

$$J_t = \sum_k x_k^2$$

where  $x_k = \sum_i x_{ik} / s$  where  $x_{ik}$  is the frequency of the  $k^{\text{th}}$  allele in the  $i^{\text{th}}$  subpopulation.

2) Genetic identity of a subpopulation,  $J_s$ . The average gene

Table 1.2. Mean allelic frequencies of 12 taxa in the D. carota complex. are labelled with the first four letters of their subspecific epithets.

[illegible]



identity within a subpopulation is

$$J_s = \sum_k x_{ik}^2$$

For studying gene diversity, a large number of loci should be examined. For a randomly breeding population in Hardy Weinburg equilibrium, it is better to study several loci in a few individuals than to study a few loci in many individuals (Nei, 1978).

3) Gene diversity in the total population,  $H_t$ :

$$H_t = 1 - J_t$$

An approximation is  $H_t = -\ln J_t$  (Nei, 1975).

4) Gene diversity in a subpopulation,  $H_s$ :

$$H_s = 1 - J_s$$

5) Average gene diversity between subpopulations,  $D_{st}$ . If  $D_{ij}$  is the gene diversity between the  $i^{th}$  and  $j^{th}$  population,  $D_{st}$  can be derived from:

$$D_{st} = (\sum D_{ij}) / s^2$$

$D_{st}$  can also be calculated by:

$$D_{st} = (1 - 1/s)(J_0 - J_1).$$

6) The relative magnitude of gene differentiation among subpopulations  $G_{st}$ , which is also called the index of gene diversity:

$$G_{st} = D_{st} / H_t$$

It can also be calculated as  $G_{st} = (H_t - H_s) / H_t$ .  $G_{st}$  is similar to Wright's  $F_{st}$  value, but it allows for more than two alleles at a locus.

7) The absolute degree of gene differentiation,  $D_m$ :

$$D_m = sD_{st} / (s-1)$$

It is an estimate of the minimum genetic diversity between subpopulations and is independent of the gene diversity within populations. It can be used to compare the degree of gene differentiation in different organisms. It is especially useful when  $s$  is small since  $G_{st}$  is only an estimate of the relative degree of gene differentiation among subpopulations.  $D_m$  is a more precise and direct approach to estimate the interpopulation gene diversity. As  $s$  increases,  $D_m$  approaches the value of  $D_{st}$ . Allelic frequencies determined through electrophoresis may not accurately describe that of the population, especially if the sample size is small. The larger the sample, the better the estimate. Sample size required to adequately represent a population in outcrossing species is generally smaller than inbreeders, as any one population is highly representative of the whole species (Crawford, 1983).

The GENESTAT program also calculated genetic identity and distance matrices based on the calculations of Nei (1972, 1973). A distance phenogram was constructed (appendix B), based on the genetic distance matrix by the unweighed pair-group method, using arithmetic averages (UPGMA; Sneath and Sokal, 1973) from the TAXON subroutine of the NT-SYS program (Rohlf et al., 1974). The accessions were compared on a one to one basis and also

grouped according to the taxonomic schemes of Heywood (1968), and Small (1978). Genetic distance matrices were constructed for these (tables 1.3 and 1.4 respectively), as well as distance phenograms (figures 1.7 and 1.8 respectively), utilizing the same sources for computations. Other clustering strategies were computed with SY-STAT (version 4).

Table 1.3

Nei's genetic distances (upper triangle) and genetic identities (lower triangle) for all pairwise comparisons of accessions within 12 groups (Heywood, 1968) of *D. carota* L. Taxa are labelled with the first four letters of their specific epithets.

	attr	atrs	caro	comm	drap	gade	ging	gumm	majo	mari	maxi	sati
attr	****	.008	.053	.025	.019	.092	.040	.039	.256	.071	.037	.021
atrs	.992	****	.052	.031	.012	.104	.049	.035	.263	.066	.030	.011
caro	.947	.948	****	.075	.086	.118	.108	.074	.170	.083	.058	.075
comm	.975	.969	.925	****	.039	.108	.059	.057	.254	.074	.048	.044
drap	.981	.988	.914	.961	****	.140	.051	.053	.320	.102	.054	.011
gade	.908	.896	.882	.892	.860	****	.153	.076	.409	.088	.098	.165
ging	.960	.951	.892	.941	.949	.847	****	.077	.364	.113	.074	.060
gumm	.961	.965	.926	.943	.947	.924	.923	****	.316	.026	.027	.044
majo	.744	.737	.830	.746	.680	.591	.636	.684	****	.313	.284	.263
mari	.929	.934	.917	.926	.898	.912	.887	.974	.687	****	.030	.083
maxi	.963	.970	.942	.952	.946	.902	.926	.973	.716	.970	****	.044
sati	.979	.989	.925	.956	.989	.835	.940	.956	.737	.917	.956	****

Table 1.4

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.

	atror	atros	carot	inlan	gingi	sativ
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	*****

Figure 1.7. Distance phenogram (UPGMA) derived from a matrix of genetic distances (table 1.3) for 12 groups (Heywood, 1968) of *D. carota* L.. Groups labelled with (C) are cultivated.

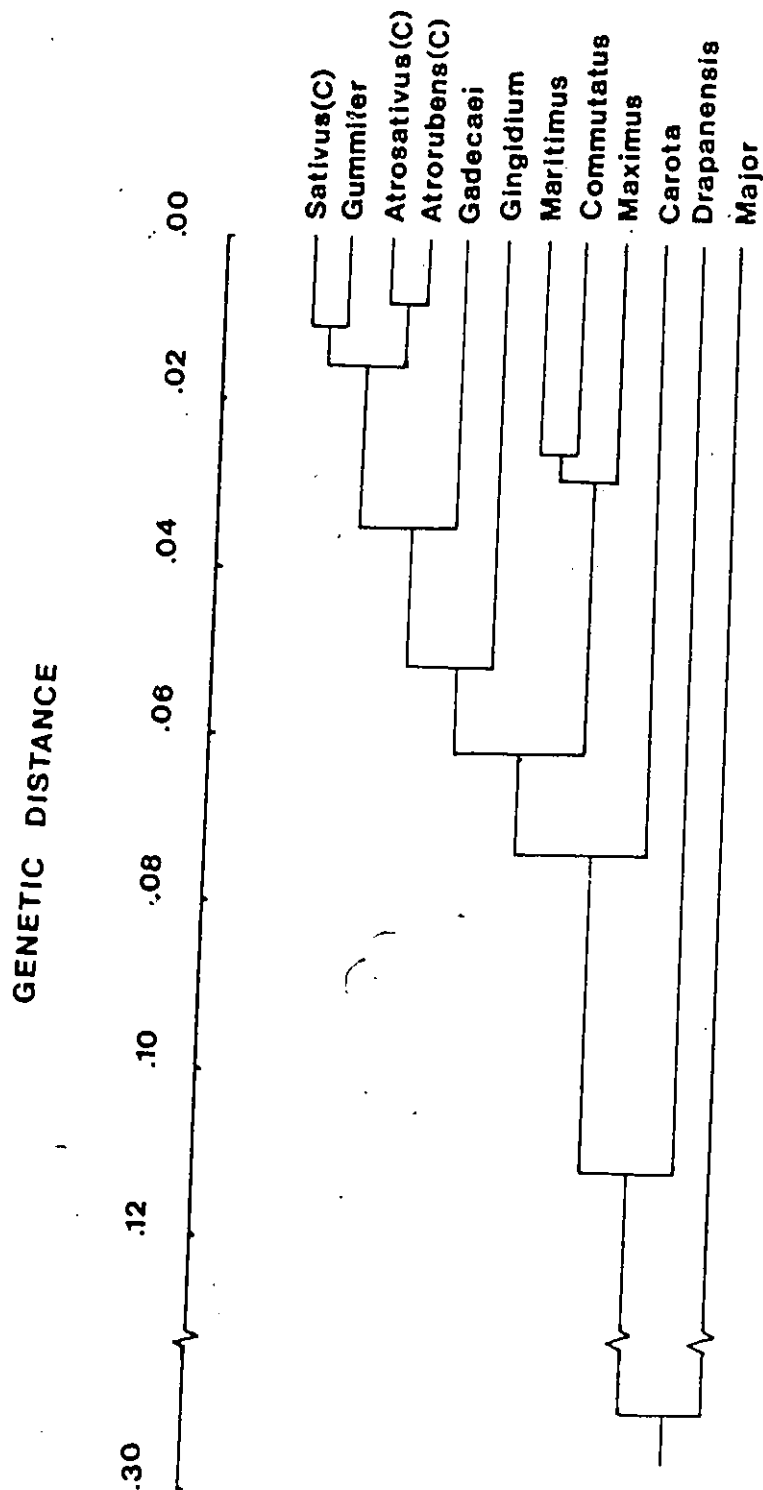
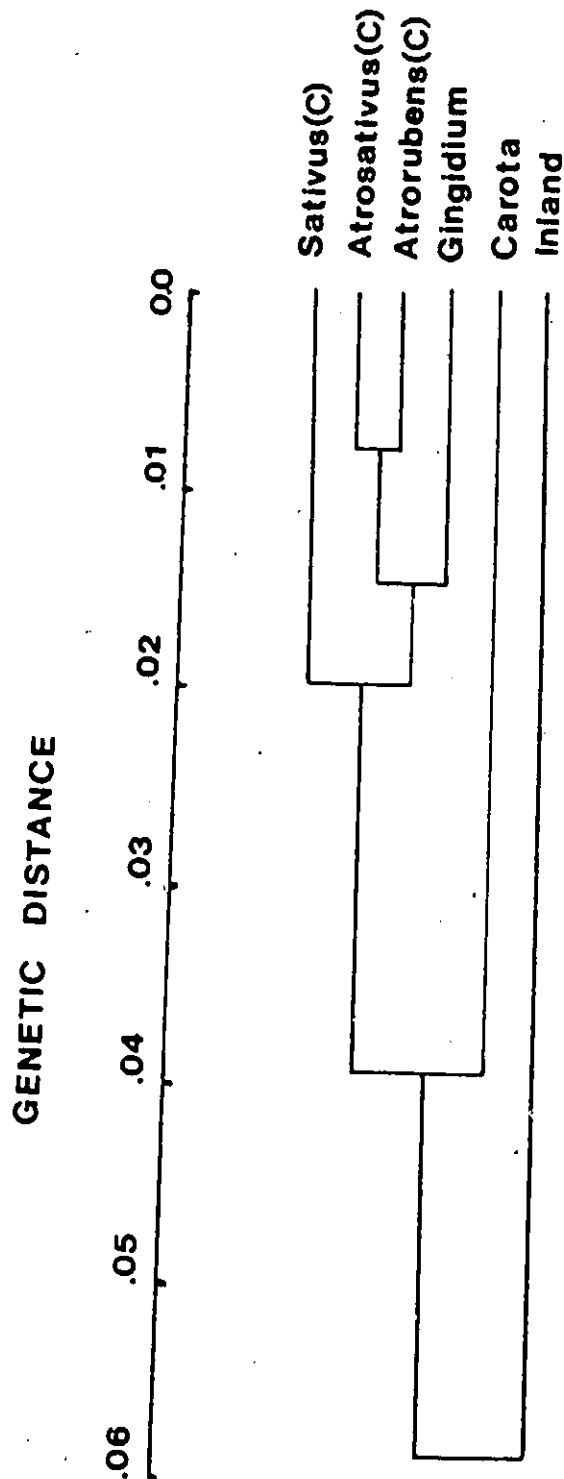


Figure 1.8. Distance phenogram (UPGMA) derived from a matrix of genetic distances (table 1.4) for 6 groups (Small, 1978) of D. carota L.. Groups labelled with (C) are cultivated.



## RESULTS

### A) Genetic Interpretation of Variable Banding Pattern

A total of 16 presumed enzyme loci coding for 34 alleles were identified (table 1.5). Figure 1.9 portrays their spatial distributions and Rf values. These isozymes were initially inferred by the segregating patterns scored on gels, but were further tested through the segregating zymograms produced by F<sub>1</sub> progeny of controlled selfings, and by the exclusion of subcellular material or the leaching of cytosolic enzymes. It has been shown (Weeden and Gottlieb, 1979; Weeden, 1983) that the cytosolic form of an enzyme can be extracted from pollen leachates. Haploid pollen of diploid species contains only one allele of each cytoplasmic gene locus. This eliminates the occurrence of interallelic heterodimers, thus facilitating the interpretation of the banding patterns. Likewise, through the extraction and isolation of intact chloroplasts, forms complementary to the cytosolic locus can be detected (Weeden and Gottlieb, 1981).

6-PGD was scored throughout the study, but is not included in the analysis due to the difficulty in interpreting the banding patterns. MDH-1, MDH-2 and MDH-3 were also scored throughout the study but also could not be included in the analysis because it was impossible to adequately interpret the five-banded pattern. Whether MDH-1 and MDH-3 are homozygous, leaving interlocus bands, or heterozygous, having overlapping interallelic and interlocus bands could not be determined. These anodal forms of MDH are probably mitochondrial in origin, as has



Table 1.5

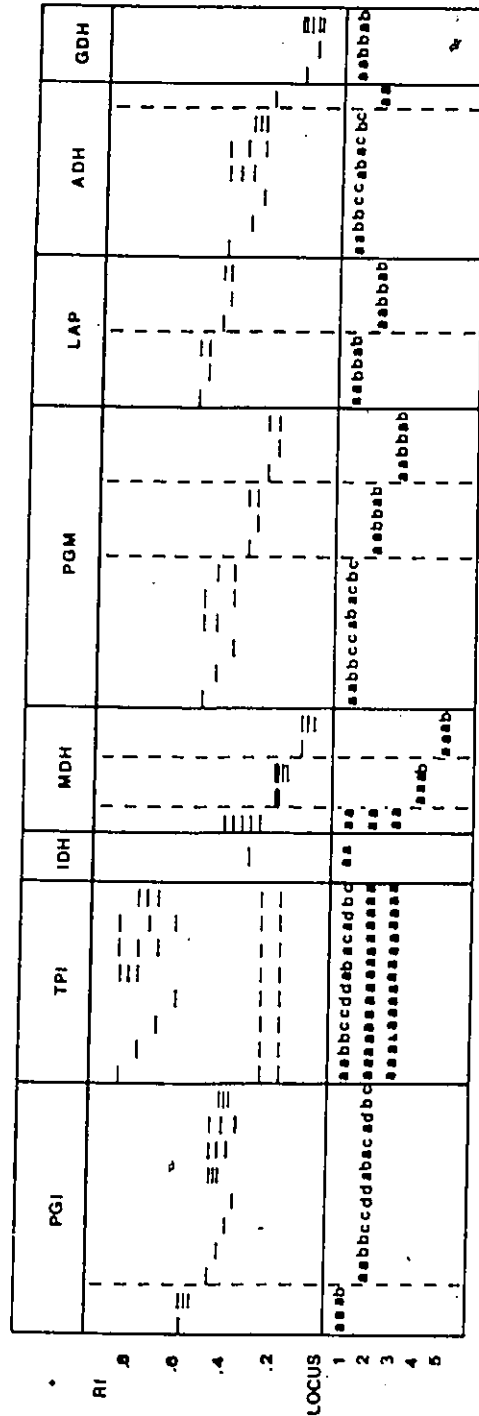
Enzyme systems utilized in the study

Locus	Alleles
-----	-----
<u>Adh-1</u>	a b c
<u>Adh-2</u>	a
<u>Gdh-1</u>	a b
<u>Idh-1</u>	a
<u>Lap-1</u>	a b
<u>Lap-2</u>	a b
<u>Mdh-4</u>	a b
<u>Mdh-5</u>	a b
<u>Pgi-1</u>	a b
<u>Pgi-2</u>	a b c d
<u>Pgm-1</u>	a b c
<u>Pgm-2</u>	a b
<u>Pgm-3</u>	a b
<u>Tpi-1</u>	a b c d
<u>Tpi-2</u>	a
<u>Tpi-3</u>	a
-----	-----
16	34

12 polymorphic loci = 0.75

Figure 1.9.

Representative illustration of banding patterns observed for eight enzyme systems in *Daucus carota* L. Inferred loci and allelic variants are designated at the bottom of the figure. Allelic designation "aa" represents the homozygous fast "a" allele, "bb" represents the homozygous slow "b" allele etc. *Mdh-4-a* is a null allele.



been found in maize (Goodman et al, 1979). Mdh-4 was the only locus that possessed a null allozyme. Goodman et. al, (1979) report null alleles for all but one Mdh locus in maize. Mdh-5 segregated in dimeric fashion.

Putative duplications of structural loci were observed in MDH, FGM, and TPI. Duplications of enzymes in subcellular compartments are most often found in dehydrogenase and isomerase enzyme systems (Gottlieb, 1982). Typically, three or four isozymes are observed for MDH in most diploid plant species (Gottlieb, 1982; Goodman et al, 1979). They are distributed in the mitochondria, microbodies and the cytosol (Gottlieb, 1982) and fail to produce interlocus bands. In our case, 3 localized groups were found, (figure 1.9) a most anodal 5 banded pattern, a central 1 or 2 banded pattern which was sometimes absent, and a cathodal group. Pollen leachates demonstrated that MDH-4, the most variable locus, was the cytosolic form of the enzyme. MDH-5 demonstrated very little genetic diversity, usually being homozygous. It was determined, through chloroplast extraction, to be the plastid form of the enzyme.

TPI is typically specified by two independently assorting nuclear loci (Gottlieb, 1982; Pichersky and Gottlieb, 1983). Daucus carota L. exhibits genetic variability at the most anodal isozyme, TPI-1, producing four allozymes, consistent with the dimeric subunit structure of the enzyme, and no variability at two monomeric cathodal bands, TPI-2 and TPI-3 (figure 1.9). These two bands were determined to be cytosolic in nature from electrophoresis of pollen leachates and are therefore presumed to be the result of a gene duplication. The absence of a hybrid

interlocus band warrants suspicion of this interpretation, however, independently assorting subcellular TPI gene duplications has been documented in species of Clarkia (Pichersky and Gottlieb, 1983). Selfing experiments in D. carota produced progeny with two monomeric bands. The two loci exhibit two of the three requirements of Gottlieb (1982) to be labelled as duplicated loci: they are true breeding and are both found in a single cellular compartment, but do not meet the third condition, as interlocus hybrid enzyme bands are absent. If, as in Clarkia these two bands assort independently, an interlocus hybrid band would not be found. The existence of an independently assorting gene duplication cannot be fully proven, owing to the monomeric nature of these putative loci, yet it appears to be the most likely explanation for the observations.

The isozymes of PGM are monomers (Gottlieb, 1981). As in most glycolytic enzymes, PGM typically possesses two independently assorting isozymes. One is derived from the plastid, and one from the cytosol (Weeden and Gottlieb, 1980; Gottlieb, 1982; Gottlieb, 1987). A third PGM isozyme, resulting from gene duplication has been reported in a few plant species (Gottlieb, 1987). Daucus carota also possesses a third locus. Pollen leachate extraction techniques show that the two most cathodal forms result from the cytosol and therefore the appearance of a third locus is due to the duplication of the cytosolic locus. The most anodal isozyme possesses three allozymes, but only one or two of them occurring in any single individual. The most cathodal allozyme of this locus quite often

approaches the most anodal allozyme of PGM-2. The slower movement of this isozyme in relation to the other two isozymes, coupled with the cytosolic origin of PGM-2 and PGM-3 confirm that the two most cathodal forms are the result of a gene duplication. Due to the monomeric structure of this enzyme, interlocus bands are not present. This scenerio, i.e., a duplication of cathodal cytosolic forms, was also observed in Lavie (Gottlieb, 1987).

