Genetic Relationships and Diversity in Carrot and other *Daucus* Taxa Based on Nuclear Restriction Fragment Length Polymorphisms

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ABSTRACT. Current classifications of the genus *Daucus* are based on morphological and anatomical characteristics. We have used single to low copy nuclear restriction fragment length polymorphisms (nRFLPs) to describe the phylogeny and relationships of eight *Daucus* species including cultivated carrot (*D. carota* L.). Parsimony analysis of 247 characters (DNA fragments from 58 probe–enzyme combinations) yielded a tree in which accessions were grouped into three major clades and phenetic analysis using Jaccard's coefficient yielded two major clusters. The phylogenetic relationships from the nuclear RFLP data generally agreed with an earlier morphological classification. Resolution and placement of *D. guttatus* and *D. muricatus* were not consistent with the morphological classifications. Molecular variation among carrot inbreds was large.

The genus *Daucus* L. includes carrot (an important vegetable source of vitamin A) and belongs to the family Umbelliferae (Apiaceae). Classical taxonomy of genus *Daucus* has used morphological characteristics of the stem, leaf, bract, inflorescence, flower, and fruit characteristics, including primary ridges, vallecular spines, and secretory vesicle anatomy (Sáenz Laín, 1981). According to Owens (1974), the early taxonomists Baillon (1880), Drude (1898) and Calestani (1905) divided what is today accepted as genus *Daucus* into various sections, subgenera and even separate genera based primarily on mericarp anatomy and morphology. The most widely accepted early classification was that of Drude (1898) who divided genus *Daucus* into three sections: *Daucus* L., *Durieuva* Boiss. and Reut., and *Ctenolophus* Batt. containing 14, seven and two species, respectively. This was used as a framework for subsequent work by Thellung (1926) who recognized 22 species in *Daucus* and divided them into six sections: *Daucus* (13 species), *Anisactis* DC. (five species), *Leptodaucus* Thell. (one species), *Chrysodaucus* Thell. (one species), *Platyspermum* (H. Hoffm.) DC. (one species), and *Pseudoplatyspermum* Thell. (one species). Heywood and Dakshini (1971) studied microcharacters of the mericarps (e.g. prominence of ridges, presence of hairs, flatness of the hairs, presence of grooves around the hairs, and vallecular ridges) as shown by the scanning electron microscope. They recognized 24 species in sections *Daucus*, *Anisactis* (= *Durieuva* Boiss. & Reut.), *Leptodaucus*, *Chrysodaucus*, and *Ctenolophus*. Before 1981, differential use of subsets of morphoanatomical characters by taxonomists had given rise to varied estimates of numbers of species in the genus: 20 (Bentham, 1867), 23 (Drude, 1898), 22 (Thellung, 1926), 24 (Heywood and Dakshini, 1971) and, using small morphological differences to demark species, 60 (Zohary, 1972).

The most recent and comprehensive morphoanatomical classification was given by Sáenz Laín (1981) (Table 1), who classified 20 species into five sections: *Daucus* L. (12 species), *Anisactis* DC. (three species), *Platyspermum* DC. (three species), *Chrysodaucus*

Thell. (one species), and *Meoides* Lange (one species). The morphological features employed for classification were characteristics of the stem, leaf, bract, umbel, and flower. Fruit features that were considered to be informative included shape, primary ridges, and vallecular spines. The shape of the secretory vesicles of essential oils (vittae) and the shape of the commissural side of the ripe mericarp (fruit) were important anatomical features measured by Sáenz Laín (1981).

