

Molecular Diversity Analysis of Cultivated Carrot (*Daucus carota* L.) and Wild *Daucus* Populations Reveals a Genetically Nonstructured Composition

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ABSTRACT. A sample of 124 *Daucus carota* L. accessions, including cultivated carrot [*D. carota* ssp. *sativus* (Hoffm.) Arcangeli] and related wild subspecies, using a variety of molecular markers was examined. Represented within the samples were wild accessions from 18 countries, 14 of 16 major root types of European origin, and examples of major North American and Asian cultivated carrot types. Amplified fragment length polymorphism (AFLP) and inter-simple sequence repeat (ISSR) markers revealed extensive variation within *D. carota*. Although cultivated carrot and wild *D. carota* subspecies can cross freely, cultivated and wild carrots clustered separately, supporting the possibility that human selection for desirable horticultural traits has artificially reduced gene flow between cultivated and wild forms. Our analyses support the likelihood that North American *D. carota* populations arose due to introduction of weedy materials rather than escape of cultivated forms. With the exception of wild vs. cultivated types, no genetic alliances were evident in dendrogram topology. Furthermore, between and even within nonmapped marker classes, dendrogram topology predictions were not consistent. Generally poor correlations among root types, geographic origin, mitochondrial, plastid, and specific nuclear diversity and AFLP/ISSR data were also observed. We concluded that genetic diversity in carrot is extensive and relatively nonstructured in nature.

Plant breeders have found multiple uses for molecular markers. Markers have been useful for linkage map development (Bradeen et al., 2001; King et al., 1998; Vivek and Simon, 1999a), marker aided selection (MAS) (Boiteux et al., 2000; Doganlar et al., 2000; Sicard et al., 1999), and map-based cloning of genes conditioning important horticultural or agronomic traits (Brommonschenkel and Tanksley, 1997; Han et al., 1999). Molecular markers have also been important tools for characterization of genetic diversity. Plant breeding relies upon successful identification and manipulation of genetic variation. Characterization of genetic variation facilitates organized application of breeding techniques to maximize effectiveness. Diverse molecular markers including restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter-simple sequence repeat (ISSR), simple sequence repeat (SSR), and derived markers such as sequence characterized amplified regions (SCARs) or cleaved amplified polymorphic sequences (CAPS) have been used to study the molecular diversity and genetic organi-

zation of a variety of crops including adzuki bean (*Vigna angularis* Willd.) (Yee et al., 1999), barley (*Hordeum vulgare* L.) (Russell et al., 1997), cassava (*Manihot esculenta* Crantz) (Sanchez et al., 1999), finger millet (*Eleusine coracana* L. Gaertn.) (Salimath et al., 1995), maize (*Zea mays* L.) (Pejic et al., 1998), onion (*Allium cepa* L.) (Bradeen and Havey, 1995), rice (*Oryza sativa* L.) (Zhu et al., 1998), soybean (*Glycine max* L. Merr.) (Maughan et al., 1996), sunflower (*Helianthus annuus* L.) (Hongtrakul et al., 1997), and wheat (*Triticum aestivum* L.) (Bohn et al., 1999), to name just a few.

Daucus carota is a morphologically diverse species found in wild or feral form throughout the Mediterranean, southwest Asia, Africa, Australia, New Zealand, and the Americas (Banga, 1957). The gene centers for the species include Asia Minor, Transcaucasia, Iran, Turkmenistan, northwest India, Afghanistan, Tadjikistan, Uzbekistan, and western Tian-Shan mountain system of central Asia (Vavilov, 1949/50). Carrot [*D. carota* ssp. *sativus* (Hoffm.) Arcangeli] is the only important cultivated form of the species and is an important vegetable crop worldwide. Carrot is an outcrossing diploid ($2n = 18$). It has been speculated that carrot may have originated in Anatolia where there is considerable phenotypic diversity (Banga, 1957) and germplasm collections are important resources for carrot improvement.

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Table 1. Carrot and other *Daucus carota* accessions.