IDH is a dimeric enzyme (Gottlieb, 1981; Gottlieb, 1987), produced in both subcellular compartments. However, in many plant species, only a single Idh locus is reported (Gottlieb, 1982). This was the case in this study. A single monomorphic isozyme was found to reside in the cytosol.

Four enzyme systems had numbers of isozymes that are typical of diploid plants (Gottlieb, 1982). These were PGI, ADH and LAP with two loci and GDH with one locus.

Two independently assorting dimeric loci were observed in PGI. Two alleles were observed at Pgi-1 while four alleles were noted at Pgi-2. The two locus system is consistent with most other diploid plant species studied (Weeden and Gottlieb, 1979; Gottlieb, 1981; Weeden and Gottlieb, 1980). PGI-1 is most often observed to be derived from plastids in plants (Weeden and Gottlieb, 1980). Chloroplast isolation and analysis, as well as pollen leachate analysis, also confirm these findings in D. carota.

Most plants contain two or three dimeric ADH isozymes, all located within the cytosol (Gottlieb, 1982). They are specified by a small group of tightly linked genes (Roose and Gottlieb, 1980), therefore they produce both inter- and intra-locus

heterodimers. Two ADH isozymes were observed. 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. The most cathodal isozyme, ADH-2, was found to be monomorphic throughout the study. Despite numerous attempts, it was not clearly discernible whether both loci result from a single cytosolic origin. They could not be resolved utilizing either subcellular localization technique. It is therefore presumed, based on previous findings (Gottlieb, 1982; Roose and Gottlieb, 1980) that both loci are cytosolic in origin.

LAP, the only variable substrate enzyme studied, is monomeric. Two polymorphic isozymes, each with two allozymes, were scored.

A single polymorphic GDH isozyme was resolved, displaying 1 or 3 bands. The two outer allozymes, which should have resolved from this tetrameric system, were usually not observed, probably due to their reduced activity within the system utilized. Three banded patterns were scored as heterozygotes.

## B) Statistical Analysis

Results of statistical analyses are presented for 12 taxa as it allows a more detailed interpretation.

### i) General

The species appears to maintain a high degree of genetic variability (table 1.6). The average number of alleles per locus ( $\bar{A}$ ) was 1.516 and the average proportion of polymorphic loci ( $\bar{P}$ ) was 0.457. Expected ( $H_{exp}$ ) and observed ( $H_{obs}$ ) heterozygosities

Table 1.6

Genetic variation in 12 subgroups of Daucus carota L. Included are: mean number of alleles per locus ( $\bar{A}$ ); proportion of polymorphic loci, where the frequency of the most common allele is less than 0.99 ( $\bar{P}$ ); observed average heterozygosity ( $\bar{H}_{obs}$ ); expected average heterozygosity ( $\bar{H}_{exp}$ ); and number of populations ( $N$ ). Pairwise values of heterozygosity are not significantly different.

Subgroup	$\bar{A}$	$\bar{P}$	$\bar{H}_{obs}$	$\bar{H}_{exp}$	$N$
-----	-----	-----	-----	-----	---
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
drapanensis	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	0.457	0.161	0.151	168

are not significantly different, with average values of 0.161 and 0.151 respectively (table 1.6). Randomly sampled populations were found to be in Hardy-Weinberg equilibrium for the majority of the polymorphic loci investigated. Genetic identity values (table 1.7), are highest among cultivated groups and lowest in the wild maritimus ( $I = 0.758$ ) and drapanensis ( $I = 0.778$ ) subspecies. When comparing on a group to group basis, wild taxa exhibit the highest and lowest values for all genetic variability estimates (table 1.6).  $A$  values are greatest in some members of the carota group, particularly subspecies major, and maximus though not significantly larger ( $P > 0.05$ , Wilcoxon two-sample test, tables 1.6 and 2.2). Values of  $P$  reach the maximum in ssp. gummifer and the minimum in ssp. drapanensis (table 1.6), both members of the gingidium group. Subspecies drapanensis has a relatively low genetic diversity, possessing the lowest values for  $A$ ,  $P$  and  $H_{exp}$  (table 1.6). However, with a sample size of two populations, any firm conclusions would be premature.  $H_{obs}$  values reach their maximums in the ssp. gadecaei population, as well as subspecies maritimus and maximus, and are lowest in ssp. commutatus (table 1.6). Expected average heterozygosity is highest in members of the carota group, especially subspecies maximus, maritimus and major and lowest in ssp. drapanensis (table 1.6).

Total genetic diversity ( $H_t$ ) varies considerably among taxa (table 1.8), and loci (table 1.9). Values of  $H_t$  among taxa range from 0.383 in ssp. carota to 0.087 in ssp. gadecaei. It is interesting to note that the highest value was observed in the most widely distributed subspecies and the lowest in an endemic



Table 1.7

Mean genetic identities and ranges of identities for comparisons of populations within 12 subgroups of Daucus carota L. The number of populations within each group is indicated by N.

Subgroup	Mean genetic identity ( $\bar{I}$ )	Range	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
drapanensis	0.778	0.778 - 0.778	2
gadecaei	-----	-----	1
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

Table 1.8

Nei's genetic diversity statistics for 12 subgroups of the D. carota complex.  $H_t$  = 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 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 gingidum groups (Wilcoxon two-sample test).

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
drapanensis	0.153	0.097	0.056	0.167
gadecaei	0.087	0.087	-----	-----
gingidum	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

Table 1.9

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

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

subspecies. The species demonstrates an overall  $H_t$  value of 0.271 (table 1.8). 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 1.9).

ii) Specific distribution of variation

A greater proportion of the total variability is observed within populations ( $H_s = 0.164$ ) than between populations ( $D_{st} = 0.107$ ) (table 1.8). This trend is observed in all taxa sampled. Therefore, a single population may be a reasonable representation of the subspecies in general. When observed in this perspective, the species is highly uniform, which conforms with morphological studies. Subspecies major shows the lowest value obtained for the portion of gene diversity occurring between populations ( $D_{st} = 0.021$ ). The proportion of allozyme diversity due to interpopulation differences ( $G_{st}$ ), is useful in estimating the relative magnitude of gene differentiation (table 1.8). Subspecies carota again, 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 of the subspecies. Excluding ssp. gadecaei, ssp. major demonstrated by far the lowest relative differentiation value ( $G_{st} = 0.054$ , table 1.8) although this value is inferred from only two accessions. The species demonstrated overall marked differences between accessions in relative differentiation ( $G_{st} = 0.403$ , table 1.8).

A phenogram of genetic distances was constructed (Appendix B) illustrating the homogeneity of the species, as well as the

lack of clearly defineable groups. Advanced carotene type carrots (group A) are the most genetically identical taxa 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 show a wider spectrum of relatedness, as they are dispersed among wild taxa. The most genetically diversified groups are members of wild taxa, especially those in group "D". Wild taxa are almost exclusively found in the lower half of the phenogram (appendix B). 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.

Phenograms of the clustered groups were also constructed (figures 1.7, 1.8, 1.10, 1.11 and 1.12). A relatively close relationship between the designated subspecies is apparent, with all but two inter-specific comparisons having values of  $\underline{D}$  less than 0.10 (table 1.7). There appears to be no clear demarcation between the two major groups carota and gingidium in figure 1.7 and, in fact, ssp. sativus (carota group) appears to be more similar to ssp. gummifer (gingidium group) than any other subspecies ( $\underline{D} = 0.011$ ). Among cultivated groups, subspecies sativus is closely associated with the other two cultivar groups (figure 1.7 and 1.10), separated by  $\underline{D} = 0.016$ . Another cluster includes subspecies maritimus, commutatus, and maximus among which maximum  $\underline{D} = 0.029$ .

The two major groups are more clearly divisible in figure 1.11 in which the cultivated forms have been excluded. Because the taxa did not maintain a uniform clustering pattern when cultivated

Figure 1.10. Distance phenogram (UPGMA) derived from a matrix of genetic distances for 123 cultivated accessions of *D. carota* L.

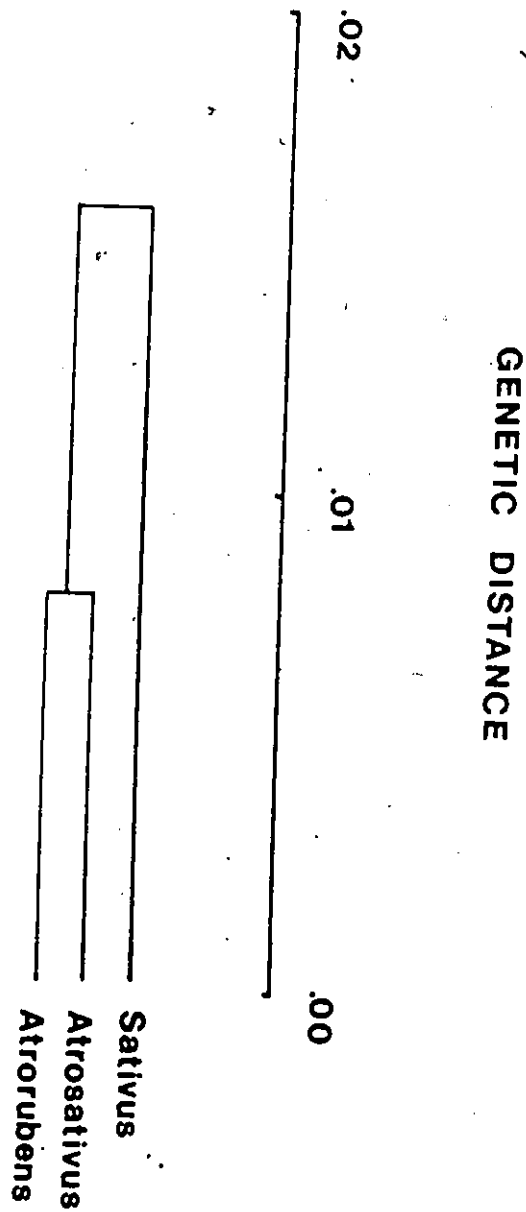


Figure 1.11. Distance phenogram (UPGMA) derived from a matrix of genetic distances for 45 wild populations of *D. carota* L.

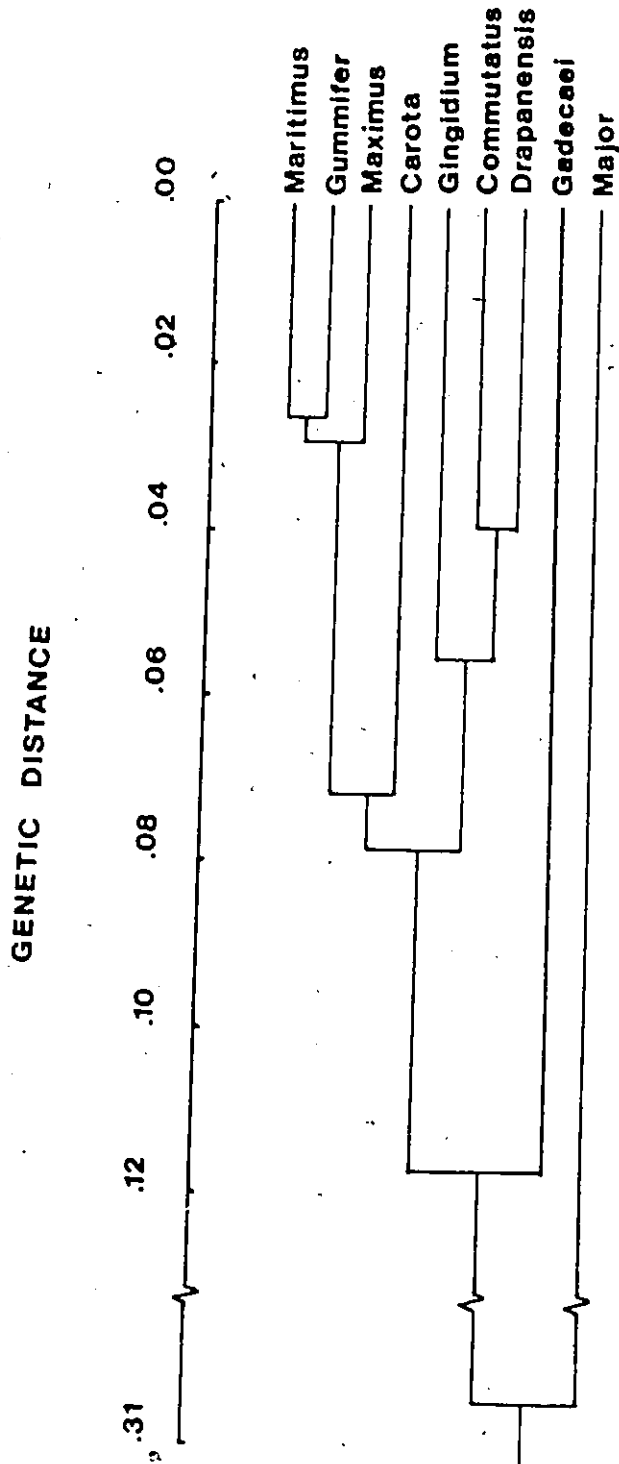
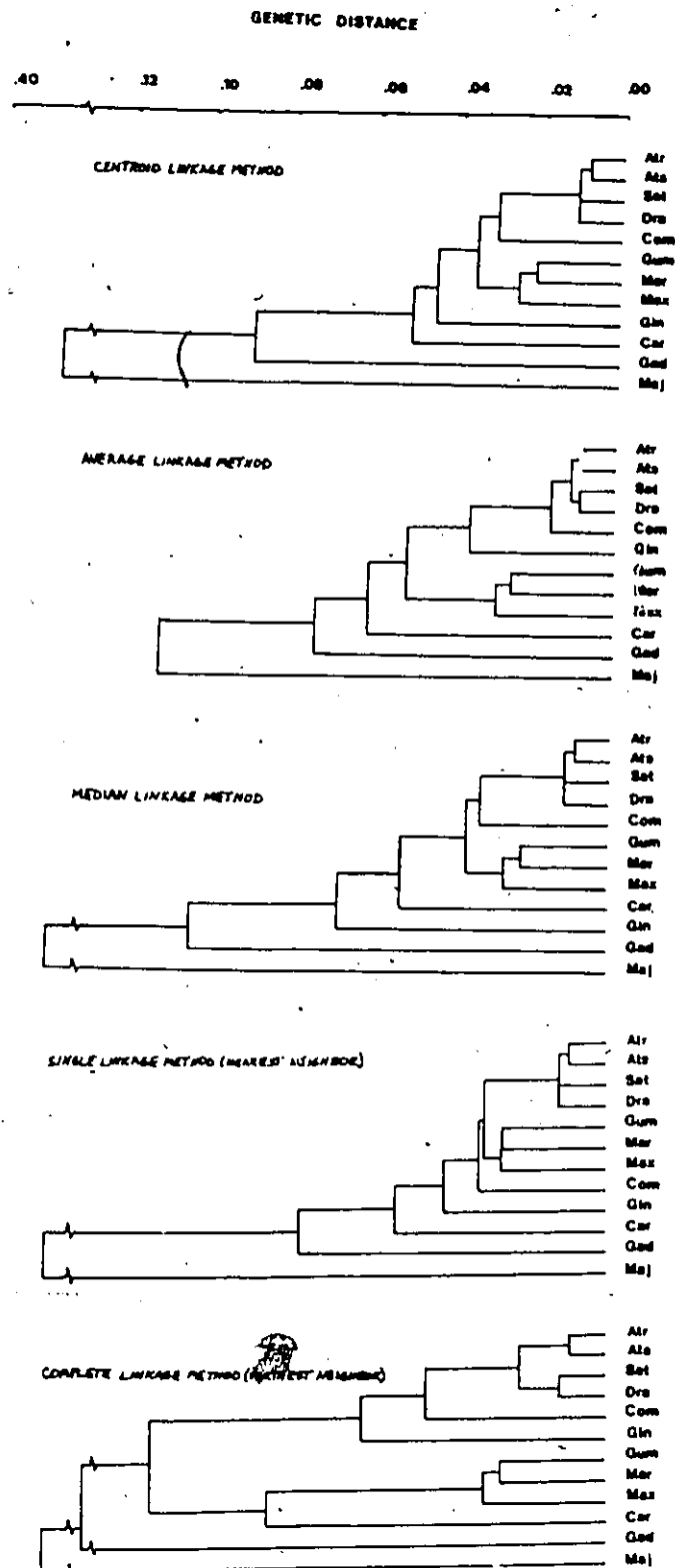


Figure 1.12. Distance phenograms produced by various clustering strategies from a matrix of genetic distances for 168 accessions of *D. carota*.





taxa were excluded (figure 1.7 versus figure 1.11), other clustering strategies were examined to see if any apparent pattern arose (figure 1.12). All five methods consistently demonstrate two clusters. The atrorubens, atrosativus, sativus and drapanensis taxa cluster out well as do the maximus, gummifer and maritimus taxa. The latter cluster is also apparent in figure 1.11. The most divergent taxon by far in all phenograms is 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 distances are less volatile when the taxa are united in Small's (1978) six general groups (figure 1.4 and 1.8). The eastern and east-west hybrid groups appear to be more genetically similar to the gingidium taxa than to the sativus taxa. This is not concordant with rare allele patterns (see chapter 3). However, all taxa are within the  $D = 0.06$  range, demonstrating a high degree of homogeneity. This is also reflected in the high  $I$  values (tables 1.7 and 1.10).

Table 1.10

Mean genetic identities and ranges of identities for comparisons of populations within six subgroups of Daucus carota L.

Subgroup	Mean genetic identity ( <u>I</u> )	Range
-----	-----	-----
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

## DISCUSSION

Morphological studies have shown the variation to be continuous from wild subspecies in the aggregate group gingidium to modern cultivars. The greatest variation in vegetative morphology occurs in wild forms. The cultivated morphotypes show less vegetative variability, yet demonstrate a diversity of root shapes and colours due to selection by man. Most cultivated accessions demonstrated relatively uniform morphotypes, but Middle Eastern and Asian semi-cultivars were extremely variable. White and purple, yellow and purple, or orange and red root colourmorphs were observed within single seed collections. This variability in semi-cultivars 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 and restriction in the systematic evaluation of a species complex.