Daucus carota L. is one of the 12 species in section *Daucus sensu* Sáenz Laín. Linnaeus first described *Daucus carota* in 1753 (cited in Heywood, 1968a). It includes several wild (weedy, semicultivated) and one cultivated form (subspecies *sativus* is the sole cultivated type in the entire genus). This species complex has been classified by Thellung (1926), Onno (1936), Heywood (1968a, 1968b), and Small (1978) [reviewed in St. Pierre et al. (1990)]. Morphological and anatomical variation in the *D. carota* complex is extensive and intracomplex hybridization may be frequent (Onno 1936). Hence, clear delineation into subspecies has been difficult (Sáenz de Rivas and Heywood, 1974; Small, 1978). Heywood (1983) refined his earlier classification and recognized two groups of *D. carota*, groups *carota* and *gingidium*, having six and seven subspecies, respectively (Table 1).

A unifying molecular marker system to resolve some of these difficulties in classification would be valuable for a difficult genus such as *Daucus*. Nuclear restriction fragment length polymorphisms (nRFLPs) have become useful tools in determining relationships within and among species (reviewed in Whitkus et al., 1994) but have not been applied to delineate the phylogeny or phenetic relationships of *Daucus*. Our objective was to describe the variation of some species of *Daucus* and germplasm accessions of carrot (*D. carota* ssp. *sativus*) with nRFLPs. General classification of *Daucus* by Sáenz Laín (1981) and that of *D. carota* by Heywood (1983) are the most recent treatments and are used for comparison to the results of this study.

Materials and Methods

PLANT MATERIAL. Seeds of 18 entries of *Daucus* and one of parsley (*Petroselinum crispum* (Mill.) Nym ex A.W. Hill) (Table 2) were obtained from i) the USDA National Plant Germplasm System's collection, Ames, Iowa (PI entries); ii) Horticulture Research International, Wellesbourne, U.K. (HRI entries); iii) the

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USDA carrot genetic program, Madison, Wis. (USDA entries); or iv) other programs as listed. Cultivated carrots (*D. carota* ssp. *sativus*) included were chosen to represent a broad range of geographic and phenotypic diversity. One wild subspecies representative (*D. carota* ssp. *drepanensis*) and all other available *Daucus* species were included. Seeds of several *Daucus* species listed in Table 1 were not known to be available. Plants were grown in pots in the greenhouse. Leaves from ≈ 15 plants of each accession were harvested in bulk 9 weeks after sowing, frozen in liquid nitrogen and dried. Voucher specimens of each accession were verified for correct classification by comparison to species descriptions (Sáenz Laín, 1981) and deposited in the University of Wisconsin Herbarium.

DNA ISOLATION. Total DNA was isolated from freeze-dried tissue by a modified CTAB extraction method (Murray and Thompson, 1980). DNA was purified on a CsCl gradient. The CsCl was removed from the DNA by dialysis against 10 mM Tris (HCl), pH 8.0, and 1 mM EDTA.

DIGESTION AND BLOTTING OF CARROT DNA. DNA was singly digested with restriction enzymes *Hind* III and *Eco*R I at 5 units/ μ g according to the manufacturer's (Promega, Madison, Wis.) recommendations. Digested DNA of each entry was electrophoretically separated in 0.8% agarose gels in 1 $\times$ TBE (Sambrook et al., 1989) at 9 mA for 18 h. The DNA was then denatured by soaking the gel in 0.4 N NaOH with 0.6 M NaCl for 20 min and blotted overnight to Zetaprobe (BioRad, Richmond, Calif.) filters by capillary transfer (Southern, 1975). The filters were rinsed in 2 $\times$ SSC and dried by baking in a vacuum oven at 80 °C for 1 h.

SOURCE AND PREPARATION OF PROBES. A *Pst* I genomic library was prepared from DNA of the carrot cultivar 'Savory'. This library was constructed as follows: genomic DNA was digested as described above and size fractionated on a sucrose gradient at 91,000 g $_n$ and 20 °C for 18 h. Fractions having DNA of size 500 bp to 2000 bp as compared to standardized size fragments on a gel, were ethanol precipitated. The precipitated fragments were cloned into the *Pst* I site in pGEM 3Zf(-) plasmid after dephosphorylation

Table 1. Classification of *Daucus* as given by Saenz Lain (1981) and *D. carota* as given by Heywood (1983).