Ref no.	Accession ^z	Seed source ^y	Molecular markers ^x			Root type ^w	Origin ^v
			Nuclear	Plastid	Nonmapped		
Cultivars							
7148	Altringham	HRIGRU 12480	J,Y	M,P	A,I	12	Cultivated
7147	Altringham Large Red	HRIGRU 12400	J,Y	M,P	A,I	12	Cultivated
1216	Baby Long	HRIGRU 9327	J,Y	M,P	A,I	8	Cultivated
1224	Birincekutskaja 415	HRIGRU 3957	J,Y	M,P	A,I	15	Cultivated
1208	Blanche à Collet Vert Hors Terre ^{*z}	HRIGRU 8124	J,Y	M,P	A,I	5	Cultivated
7154	Blanche à Collet Vert Hors Terre [*]	HRIGRU 8124	J,Y	M,P	A,I	5	Cultivated
1207	Blanche à Collet Vert Très Hors Terre [*]	HRIGRU 8115	J,Y	M,P	A,I	5	Cultivated
7153	Blanche à Collet Vert Très Hors Terre [*]	HRIGRU 8115	J,Y	M,P	A,I	5	Cultivated
1195	Camberley	HRIGRU 6045			A	11	Cultivated
7144	Carentan [*]	HRIGRU 3991	J,Y	M,P	A,I	8	Cultivated
1217	Carentan [*]	HRIGRU 3991	J,Y	M,P	A,I	8	Cultivated
7146	Carentan Kim	Pioneer Seeds	J,Y	M,P	A,I	8	Cultivated
7121	Champion Scarlet Horn	HRIGRU 3971	J,Y	M,P	A,I	2	Cultivated
1218	Champion Scarlet Horn	Pioneer Seeds	J,Y	M,P	A,I	2	Cultivated
7178	Chantenay	Vilmorin Seeds	J,Y	M,P	A,I	13	Cultivated
1198	Chantenay Kort	HRIGRU 11150	J,Y	M,P	A,I	13	Cultivated
7177	Chantenay Red Core	Peto Seeds	J,Y	M,P	A,I	13	Cultivated
7176	Chantenay Red Cored	PI 264232	J,Y	M,P	A,I	13	Cultivated
1200	Cluseed Newmodel	HRIGRU 3898	Y		A	13	Cultivated
1226	Cold King	HRIGRU 7134	J,Y	M,P	A,I	13	Cultivated
1203	Danvers Danro RS	HRIGRU 5595	J,Y	M,P	A,I	10	Cultivated
7182	Danvers 126	Asgrow Seeds	Y	M,P	A,I	10	Cultivated
1219	De La Halle	HRIGRU 6091	J,Y	M,P	A,I	8	Cultivated
1204	Duwickier	HRIGRU 6767	J,Y	M,P	A,I	2	Cultivated
7124	Early French Frame	HRIGRU 6162	J,Y	M,P	A,I	6	Cultivated
7122	Early Half Long Horn	Pioneer Seeds	J,Y	M,P	A,I	3	Cultivated
1220	Early Nantes [*]	HRIGRU 6089	J,Y	M,P	A,I	8	Cultivated
7128	Early Nantes [*]	HRIGRU 6089	Y	M	A	8	Cultivated
7137	Early Nantes	Bountiful Garden Seeds	J,Y	M,P	A,I	8	Cultivated
1205	Early Scarlet Horn [*]	HRIGRU 9311	J,Y	M,P	A,I	2	Cultivated
7120	Early Scarlet Horn [*]	HRIGRU 9311	J,Y	M,P	A,I	2	Cultivated
7125	Early Short Horn	HRIGRU 9297	J,Y	M,P	A,I	2	Cultivated
1209	Gelbe Lobbericher	HRIGRU 3922	J,Y	M,P	A,I	5	Cultivated
7156	Gelbe Rheinische	HRIGRU 3921	J,Y	M,P	A,I	5	Cultivated
7158	Gelbe Wortel	HRIGRU 11146	J,Y	M,P	A,I	5	Cultivated
7175	Giant Chantenay	PI 264238	Y	M,P	A,I	13	Cultivated
1214	Gold Pak [*]	HRIGRU 3885	J,Y	M,P	A,I	14	Cultivated
7184	Gold Pak [*]	HRIGRU 3885	J,Y	M,P	A,I	14	Cultivated
7169	Guerande	Vilmorin Seeds	Y	M,P	A,I	9	Cultivated
7183	Imperator 58	Arco Seeds	Y	M,P	A	14	Cultivated
7180	James Scarlet Intermediate	HRIGRU 6100	J,Y	M,P	A,I	4	Cultivated
7174	Kuroda	Crookham Seeds	J,Y	M,P	A,I	16	Cultivated
1201	Kuroda Chantenay	HRIGRU 3977	J,Y	P	A,I	16	Cultivated
7170	Kuroda Chantenay	Ferry Morris Seeds	J,Y	M,P	A,I	16	Cultivated
1202	Kuroda Gosun	Rogers NK Seeds	J,Y	M,P	A,I	16	Cultivated
7171	Kuroda Gosun	HRIGRU 4005	J,Y	M,P	A,I	16	Cultivated
7172	Kuroda PS [*]	Peto Seeds	J,Y	M,P	A,I	16	Cultivated
7173	Kuroda PS [*]	Peto Seeds	J,Y	M,P	A,I	16	Cultivated
7152	Lange Witte Groen Kop	PI 451752	J,Y	M,P	A,I	5	Cultivated
7150	Long Red	PI 193506	J,Y	M,P	A,I	1	Cultivated
7149	Long Red Surrey	HRIGRU 6102	J,Y	M,P	A,I	1	Cultivated
1215	Long Surrey	Crookham Seeds	J,Y	M,P	A,I	1	Cultivated
7132	Nantaise D74	PI 261613	Y	M,P	A,I	8	Cultivated
7135	Nantejska	PI 285616	J,Y	M,P	A,I	8	Cultivated
7140	Nantes	Crookham Seeds	J,Y	M,P	A,I	8	Cultivated
7134	Nantes 20	PI 225870	J,Y	M,P	A,I	8	Cultivated
7136	Nantes Britton	Rogers NK Seeds	J,Y	M,P	A,I	8	Cultivated
7141	Nantes Fancy	Johnny's Select Seeds	J,Y	M,P	A,I	8	Cultivated
7130	Nantes Munkegaard II	PI 276325	Y	M,P	A,I	8	Cultivated
7133	Nantesa	PI 249535	Y	M	A	8	Cultivated
1197	Ohne Herz	HRIGRU 8145			A	15	Cultivated
7127	Parisienne Forcer	PI 341207	Y	M	A,I	6	Cultivated
7165	Red Elephant	HRIGRU 3982	J,Y	M,P	A,I	1	Cultivated
7142	Scarlet Nantes	Alf Christiansen Seeds	J,Y	M,P	A,I	8	Cultivated

