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Mitochondrial genome diversity among cultivars of *Daucus carota* (ssp. *sativus*) and their wild relatives

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Summary. Restriction fragment patterns of mitochondrial DNAs (mtDNAs) from 13 carrot cultivars (*Daucus carota* ssp. *sativus*), wild carrot (ssp. *carota*), ssp. *gummifer*, and *D. capillifolius* were compared with each other using four restriction endonucleases. The mtDNAs of the 13 carrot cultivars could be classified into three distinct types – I, II and III – and were also clearly distinguishable from the mtDNAs of wild carrot (type IV), *gummifer* (V) and *D. capillifolius* (VI). The proportions of common restriction fragments (F values) shared by two of the three mtDNA types (I, II and III) of carrot cultivars were approximately 0.5–0.6. The F values were 0.4–0.5 for mitochondrial genomes between wild carrot, ssp. *gummifer* and *D. capillifolius*. The mitochondrial genomes between wild carrot and the carrot cultivars showed closer homologies those between wild carrot, ssp. *gummifer*, and *D. capillifolius*. The diversity of the mitochondrial genomes among the carrot cultivars is too high to presume that it was generated from the cytoplasm of only one common ancestor during the relatively short history of carrot breeding. These results suggested that the three types of cytoplasms found in the carrot cultivars might have existed in a prototype of *D. carota* in pre-historical times.

Key words: *Daucus* – Carrot – Mitochondrial DNA

Introduction

Each plant species reveals its own unique pattern of mitochondrial or plastid DNA by restriction endonuclease fragment analysis. The variation in restriction patterns of the cytoplasmic DNAs within specific taxonomic groups or related species provides useful information about phylogenetic and evolutionary relationships (eg., Atchison et al. 1976; Levings and Pring 1976; Vedel et al. 1976; Quetier and Vedel 1977).

The taxonomic relationships between wild and cultivated species of the genus *Daucus* (Small 1978) are not clearly understood, partially because of the absence of intrinsic barriers to interbreeding. For example, a wild species, *D. capillifolius* Gilli, clearly differs from domesticated carrot, *D. carota* L., in several morphological traits. The two species are readily crossable, however, and produce highly fertile hybrids (McCollum 1975). Novel studies, such as restriction fragment pattern analysis (DeBonte et al. 1984) or DNA sequence analysis of cytoplasmic genomes, are therefore expected to define phylogenetic relationships in *Daucus* species.

In *Daucus* species, the mitochondrial genomes are much less conserved with respect to their restriction patterns than are the plastid genomes (DeBonte et al. 1984; Matthews and DeBonte 1985). In this study, we compared the restriction patterns of mitochondrial DNAs (mtDNAs) between 13 carrot cultivars (*D. carota*) and 3 wild species in *Daucus* in order to investigate mtDNA variation and to classify their mtDNA types. There was a noticeable diversity of mitochondrial genomes in the *Daucus* species and even in the carrot cultivars.

Materials and methods

Plant material

Thirteen cultivars of *Daucus carota* ssp. *sativus*, and a single accession of each of *D. carota* ssp. *carota* (wild carrot), ssp. *gummifer*, and *D. capillifolius* were used in the present investigation. Their sources are as follows: 'Imperator', 'Early Chantenay', 'Heian-nagafutori-kintoki', 'Kokubu-senkodaicho', and

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'Kuroda-gosun' from the Takii Seed Co., Kyoto; 'US harumaki-gosun' from the Yokohama Ueki Co., Yokohama; 'Koyasu-sanzun' and 'Manpukuji-senkodaicho' from the Nippon Norin Seed Co., Tokyo; 'Kono-senko-sanzun' from the Takayama Seed Co., Kyoto; 'Koizumi-riso-gosun', 'Nantes Scarlet', and 'Kikuyo-gosun' from H. Ito, Chonan Breeding and Research Farm, Kyowa Seed Co., Chiba; Yagoto-gosun from S. Tasaki, Nagoya Agricultural Center, Nagoya; wild carrot (ssp. *carota*) from H. Uchimiya, University of Tsukuba, Ibaraki; and ssp. *gummifer* and *D. capillifolius* from G. D. McCollum, Agricultural Research Station, USDA, Beltsville, Maryland.