<i>D. sect. Daucus</i>	
<i>D. carota</i> L.	
	<i>carota</i> group
	(a) subsp. <i>carota</i>
	(b) subsp. <i>sativus</i> (Hoffm.) Arc.
	(c) subsp. <i>maritimus</i> (Lam.) Batt.
	(d) subsp. <i>major</i> (Vis.) Arc.
	(e) subsp. <i>azoricus</i> Franco
	(f) subsp. <i>parviflorus</i> (Desf.) Thell.
	<i>gingidium</i> group
	(g) subsp. <i>gadecaeii</i> (Rony & Camus) Heywood
	(h) subsp. <i>gummifer</i> Hooker fil.
	(i) subsp. <i>commutatus</i> (Paol.) Thell.
	(j) subsp. <i>hispanicus</i> (Gouan) Thell.
	(k) subsp. <i>hispidus</i> (Arc.) Heywood
	(l) subsp. <i>drepanensis</i> (Arc.) Heywood
	(m) subsp. <i>ruseus</i> (Onno) Heywood & Okeke
	<i>D. gracilis</i> Steinh.
	<i>D. guttatus</i> Sm
	<i>D. involucratus</i> Sm.
	<i>D. jordanicus</i> Post
	<i>D. sahariensis</i> Murb.
	<i>D. syrticus</i> Murb.
	<i>D. capillifolius</i> Gilli
	<i>D. crinitus</i> Desf.
	<i>D. tenuisectus</i> Coss.
	<i>D. montevidensis</i> Link ex Sprengel
	<i>D. pusillus</i> Michx.
<i>D. sect. Platyspermum</i> DC.	
	<i>D. muricatus</i> L.
	<i>D. bicolor</i> Sm.
	<i>D. littoralis</i> Sm.
<i>D. sect. Anisactis</i> DC.	
	<i>D. durieua</i> Lange
	<i>D. glochidiatus</i> (Labill.) Fischer & al.
	<i>D. montanus</i> Humb. & Bonpl. ex Schult.
<i>D. sect. Chrysodaucus</i> Thell.	
	<i>D. aureus</i> Desf.
<i>D. sect. Meoides</i> Lange	
	<i>D. setifolius</i> Desf.

with calf intestinal alkaline phosphatase. JM109 strain of *Escherichia coli* (Migula) Castellani and Chambers 1919,941) was transformed with the plasmids (Hanahan, 1983) and plated on LB media with 100 µg·mL⁻¹ of ampicillin and X-gal (Messing, 1983). Colorless colonies containing recombinant plasmids were individually grown overnight in LB broth with antibiotic. Plasmids were isolated using the miniprep procedure of Riggs and McLachlan (1986). Probes from the library were designated as DCG 1, DCG 2, etc. following the nomenclature of Myers and Weeden (1988). Plasmid concentration was spectrophotometrically determined at 260 nm. Plasmids digested with *Pst* I were subjected to electrophoresis to identify those containing inserts. One µg aliquots of plasmid with carrot inserts were individually spotted onto a Zetaprobe filter. Potentially single- to low-copy-number inserts were identified by the dot-blot procedure of Landry and Michelmore (1985).

Thirty carrot genomic inserts with weak signals on the dot-blot (assumed to be single to low copy nuclear DNA) were selected for the this study. Probes were prepared as described by Sambrook et al. (1989) with the following modifications. Seventy nanograms of total sheared carrot DNA (used to detect repetitive clones) or genomic insert DNA was oligolabelled with P³² using the Decaprime kit (Ambion, Austin, Texas) to a specific activity of 10⁸ cpm/µg. Probe DNA was heat-denatured and hybridized to the blots in 30 mL hybridization solution (40% deionized formamide, 1 mM EDTA, 0.25 M Na₂HPO₄, pH 7.2, and 7% SDS) overnight at 42 °C. Filters were washed twice in 0.1× SSC with 0.1% SDS for 5 and 10 min each. Filters were rinsed in 2× SSC, wrapped in plastic and placed on X-ray film (Kodak XAR-5) with intensifying screens at -70 °C for 1 to 3 d.