Nehou (1961), warns that attempts to easily identify and group geographically dispersed plants by a set of morphological characters are in vain. Many of the subspecies previously described appear to form a series of nomina confusa as various authors struggled to identify and classify them. Geographic variants within the subspecies also contribute to the confusion. Plants identified as ssp. maximus north of the Iberian peninsula possess larger fruit size and therefore should be identified as members of ssp. carota sensu lato (Sean de Rivas and Heywood, 1974). Two distinct morphotypes of ssp. gummifer occur and complicate identification of this subspecies. Atlantic forms of

ssp. gummifer are highly pubescent in comparison to Mediterranean forms (Hegi, 1956). Plants identified as ssp. maritimus possess the discriminating traits in Iberia, yet progressively resemble ssp. carota eastward (Small, 1978). Subspecies intermediate to presently acknowledged subspecies have been described, such as D. lucidus L. (Thellung, 1927; Hegi, 1926) which is intermediate in morphology to ssps. hispanicus and gummifer. Presently accepted subspecies also demonstrate some form of intermediacy among themselves based on morphology. Both subspecies major (Hegi, 1926) and ssp. sativus (Hegi, 1956; Thellung, 1927) appear to be intermediate to ssps. carota and maximus. Subspecies fontanesii, a compendium of ssps. hispidus and hispanicus, appeared to be intermediate to ssps. gummifer and maximus or gummifer and commutatus respectively (Hegi, 1926). Although not clearly described as a subspecies utilized in this study, plants resembling ssp. hispanicus were obtained and grouped in the gingidium group due to the difficulty in clearly identifying the accessions. The status of this subspecies is uncertain, as it is not recognized as a distinct subspecies by some authorities (Jury, pers. comm.). Geographic ecotypes do not warrant subspecies recognition, although ssp. rupestris, an extremely rare endemic with few distinct morphological attributes has been recognized (Heywood, 1968). Small (1978), has suggested that the Iberian ssp. maritimus is simply a coastal form of ssp. carota sensu lato. This lack of subspecies integrity and stability increases the difficulty of a definitive taxonomic treatment. Sean de Rivas and Heywood (1974) proposed elevation of ssp. maximus to the species level owing to the uniqueness of fruit

characters, particularly the size, length of spines and shape of vitae in southern accessions. Although these morphological traits suggest phylogenetic separation, electrophoretic data do not (figure 1.7). The subspecies is closely genetically related to subspecies maritimus and commutatus (maximum  $D = 0.03$ ).

D. carota is not alone, many weedy species are subject to some degree of taxonomic difficulty (McNeill, 1982). These problematic species must be delimited in order to properly define and systematically assess our world flora. The importance of adequate taxonomic measurement is even more pronounced in species harbouring a cultivated form. Taxonomic complexity is the result of several variables. First, the subspecies delineated vary in geographic distribution, some are widespread while others are endemic to a particular region or ecological niche. Second, the species is highly outbred, owing to its floral biology and diverse pollinator pool. Third, especially in the case of cultivated types, divergence appears to be relatively recent, particularly in ssp. sativus, which was not noted before the fourteenth century (Banga, 1963). Fourth, the high degree of phenotypic plasticity within the species renders delineation of taxa difficult.

Geographically widespread subspecies are subjected to a larger variety of environments and thus greater variation in selective pressures. They 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). Small populations are more

susceptible to genetic drift and fixation as a result of periodic bottlenecks. In endemic populations, a reduction in genetic variability may be due as well to strong selective pressures, to adapt to narrow ecological conditions (Kruckeberg and Rabinowitz, 1985). In either case, genetic restriction and reorganization are prevalent. This is particularly pertinent to endemic D. carota subspecies on Mediterranean islands and maritime coasts such as ssps. gadecaei and rupestris.

The genetic structure of plant populations results from interactions between mutation, migration, selection and genetic drift (Loveless and Hamrick, 1984). Plant breeding systems have been identified as major factors affecting genetic structure (Brown, 1979). The techniques utilized in developing and maintaining a cultivar can have important influences on the genetic variation found within the species (Brown, 1979). The differences observed in cultivated accessions may be due to the extreme inbreeding techniques utilized in selecting and breeding cultivars. If highly inbred lines are utilized, the resulting cultivars will demonstrate a reorganization of the isozyme distribution once a bottleneck period has been overcome (Levin, 1976). On the other hand, if the crop is developed and maintained as an open-pollinated population, as is D. carota, the genetic variation should be distributed more evenly. There should be less intra-population homogeneity and more inter-population homogeneity as gene flow occurs more easily between populations (table 1.8). 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

degree of gene flow restriction, yet still maintain an amount of genetic variability comparable to wild taxa ( $H_{obs} = 0.160$ , table 1.6). The uniform distribution of genetic diversity is apparent, as semi cultivars are intermingled between both cultivars and wild accessions (Appendix B). The relatively low species'  $D_{st}$  value of 0.107 supports this trend (table 1.8). The species strong outcrossing tendencies (approximately 95%, Thompson, 1962) and ability to hybridize readily wherever two subspecies co-exist reduces the chance of genetic differentiation, 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). Outcrossing is emphasized by the presence of both hermaphroditic and staminate flowers and a wide variety of pollinators. 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 1.8). The  $H_t$  value for D. carota sensu lato is comparable to that found in other predominantly outcrossing species (Loveless and Hamrick, 1984). The high mean value for  $H_t$  can be attributed to the wide diversity of taxa studied and the fact that both cultivated and wild forms were compared. The largest  $H_t$  value was calculated in the most widespread taxon (ssp. carota) while the lowest value was calculated in the endemic coastal ssp. gadecaei, table 1.8). The  $G_{st}$  value however is comparable to inbreeders. Loveless and

Hamrick (1984) did not report a consistent decline in  $G_{st}$  values with an increasing outcrossing rate, demonstrating that other ecological factors affect this gene statistic. Daucus carota L. values for genetic variability are compared to the two main breeding systems in table 1.11.

Gottlieb (1981) concluded that outcrossing species have approximately three times more polymorphic loci per species, 1.3 times more alleles at these loci, more than 8 times more loci polymorphic per population, and a mean observed heterozygosity per individual about 90 times greater than that observed in selfers. The  $P$  value for D. carota in table 1.11 is similar to other outcrossers (Tukey's test; Sokal and Rohlf, 1981) however, the  $H_{obs}$  value, is particularly high. The low levels of  $I$  in ssps. maritimus (0.758) and drapanensis (0.778) are similar to mean genetic identity values for different species (Crawford, 1983; Loveless and Hamrick, 1984). Factors which may have influenced these values include the accessions studied and the enzyme systems utilized in making these estimates. Not all loci diverge at the same rate; those involved in glycolysis are highly conserved (Gottlieb, 1981). At least 9 subspecies consisting of a large number of geographic and morphological variants were utilized. Also, the enzyme systems observed were usually polymorphic; only 4 of 16 of those scored were monomorphic (table 1.5 and 1.9). Other enzyme systems not utilized in this analysis (6-PGD, MDH-1, MDH-3, ACP, ME, and SOD) have all demonstrated various degrees of polymorphism. Therefore, by utilizing the 4 monomorphic isozymes TPI-2, TPI-3, IDH-1 and ADH-2, and several enzymes involved in the glycolytic pathway, the estimates of



Table 1.11.

A comparison of the genetic variability between D. carota and various breeding systems. Values for selfers and outcrossers are means of species and subspecies compiled by Gottlieb (1981) (\*). Values for D. carota are means of the 12 taxa studied. To test for statistical significance, a one-way ANOVA was undertaken. As a post hoc test, Tukey's test of significance of differences among group means were calculated and are denoted by letters of the alphabet.

	Pop'ns	Enz	loci	A	P	H <sub>obs</sub>	I
	-----	----	-----	----	----	-----	----
* selfers	14.04	8.96	16.61	2.26a	.044a	.001a	.975a
S.E.	3.62	0.66	1.05	.099	.014	.0004	0.01
n	28	28	28	19	28	28	13
*outcrossers	11.43	9.86	17.57	2.90b	.370b	.086b	.956a
S.E.	3.62	0.75	0.93	0.17	0.05	.017	.011
n	21	21	21	20	21	21	14
<u>D. carota</u>	14.0	7.83	15.50	2.05a	.457b	.161c	.884b
S.E.	.366	.022	.031	.026	.016	.009	.017
n	12	12	12	12	12	12	11

Enz = number of enzyme systems observed

A = mean number of alleles at polymorphic loci

P = mean proportion of loci polymorphic in an average population

H<sub>obs</sub> = observed mean heterozygosity in the average individual

I = mean genetic identity of all pairs of populations.

variability are probably conservative. It is evident (table 1.9) that various loci yield a diversity of genetic variability estimates.

Populations of *D. carota* have a relatively high value for heterozygosity, which is partitioned unevenly between the loci observed (table 1.6). The potential ecological and life history traits which 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, its promiscuous and general entomophilous pollinators and its large population sizes (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. These processes prevent genetic drift and divergence.

The division of landraces and cultivars into several taxa (Zagorodskikh, 1939; Rubashevskaya, 1931; Alefeld, 1866; Matzkevitch, 1929; and others) is based on a few highly variable traits. Various forms represent ecological adaptations to the major environmental stresses in these areas, as well as the artificial agricultural stresses to which they are exposed. Cultivated taxa are highly uniform genetically, which is demonstrated in low  $D$  and  $D_{st}$  values (figure 1.10 and table 1.8). Artificial selection and manipulation by man has rendered morphological traits highly stochastic and volatile among regions. Because the western group is such a recent agronomic development, and it presumably originates from few individuals (Banga, 1957), genetic variability does not correlate with

morphological variability. The taxon has only recently been of major agricultural importance and therefore the various morphotypes have not yet had time to adequately diverge within these regions. Therefore, from a genetic perspective, the eastern and western divisions are largely artificial. The morphological traits utilized to distinguish them, particularly root pigmentation offer additional clues to further evaluate the taxa.

Phenotypic plasticity and genetic variability are the major methods in which plant species adapt themselves to variable environments (Schlichting, 1986). Hamrick et al, (1979) concluded that seed plants as a group maintain a relatively high degree of genetic variation. Plants maintain this variation due to the constraints imposed upon them. Because they are immobile, they have developed the ability to phenotypically adjust to varying environments without requiring large amounts of genetic variability. Plasticity may also have a stabilizing selective effect, allowing different genotypes to assume the same phenotype (Bradshaw, 1965) thus uniting various genotypic taxa or subspecies. This phenotypic plasticity allows for the formation of locally adapted ecotypes in corresponding microhabitats, and allows for the preservation of high degrees of genetic variability within the species. Daucus carota is a Paleotemperate species which has become Holarctic, therefore it is no surprise that with such wide ecological conditions, so many phenotypes have been described (Nehou, 1961).

## PHENETICS

The delimitation of a species or subspecies involves the evaluation of philosophical and taxonomic data made available to the taxonomist. The International Code of Botanical Nomenclature carefully avoids including a species concept within its rules (Zander, 1985). Therefore, delineations of species or subspecies complexes may be as diverse as the number of studies undertaken. Geographic distribution and ecological preferences alone are not enough in delimiting a species or even a subspecies, although delineation of the latter is less formal and structured than that of the former (Zander, 1985). Nevertheless, the best classification scheme would involve a careful genetic analysis of a species throughout its geographic range, and would demonstrate as closely as possible the morphological and ecological diversity within the various taxa, as this would more closely reflect its evolutionary history. The general scheme proposed by Thellung (1926b) for D. carota taxonomy is a plausible one, based on morphological data, as two major phyletic groups are distinguishable. However, no clear pattern of division is consistent among phenograms (figures 1.7, 1.11, 1.12). Small (1978), divided these two groups utilizing morphological traits, however appropriate character weighting was necessary. Enzyme electrophoresis utilizes unweighted genetic characters, resulting in unbiased results. 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 is also unwarranted. Additional populations, especially of the three subspecies which were not clearly identifiable in this study may help to fortify the existing database. As it stands, many of Heywood's subspecies (1968) could be collectively grouped into fewer subgroups. This is supported by the large amounts of genetic variability within accessions in relation to the smaller amounts of genetic variability between accessions, as well as the inconsistent morphological diversity within groups.

The inconsistent clustering patterns suggest that the species cannot be clearly divided based on electrophoretic data. Utilizing the UPGMA method of clustering (figure 1.7), the cultivated taxa cluster tightly with ssp. gummifer. This pattern is not observed in figure 1.12 where the cultivated taxa cluster with ssp. drapanensis and ssp. gummifer is clustered with ssps. maritimus and maximus in all cases. Two trends are apparent in all phenograms. The cultivated taxa form a united group and ssps. 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 (2 accessions).

The taxonomic divisions of Small (1978), although not intended to formally delineate taxa, do not adequately encompass the morphological and genetic variability observed. When clustered into these 6 groups (figure 1.8), very little genetic

differentiation of groups was observed. This is attributed to the large amounts of genetic variation within ~~the~~ supposed to among groups. However, it does serve as a useful guide in separating cultivated forms, and thus was utilized in this study.

Electrophoretic data do very little in further classifying cultigens. It does demonstrate a closer homology of the atrosativus group to the atrorubens group than the sativus group (figures 1.7, 1.8, and 1.10) and all could be grouped as a single taxon. This is due to their high  $I$  and extremely low  $D_{st}$  values. This is further elaborated in chapter 2. Root pigmentation, although a highly variable character, prone to mutations and reversions as it is based on only a few dominant genes, appears to be the most sensitive trait in following the relationships of cultivated forms. This is discussed in chapter 3.

Archeobotanical, ecological, karyotype, chemotaxonomic, quantitative DNA analysis and morphological studies have proven to be of limited use in further classifying the species complex. Electrophoretic data does not provide evidence for grouping the taxa into widely separable groups. The taxa are not genetically differentiated because they are con-specific. The considerable karyotype variability noted by Owens (1974), as compared to electrophoretic differences is not surprising. Only a small percentage of the DNA in plants is considered to be involved in the coding of proteins (Price et al., 1984). Most of the DNA variation is due to the amplification of nucleotide sequences within chromosomes and is independent of the number of chromosomes (Narayan, 1985). Closely related species may vary

from three to six fold in nuclear DNA amount (Narayan, 1985), therefore it is a poor variable to base conclusions upon.

Knowledge of the linkage relationships of various enzyme systems and the presence of duplicated loci are of great importance for phylogenetic purposes. They allow the inference of lineages, since chromosomal rearrangements are necessary for these to occur. The utility of duplicated loci in D. carota, however, is limited, as they are found throughout the complex. It would be interesting to compare these gene duplications with the genetic structure of the same enzyme systems in other Daucus species. This might aid in the delineation of the genus, which is in need of further taxonomic study. The genetic relationship of D. capillifolius to D. carota would be of interest as they cross readily and demonstrate a high nucleotide sequence homology (Matthews et al, 1984).

The phylogenetic delineation of a species should encompass a conservative classification system which reflects the evolutionary history of that species group. Results from electrophoretic data tend to unite the morphological and ecological groupings into a more cohesive, unified system. The 11 subspecies delineations should be maintained to describe the diversity observed. Although not applicable in all plant groups, the biological species concept of Mayr (1963) states that a species is defineable as a group of populations that is reproductively isolated from all others. The occurrence of hybridizations both between wild and cultivated taxa as well as within wild taxa and the degree of microevolution among wild forms and among cultivated forms, plus the narrow genetic

distances observed suggest that the various taxa should be ranked at the level of subspecies, and local variants given the rank formae speciales.



II. THE IMPACT OF DOMESTICATION ON THE GENETIC VARIABILITY IN  
CULTIVATED D. CAROTA SSP. SATIVUS AND THE GENETIC  
HOMOGENEITY OF VARIOUS CULTIVARS.

## INTRODUCTION.

The introduction of isozyme studies of plants has revealed considerable variability both within and among plant species (Brown, 1983). It is this variability which allows us to appreciate the genetic importance of particular populations, and the usefulness of their preservation.

### A) Importance of Germplasm Centres and Gene Conservation

Domestication has almost never led to the speciation of a crop, therefore it is still possible to hybridize cultivated and wild forms. Centres of genetic and morphological diversity do exist for most crop species, but many of these are being wiped out by advancing civilization, particularly the modern agricultural revolution (Harlan, 1975; Hedrick, 1975). Today, fewer varieties of crops are cultivated, tending towards monoculture of high yielding, uniform varieties. This has caused a restriction in the gene pool of species as local landraces and endemic varieties, genetically suited to a particular agroecosystem, are no longer grown. Therefore several potentially economically important traits are only found in weedy relatives.

Obviously, as many populations as possible should be preserved, but this is not feasible owing to financial and space restrictions. The use of electrophoretic markers to sample populations would aid in the selection of populations which maintain rare genetic properties useful in local adaptation. There is much greater variability in exotic germplasm than has already been acquired (Tanksley, 1983; Peeters and Galwey, 1988). Plant gene resource centres have been established in many

developed countries, and a world headquarters, the International Board for Plant Genetic Resources (IBPGR), has been founded in Rome, Italy to orchestrate their operation.

#### B) Isozymes in Breeding

Isozymes have many advantages in a breeding program. It is believed that certain isozymic markers are tightly linked to genes responsible for physiological and morphological traits. Isozyme markers have been shown to be closely linked with monogenic traits such as those controlling the number of ears and yield in corn (Zea mays, Tanksley, 1983); storeability, nitrogen fixation, and disease resistance in soybean (Glycine max, Kiang and Gorman, 1983); root knot nematode resistance and male sterility in tomato (L. esculentum, Rick, 1983). Tree fruits have long juvenile periods. Isozyme markers tightly linked to specific genes controlling desirable traits could reduce the time and space needed to maintain these breeder lines, making the selection process more efficient. A sex linked gene tightly linked to an isozyme marker is being sought in date palm, as it is a long lived dioecious monocot, and pistillate, fruit-bearing trees are of primary interest (Torres and Tisserat, 1980).

Isozyme markers are used to ensure the transfer of desired traits through interspecific or other crosses into useful cultivars. This requires screening the F<sub>1</sub> population for individuals which inherit most of their genes from the cultivated parent, yet possess the genes controlling the desired trait from the donor plant.

Detection of unintended hybridizations and hybrid purity is

of concern to plant breeders. Isozyme markers have been utilized to monitor specific crosses in date palm (Torres and Tisserat, 1980) and interspecific hybridizations in tomato (Rick, 1983); to ensure seeds are the result of sexual recombinations, rather than asexually derived in polyembryonic citrus species (Torres et al, 1982); to determine the genetic distance between parents in soybean, as greater distance between parental strains is associated with greater productivity of hybrids (Kiang and Gorman, 1983); to assess the genetic purity of cole crops, due to the importance of uniformity in mechanical harvesting (Arus and Shields, 1983); and many other applications.

Isozyme electrophoresis has been remarkably successful in cultivar fingerprinting. The distinctiveness of a new crop variety must be established prior to its registration (Bailey, 1983). It has been utilized to fingerprint clonal apple cultivars (Chevreau and Laurens, 1987), apple rootstocks (Menendez et al, 1986a, 1986b), celery (Quiros et al, 1987), rye (Adams et al, 1987), white bean (Weeden, 1984), and radish varieties (Ellstrand and Marshall, 1985).

### C) Isozyme Variability in Cultivated Crops

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 (21 species observed) had an  $H_{exp}$  of 0.172 while wild species (89 species observed) had an  $H_{exp}$  of 0.136. The greater genetic variability in cultivated forms is due to artificial genetic manipulation.