Isolation of mtDNAs

MtDNAs were isolated from carrot cell suspensions initiated from hypocotyl-derived calli as described by Ichikawa et al. (1987).

Restriction endonuclease analysis

MtDNA (1–2 μg) was digested with restriction enzymes according to the supplier's recommendations for at least 4 h at 37 °C. The restriction fragments were separated on 0.5% agarose gels at 40V for 16 h. The gels were stained with 1 μg/ml ethidium bromide and subsequently photographed. Minimum molecular sizes of the mtDNAs were estimated from the addition of restriction fragment sizes without considering their stoichiometric differences. HindIII-digested lambda DNA was used as a molecular size marker.

Results

The restriction patterns of mtDNAs from 13 cultivars of *D. carota* ssp. *sativus*, wild carrot (ssp. *carota*), ssp. *gummifer*, and *D. capillifolius* were analyzed with four restriction endonucleases, PstI, SalI, XbaI, and XhoI. The representative patterns are shown in Figs. 1 and 2. Approximately 30 fragments (from 20 to 40) were detected with each restriction enzyme. From these restriction patterns, mitochondrial genomes in the carrot cultivars were classified into three typical groups: I, II and III (Table 1). Minor differences in restriction patterns were observed among cultivars of the same type, especially in type III; for example, between 'Kuroda-gosun' and 'Early Chantenay'. Many of these differences could have occurred as a result of changes in the stoichiometry of specific DNA fragments and partly because of base substitutions. Moreover, each mtDNA restriction pattern of these three types was clearly distinguishable from those of wild carrot (type IV), ssp. *gummifer* (V), and *D. capillifolius* (VI).

The densitometer scan of each mtDNA pattern showed several fold changes in fragment intensity (data not shown). This result indicated that the *Daucus* mitochondrial genome should have as complex a multipartite organization as described for *Brassica campestris* (Palmer and Shields 1984), maize (Lonsdale et al. 1984), and spinach (Stern and Palmer 1986).

The molecular weights were calculated and compared for all SalI, PstI, and XbaI restriction fragments greater

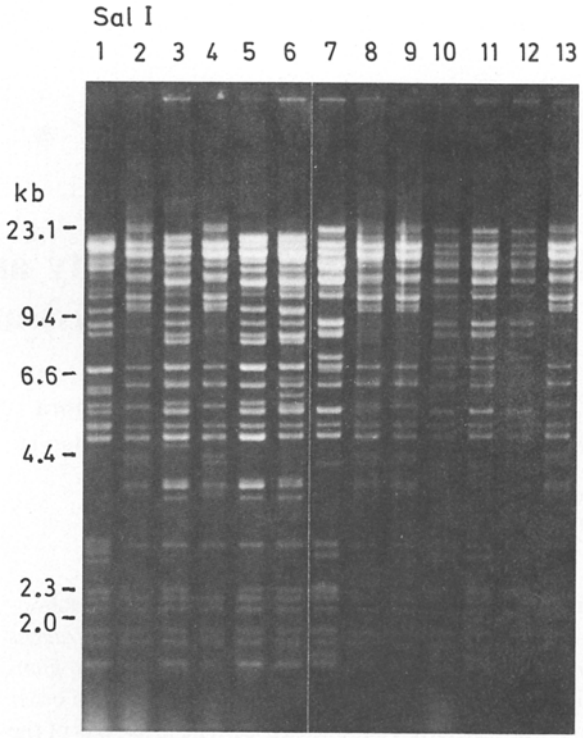

Fig. 1. SalI restriction fragment patterns of mtDNAs from 13 cultivars of *D. carota* ssp. *sativus*. *Lanes 1–13:* wild carrot, 'Koizumi-riso-gosun', 'Kikuyo-gosun', 'Yagoto-gosun', 'Kuroda-gosun', 'Early Chantenay', 'US Harumaki-gosun', 'Koyasu-sanzun', 'Kono-senko-sanzun', 'Manpukuji-senkodaicho' 'Kokubu-senkodaicho', 'Heian-nagafutori-kintoki', and 'Imperator'