DATA SCORING AND ANALYSIS. For each probe–enzyme combina-

tion, restriction fragments among all accessions were assigned numbers (1, 2, 3 ...n) according to decreasing molecular weights. Fragment sizes were estimated from autoradiographs using a computer program that interpolates from a standard curve of end-labelled fragments of *Hind* III digest of the bacteriophage lambda (Schaffer and Sederoff, 1981). The presence of a band in a particular accession was scored as 1 and absence as 0. Since these nRFLPs are not mapped, an evaluation of restriction fragments was made, rather than restriction sites. Each fragment was treated as a unit character.

Cladistic and phenetic analyses of nRFLP data were performed. Cladistic analysis attempts to identify the level of divergence among a group of related taxa based upon character states (e.g., presence or absence of given nRFLP fragments) relative to an outgroup (e.g., a related genus). Phenetic analysis uses a measure of similarity or dissimilarity calculated from shared polymorphisms and a clustering algorithm.

The computer program Phylogenetic Analysis Using Parsimony (PAUP version 3.1) developed by D.L. Swofford (Illinois Natural History Survey, Champaign, Ill.) was used to generate the phylogenetic trees for cladistic analysis. Parsley (*Petroselinum crispum*), an Apiaceae distant to *Daucus*, and *D. glochidiatus*, the most distant of the *Daucus* species (Sáenz Laín, 1981), were used to root the trees. A separate analysis including only 2 of the 10 cultivated carrots was performed. Search strategies and protocols were used as reviewed in Olmstead and Palmer (1994). Only the phylogenetically informative data were used. One hundred bootstrap replicates (data sampling with replacement) were run and decay analysis was performed to obtain a relative measure of statistical strength for the delineated clades.

Table 2. List of *Daucus* species, carrot inbreds and cultivars used to estimate diversity.

Section ²	Accession	Description/origin	Seed source
(Outgroup)	<i>Petroselinum crispum</i> ((Mill.) Nym ex A. W. Hill)	Parsley	Agricultural University, Krakow, Poland
<i>Anisactis</i> (A)	<i>Daucus glochidiatus</i> (Labill.) Fischer et al.	Australian sp.	HRI ³ 8251
<i>Platyspermum</i> (P)	<i>D. muricatus</i> L.	Mediterranean sp.	PI ⁴ 295863
	<i>D. littoralis</i> Sm.	N. African sp.	PI 341902
	<i>D. broteri</i> Ten. (<i>D. bicolor</i> Sm.)	N. African sp.	PI 295857
<i>Daucus</i> (D)	<i>D. guttatus</i> Sm.	E. Mediterranean sp.	PI 279798
	<i>D. gracilis</i> Steinh.	N. African sp.	HRI 6677
	<i>D. capillifolius</i> Gilli	N. African sp.	PI 279764
	<i>D. carota</i> ssp. <i>drepanensis</i>	Mediterranean type	PI 279762
	<i>D. carota</i> ssp. <i>sativus</i> (cultivated)		
	Brasilia	Brazilian cultivar	EMBRAPA, Brazil
	Danvers	Euro–American cultivar	Alf Christianson Seeds
	Savory	U.S. hybrid cultivar, Euro–American origin	USDA
	HCM	High carotene, Asian–American origin	USDA
	Kokubu	Japanese cultivar	PI 264543
	B5280	U.S. Inbred (Asian–European origin)	USDA
	B8245	U.S. Inbred (Asian–American origin)	USDA
	B6274	U.S. Inbred (European–American origin)	USDA
	B9304	U.S. Inbred (European origin)	USDA
	B7262	U.S. Inbred (Turkish–European origin)	USDA

²Classification by Sáenz Laín (1981).