Allozyme surveys of cultivated plants generally report little within cultivar variation, but considerable among cultivar variation (Tanksley and Orton, 1983, Hamrick et al, 1979). This trend is not typical of wild populations. In them, the majority of allozyme variation is found within rather than among populations (Brown, 1979). Nevertheless, 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. Under the influence of human manipulation, several crop species have evolved radically different morphologies, with very little fundamental chromosome repatterning (Harlan, 1975).. Although their morphologies have changed, wild and cultivated forms are still highly interfertile, producing viable offspring (Harlan, 1975; Laferriere, 1986). Studies of genetic variability in various outcrossing crop species such as corn (Kahler et al, 1986), sunflower (Laferriere, 1986; Dry and Burden, 1986), crucifers (Surrs, 1986), celery (Quiros et al, 1987), cucurbits (Kirkpatrick et al, 1985; Decker and Wilson, 1987; Decker, 1985, 1988), Citrullus spp. (Navot and Zamir, 1987), Cucumis spp. (Staub et al, 1987) have been undertaken. A cultivated crop's genetic diversity may vary considerably from its progenitor, depending on the length and degree of bottleneck the species was subjected to, as well as the degree of introgression with wild populations. The latter point is particularly of concern to outcrossing species, as it has been shown that the introduction of only one migrant every two generations is sufficient enough to

obscure any genetic differentiation caused by drift (Wright, 1931). Therefore, little differentiation would be expected between widely separated populations (Loveless and Hamrick, 1984). Wild subspecies carota is a cosmopolitan anthropochorous, weedy species and therefore great care is necessary to ensure that commercial seed plots are properly isolated from any wild populations. Introgression of cultivated crops with wild species is a problem in several crops and has been noted in Helianthus annuus, Lactuca sativa, and Raphanus sativus (Panetsos and Baker, 1967). Consequently, it is quite likely to occur in D. carota as well.

The improvement of outcrossing species is a slow and laborious task, requiring mass selection techniques to maintain overall crop vigour. This, however, reduces the control of uniformity, a feature often lacking in most outcrossing species. These species are typically subject to relatively serious inbreeding depression when selfing or maintainer lines are produced. In fact, inbreeding depression is the leading limiting factor in maintaining intensive breeding programs on these crops (Orton, 1983). Many outcrossing species have developed methods of preventing selfing such as dioecy, allogamy or protandry in order to maintain heterozygosity and overall genetic diversity. These mass populations of breeder lines represent a wide variety of genotypes which demonstrate uniform phenotypes under agronomic conditions. Isozyme polymorphisms act as useful markers to estimate the genetic diversity found in these populations.

Genetic purity of commercial seed lots is of vital

importance for commercial seedsmen, as their reputation is determined by the performance of the crop species. 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 in various crop species. It is also of use to ensure  $F_1$  hybrid purity (Arus, 1983; Arus and Shields, 1983; Surrs, 1986).

The purpose of this section is twofold. First, if D. carota is the result of selection followed by mutation and manipulation, then a genetic bottleneck has occurred, causing a pattern of genetic reorganization in resulting cultivars subsequently. Has this occurred and to what degree? Secondly, electrophoresis was used to determine the genetic integrity of four commercial varieties by looking at 13 lines of commercial carrot cultivars.

#### MATERIALS AND METHODS

Seeds representing 4 commonly grown carrot varieties from various morphological groups (figure 2.1) were analyzed using at least 3 different sources (table 2.1). These represent both bunching and commercial processing types. "Red Cored Chantenay", "Scarlet Nantes", and "Danvers Half Long" are varieties maintained by open pollination, while "A-Plus" is an  $F_1$  hybrid variety. Seeds were sown in the University of Windsor greenhouses and allowed to grow to maturity. Results were then compared to data gathered from wild populations. Methodologies, electrophoretic procedures and statistical manipulations were

Figure 2.1. Mature root silhouettes of the four carrot cultivars examined in chapter two.

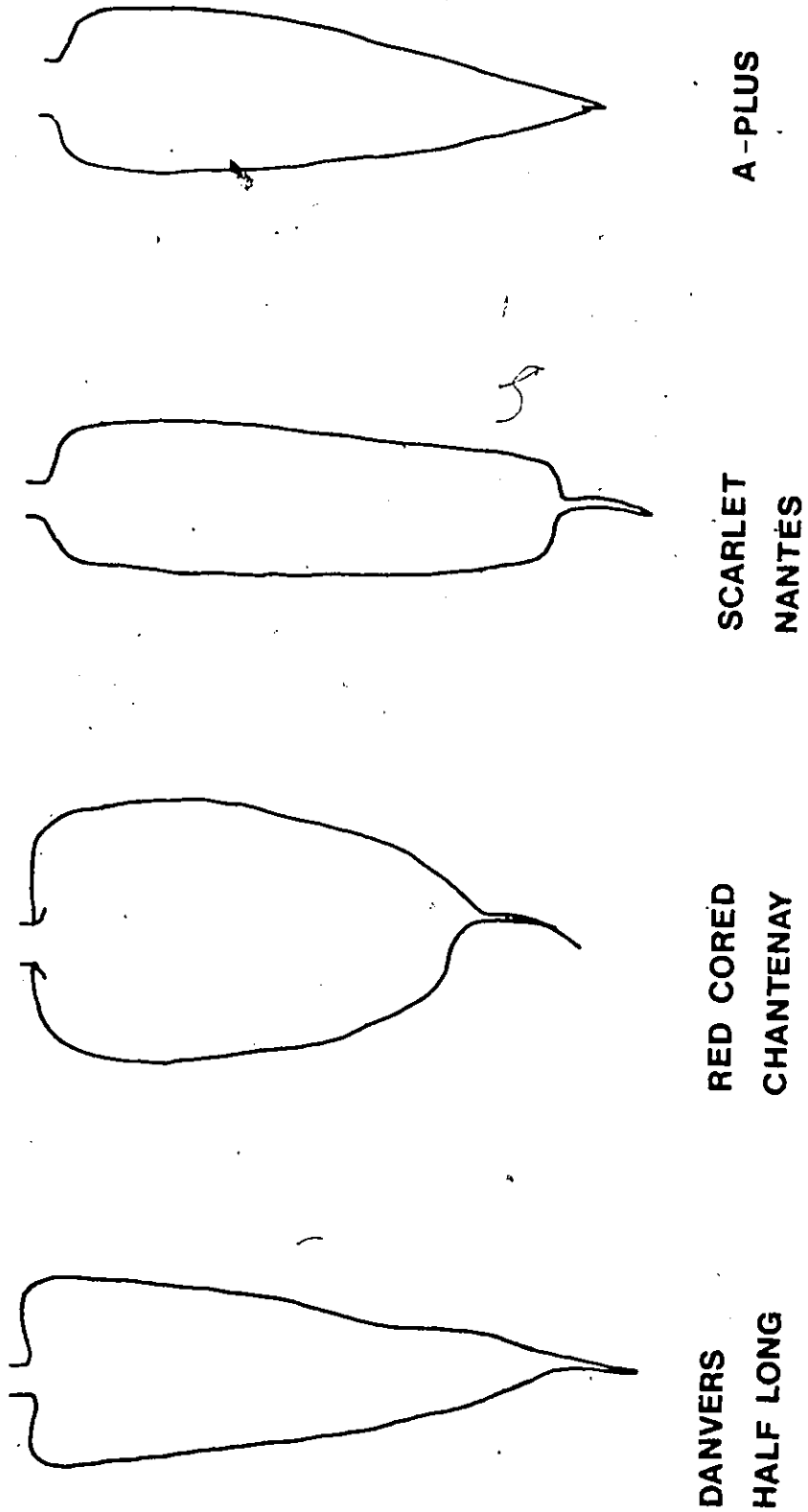




Table 2.1.

Genetic variation in 13 accessions of 4 orange rooted carrot cultivars. Included are: mean number of alleles per locus ( $\bar{A}$ ); proportion of polymorphic loci, where the frequency of the most common allele is less than 0.99 ( $\bar{P}$ ); observed average heterozygosity ( $H_{obs}$ ); expected average heterozygosity ( $H_{exp}$ ); Pairwise values of heterozygosity are not significantly different. Twenty five individuals were examined from each accession.

Cultivar	Source	$\bar{A}$	$\bar{P}$	$H_{obs}$	$H_{exp}$
Scarlet Nantes	Jung	1.565	0.560	0.186	0.255
	Dominion	1.510	0.500	0.169	0.164
	Harris-	1.454	0.378	0.116	0.151
	Moran				
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

identical to those described in chapter 1.

## RESULTS

### A) General

A great deal of genetic variability is maintained in all accessions of the four cultivars (table 2.1). 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 largely due to the method in which the cultivars are maintained.

Both cultivated and wild forms demonstrated a relatively large genetic variability. Wild forms demonstrate greater variability though not significantly so (Wilcoxon two-sample test; table 2.2). The proportion of polymorphic loci,  $P$ , maintained in cultivated forms (0.451) is less than that in wild accessions (0.474, table 2.2). This reduction in variability is also observed in  $A$ , the mean number of alleles per locus, estimated at 1.503 for cultivated and 1.551 for wild forms. Total genetic diversity,  $H_t$ , in wild subspecies (0.307) is not significantly larger than that observed in cultigens and landraces, (0.230, table 2.2). The proportion of polymorphic loci in the 4 cultivars ranges from 0.457 in "A-Plus" to 0.564 in "Red Cored Chantenay" (table 2.3). 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 2.1) are not significantly different and conform well with those of the species in general.

Table 2.2

Genetic variation and Nei's genetic diversity statistic averages for wild and cultivated Daucus carota L. taxa. Presented are: total gene diversity within a taxon ( $H_t$ ); gene diversity within populations of a taxon ( $H_s$ ); gene diversity between populations within a taxon ( $D_{st}$ ); coefficient of gene differentiation ( $G_{st}$ ); proportion of polymorphic loci, where the frequency of the most common allele is less than 0.99 ( $P$ ); mean number of alleles per locus ( $A$ ); and observed average heterozygosity ( $H_{obs}$ ). All paired values are not significantly different ( $P > 0.05$ ) utilizing the Wilcoxon two-sample test.

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

## 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 2.2). The proportion of allozyme diversity due to interpopulation differences,  $G_{St}$ , estimates the relative magnitude of gene differentiation among populations. Wild forms show a greater differentiation (table 2.2). Twenty eight percent of the allozyme variation at polymorphic loci is due to interpopulation variation, as opposed to 24 % for cultigens. A greater proportion of genetic variation is found within both wild and cultivar accessions rather than among them.

Three of the four cultivars demonstrate higher than average  $H_S$  values (table 2.3) 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 a more uniform crop. The 4 varieties demonstrate fairly low  $D_{St}$  values (table 2.3), therefore only a small fraction of their genetic variability occurs between accessions. This is reflected in their small  $G_{St}$  values (table 2.3), which indicate 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 as compared to wild taxa. A second useful technique to determine the degree of genetic reorganization in cultivated forms is to measure the

Table 2.3.

Genetic variation and Nei's genetic diversity statistics within and among 4 cultivars of Daucus carota L. Presented are: total gene diversity within a taxon ( $H_t$ ); gene diversity within populations of a taxon ( $H_s$ ); gene diversity between populations within a taxon ( $D_{st}$ ); coefficient of gene differentiation ( $G_{st}$ ); proportion of polymorphic loci, where the frequency of the most frequent allele is less than 0.99 ( $P$ ); and mean number of alleles per locus ( $A$ ).

Cultivar	$H_t$	$H_s$	$D_{st}$	$G_{st}$	$P$	$A$
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

divergence of cultivated forms from wild populations through cluster analysis of values of  $D$  (figure 1.8). As can be seen, wild forms cluster fairly well from cultivated forms, demonstrating some degree of divergence as discussed in chapter 1. The taxa are only loosely separated, with the cultivated forms diverging at a genetic distance of 0.02 (figure 1.8) and the 4 cultivars diverging at a genetic distance of 0.03 (figure 2.2; table 2.4). Due to the relatively high  $H_t$  levels obtained, resulting from the higher degree of variability within taxa rather than among them, the division of clusters are almost arbitrary.

The same pattern is observed in the 4 cultivar groups (figure 2.2; table 2.5), as all pairwise comparisons have genetic distances of less than 0.04. This high degree of genetic similarity (table 2.5) demonstrates the uniformity within and among cultivars. When all 13 accessions are clustered (figure 2.3) utilizing various clustering strategies, 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). Some of these types, including the varieties "Long Orange" and "Early Scarlet Horn" were analyzed in this study, but their genotypes do not demonstrate an affinity to any particular taxa. Through time, the list of available cultivars has risen,

Figure 2.2. Distance phenogram (UPGMA) derived from a matrix of genetic distances for 13 accessions of four orange rooted D. carota cultivars.

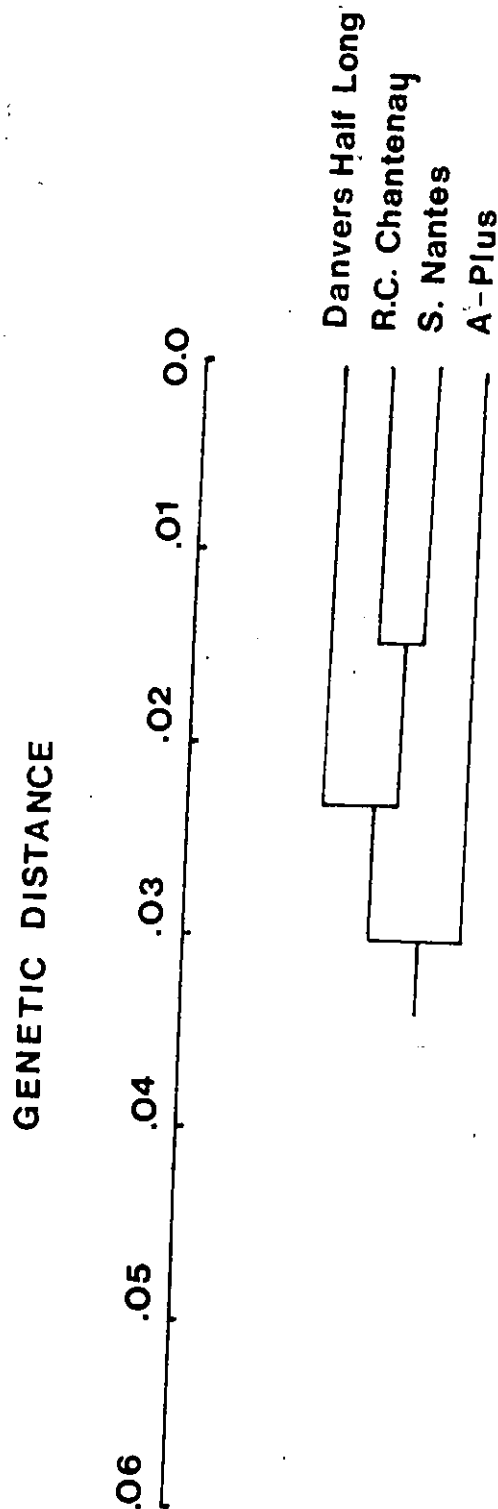


Table 2.4

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	*****

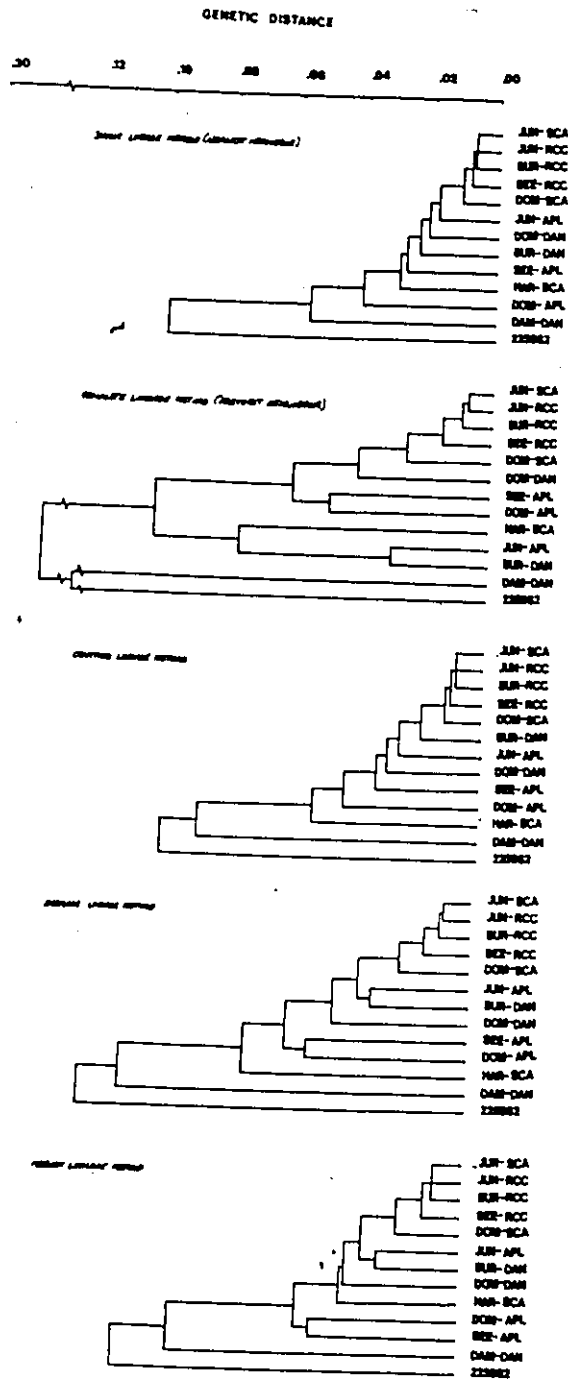


Table 2.5

Mean genetic identities and ranges of identities for pairwise comparisons of populations within 4 cultivars of D. carota L.

Cultivar	Mean Genetic Identity ( <u>I</u> )	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

Figure 2.3. Distance phenograms produced by various clustering strategies from a matrix of genetic distances for 13 cultivated accessions of *D. carota*.



from a few unspecialized varieties (Villmorin Andrieux, 1885; Goff, 1888; Vilmorin, 1890), to hundreds of specialized varieties (Magruder et al., 1940; Babb et al., 1950 and others). Carrot breeding has drastically changed recently. Early methods included mass selection or combined mass pedigree selection. The techniques utilized in developing and maintaining a cultivar have an important influence on the genetic variation found within that cultivar (Brown, 1979). If highly inbred lines are utilized, the resulting cultivar will demonstrate a reorganization of gene frequencies once the genetic bottleneck has been overcome (Levin, 1976). The resulting cultivar will demonstrate a more uniform genome. If on the other hand, the crop is developed and maintained as an open pollinated population, as in most carrot cultivars, genetic variation will be distributed more evenly. There will be less intra-population and more inter-population homogeneity, as gene flow occurs more easily. Relatively little genetic variability is usually observed in outcrossing crop species populations and this may be attributed to selection (Allard et al., 1972) and to genetic drift due to the restriction of population size (Brown and Allard, 1971). 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 wild subspecies ( $H_{obs} = 0.160$ ) (table 2.2). 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 species and rogues are removed to prevent genetic contamination.