Ref no.	Accession ^z	Seed source ^y	Molecular markers ^x			Root type ^w	Origin ^v
			Nuclear	Plastid	Nonmapped		
7139	Scarlet Nantes 616	Stokes Seeds	J,Y	M,P	A,I	8	Cultivated
7166	St. Valerio	PI 261614	J,Y	M,P	A,I	1	Cultivated
7162	St. Valery	HRIGRU 9313	J,Y	M,P	A,I	1	Cultivated
7164	St. Valery	Pioneer Seeds	Y	M,P	A	1	Cultivated
7159	Topweight	PI 306810	J,Y	M,P	A,I	11	Cultivated
7160	Topweight	Jim Henry Seeds	J,Y	M,P	A,I	11	Cultivated
7161	Topweight	Yates Seeds	J,Y	M,P	A,I	11	Cultivated
1212	White Belgian	HRIGRU 8720	J,Y	M,P	A,I	5	Cultivated
7151	White Belgian	HRIGRU 8112	Y	M,P	A,I	5	Cultivated
Wild populations							
7188	<i>Daucus carota</i>	HRIGRU 8001	Y	M	A	Wild	U.K.
7191	<i>Daucus carota</i> ssp. <i>azoricus</i>	HRIGRU 6667	J,Y	M,P	A	Wild	Spain
7193	<i>Daucus carota</i> ssp. <i>gingidium</i>	HRIGRU 7159	Y	M	A	Wild	Portugal
7194	<i>Daucus carota</i> ^a	HRIGRU 7188	Y	M	A	Wild	Portugal
7195	<i>Daucus carota</i> ssp. <i>hispidifolius</i>	HRIGRU 7189	Y	M,P	A	Wild	Chile
7196	<i>Daucus carota</i> ssp. <i>commutatus</i>	HRIGRU 7386	Y	M,P	A	Wild	Italy
7198	<i>Daucus carota</i> ssp. <i>maritimus</i>	HRIGRU 7388	Y	M,P	A	Wild	France
7200	<i>Daucus carota</i> ssp. <i>commutatus</i>	HRIGRU 7999	Y	M,P	A	Wild	Germany?
7201	<i>Daucus carota</i> ssp. <i>hispanicus</i>	HRIGRU 8000	Y	M,P	A	Wild	Germany?
7202	<i>Daucus carota</i> ssp. <i>hispanicus</i>	HRIGRU 8232	Y	M,P	A,I	Wild	Germany
7204	<i>Daucus carota</i>	HRIGRU 5785A	J,Y	M,P	A,I	Wild	Czechoslovakia
7205	<i>Daucus carota</i> ssp. <i>carota</i>	HRIGRU 5786	Y	M,P	A	Wild	Czechoslovakia
7207	<i>Daucus carota</i> ssp. <i>carota</i>	HRIGRU 6666	J,Y	M,P	A,I	Wild	Ireland
7209	<i>Daucus carota</i> ssp. <i>carota</i>	HRIGRU 6672	Y	M,P	A	Wild	U.K.
7210	<i>Daucus carota</i> ssp. <i>carota</i>	HRIGRU 6673	Y	M	A	Wild	U.K.
7211	<i>Daucus carota</i> ssp. <i>carota</i>	HRIGRU 6674	J,Y	M,P	A	Wild	Malta
7213	<i>Daucus carota</i> ssp. <i>carota</i>	HRIGRU 6676	Y	M,P	A	Wild	U.K.
7214	<i>Daucus carota</i> ssp. <i>carota</i>	HRIGRU 6678	Y	M,P	A	Wild	Spain
7217	<i>Daucus carota</i> ssp. <i>carota</i>	HRIGRU 6681	Y	M,P	A,I	Wild	Poland
7218	<i>Daucus carota</i>	HRIGRU 7157	Y	M,P	A,I	Wild	Malta
7219	<i>Daucus carota</i> ssp. <i>carota</i>	HRIGRU 7158	J,Y	M,P	A,I	Wild	Morocco
7220	<i>Daucus carota</i> ssp. <i>gadecaei</i>	HRIGRU 7160	J,Y	M,P	A,I	Wild	France
7223	<i>Daucus carota</i>	HRIGRU 7186	Y	M	A,I	Wild	Portugal
7225	<i>Daucus carota</i>	HRIGRU 7191	Y	M,P	A,I	Wild	Spain
7226	<i>Daucus carota</i>	HRIGRU 7192	Y	M,P	A,I	Wild	Spain
7227	<i>Daucus carota</i>	HRIGRU 7193	J,Y	M,P	A	Wild	Spain
7228	<i>Daucus carota</i>	HRIGRU 7194	Y	M,P	A	Wild	Pakistan
7229	<i>Daucus carota</i> ssp. <i>carota</i>	HRIGRU 7380	J,Y	M,P	A	Wild	Syria
7230	<i>Daucus carota</i> ssp. <i>carota</i>	HRIGRU 7381	Y	M,P	A	Wild	Syria
7231	<i>Daucus carota</i> ssp. <i>carota</i>	HRIGRU 7382	Y	M,P	A	Wild	Syria
7232	<i>Daucus carota</i> ssp. <i>carota</i>	HRIGRU 7383	Y	M,P	A	Wild	Syria
7233	<i>Daucus carota</i> ssp. <i>carota</i>	HRIGRU 7384	Y	M	A	Wild	Germany
7234	<i>Daucus carota</i>	HRIGRU 8710	Y	M,P	A	Wild	U.K.
7235	<i>Daucus carota</i>	HRIGRU 8715	J,Y	M,P	A	Wild	U.K.
7239	<i>Daucus carota</i> ssp. <i>carota</i>	HRIGRU 7385	J,Y	M,P	A,I	Wild	Italy
7240	<i>Daucus carota</i>	HRIGRU 7389	J,Y	M,P	A,I	Wild	China
7241	<i>Daucus carota</i>	HRIGRU 8692		M	A	Wild	U.K.
7243	<i>Daucus carota</i> ssp. <i>carota</i>	HRIGRU 8696	Y	M,P	A	Wild	U.K.
7244	<i>Daucus carota</i> ssp. <i>carota</i>	HRIGRU 8698	Y	M,P	A	Wild	U.K.
7248	<i>Daucus carota</i>	HRIGRU 8708	J,Y	M,P	A	Wild	U.K.
7250	<i>Daucus carota</i>	Vilmorin Seeds	Y	M,P	A	Wild	France
7252	<i>Daucus carota</i>	Vilmorin Seeds	J,Y	M,P	A	Wild	France
7254	<i>Daucus carota</i>	Daehnfeltd Seeds	J,Y	M,P	A	Wild	Denmark
7255	<i>Daucus carota</i>	Daehnfeltd Seeds	J,Y	M,P	A	Wild	Greece
7257	<i>Daucus carota</i>	Daehnfeltd Seeds	Y	M,P	A,I	Wild	Greece
7259	<i>Daucus carota</i>	Daehnfeltd Seeds	J,Y	M,P	A	Wild	Greece
7260	<i>Daucus carota</i>	Daehnfeltd Seeds	J,Y	M,P	A	Wild	Denmark
7262	<i>Daucus carota</i>	Daehnfeltd Seeds	Y	M,P	A	Wild	Denmark
7264	<i>Daucus carota</i>	P.W. Simon	Y	M,P	A,I	Wild	Wisconsin
7266	<i>Daucus carota</i>	M.R. McDonald	J,Y	M,P	A,I	Wild	Canada
7267	<i>Daucus carota</i>	P.W. Simon	J,Y	M,P	A	Wild	Washington

^zAn asterisk following an accession name indicates independently duplicated accessions. See Materials and Methods for details.