³HRI = Horticulture Research International (Wellesbourne, U.K.).

⁴PI = USDA plant introduction.

Fig. 1. Strict (100%) consensus tree of four most parsimonious trees generated from nuclear RFLP data. The numbers on the branches indicate the bootstrap values supporting the clades (percentages) and decay indices (d). Only the values >50% have been included. Letters in parentheses denote sections of Sáenz Laín (1981). Clades are indicated by roman numerals.

Jaccard's (1908) similarity coefficients were calculated on the data. Cluster analysis on these matrices was done using the unweighted-pair-group method (UPGMA) (Sneath and Sokal, 1973) with NTSYS version 1.7 (Rohlf, 1992).

Results and Discussion

CLADISTIC ANALYSIS. Among 247 nuclear DNA fragments recorded from the combination of 30 probes and two endonucleases, one fragment was present in only one accession but absent in all others, 77 fragments were common to all accessions (31.2%), and the remaining 169 fragments were phylogenetically informative (68.5%). Phylogenetic trees were generated from these data by PAUP. Relationships of the accessions are indicated by the topology of the tree. Each of the four shortest (most parsimonious) trees had a length of 673 steps.

A strict (100%) consensus tree was generated from the four parsimonious trees (Fig. 1). The statistical consensus among the four trees for each branch is indicated by bootstrap values. Using minimum bootstrap values of 50%, three principal clades were observed in this consensus tree. Two species, *D. glochidiatus* (from Australia) and *D. guttatus* (from the eastern Mediterranean), were unresolved. *Daucus glochidiatus* belongs to section *Anisactis* (Sáenz Laín, 1981) and was the only representative of this section in our study. *Daucus guttatus* is separated from the other members of its section (section *Daucus*). This is interesting and calls for further investigation as it suggests polyphyly.

Clade I was represented by *D. littoralis* and *D. broteri* with a bootstrap value of 87%. Both belong to section *Platyspermum* (Sáenz Laín, 1981).

The North African species *D. capillifolius* and *D. gracilis* and the Mediterranean taxa *D. carota* ssp. *drepanensis* and *D. muricatus* were in clade II supported by a bootstrap value of 51%, only slightly exceeding the minimum value we used to define clades. The three species were separated from *D. carota* ssp. *drepanensis* with a bootstrap value of 83%. All these species belong to section *Daucus* (Sáenz Laín, 1981) except *D. muricatus*, a Mediterranean

species, which Sáenz Laín placed in section *Platyspermum*. Notably, *D. muricatus* was not grouped with other members of this section, *D. broteri* and *D. littoralis*. Our results lend support to Heywood's (1977) placement of *D. muricatus* and *D. broteri* (*D. bicolor*) in different sections.

Accessions in clade III were supported by a bootstrap of 91%. All these are cultivated carrots and have been classified under section *Daucus* by Sáenz Laín (1981). Wide variation among these carrots was seen. Inbred B7262 (a purple-rooted carrot with Turkish parentage) was outside clade III in this consensus tree.

To reduce the large representation of cultivated carrots in the phylogenetic analyses, only two cultivated carrots, 'Brasilia' and B7262 were retained in a separate analysis. The same three principal clades were observed in the resulting consensus tree. Again, the distinction between clades II and III of Fig. 1 was weak, supported with a bootstrap of 54% (data not presented).

Daucus littoralis and *D. broteri* remained in clade I while *D. glochidiatus* and *D. guttatus* remained unresolved.

Daucus capillifolius readily intercrosses with *D. carota* (McCollum, 1975) as does *D. carota* ssp. *drepanensis* (Onno, 1936). Crosses between *D. carota* and *D. gracilis* are not reported, whereas crosses between *D. carota* with *D. muricatus* have been reported (Boblenz et al., 1990), but later questioned (Steinborn et al., 1995). In light of these reports, it is interesting to note the proximity of clade II (which includes *D. capillifolius* and *D. muricatus*) to clade III (which includes *D. carota*).