Even though some genetic re-organization within carrot cultivars is observed, it is considerably less than that typically found after domestication (Brown, 1978). A large percentage of the variability in wild populations must be in members of the gingidum group as significantly different  $H_t$  ( $P < 0.05$ ) and  $H_s$  ( $P < 0.01$ ) values were obtained in comparison to members of the carota group. 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 2.2). 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 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). The hybrid "A Plus" cultivar has a unique origin. It is an  $F_1$  hybrid between two inbred lines. 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 both the male sterile female line and the pollen donor male line used to produce it. The parental lines were not highly inbred as fixed heterozygosity

was not observed at any locus from any accession. Both parental lines are inbred, but still maintain genetic variability. Cultivars with a high  $H_t$  value are probably more universally acceptable. Generally, the more heterozygous an organism, the more genetically diverse it is, and, the more environmental situations in which it will remain uniform. (Bradshaw, 1965). This is the principle of hybrid vigour and is manifested in the high proportion of polymorphic loci in most of the cultivars. They have been bred to be uniform in a variety of situations. Irregular morphotypes are rogued out, in fields for seed production. The low  $D_{st}$  and  $G_{st}$  values observed in cultivars is a good reflection on the companies producing these seeds. Seed company policy restricts public knowledge as to their sources for seed. It is however common knowledge that most eastern companies buy large seed lots from western producers. 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.

In conclusion, no real pattern of genetic differentiation is observed among cultivars. Therefore, the maintenance and domestication of cultivated carrots has resulted in 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. The distribution of genetic variability in carrot is not consistent with most wild-cultivar genetic studies because more variability is observed in wild accessions (Hamrick et al., 1979). However, most wild progenitor species do not demonstrate the morphological, geographic, and genetic variability observed in Daucus carota L. The cultivars appear to have evolved parallel to one another following the initial genetic bottleneck of domestication.

III. GEOGRAPHIC DISTRIBUTION OF GENETIC VARIABILITY AND  
POTENTIAL ROUTES OF DOMESTICATION.

## INTRODUCTION

Determining the origin and centers of genetic diversity of a crop species is of great importance in understanding its history and phylogenetic makeup. It is also important to plant breeding programs for the accession of varied germplasm. Wild forms are usually more genetically diverse because of the various ecological and edaphic factors to which they have been exposed, the greater possibility of genetic drift and testing of potential recombinations in marginal or stressful environments. Plant breeders and taxonomists studying a sympatric wild/domesticated species group should attempt to determine the presence of intermediate forms, the impact of domestication on the cultivated population, and the length of time that cultivated type been known (Brandenburg, 1981).

Two centers of origin for the Apiaceae have been proposed: Pacific North and Central Americas and the Mediterranean (Moore, 1971). These two areas possess morphologically different genera and are distinctly divided. The Mediterranean centre is more important, as it possesses approximately three times as many species, and several more generic diploid endemics than the American centre (Moore, 1971). It is in the Mediterranean centre that D. carota is believed to have originated.

Currently, there are two opposing hypotheses as to the method by which to determine the area of origin of a species. Matthew (1915 in Brown and Gibson, 1983), believed that newer, evolutionarily superior species would supplant the original ones, forcing them to less than optimal sites. Therefore the centre of origin is where the derived forms are found. In contrast,



another theory states that the centre of origin is where the primitive forms are still found. Ancestral populations remain in the same area while derived forms disperse radially. In actuality, either theory may apply, depending on the taxon, method of speciation, dispersal, and the way it reacts to its environment (Brown and Gibson, 1983). Vavilov's thesis encompasses these theories: the area of greatest diversity is also the primary centre of dissemination (Vavilov, 1949-50).

Information on the origin of the domestic carrot is very sparse. Archeological records are limited, therefore most theories have been constructed based on written records and contemporary paintings (eg., Time, Feb.16, 1976). Old records are highly unreliable because the term "pastinaca" was used interchangeably for parsnips and carrots (Banga, 1976; Brandenburg, 1981; Helweg, 1908). It is believed that D. carota was initially utilized for medicinal purposes because of the strong fragrant scent of the fruit, the sharp taste of the root and the pungent odours of the leaves. It was valued by Greek physicians as a stomach tonic (Boswell, 1947), and was noted for its pungency (Banga, 1963a). It was not widely known as a food plant until the end of the nineteenth century (Banga, 1963a). In America, it rose from a delicacy for horses to an important human food crop when its value as a source of carotene or provitamin A was found in the early twentieth century (Boswell, 1947).

Various origin theories have been proposed (figure 3.1) based on 3 general themes, hybridization, selection and mutation.

Figure 3.1. Proposed theories of origin for the cultivated carrot.

## A) Hybridization

- i) Adapted from Helweg (1908). Hybridization of semi cultivars.  
violet carrot X white or yellow carrot ---> European red carrot in 1 generation
- ii) Adapted from Thellung (1926). Hybridization of 2 wild subspecies, root swelling due to hybrid vigour. It is morphologically intermediate between ssp. carota and maximus.  
D. carota x D. carota -----> D. carota  
ssp. carota ssp. maximus ssp. sativus
- iii) Adapted from Matzkevitch (1929). Adopted Thellung's hybridization theory but added a third parent in the hybridization scheme - the anthocyanin carrot of Afghanistan (ssp. "afghanicus").  
D. carota ssp. carota  
D. carota ssp. maximus  
D. carota ssp. afghanicus ] ---> hybridizations ---> D. carota  
ssp. sativus
- iv) Adapted from Zagorodskikh (1939). Hybridization scheme in the Mediterranean. Wild European forms were improved over centuries by man and intercrossed with Asian forms.  
wild European form --> selection --> advanced white coloured form X wild and cultivated Afghan forms ---> carotene forms  
yellow carrot X white coloured wild and cultivated Afghan forms ----> carotene forms

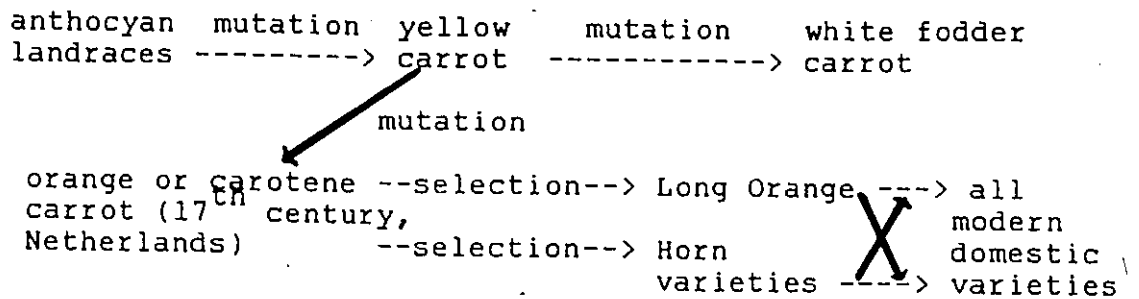
## B) Selection

- i) Adapted from Vilmorin (1840). Direct selection from wild forms grown in "cultivated" environments.  
wild ---> successive selection for desired traits (straight, large, red, tender) ---> high quality carotene cultivars
- ii) Adapted from Vavilov and Bukinich (1929). Selection in S. Afghanistan to N. India of wild anthocyan types.  
wild Afghan forms ---> selection ---> cultivated anthocyan Afghan forms

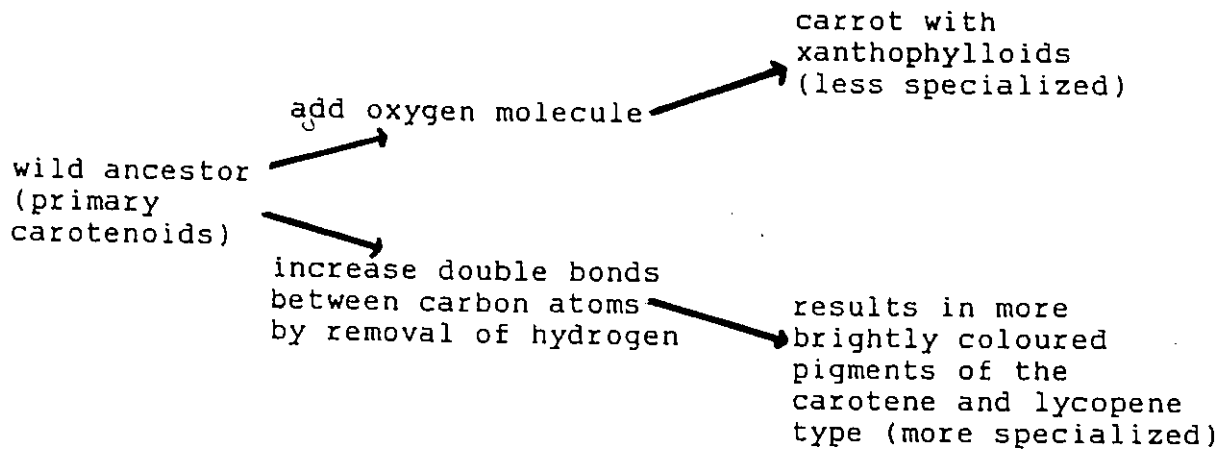
Figure 3.1 cont'd

C) Mutation

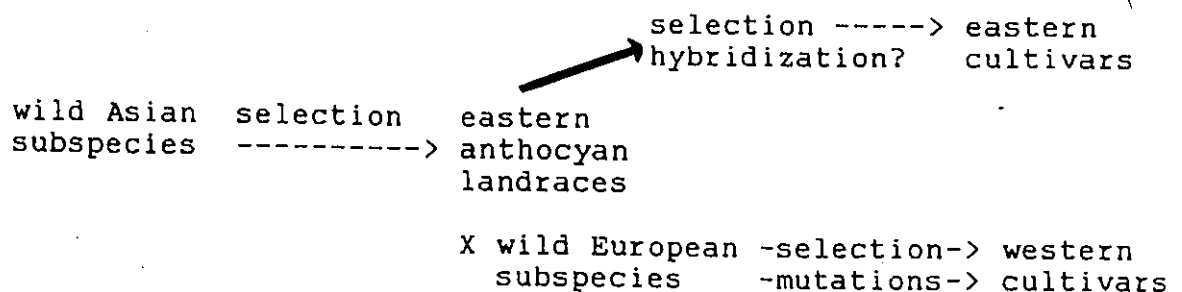
- i) Adapted from Banga (1963). Anthocyanin carrot originated in Afghanistan. The carotene form is the result of successive mutations and selections of these. It spread east and west in the tenth to twelfth centuries, arrived in Europe in the fourteenth and fifteenth centuries, China in the thirteenth century and Japan in the seventeenth century



- ii) Adapted from Lyubimenko et al., (1936). Mutations occurring in pigmentation production pathways.



- D) Present study: combination of selection, hybridization and mutations.



#### A) Hybridization:

1- hybridization processes only- mainly intercrossing of wild subspecies: Braun (1842), Krause (1904), Helweg (1908), Baur (1924), Thellung (1926), Rasmuson (1926), Matzkevitch (1929).

2- direct selection from wild (probably ssp. carota) and hybridization with wild and cultivated forms: Zagorodskikh (1939), Hiroe (1962).

#### B) Selection and/or mutation:

1- origin directly from wild subspecies, by selection and culture over a long period of time: Hoffman (1816), Vilmorin (1840), Rubashevskaya (1931).

2- resulted from a series of mutations and selections to the present day form: Banga (1963a), Lyubimenko et al., (1936).

The Middle East through Asia Minor includes the world's most heavily travelled corridor of human migration. Along the corridor a wide range of climates, soil types, and other geographic variables are encountered (Boswell, 1949; Zohary, 1973). Also, physical barriers such as mountain ranges and islands are prevalent. These are factors which restrict gene flow and create endemic populations susceptible to genetic drift. Ecotypes may persist in areas which were inhospitable to original colonists.

Banga (1963a, 1957), suggested that anthocyanin containing carrots were domesticated in Afghanistan and spread westward south of the Mediterranean with the influence of the Moors in the tenth to twelfth centuries, and to western Europe in the fourteenth and fifteenth centuries. Zagorodskikh (1939) suggested two western routes of dissemination, the former plus a

pathway north of the Mediterranean, via Iran, Turkey etc. The anthocyanin carrots reached China at the end of the thirteenth century, and Japan in the seventeenth century (Banga, 1963a). Banga (1963a) suggested that a yellow variant accompanied the purple type and that white and orange carrots are mutations of this yellow variant. Orange carrots were selected in the Netherlands in the seventeenth century (Banga, 1963a).

A great deal of pioneering work was sparked by the theories and findings of the great Russian agronomist N.I. Vavilov. Several Russian investigators have intensely studied the great diversity of wild and cultivated Asiatic forms. According to Matzkevitch (1929) the region in which the greatest morphological diversity occurs is in Afghanistan and surrounding regions, particularly at the junction of the Hindu Kush and Himalaya mountain ranges. The carrot is the most common food crop in Afghanistan (Vavilov and Bukinich, 1929), where it grows in mountainous regions up to 2650 meters above sea level. Vavilov and Bukinich (1929) believed that the carrot of southern Afghanistan and surrounding areas represents one of the basic world sources of the cultivated carrot. In this clearly defineable geographic zone are found many original forms, including the endemic violet carrot. The plants of this area have characters which are found in both cultivated and wild forms. Asia Minor (Turkey) is the area of convergence of European, Mediterranean, and Asiatic forms, resulting in a hybridization zone and an important secondary source of variability (Vavilov and Bukinich, 1929).

Other scientists (Matzkevitch, 1929; Zagorodskikh, 1939) believed the anthocyanin carrot was domesticated in Afghanistan and then spread eastward to Asia Minor where it hybridized with D. carota ssp. maximus. They suggested that white, yellow and orange fleshed carrots segregated from this hybrid mixture and were later domesticated.

Supporters of the hybridization theory include Thellung (1927), who postulated that ssp. sativus is the result of a ssp. carota x ssp. maximus hybridization in the Mediterranean region because it appears morphologically intermediate and Krause (1904) who believed that ssp. sativus results from a ssp. carota X ssp. major hybridization for the same reasons.

Still another theory states that the wild carrot can be converted into domestic forms in 3 or 4 generations under cultivated conditions (Vilmorin, 1840) and vice versa (Thellung, 1926b). Vilmorin's experiments have never been repeated, and it is highly unlikely that any changes would occur. Wild plants in a cultivated environment would still remain a "wild" plant (Boswell, 1947; Domin, 1943). Plants may grow larger and produce larger yields, but their genetic makeup is the same. The difference between cultivated and wild plants is that wild plants undergo random mating and natural selection, while cultivated plants are the result of many generations of controlled breeding and selection. Geneticists simply recombine the existing gene pool into desired combinations through controlled pollinations, and wise selection. A "degenerans" form has been described by Thellung (1926b) and others, which is postulated to be a reversion of the cultivated form into a "wild" form possessing

thin white roots, but this is unlikely. A more realistic explanation would be that these plants are the result of cultivated X wild subspecies hybridization, thereby possessing traits found in both parents.

There exist in both eastern and western wild groups, forms which tend to have a more cultivated habit. Zagorodskikh (1939) points out that roots of wild Asiatic types with a white surface, when uprooted, develop an anthocyanin colouration after a few days of exposure to light. This colouring does not occur in wild European forms. We can surmise that wild Asiatic forms with an innate capacity to produce anthocyanin pigmentation are the most probable ancestors of the Asiatic cultivated anthocyanin carrot. Brandenburg (1981) suggests that the wild European form may have played a minor role in the development of the modern cultivated form due to the occurrence of large fleshy white cultivars.

Until the seventeenth and eighteenth centuries, "red" carrots were actually purple in colour, as red cabbage and red beets are today (Banga, 1957, 1963a). Later, other carotene varieties were also called "red" such as the orange variety "Red Cored Chantenay" and the "carotte rouge a collet vert" (Wittmack, 1904). This source of confusion again renders historical notes difficult to interpret. The yellow type was preferred over the anthocyanin type for aesthetic reasons, as the water soluble anthocyan pigments would discolour dishes (Banga, 1963b). The yellow type therefore became the leading variety by the end of the eighteenth century. White types were first described at the end of the seventeenth century, but were largely used as cattle

feed. Orange carotene carrots first appeared in seventeenth century Dutch paintings. Eighteenth century writings described 2 types: a) a large, long, pale orange winter type carrot from which the "Long Orange" variety was developed, and b) a smaller, finer, strain of darker orange colour from which the "Horn" varieties, named after Horn, Netherlands, were selected (Helweg, 1908). All commercial carotene carrots arose from these two selections (Banga, 1963a, 1976) (figure 3.2). Anthocyan forms were introduced to Japan in the seventeenth century, and later the carotene type. Their introduction led to hybridizations, producing blood red root colouration, due to the large amounts of lycopenoid pigmentation (Zagorodkikh, 1939). The high humidity and deep tillage by hand favoured the production of long roots, resulting in Japanese cultivars with roots capable of attaining 1 meter in length.

Most authors agree with Matzkevitch that Afghanistan is the primary centre of dissemination, based on the great morphological diversity found in this area. However, no conclusive evidence has been found to prove either the hybridization, selection or mutation theories. It is hoped that a genetic study such as isozyme electrophoresis may help clarify the situation.

#### MUTATION THEORY

Carotene and anthocyan-anthochlor pigmentations vary in structure and cellular localization. Carotenes are membrane bound, hydrophobic molecules containing oxygen molecules in their structure. Anthocyanins are water soluble molecules, readily leached out, lacking oxygen molecules in their chemical makeup.



Figure 3.2 . Proposed routes of development of modern carotene carrot varieties. "Long orange" and the "Horn" varieties were selected from segregating carrot populations (Banga, 1963,1976).

- A- Long Orange --->Brunswick ---> Flanders, Flakkee, St.Valery, Bauers Kieler Rote, Meaux etc.
- B- Horn varieties i) Late Half Long Horn ---> James Intermediate, Langendijker, Danvers, Luc, LaMerveille, Grosse Normande, Guerande, Chantenay etc.
- ii) Early Half Long Horn ---> Croissy, Montesson, Utrecht Forcing, Vertou, Amsterdam Forcing, Nantes etc.
- iii) Early Short Horn ---> Grelot, Parisienne, Davanture, Planet etc.

It is evident that these represent two distinct groups of diverse origin. It is possible that, through point mutations, the biosynthetic pathway leading to pigmentation production is blocked or altered, creating various colour morphs. Instability in colour patterns, morphological habit, and fruit shape, have been reported in cultivated crops (Pearson, 1968; Mutschler and Pearson, 1987). Mutations affecting carotene biosynthesis in microorganisms have been investigated (Jensen et al., 1958). Subtle physiological differences such as changes in temperature and photoperiod, may effect the conversion of carotenoids (Lyubimenko et al., 1936), as heat increases the frequency of off-types in unstable crop populations (Mutschler and Pearson, 1987). It has been suggested that white and yellow rooted carrots have biosynthetic blocks at different points in the sequential development of carotene bodies from proplastids (Ben Shaul and Klein, 1965). Biosynthetic pathways determine the final pigmentation of a particular cell.

A distinctive carotenoid was found in wild forms and is considered the initial pigment in the development of the various carrot pigmentations (Lyubimenko et al., 1936). Xanthophylls were determined to be the least modified derivative of this primary carotenoid. This is supported by the observation that an increase in xanthophyll is accompanied by a decrease in alpha carotene (Imam and Gabelman, 1968). Also, the conversion of lycopenes to beta carotenes in leaf chloroplasts of carrots has been demonstrated, showing their close relationship (Umiel and Gabelman, 1972).