^yHRIGRU accessions are from the Horticulture Research Institute, Genetic Resources Unit, Wellesbourne, Warrick, United Kingdom. PI accessions are from the USDA *Daucus* collection, Ames, Iowa.

^xMarker data available are listed for each accession. A = AFLP, J = SCAR marker Mj-1, I = ISSR, M = mitochondrial markers, P = P10 marker, Y = Y2 marker. See Materials and Methods for details.

^w1 = Long Orange, 2 = Early Short Horn, 3 = Early Half Long Horn, 4 = Late Half Long Horn, 5 = Yellow Belgian, 6 = Paris Market, 7 = Amsterdam Forcing, 8 = Nantes, 9 = Oxheart, 10 = Danvers, 11 = Flakkee, 12 = Altringham, 13 = Chantenay, 14 = Imperator, 15 = Berlicum, 16 = Kuroda. Illustrated by Rubatzky et al. (1999).

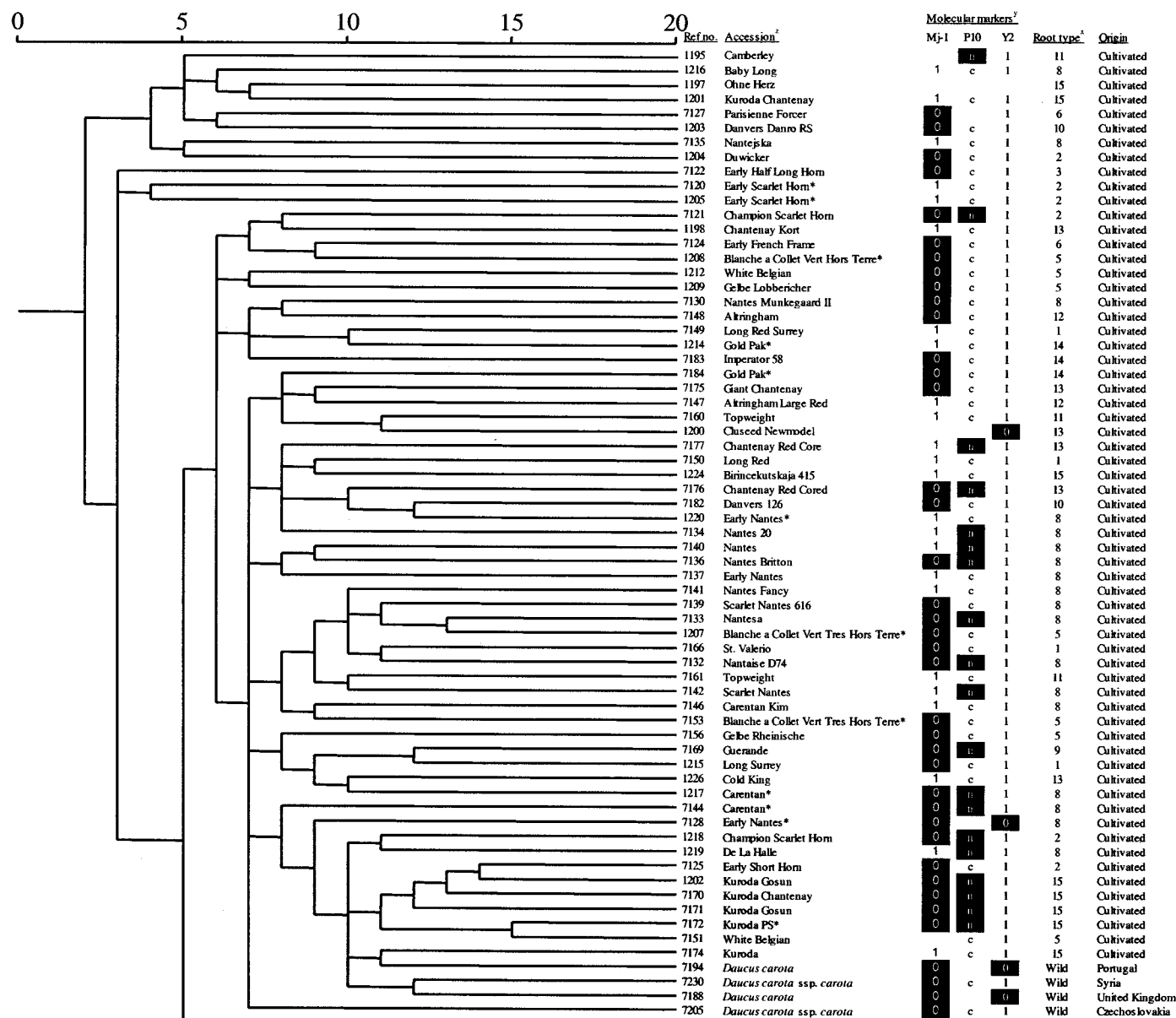
^vFor wild materials, country of origin is listed.

^aAccession 7194 was received as *D. carota* ssp. *duriensis* lange. This subspecific name could not be confirmed.