than 1.0 kilobase pairs (kb) from mtDNA digests of 'Kokubu-senkodaicho' (type I), 'Imperator' (II), and 'Kuroda-gosun' (III). This analysis ignored the stoichiometry of the DNA fragments. A total of 41 restriction fragments were shared in common by the three (I, II and III) mtDNA types of the carrot cultivars. MtDNAs from wild carrot (type IV), ssp. *gummifer* (V), and *D. capillifolius* (VI) shared 38, 27, and 31 of the 41 fragments common to all three (I, II and III) mtDNA types, respectively.

The proportions of shared fragments (F values) between two types of mtDNAs were calculated according to Nei and Li (1979) (Table 2). In this calculation, all DNA divergences between two populations are assumed to have derived from nucleotide substitutions. This does not hold true in plant mtDNA restriction patterns because of the complex molecular structure of plant mtDNA (Lonsdale 1984). However, as described in some research articles on higher plant mtDNAs (Borck and Walbot 1982; Holwerda et al. 1986; Terachi and Tsunewaki 1986), this value is regarded as a meaningful index when evaluating similarities between two mtDNAs. The F values obtained between the three mtDNA types (I, II and III) of cultivated carrot were about 0.5–0.6. The

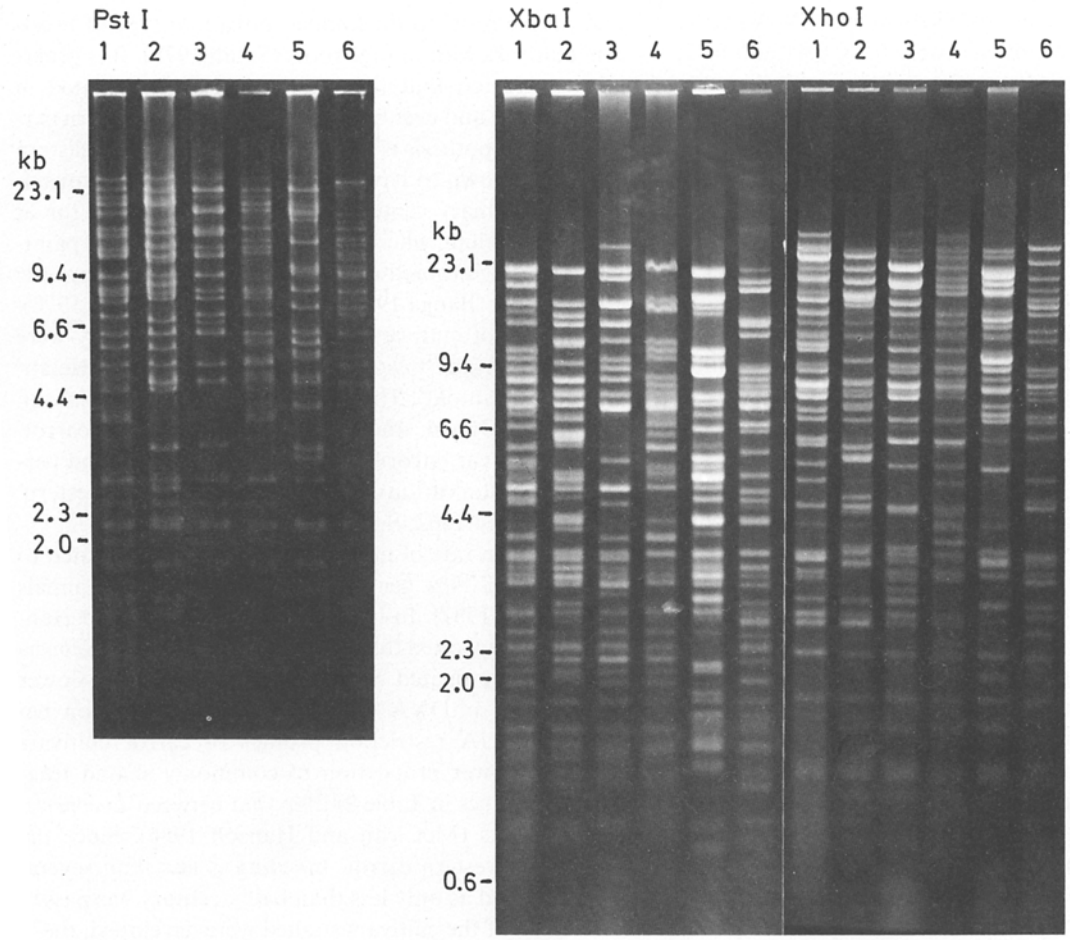

Fig. 2. PstI, XbaI, and XhoI restriction fragment patterns of mtDNAs from six accessions of *Daucus* representing type I–VI mtDNAs. *Lanes 1–6:* 'Kokubu-senkodaicho' (type I), 'Imperator' (II), 'Kuroda-gosun' (III), wild carrot (IV), ssp. *gummifer* (V), and *D. capillifolius* (VI)

Table 1. Classification of mtDNAs from cultivated carrots and their wild relatives by restriction fragment patterns

Type of mtDNA	Species, subspecies or cultivar[a]
I	Heian-nagafutori-kintoki
	Kokubu-senkodaicho
	Manpukuji-senkodaicho
	US harumaki-gosun
II	Imperator
	Koizumi-riso-gosun
	Kono-senko-sanzun
	Koyasu-sanzun
	Yagoto-gosun
III	Early Chantenay