Pigment development is believed to follow two basic

directions in carotene type carrots: 1) towards the formation of xanthophyll and xanthophylloids through the introduction of an oxygen molecule, and 2) towards the development of more brightly coloured pigments of the carotene and lycopene type through the increase in number of double bonds by the removal of hydrogen (Lyubimenko et al., 1936, figure 3.1).

There may be genes controlling the hydroxylation or dehydrogenation of particular pigments which are independent of pigment distribution, as has been postulated for the anthocyanin production in radish (Harborne and Paxman, 1963). An example is the conversion of phytoene to lycopene which involves a stepwise dehydrogenation through the removal of two hydrogen atoms and the formation of a double bond (Umiel and Gabelman, 1972). Understanding the biosynthetic pathways in pigmentation production may help answer questions on carrot origin and distribution. The isolation of enzymes such as cyanidin 3-o-glucosyltransferase from an anthocyanin containing carrot (Petersen and Soitz, 1986) is well on the way to understanding these pathways.

Environmental constraints may also affect root coloration. Banga and DeBruyn (1964) indicate that higher temperatures (greater physiological activity) and greater root weight (more available carbohydrates), as well as increased relative growth rate and available plant nutrients, increase the production of root carotene pigmentations. The intensification of root colour observed in cultivated varieties can be attributed to an increase in the concentration of soluble carbohydrates within

cells (Lyubimenko et al., 1936). An excess of sugars and other intermediate compounds of general carbohydrate metabolism may alter intracellular physiological relations, favouring pigment synthesis (Lyubimenko et al., 1936; Banga and DeBruyn, 1964). When there is competition for these materials, protein synthesis takes priority (Banga and DeBruyn, 1964). Environmental stress and less fertile soils such as those encountered by wild forms, may partially explain the reduction in pigmentations found in these populations.

Carbohydrate allocations vary among carrot groups. Sugar content varies from 4.8 to 5.1% in anthocyan types, from 5.1 to 6.0% in yellow types, and from 8.1 to 8.3% in carotene types (Vavilov and Bukinich, 1929. Sizova (1957) found the largest and the widest spectrum of deposition of starch granules in a semi-cultivated accession from Asia Minor.

In order to clarify theories of origin, isozymes could be utilized as genetic markers to trace gene flow and pinpoint regions with rare and unique alleles. This method has been successfully used with other crop species (Gepts et al., 1986, McLeod et al., 1982)

#### METHODS

Isozymes were utilized as genetic markers to construct maps of spatial distribution of gene frequencies. Allelic frequency data from chapter 1 were utilized to construct maps bearing pie chart configurations representing the allelic frequencies of each accession surveyed. Maps of Eurasian cultivar-landraces, wild Eurasian accessions, and other wild and cultivated accessions

were constructed. Alleles used to construct these maps were among the most consistently resolved polymorphic loci. A common allozyme (TPI-1-D), two less frequent allozymes (PGM-1-B, PGM-1-C), and two very infrequent allozymes (PGI-2-A, TPI-1-C), were included (figures 3.3 to 3.7) in an attempt to assess possible geographic patterns in allelic frequencies and distribution.

## RESULTS AND DISCUSSION

### a) Cultivated Accessions

TPI-1-D is a common allozyme, occurring at high frequencies in some populations (figure 3.3 a) but absent in others. No geographic pattern is distinguishable. Populations in adjacent areas may be either fixed or lack this allele, particularly in Middle Eastern and Asian accessions.

Utilizing less frequent allozymes, a more distinctive trend is apparent. Allozymes PGM-1-B and PGM-1-C are found in three geographic regions (figure 3.4 a and 3.5 a respectively). One area includes Afghanistan and surrounding areas (1 Afghan, 1 north Indian, and 2 southern Russian provinces bordering Afghanistan for PGM-1-C, as well as 1 Afghan, and 2 Indian accessions for PGM-1-B). The second area includes Turkey and Iran (3 Turkish and 1 west Iranian accession for both loci). The third area includes the Netherlands and other early cultivar developing countries (3 Dutch accessions for PGM-1-C and 1 Danish and 4 North American cultivars having PGM-1-B).

Very infrequent allozymes (figure 3.6 a, 3.7 a) give a small yet important piece of information. TPI-1-C is only present in 1

Figure 3.3. Maps of the spatial distribution of TPI-1-D. Pie chart representation of the allozyme's frequency are shown for Eurasian cultivated (3.3 A), Eurasian wild (3.3 B), and global wild and cultivated (3.3 C). North American populations in figure 3.3 C are wild ssp. carota, all other populations are cultivars.

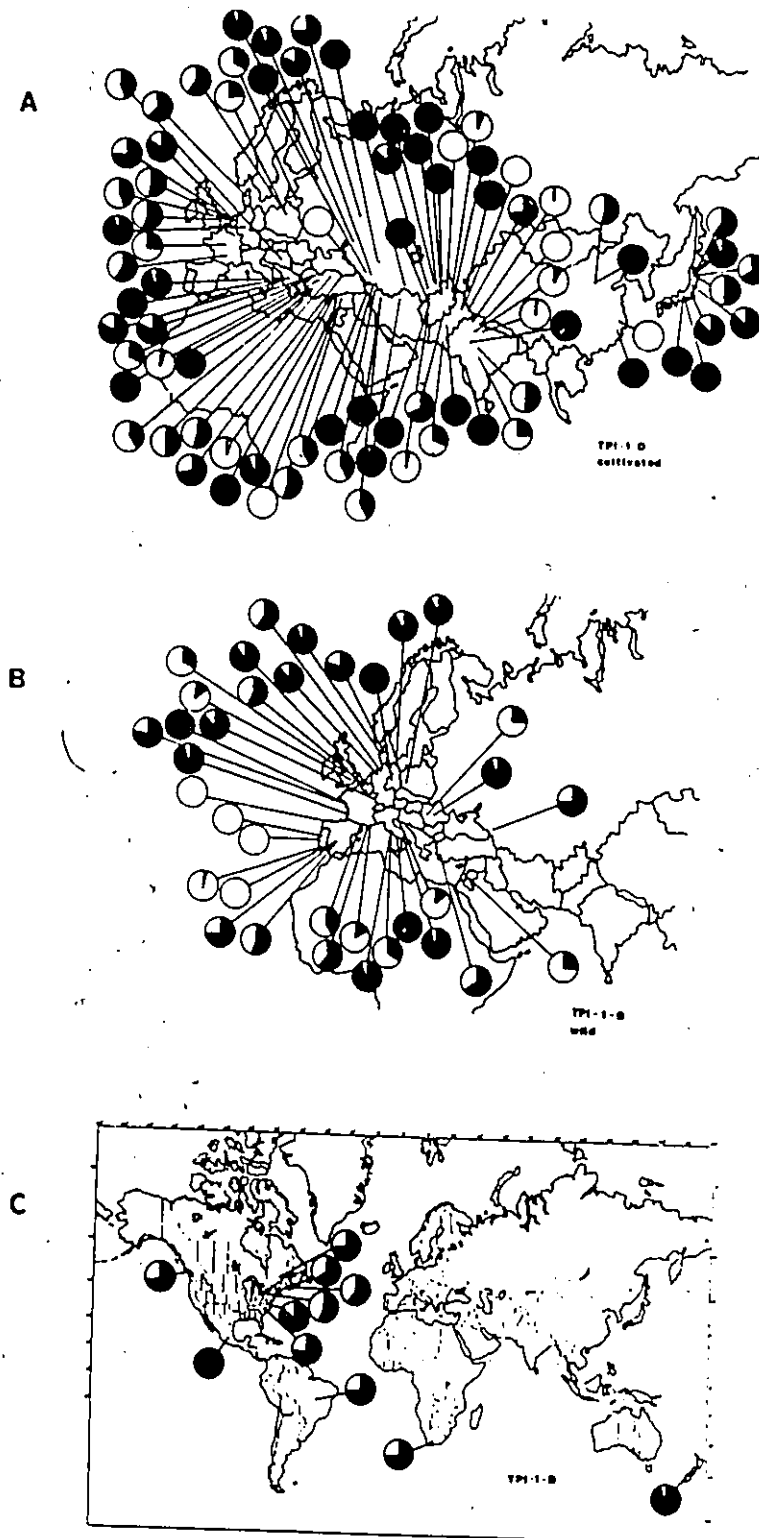


Figure 3.4. Maps of the spatial distribution of PGM-1-B. Pie chart representation of the allozyme's frequency are shown for Eurasian cultivated (3.4 A), Eurasian wild (3.4 B), and global wild and cultivated (3.4 C). North American populations in figure 3.4 C are wild ssp. carota, all other populations are cultivars.

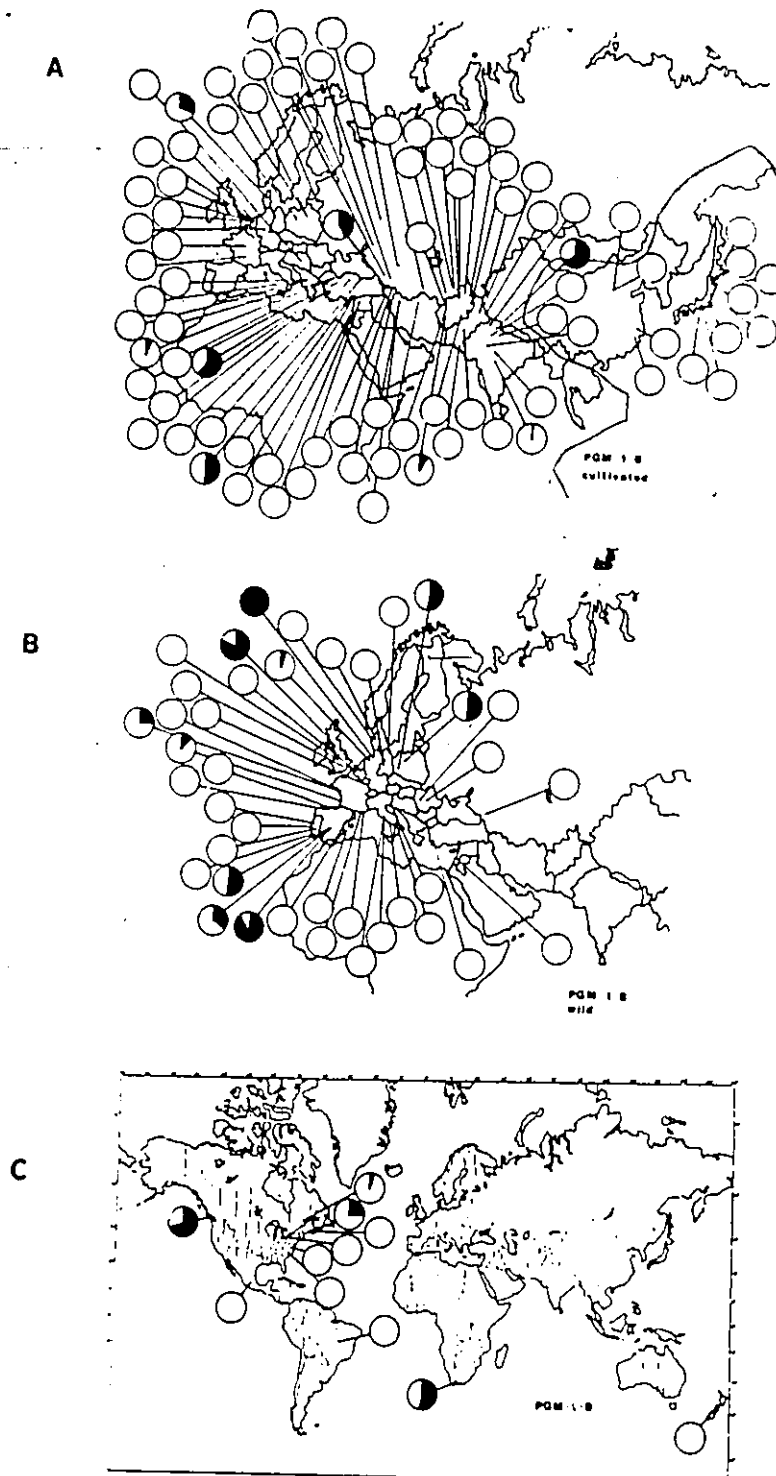


Figure 3.5. Maps of the spatial distribution of PGM-1-C. Pie chart representation of the allozyme's frequency are shown for Eurasian cultivated (3.5 A), Eurasian wild (3.5 B) and global wild and cultivated (3.5 C). North American populations in figure 3.5 C are wild ssp. carota, all other populations are cultivars.

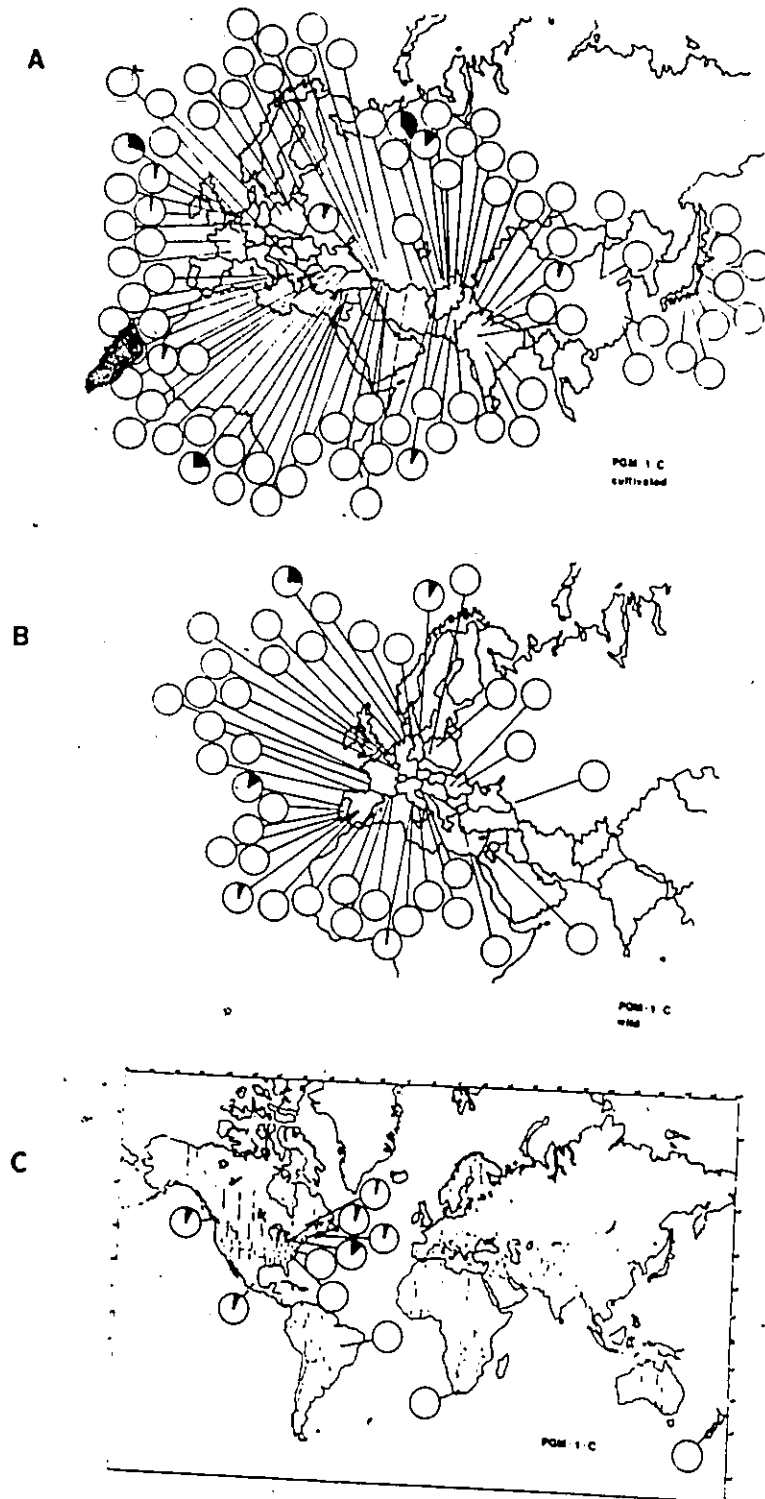




Figure 3.6. Maps of the spatial distribution of TPI-1-C. Pie chart representation of the allozyme's frequency are shown for Eurasian cultivated (3.6 A), Eurasian wild (3.6 B) and global wild and cultivated (3.6 C). North American populations in figure 3.6 C are wild ssp. carota, all other populations are cultivars.