Although modest compared to the number of accessions that have been collected for major agronomic crops, the U.S. Department of Agriculture, Agricultural Research Service Germplasm Resources Information Network (USDA, ARS GRIN) currently lists more than 1100 *D. carota* accessions. These are maintained as a working collection in Ames, Iowa. Additionally, the Horticulture Research Institute Genetics Resources Unit in Wellesbourne, United Kingdom maintains 1344 *Daucus* L. accessions. These numbers will increase with continued collection of cultivated and wild carrot germplasm, making effective germplasm utilization increasingly costly and difficult. Previous attempts to describe diversity organization in *D. carota* have included numerical analysis of 44 morphological characters for 437 wild and cultivated accessions (Small, 1978) and evaluation of nine isozyme systems in 168 wild and cultivated accessions (St. Pierre and Bayer, 1991; St. Pierre et al., 1990). In the present study a much wider variety of molecular markers including nonmapped, organellar, and nuclear marker types are used to characterize diversity within 124 open-pollinated carrot cultivars and wild accessions from 18 countries in an attempt to correlate molecular diversity with morphological and geographical data.

Materials and Methods

PLANT MATERIALS AND DNA EXTRACTION. Accessions used in this study are listed in Table 1. Accessions were received from the Horticulture Research Institute, Genetic Resources Unit, Wellesbourne, United Kingdom, USDA North Central Regional Plant Introduction Station, Ames, Iowa, and commercial seed sources. Included were 73 open-pollinated carrot cultivars including representatives from 14 of the 16 European primary cultivars (Simon, 2000) and the predominant cultivars from North America ('Imperator') and Asia ('Kuroda') and 51 wild *D. carota* populations (Table 1). Accessions from the Horticulture Research Institute, Genetic Resources Unit and the USDA North Central Regional Plant Introduction Station have been propagated approximately once every 10 years since the date of their collection by random pollinations among a 20 to 50 plants (D. Astley and M. Widrechner, personal communications). For DNA extractions, plants were grown in greenhouses in Madison, Wis. and, for cultivated types, in commercial carrot fields in Wisconsin and California for evaluation of morphological variation. DNA for marker analyses was extracted (Murray and Thompson, 1980) in bulk from the leaves of 10 to 20



greenhouse grown seedlings for each accession. For seven of the open-pollinated cultivars ('Blanche à Collet Vert Hors Terre', 'Blanche à Collet Vert Très Hors Terre', 'Carentan', 'Early Nantes', 'Early Scarlet Horn', 'Gold Pak', and 'Kuroda PS'), representing five different root types (Table 1), a second bulked DNA sample from a different group of 10 to 20 plants each from the same seed source was prepared and evaluated independently to provide an estimate of intra-accession variation. DNA concentrations were estimated via fluorometry following manufacturer's (Hoefer Scientific Instruments, San Francisco) instructions.

MOLECULAR MARKERS. Markers generated included nonmapped (AFLP, ISSR), specific organellar (chloroplast P10, mitochondrial markers linked to *atp1*, *atp6*, *atp8*, *atp9*, *cob*, *cox1*, and *nad9*), and specific nuclear markers linked to root core pigmentation (Y2) and

nematode resistance (*Mj-1*). Generation of AFLP (Bradeen and Simon, 1998), plastid (Vivek and Simon, 1999b), mitochondrial (Bach, 2000), Y2 (Bradeen and Simon, 1998), and *Mj-1* markers (Boiteux et al., 2000) used previously described protocols. AFLP markers were generated using primer pairs E-AGG × M-CTC (primer pair A), E-ACT × M-CAG (primer pair B), E-AGC × M-CTA (primer pair C), E-AAG × M-CAG (primer pair D), and E-ACT × M-CAA (primer pair E).

ISSR markers were generated using primers ISSR4 [(GACA)₄] and ISSR5 [VHV(CT)₈] where V = A, C, or G and H = A, C, or T]. Total reaction volumes were 20 µL and included 1.6 units Taq DNA polymerase Goldstar (Eurogentec, Angers, France), 1× reaction buffer containing 1 mM MgCl₂ (Eurogentec), 0.2 mM each dNTP, 0.5 µM of a single primer, and 20 ng template DNA. Thermocycler (Hybaid, Franklin, Mass.) conditions for ISSR5 were 5 min at 94 °C, 40 cycles of 30 s at 94 °C, 45 s at 50 °C, 2 min at 72 °C, with a final 7 min extension at 72 °C. For ISSR4, the program was modified to include 45 cycles and an annealing temperature of 45 °C. Amplification products were electrophoresed through a 1.6% agarose gel at 100 V, stained with ethidium bromide, and visualized via ultraviolet light. Individual ISSR fragments were scored as band present or absent for each accession.