We had some concern about using parsley as an outgroup in these studies, due to its distance from *Daucus* within the Apiaceae. To provide an analysis with a relative closer than parsley, the most disparate *Daucus* species studied, *D. glochidiatus*, was used as an outgroup in an additional PAUP analysis of our data. General trends were identical in both analyses (data not presented).

PHENETIC ANALYSIS. Accessions were separated into two major groups with the cluster analysis (Fig. 2). As with cladistic analysis, *D. glochidiatus* was not part of these major groups. Group I had

Fig. 2. UPGMA dendrogram (Sneath and Sokal, 1973) of clusters among carrot entries, *Daucus* species and parsley (*Petroselinum*) as determined by nuclear RFLPs. Jaccard's (1908) coefficient was used to determine the similarity matrix. Cluster numbers are indicated by roman numerals.

both members of section *Platyspermum* (*D. littoralis* and *D. broteri*) as well as *D. guttatus* of section *Daucus* (which was not grouped with members of section *Platyspermum* in our cladistic analysis). We found *D. muricatus* was not clustered with the other members of section *Platyspermum* in the phenetic analysis. *Daucus littoralis* and *D. broteri* consistently grouped together.

Group II had only members of section *Daucus*. *Daucus capillifolius*, *D. carota* ssp. *drepanensis*, *D. gracilis* and B7262 were members of cluster II in this phenetic analysis, unlike the cladistic analysis. Phenetic analysis should provide particularly useful information about relationships among cultivated carrots, which interbreed. Wide variation was seen among the cultivated carrots with inbred B7262 being closer to ssp. *drepanensis* than to other cultivated carrots.

Thus, there was general congruence of systematic relationships, by either cladistic or phenetic analyses of nRFLP data, with classical taxonomic treatments. The exceptions in both analyses were the unexpected grouping of *D. muricatus* away from other members of section *Platyspermum* and of *D. guttatus* from other members of section *Daucus*. An evaluation of more accessions of the species included in this study, as well as more molecular marker classes, would allow a better comparison of our molecular phylogeny with traditional morphological classification.