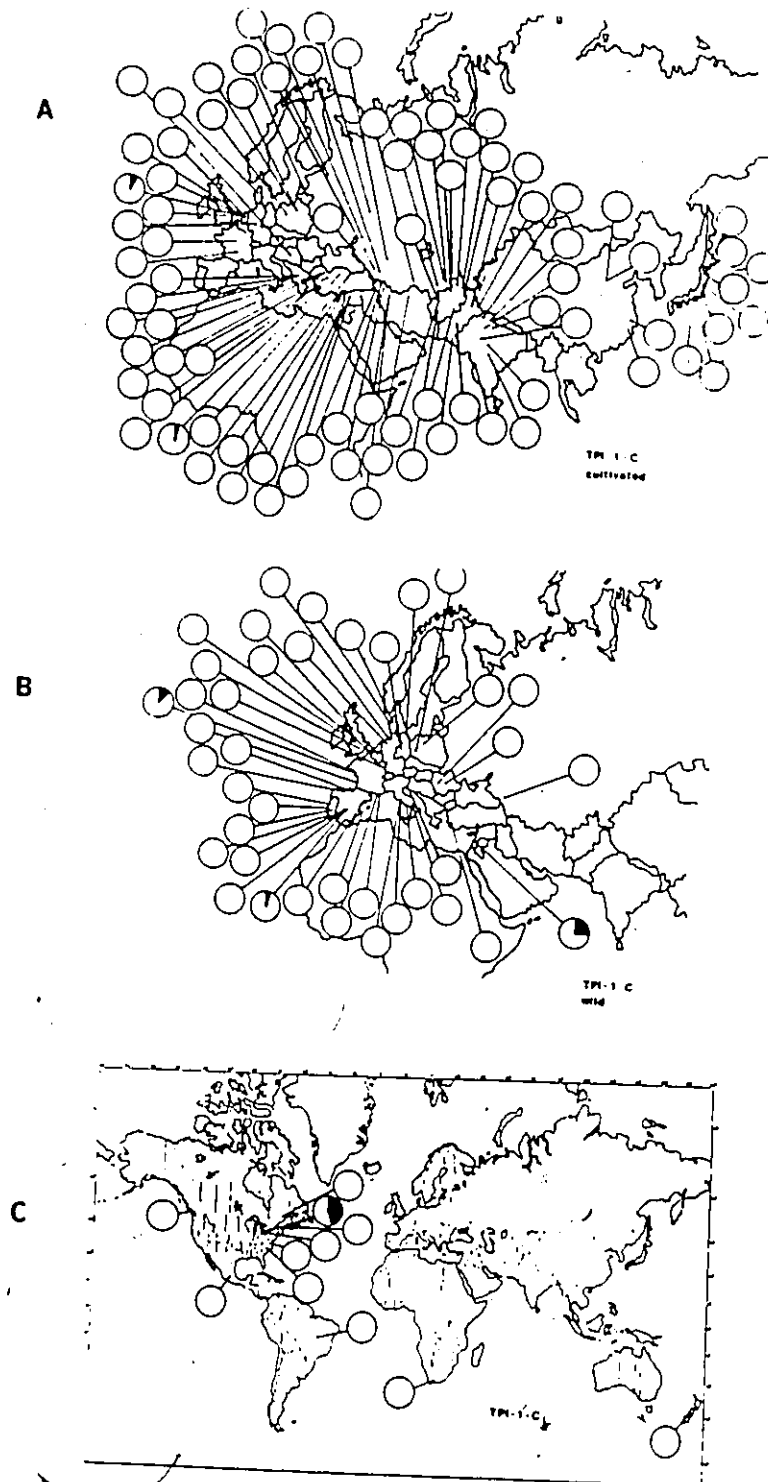
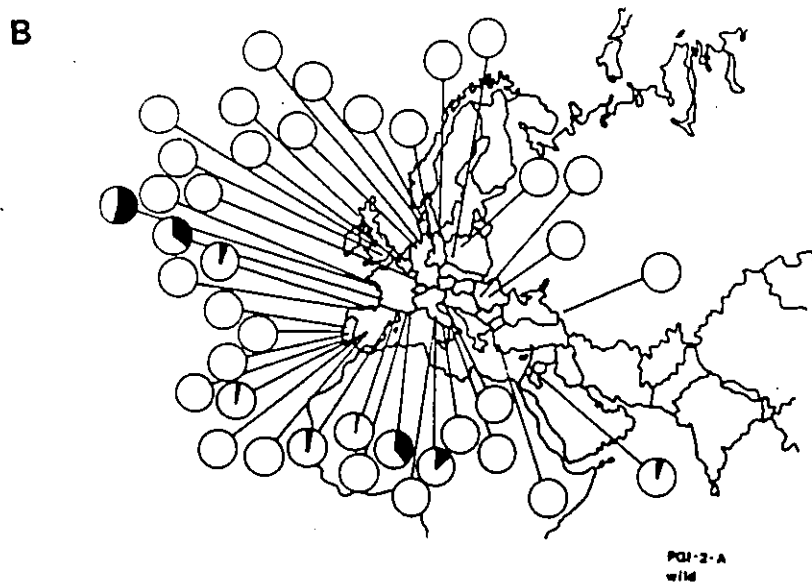
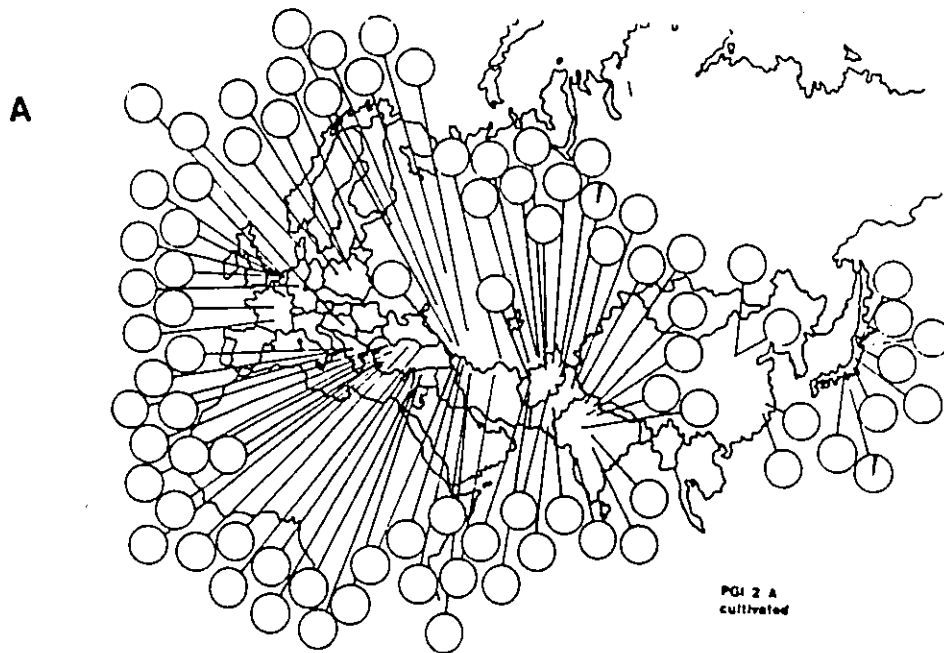


Figure 3.7. Maps of the spatial distribution of PGI-2-A. Pie chart representation of the allozyme's frequency are shown for Eurasian cultivated (3.7 A) and Eurasian wild (3.7 B). The allozyme was not observed in other populations.



Turkish, 1 Dutch and 2 North American accessions, while PGI-2-A, an allozyme found in wild coastal species, is extremely rare in cultivated accessions, occurring only in 1 Turkish and 1 Dutch accession. In both cases it is a very infrequent allozyme, with  $q = 0.02$ .

#### B) Wild Accessions

Again, TPI-1-D is a common and widespread allozyme in most accessions. This allozyme variant is virtually lacking (figure 3.3 b) in the Iberian peninsula, particularly in Portugal. It is present in every other wild accession analyzed.

The two less frequent allozymes, PGM-1-B and PGM-1-C, are scattered throughout wild European accessions, producing little or no pattern (Figures 3.4 b and 3.5 b respectively). These two allozymes are scattered throughout Europe in wild accessions in low frequencies. PGM-1-B does not occur in many populations, but it is relatively common in the populations in which it does occur, and is fixed in 1 West German accession. They nevertheless demonstrate a pattern in North America, where they are present in most (PGB-1-B), or all (PGM-1-C) wild Canadian populations, yet are both lacking in the two American populations.

In wild accessions, the very infrequent PGI-2-A allozyme is exclusively found in Atlantic and Mediterranean coastal populations, principally subspecies in the gingidium complex, or members of the carota complex in this area. It is totally absent in populations outside of Eurasia. TPI-1-C occurs in 4 wild accessions, 1 French, 1 Spanish, 1 Israeli, and 1 Canadian, showing no real pattern.

It has been suggested, that the degree of polymorphism decreases from the centre of the distribution of a species to the periphery of its range (Mayr, 1963). Hedrick (1985) introduced the concept of the probability of occurrence of unique genotypes (U) to the centre-of-origin argument. U allows one to infer the ancestry of a population or species. The population with the greatest number of unique or rare alleles is assumed to be the most ancestral, as all other populations are a subset of this. The U concept, species or subspecies pairing of similar rare alleles and morphological and cytological data are tools utilized to infer centres of origin or areas of divergence in several crop species. This includes Glycine max (Kiang and Gorman, 1983), Phaseolis vulgaris (Gepts et al., 1986; Gepts and Bliss, 1986), Cicer arietinum L. (Tuwafé et al., 1988), Cucurbita pepo L. (Decker, 1988), Capsicum sps. (McLeod et al., 1982, 1983) and Oryza sps. (Glaszmann, 1987; Second, 1982).

Wild progenitor-derivative species pairs have also been deduced or substantiated through electrophoretic variants. Derivative species are less variable genetically (50% less in Lasthenia maritima (Crawford et al., 1985)), have fewer polymorphic loci and lower observed heterozygosity (Loveless and Hamrick, 1984), and fewer alleles per locus (Nakai, 1981) than their progenitor species. Differences in environmental variation and differential selection pressures account for genetic and spatial patterns observed (Loveless and Hamrick, 1984).

Applying these concepts to cultivated D. carota, we find clues from each allozyme observed, suggesting possible gene flow patterns. Electrophoretic data tends to support morphological

studies, in that Afghanistan and Turkey not only act as centres of morphological diversity, but also centres of maximum allelic variation. Many allelic variants, (PGM-1-B, PGM-1-C, PGI-2-A and TPI-1-C) rarely observed in the species as a whole, are found in these two regions. Introgression of these very infrequent allozymes is slight, therefore their presence in the Netherlands, Denmark and Japan seem to substantiate earlier beliefs that germplasm from these two centres were transported to areas of varietal development and distribution. Radiation of cultivar forms in secondary zones is well documented (Banga, 1963a; Hiroe, 1962; Small, 1978).

The patchy distribution of TPI-1-D in Middle Eastern and Asian accessions may be attributed to barriers to gene flow resulting from the geographic relief of these areas. Artificial manipulation and distribution of the crop by man could also cause the erratic pattern observed. Seeds of desirable landraces have undoubtedly been dispersed by man through commerce for centuries, producing a mosaic of genotype distribution worldwide.

Wild subspecies do not necessarily form distinct groups with particular allozymes. PGI-2-A is found in 9 accessions, all originating from the Mediterranean or Atlantic coasts, but these include ssp. commutatus (2), ssp. maximus (2), ssp. gadecaei (1), ssp. carota (1), ssp. major (1), ssp. gummifer (1), and subspecies of the gingidium group (1). In the populations in which it does occur, a 10 to 20 fold increase in frequency is observed in members of the gingidium complex as compared to members of the carota complex. This allozyme was not found in

every accession of the gingidium complex, therefore it cannot be labelled as a diagnostic feature of the group; it does, however, characteristically occur in higher frequencies in these accessions. Its occurrence in members of the carota group is probably due to introgression of germplasm in sympatric populations. Its extremely infrequent occurrence in cultivated material supports Small's (1978) contention that members of the gingidium complex contributed very little to the evolution of the cultivated carrot. The absence of TPI-1-D in most Portuguese and Spanish accessions may suggest a genetic bottleneck in these accessions. The peninsula and the arid interior climate of this region probably restricts the number of suitable areas for plant establishment and gene flow. North African accessions would have been useful in determining the relative genetic variability in this area.

Additional wild Asiatic material would have been useful in estimating the actual distribution of infrequent alleles. At present, all infrequent alleles observed in cultivated Asian and European accessions are available in wild accessions from Europe, the Middle East and Asia. Perhaps additional allelic forms, not found in any European material exist in wild Asian germplasm. If so, they were not detected in landraces and semi-cultivars of this region which have most likely been derived from the wild populations. The fact that no unique alleles were found in the few wild Asian or eastern atrorubens type does not exclude, but certainly does not give reason to expect the occurrence of additional allelic forms in wild Asian populations.

It is plausible to suggest, based on the geographic

distribution of PGM-1-B and PGM-1-C, that D. carota ssp. carota arose and radiated in North America from multiple introductions. These two allelic variants were observed in northern populations, but not in southern populations. Additional southeastern accessions would be helpful in estimating the distribution pattern of this allozyme.

When specific allelic markers are available for each species or subspecies group, electrophoresis is an excellent technique to test the hypothesis of hybrid origin (Crawford et al., 1985). Marker alleles were not available in this study, largely due to a lack of gene flow barriers between the groups. A few allozymic markers can be utilized to make broad generalizations about potential taxa involved in the development of cultivated forms. PGI-2-C is found in all members of the carota complex at frequencies less than 0.10, (0.01 in sativus and 0.04 in atrorubens), except in ssp. maximus, in which it is found at a frequency of 0.295 (table 1.2). This allozyme is completely lacking in all members of the gingidium group. Therefore, ssp. maximus may have contributed to the germplasm of cultivars. Likewise, members of the gingidium group are excluded. MDH-5-A is relatively infrequent in all groups (less than 0.09 and only 0.019 in domesticated groups), except ssp. maritimus (0.278), ssp. gingidium (0.165), and ssp. gummifer (0.359). It is completely lacking in ssps. drapanensis, commutatus, and major (table 1.2). This suggests ssp. maritimus may have contributed to the cultivated carrot's germplasm as ssps. gingidium and gummifer were previously excluded. PGM-1-C occurs at a frequency

of 0.029 in cultivated forms. It is found at a frequency of 0.035 in ssp. maritimus, 0.241 in ssp. carota, 0.009 in ssp. commutatus, 0.107 in ssp. gummifer and is absent in all other subspecies (table 1.2). Subspecies carota maintains this allozyme in a high frequency and thus may be involved in the development of cultivated forms. PGM-1-B occurs at a frequency of 0.06 in cultivated forms. It is also found in ssp. maritimus (0.340), ssp. maximus (0.213), ssp. carota (0.417), ssp. gummifer (0.107), and ssp. gadecaei (0.10) (table 1.2). Again, ssps. maritimus, maximus and carota may have been involved in the introgression of this allele in the cultivated form. ADH-1-B occurs at a frequency of 0.093 in cultivated forms. It occurs in ssp. maximus (0.034), ssp. major (0.555), ssp. carota (0.140), and ssp. gummifer (0.095), and absent in all other subspecies (table 1.2). Subspecies major maintains this allele in high frequency.

Subspecies which maintain relatively high allelic frequencies and thus have potentially contributed to the cultivated forms' germplasm include ssps. maximus (PGI-2-C, PGM-1-B), maritimus (MDH-5-A, PGM-1-C, PGM-1-B), carota (PGM-1-C, PGM-1-B, ADH-1-B), and major (ADH-1-B). However, all subspecies have been excluded due to the absence of one or more alleles except ssp. carota. Allelic forms which are rarely observed in cultivated forms yet are observed in gingidium type subspecies, cannot be attributed to these morphotypes because PGI-2-A, which is found in many of these accessions, is not found in cultivated forms.

One may argue that these alleles may be present in a single



subspecies population, which was not included in this analysis, leading to the selection or mutation of this particular form, and subsequently evolving to the present form. One might also argue the converse, the absence of an allozyme in a wild subspecies but its presence in cultivated forms does not exclude their contribution. Both are highly unlikely, as it would take a highly variable form to maintain this genetic diversity. Instead, a more reasonable explanation would be that the domesticated forms arose through selection, mutation and, intentionally or unintentionally, hybridization subsequently occurred with wild subspecies.

A unique allele was observed in a single ssp. sativus accession. A North American carotene cultivar possesses an ADH-1-C allele, not recorded in any other population (table 1.2). This may be the result of a mutational event during the evolution of this variety. Accessions which maintain rare or unique alleles may also possess useful and unique morphological traits. They represent potential sources of germplasm for exploitation in cultivated forms. Major sources for each infrequent allozymic form are listed in table 3.1. The rare allele may not benefit the plant in any any useful agronomic way, but it represents a deviation from the average genotype and thus potentially useful genetic and morphological traits may be present in these accessions.

Because all groups studied are subgroups within a single species, genetic similarity is evident, making it difficult to find unique allelic forms associated with any particular

Table 3.1.

Potential sources of infrequently found allozymes in cultivated taxa of D. carota for germplasm exploitation. Presented are average frequencies of these allozymes in cultivated and wild taxa as well as the subgroup which displayed the highest frequency of the allozyme in table 1.2.

allozyme	Frequency in cultivated	Frequency in wild	Major source	Value
-----	-----	-----	-----	-----
ADH-1-C	0.001	0.000	sativus	0.001
GDH-1-A	0.039	0.093	maritimus	0.200
MDH-5-A	0.019	0.109	gummifer	0.359
PGI-2-A	0.000	0.034	gummifer	0.210
PGI-2-C	0.048	0.048	maximus	0.295
PGM-1-B	0.060	0.100	maritimus	0.340
PGM-1-C	0.029	0.020	gummifer	0.107
TPI-1-A	0.000	0.014	maritimus	0.072
TPI-1-C	0.001	0.012	maximus	0.060

subspecies. Allelic forms which are common in cultivated forms are also common in wild accessions. Alleles, rarely observed in cultivated forms, are common in several different wild subspecies.

Determining centres of genetic variability requires a thorough knowledge of the crop and its wild relatives, and their variability must be studied carefully (Vavilov, 1949-50). The domestication pattern for D. carota does not follow the models of Vavilov (1949-50). Rather, it is that of a polycentric crop (Harlan, 1975) because there is a core area of wide genetic variability (Afghanistan) where probable progenitors are endemic, based on morphological data (Matzkevitch, 1929; Vavilov and Bukinich, 1929; Small, 1978), and the occurrence of infrequent alleles. However, a second area (Turkey) appears to maintain as much genetic variability, and can therefore be considered as a centre of diversity (Harlan, 1971). Rare alleles are further detected in European and North American germplasm, where the crop has been distributed and further domesticated. This pattern of radiation and further domestication in other continents is certainly non centric in nature. Wild relatives of the early cultivars are widely distributed around the Mediterranean and in Asia Minor, and it is in Turkey and surrounding areas where eastern landraces and Eurasian wild species co-exist. Reports suggest their co-occurrence is a recent one, occurring during the Pliocene, and not earlier, when the western part of the Middle East was invaded by Mediterranean vegetation coming from the north-west (Zohary, 1973).

It is quite probable that the two distinctive cultivated

forms arose from two different areas. Multiple centres of origin have been described for many crop species, including Phaseolus vulgaris (Gepts et al., 1986; Gepts and Bliss, 1986), Cucurbita pepo (Decker, 1988), Capsicum sps. (McLeod et al., 1982, 1983) and Oryza sps. (Second, 1982; Glaszmann, 1987). These species show many variants, owing to the wide variability found in wild progenitor species.

It is unfortunate that allelic variants specific to each group were not detected. However, with a mean genetic distance of 0.016, that is understandable. The high level of intra-sub-specific genetic variability and the low  $D_{st}$  values among subspecies makes it difficult to utilize this data for phylogenetic assessments. There are no allelic variants which are found solely in cultivars and landraces, therefore we cannot presume that western varieties arose solely from eastern varieties.

The two cultivated forms approach one another in genetic variability, yet certain morphological traits divide them. Therefore, it is reasonable to suggest two alternate routes of domestication for the two major cultivar groupings. Speculation is required, as allelic frequency data does not substantiate a solid conclusion. The domestication of the eastern group is a simple one. Endemic anthocyan and anthochlor landraces of the southern Russian province of Tadzhik, Afghanistan, West Pakistan and north west India (Vavilov and Bukinich, 1929) may have been gradually improved and domesticated by the people of these regions through selection and hybridization with local wild

subspecies. These may represent the earliest cultivars and were thus distributed across the continent to Turkey by the eleventh century and to Japan by the seventeenth century (Banga, 1957). They were distributed to Europe by the thirteenth to fourteenth centuries (Banga, 1957). These carrots were of low quality, being low in soluble sugars, having water soluble pigments and bolting in the first year. Therefore, they produced only mediocre root size and had poor overwinter keeping qualities. Consequently they were not a highly valued crop and were probably reserved for lower income people as a food source and for animals as a fodder crop. With the introduction of carotene varieties in Europe, the anthocyan types were discontinued by European seedsmen (Vilmorin-Andrieux, 1885) and their popularity was restricted to the Middle East, the Orient and Asian countries, which had always utilized them in staple dishes including 'plov' (Matzkevitch, 1929).

The domestication of the western grouping requires more speculation. It has the root size and shape of eastern cultigens, yet possesses morphological and pigmentation traits available in wild Mediterranean subspecies. Electrophoretic data demonstrates a higher degree of genetic homology between eastern and western cultivars and to a lesser extent, though highly significant, to wild subspecies in the D. carota complex. The most likely occurrence would be the development of western type cultigens in a zone of hybridization, where many of the subspecies occur in sympatry, such as Asia Minor. This would support the theory that Turkey and surrounding areas act as a zone of differentiation.