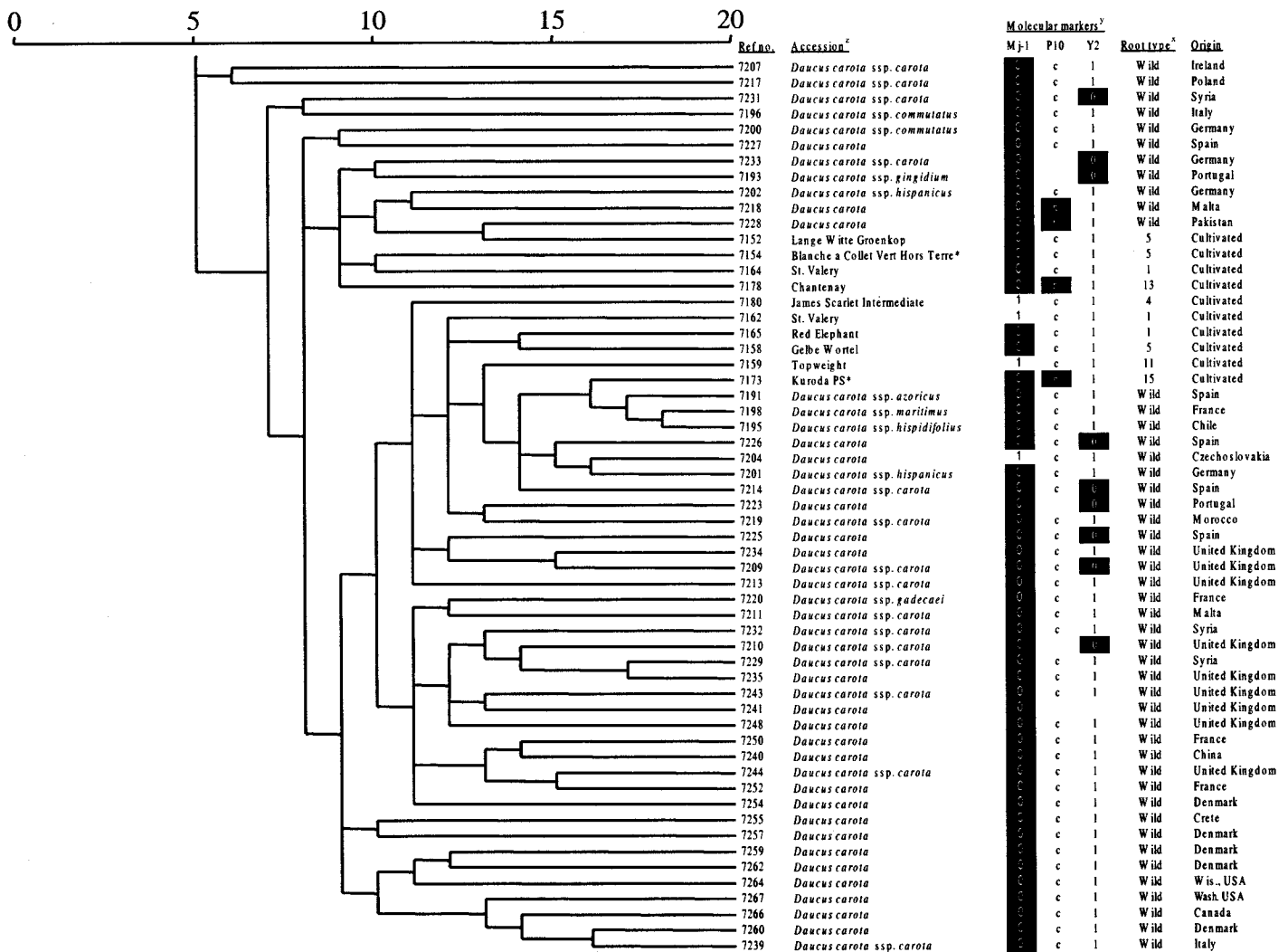
DATA ANALYSIS. Data from nonmapped marker classes and mitochondrial markers were used to calculate Jaccard's (1908) similarity coefficients for all accessions using a macro written for Microsoft Excel 2000. Jaccard's (1908) coefficients were calcu-

Fig. 1. *Daucus carota* dendrogram based on AFLP and ISSR data. Distance measurement is Jaccard's (1908) similarity coefficient.

*An asterisk following an accession name indicates independently duplicated accessions. See Materials and Methods for details.

¹Molecular markers: *Mj-1*: dominantly scored as fragment present (1) or absent (0); P10: codominant marker, scoring reflects present (c) or absence (n) of internal BglII fragment; Y2: dominantly scored as fragment present (1) or absent (0).

²Root type: 1 = Long Orange; 2 = Early Short Horn; 3 = Early Half Long Horn; 4 = Late Half Long Horn; 5 = Yellow Belgian; 6 = Paris Market; 7 = Amsterdam Forcing; 8 = Nantes; 9 = Oxheart; 10 = Danvers; 11 = Flakkee; 12 = Altringham; 13 = Chantenay; 14 = Imperator; 15 = Berlicum; 16 = Kuroda. Illustrated by Rubatzky et al. (1999).



lated from data from each separate AFLP primer pair, all AFLP primer pairs together, all ISSR markers alone, and all AFLP primer pairs and all ISSR markers together. Jaccard's (1908) coefficients were also calculated for mitochondrial data. Dendrogram construction (neighbor-joining), comparison of dendrogram topologies via cophenetic correlation, and principal component analyses were completed using NTSys-pc (version 1.70, Exeter Software, Setauket, N.Y.) software. Molecular phenotypes for nuclear and chloroplast markers are superimposed on the nonmapped data dendrogram and are discussed within the context of that dendrogram. Comparison of pairwise similarity matrices for nonmapped marker types included Spearman's rank order correlations (Spearman, 1904). Analysis of molecular variance (AMOVA) was performed upon Jaccard's (1908) distance calculations of AFLP and ISSR data (generated using a macro written for Microsoft Excel 2000) using WinAmova 1.55 (Excoffier et al., 1992).

Results and Discussion

Root phenotypes of cultivated carrots grown in field trials in Wisconsin and California were consistent with those of the designated root type (Table 1). Subspecific designations for wild carrots were provided with seed samples from the germplasm collections and verified morphologically.

A total of 140 reliable, reproducible AFLP markers, two mapped nuclear markers (*Mj-1* and *Y2*), a plastid polymorphism (P10), 20 mitochondrial markers, and 23 ISSR markers were scored for the accessions in this study, as indicated in Table 1. AFLP and ISSR markers were considered reliable and repeatable if the presence or absence of the fragment could be determined visually without ambiguity. A limited number of duplicate reactions was included to further confirm repeatability.

Jaccard's (1908) coefficient is a conservative estimate of genetic similarity. Because the calculation considers only the shared presence of a fragment but not the shared absence of a fragment as informative, Jaccard's (1908) may underestimate the true genetic similarity between two accessions. The dominant nature of AFLP and ISSR markers prevented us from ascribing confidently a genetic basis to the absence of a marker. Santos and Simon (2001) reported that similarly sized AFLP fragments shared between two nonrelated carrot F_2 populations are highly similar (>91% homology for 26 out of 31 samples) at the sequence level. We can be reasonably confident, therefore, that the shared presence of a fragment between two accessions indicates a genetic relationship. However, the underlying cause of the absence of a fragment is unknown. Because any one of a number of underlying causes may be responsible for the absence of a fragment, to consider that the shared absence of a fragment between two accessions is indicative of genetic similarity can be very misleading. A similarity coefficient that includes both the presence and absence of a dominant marker in its calculation (e.g., simple matching coefficient) may provide very inflated estimates of similarity, particularly for very divergent accessions, such as those in this study. To avoid the potential for inflated similarity estimates, we have opted for the more conservative Jaccard's (1908) coefficient.