	Kikuyo-gosun
	Kuroda-gosun
	Nantes Scarlet
IV	ssp. *carota* (wild carrot)
V	ssp. *gummifer*
VI	*D. capillifolius*

[a] All cultivars belong to *Daucus carota* ssp. *sativus*

Table 2. The proportion of PstI, SalI, and XbaI restriction fragments (F) shared in common by all possible pairs of six *Daucus* mtDNA types[a]

Type	I	II	III	IV	V
II	0.51				
III	0.59	0.55			
IV	0.63	0.58	0.69		
V	0.46	0.43	0.33	0.40	
VI	0.45	0.46	0.51	0.49	0.50

[a] The proportion of fragments (F value) shared in common by two mtDNA types was calculated after Nei and Li (1979), using the formula $F = 2N_{XY}/(N_X + N_Y)$, where N_X and N_Y are the total fragment numbers in three restriction patterns of two types, X and Y, respectively, whereas N_{XY} is the number of fragments shared in common by the two types

average F values between mtDNAs of type IV, V, or VI and type I–III of the cultivars were 0.63, 0.41 and 0.47, respectively. Although the difference in morphology between *D. capillifolius* (type VI) and *D. carota* is more distinct than that between ssp. *gummifer* (type V) and *D. carota*, the homology in mtDNAs is higher between the former than the latter two.

Discussion

Suspension cell cultures were used for isolating mtDNAs in this study, since there was a difficulty in isolating them from carrot leaf materials. The reproducibility in mtDNA restriction fragment patterns of carrot suspension cultures was first examined. The mtDNA restriction patterns of two *D. carota* cell lines had not changed over a subculture period of 1.5 years (data not shown). Matthews and DeBonte (1985) have also pointed out the stability of mitochondrial and plastid DNA restriction patterns from *Daucus* cell cultures. The patterns of mtDNAs from green carrot plants and cell cultures were the same. These results indicate that spontaneous changes in mtDNA restriction patterns occur only rarely during cell culture of *Daucus* species.