Mutants are prevalent in the species, noted in wild subspecies by the unique disaccharide pigment in ssp. maritimus, in cultivated forms by the unique allelic form ADH-1-C in the cultivar "Chantenay Long", their transition from annual to biennial habit, and the presence of red lycopoid carrots in Japan. The red locus is epistatic to the orange locus even when it is homozygous recessive. Infrequent alleles, alien to parental populations or in much greater frequencies in these populations may have been instilled in subsequent hybrid swarm populations. Numerous agroecotypes may have been selected for at this time, allowing for a wide dispersion of carrot cultivation in ecologically and anthropologically diverse environments. Introgression of genes from wild subspecies has undoubtedly contributed to the genetic variability of the domesticated carrot, allowing for the production of highly adaptive and valuable varieties.

## CONCLUSION

The breeding system of a plant species is the major factor influencing its genetic structure. Predominantly outcrossing species maintain higher levels of intrapopulation genetic diversity than predominantly inbreeding species (Brown, 1979; Hamrick et al, 1979; Gottlieb, 1981). Factors promoting pollen and seed dispersal between populations enhance the dispersal of alleles among widely separated individuals and reduce the differentiation within the species (Loveless and Hamrick, 1984). New or rare alleles are introduced into populations which encounter varying selective pressures and stochastic events. The breeding system, ecological habits and life history traits of D. carota provide potential for high genetic diversity. These factors help explain the relatively high genetic diversity values encountered. A majority of the variability within the complex is maintained within rather than among taxa. The taxa surveyed represent one morphologically and genetically diverse species and thus should be delimited at the subspecies and forma speciales levels. Cultivated accessions represent a genetically uniform taxon. They possess a reduction in genetic variability in all aspects when compared to the genetically more diverse wild taxa, indicating the occurrence of a bottleneck period and the strict selective pressures maintained in an agricultural environment. The four varieties observed demonstrate highly uniform, genetically similar lines, suggesting that the seed companies maintain their genetic integrity.

Spatial distribution of allelic frequency data tend to support the historical distribution of carrot germplasm schemes

proposed by Banga (1963a) and others. Two areas, Afghanistan and Turkey seem to display the greatest amounts of genetic variation within the complex. These two areas possess the greatest degree of morphological diversity and therefore are considered key areas of germplasm accession and preservation. No doubt evolutionary changes in D. carota continue and are multifaceted, particularly in cultivated populations, and the species will continue to diverge as spatial and temporal variables affect it.





Appendix A. Germplasm sources and taxonomic assessments. Where "A" is the taxonomic assessment according to the scheme of Heywood (1968), (fig. 1.3), "B" is the taxonomic assessment according to the scheme of Small (1978), (fig. 1.4), and "Status" represents the cultivated status of each accession (W = wild, S-CV = semi-cultivated, and CV = cultivated).

Acc. No.	Source*	Site of Collection or "Cultivar Name"	ID Number	A	B	Status
1	1	Univ.Sienna, Italy	127/83	COM	F	W
2		Frankreich, GDR	124/80	MAR	D	W
3		Suditalen	215/84	CAR	D	W
4		Univ.Halle, FRG	126/85	CAR	D	W
5		Univ.Frankfurt, GDR	134/77	GUM	F	W
6		Leningrad, USSR	214/83	ATS	B	S-CV
7	2	"Lucky's Gold"		SAT	A	CV
8		"A-Plus"		SAT	A	CV
9		"Candy Pak"		SAT	A	CV
10		"Amsterdam Minicor"		SAT	A	CV
11		"Scarlet Nantes"		SAT	A	CV
12		"R. Cored Chantenay"		SAT	A	CV
13	3	"Goldpak"		SAT	A	CV
14		"R. Cored Chantenay"		SAT	A	CV
15		"Oranza"		SAT	A	CV
16		"Nantucket F1"		SAT	A	CV
17		"Mokum F1"		SAT	A	CV
18		"A-Plus"		SAT	A	CV
19	4	"Danvers H. Long"		SAT	A	CV
20		"R. Cored Chantenay"		SAT	A	CV
21		"Short'n Sweet"		SAT	A	CV
22	5	"Lindoro"		SAT	A	CV
23		"A-Plus"		SAT	A	CV
24		"Chantenay Long"		SAT	A	CV
25		"Red Chantenay"		SAT	A	CV
26		"Danvers H. Long"		SAT	A	CV
27		"Imperator"		SAT	A	CV
28	6	"Scarlet Nantes"		SAT	A	CV
29		"Touchon"		SAT	A	CV
30		"Spartan Delight"		SAT	A	CV
31		"Planet"		SAT	A	CV
32		"Mini Express"		SAT	A	CV
33		"Little Finger"		SAT	A	CV
34		"Pioneer"		SAT	A	CV
35		"Sucram"		SAT	A	CV
36	6	Charlottetown, PEI	81-122	CAR	D	W
37	7	Kentville, NS		CAR	D	W
38	8	St.Jean, PQ		CAR	D	W
39	9	Vancouver, BC		CAR	D	W
40	10	St.Joachim, ON		CAR	D	W
41	11	"Scarlet Nantes"		SAT	A	CV
42	12	"BETA III"		SAT	A	CV
43	13	Lahore, PAK	163235	ATS	B	S-CV
44		Delhi, INDIA	163238	ATS	B	S-CV

## Appendix A cont'd

45	Amritsar, INDIA	163240	ATS	B	S-CV
46	Jullundur, INDIA	163241	ATS	B	S-CV
47	Khandwa, INDIA	164136	ATS	B	S-CV
48	Jakhal, INDIA	164388	ATS	B	S-CV
49	Jaipur, INDIA	164484	ATS	B	S-CV
50	Hubli, INDIA	164689	ATS	B	S-CV
51	Ankara, TURKEY	165051	ATS	B	S-CV
52	INDIA	165522	SAT	A	CV
53	Adana, TURKEY	167082	ATS	B	S-CV
54	Mersin, TURKEY	167211	ATR	C	S-CV
55	Mugla, TURKEY	169480	ATS	B	S-CV
56	Soma, TURKEY	169482	ATS	B	S-CV
57	Bergama, TURKEY	169483	ATS	B	S-CV
58	Istanbul, TURKEY	169484	ATR	C	S-CV
59	Babaeski, TURKEY	169486	ATS	B	S-CV
60	Ezine, TURKEY	169488	ATS	B	S-CV
61	Bursa, TURKEY	169490	ATR	C	S-CV
62	Turhal, TURKEY	171641	ATS	B	S-CV
63	Tokat, TURKEY	171642	ATS	B	S-CV
64	Dehra Dun, INDIA	174828	ATS	B	S-CV
65	Bareilly, INDIA	175132	ATS	B	S-CV
66	Mirpurkhas, PAK	181052	ATS	B	S-CV
67	Chtura, LEBANON	181765	SAT	A	CV
68	Chtura, LEBANON	181766	ATS	B	S-CV
69	Aleppo, SYRIA	181880	ATS	B	S-CV
70	AFGHANISTAN	200876	ATS	B	S-CV
71	Faizabad, AFGH	211590	ATR	C	S-CV
72	Mozari-Sharif, AFGH	211591	ATS	B	S-CV
73	Herat, AFGH	211592	ATS	B	S-CV
74	Herat, AFGH	220657	ATR	C	S-CV
75	Kunduz, AFGH	220795	ATR	C	S-CV
76	Gardez, AFGH	221924	ATR	C	S-CV
77	Tehran, IRAN	222249	ATR	C	S-CV
78	Tehran, IRAN	222250	ATS	B	S-CV
79	Bezaiyeh, IRAN	222794	ATR	C	S-CV
80	Tabriz, IRAN	223360	ATR	C	S-CV
81	Ardabil, IRAN	223361	ATR	C	S-CV
82	Khoy, IRAN	223362	ATR	C	S-CV
83	Jalalabad, AFGH	223504	ATS	B	S-CV
84	Copenhagen, DEN	225868	SAT	A	CV
85	Izumi, JAPAN	226043	ATS	B	S-CV
86	Warmenluzen, NETH	230723	SAT	A	CV
87	Paris, FRANCE	261783	ATS	B	S-CV
88	Osaka, JAPAN	264543	ATS	B	S-CV
89	Hangu, W.PAK	269485	ATR	C	S-CV
90	Warsaw, POLAND	285620	SAT	A	CV
91	Warsaw, POLAND	285621	SAT	A	CV
92	Hiratsuka, JAPAN	294080	SAT	A	CV
93	Hiratsuka, JAPAN	294084	ATS	B	S-CV
94	Hiratsuka, JAPAN	294087	SAT	A	CV
95	Hiratsuka, JAPAN	294090	SAT	A	CV
96	Kyoto, JAPAN	319860	ATS	B	S-CV
97	Lithuania, USSR	325987	ATS	B	S-CV
98	YUGOSLAVIA	357977	SAT	A	CV

## Appendix A cont'd

99		YUGOSLAVIA	357981	SAT	A	CV
100		YUGOSLAVIA	357988	SAT	A	CV
101		Genebank GDR	378533	SAT	A	CV
102		Hangchow, CHINA	419042	ATS	B	S-CV
103		Peking, CHINA	419084	ATS	B	S-CV
104		Azerbaijan, USSR	430524	ATR	C	S-CV
105		Uzbek, USSR	430528	ATR	C	S-CV
106		Uzbek, USSR	430529	ATR	C	S-CV
107		Tadzhik, USSR	430530	ATS	B	S-CV
108		Azerbaijan, USSR	430531	ATR	C	S-CV
109		Daghestan, USSR	430532	ATR	C	S-CV
110		USSR	430533	ATS	B	S-CV
111		Peking, CHINA	432901	SAT	A	CV
112		Wageningen, NETH	451752	SAT	A	CV
113		Wageningen, NETH	451755	SAT	A	CV
114		Wageningen, NETH	451757	SAT	A	CV
115		Wageningen, NETH	451758	SAT	A	CV
116		Sinkiang, CHINA	478370	ATS	B	S-CV
117		MEXICO	226310	ATS	B	S-CV
118		S. AFRICA	272258	ATS	B	S-CV
119		DENMARK	279762	DRA	F	W
120		HUNGARY	279782	GIN	E	W
121		DENMARK	279784	GIN	E	W
122		SPAIN	295862	MAX	D	W
123	14	BRAZIL		ATS	B	S-CV
124	15	"Scarlet Horn F."		SAT	A	CV
125	16	Linz, AUSTRIA		SAT	A	CV
126	17	JAPAN		ATS	B	S-CV
127	18	Rome, ITALY	919	DRA	F	W
128		Coimbra, PORTUGAL	39.4	MAR	D	W
129		Madrid, SPAIN	149-77	MAX	D	W
130		Madrid, SPAIN	451	MAR	D	W
131		Madrid, SPAIN	452	MAX	D	W
132		Izmir, TURKEY		CAR	E	W
133		Siena, ITALY		COM	F	W
134		POLAND		ATS	B	S-CV
135		Tel-Aviv, ISRAEL	5.85	MAX	D	W
136		Brussell, BELGIUM		CAR	D	W
137		Le Cave di Miano, ITA		MAX	D	W
138	19	Geogian SSR	1680	CAR	D	W
139		Turkmenian SSR	2350	ATS	B	S-CV
140		Uzbek SSR	2310	ATS	B	S-CV
141		Kazakh SSR	2308	ATS	B	S-CV
142	20	"White Belgian"		SAT	A	CV
143	21	"Dutch Horn"	677901	SAT	A	CV
144		"James Intermediate"	618401	SAT	A	CV
145	22	E. Sussex, ENGLAND	52043	CAR	D	W
146		Puszczykowko, POLAND	50658	CAR	D	W
147		San Vincente, SPAIN	60512	CAR	D	W
148		Montjuic, SPAIN	20389	MAJ	D	W
149		Lousa, PORTUGAL	20378	CAR	D	W
150	23	Greensboro, NC, USA		CAR	D	W
151		Charlottesville, VA, USA		CAR	D	W
152	24	IRAN	3932	ATR	C	S-CV

## Appendix A cont'd

153		NEW ZEALAND	6330	SAT	A	CV
154		FRANCE	3919	SAT	A	CV
155	25	Lisboa, PORTUGAL		MAR	D	W
156	26	"Danvers Half Long"		SAT	A	CV
157	27	Herault, FRANCE		MAJ	D	W
158		Pyrenees O. FRANCE		COM	F	W
159		Finistere, FRANCE		GAD	F	W
160	28	Liverpool, ENGLAND		CAR	D	W
161	29	Godollo, HUNGARY		CAR	D	W
162	30	Trieste, ITALY		CAR	D	W
163	31	Strasbourg, FRANCE		CAR	D	W
164	32	Foz-Tua, PORTUGAL		MAR	D	W
165	33	Marseille, FRANCE		GUM	F	W
166	34	Nantes, FRANCE		GUM	F	W
167		Nantes, FRANCE		GUM	F	W
168	35	Antwerpen, BELGIUM		CAR	D	W

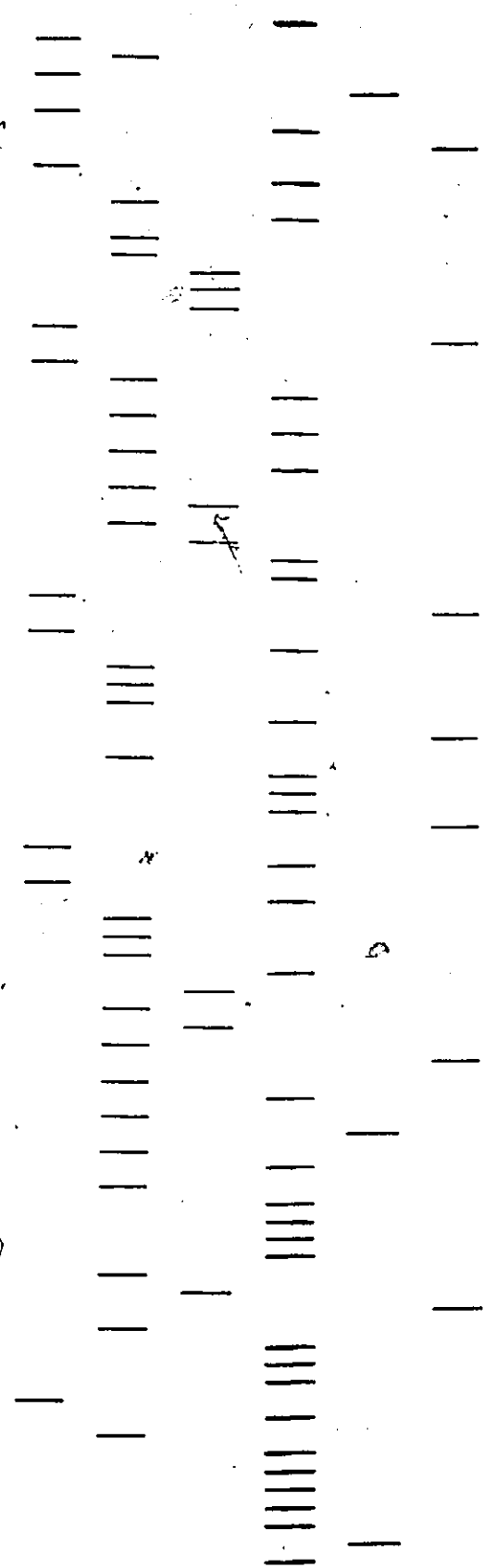
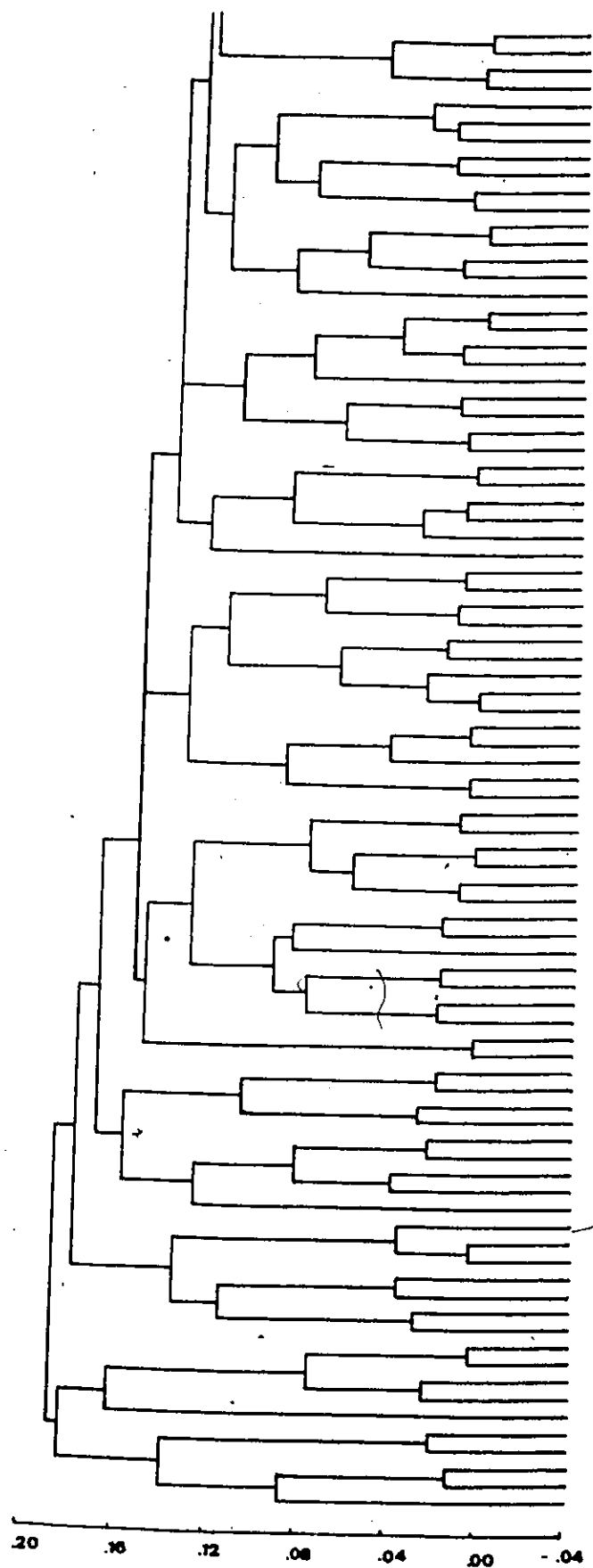
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- 6- Agriculture Canada, Charlottetown, PEI
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- 30- Civico Orto Botanico, Trieste, Italy
- 31- Jardin Botanique de l'Univ. Louis Pasteur, France
- 32- Hortus Botanicus Univ. Portucalensis, Portugal
- 33- Jardin Botanique Ville de Marseille, France
- 34- Jardin Botanique, Nantes, France
- 35- Hortus Botanicus Antverpiensis, Antwerpen, Belgium



Appendix B. Distance phenogram (UPGMA) derived from a matrix of genetic distances for 168 accessions of *D. carota* L. Lines to the right indicate membership in Small's (1978) (figure 1.4) taxonomic groups.





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