Similarity coefficients estimated in this study ranged from ≈ 0.3 to ≈ 0.8 for cultivated carrot and from ≈ 0.2 to ≈ 0.7 for wild *Daucus* populations (Fig. 1). Direct comparison of these similarity values with those estimated from similar collections in other crop species is difficult because of the wide variety of distance

and similarity measures employed (Fang et al., 1997; Hartl and Seefeldt, 1998; Paul et al., 1997; Perera et al., 1998; Yang et al., 1996). Nevertheless, it is obvious that carrot cultivars and *Daucus* populations are broadly diverse with relatively little evidence for reduction in allelic diversity during development of open-pollinated cultivars. Principal component analysis (Fig. 2) supports this conclusion, with wild *Daucus* accessions encompassing only a slightly broader statistical space than cultivated carrot accessions (0.355 vs. 0.277 for the first principal component for wild and cultivated accessions, respectively). Similarly, St. Pierre and Bayer (1991) reported, based upon analyses of nine isozyme systems, that wild *D. carota* types were slightly more variable than cultivated forms, but not significantly so. Indeed, even within the seven independently duplicated cultivars in this study, nonmapped marker variation is evident and occasionally substantial [Fig. 1; pairwise Jaccard's (1908) similarity coefficients ranged from 0.481 for 'Early Nantes' to 0.800 for 'Carentan'], indicating that even at the intra-population level allelic diversity is extensive. Similarly, Grzebelus et al. (2001) reported substantial allelic diversity within carrot inbred lines. Although use of AMOVA for dominant marker types requires careful interpretation, results of AMOVA based upon the AFLP and ISSR data reported herein are consistent with our conclusion that intra-population allelic diversity is extensive: 81.6% of the observed molecular variation is partitioned within class (i.e., within cultivated types and within wild types) vs. 18.4% between class. This supports the conclusion of St. Pierre and Bayer (1991) that more genetic variation exists within both wild and cultivated accessions than among them, based upon isozyme sampling of 123 cultivated carrot and 45 wild *D. carota* samples.

There was generally poor agreement both within (e.g., AFLP primer pair A vs. AFLP primer pair B) and between (e.g., AFLP vs. ISSR) nonmapped marker types used in this study, with each marker type suggesting a different pattern of accession relationships, as evidenced by generally low Spearman's rank order (Spearman, 1904) and cophenetic correlations (Table 2). In generating these data, extreme care was taken to assure accuracy both in sample preparation and in data collection. Because data derived from individual AFLP primer pairs did not correlate well with each other, we ruled out systematic error in sample preparation or data collection. It was likely that random experimenter

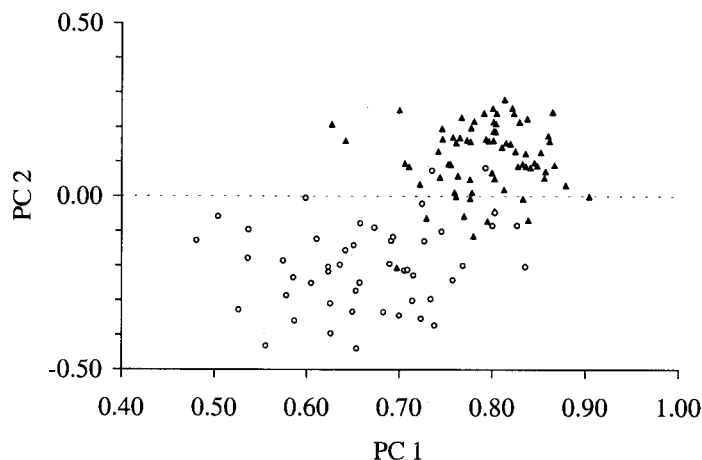


Fig. 2. Plot of the first two principal components calculated from Jaccard's (1908) similarity coefficients from 124 *Daucus carota* accessions based on 140 AFLP and 23 ISSR markers. Cultivated carrot accessions are illustrated as dark triangles; wild *Daucus* accessions are hollow symbols.

Table 2. Comparison of patterns of *Daucus carota* diversity predicted by nonmapped markers. Phenogram topologies (cophenetic correlations) above the diagonal; Spearman's rank order correlations (Spearman, 1904) below the diagonal.

			AFLP primer pairs ^z					
Primer		ISSR	A	B	C	D	E	All AFLP
ISSR			0.241	0.251	0.218	0.317	0.330	0.305
AFLP primer pairs	A	0.525		0.436	0.399	0.574	0.456	---
	B	0.369	0.634		0.490	0.423	0.301	---
	C	0.379	0.529	0.466		0.388	0.360	---
	D	0.367	0.639	0.524	0.508		0.708	---
	E	0.225	0.428	0.563	0.347	0.547		---
All AFLP		0.411	---	---	---	---	---	

²AFLP primer pairs are as listed in the Materials and Methods.

error occurred to a small degree, but the effects of random errors should be considerably ameliorated by the expansiveness of our data set. We believe that the poor agreement observed between and within markers is not due to error or to the inappropriateness of AFLP or ISSR markers for diversity analysis. In fact, both marker types have been used extensively for diversity analyses for many plant species (e.g., Fang et al., 1997; Hartl and Seefelder, 1998; Paul et al., 1997; Perera et al., 1998; Tsumura et al., 1996; Yang et al., 1996). Instead, we suggest our data reflect the true relationships within the species; *D. carota* is a diverse species with few well-defined genetic alliances. For the purposes of this study, we refer to this situation as genetically nonstructured.