Literature Cited

- Baillon, H.E. 1880. Umbelliferae, p. 84–256. Histoire Naturelle des Plantes. Hachette, Paris.
- Bentham, G. 1867. *Daucus*, p. 928. In: G. Bentham and J.D. Hooker (eds.). Genera Plantarum 1:859–931.
- Boblenz, K., T. Nothnagel, and M. Metzlauff. 1990. Paternal inheritance of plastids in the genus *Daucus*. Mol. Gen. Genet. 220:489–491.
- Calestani, V. 1905. Contributo alla systematica delle Umbellifere d'Europa. Webbia 1:259–264.
- Drude, C.G.O. 1898. Umbelliferae (in German), p. 63–250. In: A. Engler and K. Prantl (eds.). The natural plant families. Engelmann, Leipzig.
- Hanahan, D. 1983. Studies on transformation of *E. coli* with plasmids. J. Mol. Biol. 166:557–580.
- Heywood, V.H. 1968a. *Daucus*, p. 373–375. In: T.G. Tutin, V.H. Heywood, N.A. Burges, D.M. Moore, D.H. Valentine, S.M. Walters, and D.A. Webb (eds.). Flora Europaea. University Press, Cambridge.
- Heywood, V.H. 1968b. The *Daucus carota*–*gingidium* complex. Flora Europaea, Notulae Systematicae 7:65–68.
- Heywood, V.H. 1977. Multivariate taxonomic synthesis of the tribe Caucalideae, p. 727–736. In: A.-M. Cauwet-Marc and J. Carbonnier (eds.). Les Umbelliferae. Missouri Botanical Garden, St. Louis.
- Heywood, V.H. 1983. Relationships and evolution in the *Daucus carota* complex. Isr. J. Bot. 32:51–65.
- Heywood, V.H. and K.M.M. Dakshini. 1971. Fruit structure in the Umbelliferae–Caucalidae. Bot. J. Linnean Soc. 64(Suppl. 1):215–232.
- Jaccard, P. 1908. Nouvelles recherches sur la distribution florale. Bul. Soc. Vaudoise Sci. Nat. 44:223–270.
- Landry, B. and R. Michelmore. 1985. Selection of probes for restriction fragment length analysis from plant genomic clones. Plant Mol. Biol. Rpt. 3:174–179.
- McCollum, G.D. 1975. Interspecific hybrid *Daucus carota* x *Daucus capillifolius*. Bot. Gaz. 136:201–206.
- Messing, J. 1983. New M13 vectors for cloning. Methods Enzymol. 101:20–78.
- Murray, M.G. and W.F. Thompson. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8:4321–4325.
- Myers, J. and N. Weeden. 1988. Proposed revision of guidelines for genetic analysis in *Phaseolus vulgaris*. Annu. Rev. Bean Improv. Coop. 31:16–19.
- Olmstead, R.G. and J.D. Palmer. 1994. Chloroplast DNA systematics: a review of methods and data analysis. Amer. J. Bot. 81:1205–1224.
- Onno, M. 1936. The wild forms of *Daucus* sect. *Carota* (in German). Beih. Bot. Centralbl. 56:83–136.
- Owens, S.J. 1974. An examination of the floral biology, breeding system and cytology in species of the genus *Daucus* and related genera in the tribe Caucalideae (Umbelliferae). PhD thesis. Univ. Reading, Reading, Berkshire, U.K. p. 193.
- Riggs, M. and A. McLachlan. 1986. A simplified screening procedure for large numbers of plasmid mini-preparations. BioTechniques 4:312–313.
- Rohlf, F. 1992. NTSYS-pc: Numerical taxonomy and multivariate analysis system. Exeter Software, Setauket, N.Y.
- Sáenz de Rivas, C. and V.H. Heywood. 1974. Preliminary study on the *Daucus* species of peninsular Spain. Anales Inst. Bot. A.J. Cavanilles 31:97–118.
- Sáenz Lán, C. 1981. Research on *Daucus* L. (Umbelliferae). Anales Jardin Botanico De Madrid 37:481–533.
- Sambrook, J., E. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.
- Schaffer, H. and R. Sederoff. 1981. Improved estimation of DNA fragment lengths from agarose gels. Anal. Biochem. 115:113–122.
- Small, E. 1978. A numerical taxonomic analysis of the *Daucus carota* complex. Can. J. Bot. 56:248–276.
- Sneath, P.H.A. and R.O. Sokal. 1973. Numerical taxonomy. WH Freeman & Co., San Francisco.
- Southern, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503–517.
- St. Pierre, M.D., R.J. Bayer, and I.M. Weis. 1990. An isozyme-based assessment of the genetic variability within the *Daucus carota* complex (Apiaceae: Caucalideae). Can. J. Bot. 68:2449–2457.
- Steinborn, R., B. Linke, T. Nothnagel, and T. Borner. 1995. Inheritance of chloroplast and mitochondrial DNA in alloplasmic forms of the genus *Daucus*. Theor. Appl. Genet. 91:632–638.
- Thellung, A. 1926. *Daucus* (in German), p. 1501–1526. In: G. Hegi (ed.). Illustrierte Flora von Mittel europa. Verlag Paul Parey, Berlin.
- Whitkus, R., J. Doebley, and J.F. Wendel. 1994. Nuclear DNA markers in systematics and evolution, p. 116–141. In: R.L. Phillips and I.K. Vasil (eds.). DNA-based markers in plants. Kluwer Academic Publishers, Netherlands.
- Zohary, M. 1972. *Daucus*. Israel Acad. Sci. Humanities 2:448–458.