Though the similarity in mtDNAs is low between normal and male sterile lines of maize (Borck and Walbot 1982) and sugar beet (Powling 1982; Mikami et al. 1985), a much higher similarity has been found between normal cytoplasms of maize (Levings and Pring 1977), six *Brassica* species (Lebacq and Vedel 1981), *Hedysarum* species (Baatout et al. 1985), *Lycopersicon* and the related *Solanum* species (McClean and Hanson 1986), *Hordeum vulgare* and *H. spontaneum* (Holwerda et al. 1986), and *Aegilops* species (Terachi and Tsunewaki 1986). For example, Levings and Pring (1977) reported that differences found between the mtDNA restriction patterns of normal maize cytoplasms ranged from zero to four bands. Compared to the diversity in maize mtDNAs, the diversity found in carrot mtDNAs is much more remarkable (Figs. 1 and 2, and Table 2). Our results definitely demonstrate that distinct cytoplasmic diversity is present among normal cultivars of this important crop plant.

Chloroplast genomes are much more conserved than mitochondrial genomes in *D. carota* and its wild relatives (DeBonte et al. 1984). Accordingly, mitochondrial and chloroplast genomes seem to be evolving in *Daucus* at different rates and by different mechanisms, as seen in *Lycopersicon, Solanum,* and *Aegilops* (McClean and Hanson 1986; Terachi and Tsunewaki 1986).

Both wild and domesticated carrots, which belong to *D. carota,* contain numerous intergrading variants. More than 60 species have been proposed for the variants of the *D. carota* complex alone (Small 1978). Domesticated carrot (*D. carota* ssp. *sativus*) shows a variation ranging from the primitive Asian ('eastern') variants with yellow and purple roots to the familiar advanced type of 'western' cultivars with orange roots (Small 1978). It is generally considered that the 'eastern carrot' originated in Afghanistan and eventually gave rise to the 'western carrot'. This hypothesis is based on the fact that the 'eastern carrot' is known to have existed in Afghanistan, considered the primary center of diversity for carrots, for at least a millenium, whereas no reliable literature or paintings of orange-rooted carrot are to be found before the 17th century (Banga 1957; Katsumata 1968; Small 1978). All the carrot cultivars used in the present study were bred only from 'western carrots', except for 'Heiannagafutori-kintoki'. This cultivar is one of the 'Kintoki' cultivars in Japan, and belongs to the 'eastern carrot' (ssp. *sativus* var 'atrorubens') (Katsumata 1968 and personal communication), or to an 'eastern' × 'western' hybrid (Hiroe 1962; Small 1978).

The mean rate of mtDNA divergence is estimated to be about 2%–4% per million years in various animals (Cann et al. 1987). In higher plants, McClean and Hanson (1986) suggested that mtDNA divergence in *Lycopersicon* and the related *Solanum* species would be slower than animal mtDNA divergence. The comparison between mtDNA restriction profiles of carrot cultivars showed a lower proportion of commonly shared fragments (F values in Table 2) than that between *Lycopersicon* mtDNAs (McClean and Hanson 1986). Since the history of 'western carrot' breeding is less than several centuries, and as only less than half a century has passed since most of the cultivars studied were developed, the F values obtained between mtDNAs of the carrot cultivars are much too low for such a short time after their breeding. Consequently, it is strongly suggested that the diversity of mtDNAs found among carrot cultivars existed before the domestication of *D. carota*.

In some higher plants, mtDNAs possess direct repeat elements which lead to intramolecular recombination (Lonsdale et al. 1984; Palmer and Shields 1984). If the carrot mitochondrial genome also contains such elements, rearrangements might occur by intramolecular recombination. Through this mechanism, divergent mtDNA molecules would have been produced in a putative prototype of the *D. carota* complex. Our hypothesis is that the ancestors of cultivated carrots possessed divergent mitochondrial genomes and transmitted them to the present-day domesticated carrots.

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