Carrot is an outcrossing species. Before the beginning of concerted carrot breeding efforts in the 1950s, little control of pollinations was exercised during seed production. Consequently gene flow among cultivars was likely widespread. Wild carrot populations occurred over most seed production areas used up to that time and gene flow between cultivated and wild carrot could also have occurred regularly throughout most of the history of this crop (Simon, 2000). However, intentional human selection against nonadapted phenotypes may have artificially suppressed gene flow between wild and cultivated types. Brown (1989) suggested that outcrossing species show less intense population differentiation and more uniform distribution of genetic diversity than inbreeding species. Isozyme data agree (Hamrick and Godt, 1990). Our conclusion based on DNA markers that genetic diversity in carrot is by nature nonstructured supports these opinions. The accessions analyzed in this study represent a broad collection of cultivated and wild carrot populations and subspecies, particularly of European and North American origin. Accessions of Asian or Middle Eastern origin are represented to a lesser degree. We speculate that conclusions drawn from this study will apply generally to other *D. carota* accessions from Europe and North America and possibly to accessions of Asian or Middle Eastern origin.

While AFLP and ISSR data resolved few alliances within the accessions used in this study, they could, with few exceptions, successfully separate cultivated carrot from wild carrot and related subspecies (Figs. 1 and 2). Carrot is an Old World crop. It is likely that all wild *Daucus* populations in North America resulted either from unintentional introduction of weedy material from Europe or from carrot cultivars escaping cultivation. In this analysis, wild populations from North America are associated on the dendrogram not with cultivated carrot, but with their weedy European counterparts. Additionally, AMOVA demonstrates significant ($P < 0.001$) differences between wild and cultivated types. Similarly, St. Pierre and Bayer (1991) noted distinctions between wild and cultivated samples based upon isozyme analy-

ses and Small (1978) found cultivated carrot to be sharply discontinuous from wild *D. carota* accessions based upon numerical analysis of 44 morphological characters. These data are all consistent with the possibility that wild carrot populations in North America resulted from the introduction of weedy or wild materials. Because cultivated carrot and wild populations form nearly independent groupings, our data also argue against extensive gene transfer between cultivated and wild populations. It is likely that intentional human selection against off-types severely and artificially limited gene flow between wild and cultivated carrot, but the effects cannot be measured directly.

Among historic carrot cultivars, different populations maintained under a common cultivar name were sometimes, but not always, closely associated with one another (Fig. 1). For example, all three 'Early Scarlet Horn' accessions examined are closely associated (Jaccard's (1908) similarities from 0.661 for 1205 vs. 7125 to 0.786 for 1205 vs. 7120; Fig. 1). In contrast, accessions of 'Kuroda' (Jaccard's (1908) similarity of 0.326 for 7172 vs. 7174), 'Kuroda Gosun' (Jaccard's (1908) similarity of 0.477 for 1202 vs. 7171), and 'Kuroda Chantenay' (Jaccard's (1908) similarity of 0.513 for 1201 vs. 7170) were less closely related. In no case were any two accessions identical, regardless of their names or origin. This is true even for the seven independently duplicated accessions. Previously, St. Pierre and Bayer (1991) noted that certain carrot accessions sharing a common cultivar name are similar at the molecular level while others are not, based upon isozyme analyses. It is likely that, in general, different populations maintained under a similar cultivar name share common morphological features such as root shape and color, regardless of their genetic relationship. Consistent with this suggestion, there was generally poor correlation between AFLP/ISSR data and root type (Fig. 1), suggesting that root phenotype is not a good predictor of genetic relationships, that root phenotype may be under the control of a relatively small number of genes, and that various root phenotypes can be derived from genetically diverse populations through selection. This latter suggestion has been observed previously by carrot breeders and has been utilized for inbred development (Rubatzky et al., 1999). Control of root phenotype by relatively few genes combined with the observed sensitivity of carrot to inbreeding (Rubatzky et al., 1999; Simon, 2000) may account for low levels of similarity among cultivars.

In addition to root characteristics, nonmapped AFLP/ISSR data were compared to organellar data, specific nuclear data, and, for wild carrot materials, geographic origin. The topology of a phenogram constructed using mitochondrial data (not presented) does not correlate well with that of the AFLP/ISSR phenogram (cophenetic correlation = 0.419). Figure 1 illustrates that mapped nuclear and plastid markers *Mj-1*, P10, and Y2 are equally poor

predictors of the AFLP/ISSR phenogram topology. Finally, nonmapped molecular data failed to divide wild carrot populations and related subspecies into groups that reflected their geographic origin (Fig. 1). These results are consistent with the conclusion that there is little genetic structure within *Daucus* and that the accessions examined in this study lack clearly defined alliances or subgroups, with the exception of wild vs. cultivated materials.

Molecular characterization of crop plant diversity holds great potential for improving plant breeding efficiency. Understanding how individual accessions are related can aid the plant breeder in selecting appropriate crosses, in identifying unique genes for disease resistance or other traits, and in predicting heterotic effects. As an outcrossing species, cultivated carrot seems largely genetically nonstructured and molecular phenotype is a poor predictor of germplasm origin and plant phenotype. More precise determination of the subspecific classification of the wild carrot accessions examined in this study or additional examination of a larger collection of well classified wild carrots will allow evaluation of genetic structure for wild carrot subspecies. This study examined mostly nonmapped markers and only a few markers linked to traits of interest. Because many more AFLP markers than ISSR markers were employed, a balanced comparison of the relative usefulness of each marker type was not possible. Future examination of nonmapped marker types for use in carrot diversity assessment may be warranted. Future analyses utilizing nonmapped markers might also include characterization of a large number of individual plants from each accession, allowing conclusion about intrapopulation variation and allelic frequencies between populations. As markers linked to genes conditioning specific traits are identified in carrot, they may be useful in diversity assessment and in guiding germplasm selection in breeding programs. Such a targeted approach to germplasm selection may prove useful for genetically nonstructured species, such as carrot.

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