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# Evidence for maternal inheritance of the chloroplast genome in cultivated carrot (*Daucus carota* L. ssp. *sativus*)

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**Abstract** The incidence and inheritance of a chloroplast DNA (cpDNA) mutation/marker, BP10U, was studied in crosses among cultivated carrots (*Daucus carota* ssp. *sativus*). BP10U is about 400 bp larger than the more common BP10L allele. The occurrence of BP10U among carrot inbreds was widespread. Individual plants exhibited only one form of BP10, and cpDNA inheritance was strictly maternal. BP10U only occurred in male-fertile plants. Some male-fertile inbreds and all cytoplasmically male-sterile (petaloid) carrots had the BP10L allele. Alloplasmic cpDNA variation has been reported previously in *Daucus*, but this is the first report of variation and inheritance of cpDNA within cultivated carrot.

**Key words** Chloroplast DNA · *Daucus carota* L. ssp. *sativus* · Maternal inheritance

## Introduction

Plastid inheritance has been studied using phenotypic characters (reviewed in Smith 1989) as well as protein and DNA markers (e.g. Conde et al. 1979; Pring et al. 1982). The transmission of chloroplast (cp) DNA is also determined by evaluating pollen stained with a plastid dye, 4′,6-diamidino-2-phenylindole (DAPI), to enable cytological study of chloroplast inheritance (Corriveau and Coleman 1988). Based on all these lines of evidence plastid inheritance was categorized by Hagemann (1992) into uniparental-maternal, uniparental-paternal, and non-Mendelian biparental types. Further, three types of non-Mendelian biparental plastid inheritance were recognized; subtype (a), having a bias towards the transmission of maternal plastids; subtype (b), having equal transmission of plastids from both parents; and subtype (c), having a bias towards the transmission of paternal plastids.

The DAPI cytological evaluation of 235 angiosperm species (Corriveau and Coleman 1988) is by far the most extensive report to date on the possible modes of plastid inheritance. In this study, biparental plastid inheritance with a predominantly maternal bias was indicated in 18% of the angiosperm species, while 82% of the species appeared to have exclusive maternal inheritance. However, the presence of plastids in the pollen of 18% of these species is not considered to be conclusive evidence for paternal contribution because plastids in the pollen generative cells can be excluded, eliminated, or altered during pollen development, maturation, male gametophyte development, or at stages following fertilization (reviewed in Smith 1989). Strict paternal plastid inheritance, although common in gymnosperms, has not been reported for angiosperms.

The mode of chloroplast inheritance in genus *Daucus* has been studied both cytologically and with molecular markers, and yielded contradictory results. Cytological observations using DAPI and epifluorescence microscopy failed to detect the presence of any plastid in *Daucus carota* pollen (Corriveau and Coleman 1988). That would suggest maternal plastid inheritance. Molecular evidence based on restriction enzyme pattern analyses of the progeny of a purported interspecific cross (*Daucus muricatus* × *D. carota* ssp. *sativus* (cultivated carrot)) appeared to demonstrate that plastids could be paternally inherited (Boblenz et al. 1990), contrary to the DAPI results. Steinborn et al. (1995) reinvestigated their same filial material using cpDNA and

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mitochondrial (mt) DNA probes. Their observations confirmed that cpDNA restriction fragment length polymorphisms (RFLPs) of the progeny were similar to those of the putative paternal parent. However, mtDNA RFLP patterns in the progeny included several that were absent in either of the putative parents, leading these investigators to doubt the heritage of the $F_2$ plants. Both this interspecific cross and several other interspecific and intersubspecific crosses were also investigated, and while variation for a 1.3-kb fragment was noted in the pattern of *Bgl*II-digested DNA (Borner et al.1995; Steinborn et al. 1995), intraspecific inheritance patterns were not noted. Thus the mode of plastid inheritance in carrot remains unresolved.

We found a cpDNA RFLP within *D. carota* ssp. *sativus* (Vivek and Simon 1998) which gave us a unique opportunity to study cpDNA inheritance within cultivated carrot where crosses are readily achieved. Thus, the objective of this study was to determine the mode of inheritance of this cpDNA marker (BP10) in cultivated carrot (*Daucus carota* ssp. *sativus*) and to determine the incidence of this mutation in an array of carrot inbreds. The findings presented below are the first report on the inheritance of cpDNA in cultivated carrots.

## Materials and methods

### Plant materials

The carrot entries evaluated included 16 male-fertile maintainer lines (B-lines), their male-sterile counterparts (A-lines), two male-fertile restorers (C-lines), 12 male-sterile × male-fertile $F_1$ hybrids, four male-fertile × male-fertile $F_1$ hybrids, and two $F_2$ populations (Tables 1 and 2). Plants were grown from seed in pots, and DNA was extracted from each individually harvested plant. For each entry in Tables 1 and 2, DNA of 3–5 individually extracted samples was analyzed, with one exception. For B2566A × B6274B, 300 plants (30 bulked samples of 10 plants each) were extracted to evaluate a larger sample of 1 entry.

### DNA extraction, digestion, and blotting

The procedures followed were as described by Vivek and Simon (1998) and are briefly listed below. Total DNA was isolated from lyophilized leaves with a modified CTAB method and digested with *Bgl*II restriction enzyme at 5 U/μg according to the manufacturer's specifications (Promega, Madison, Wis.). Electrophoresis of digested DNA was on 0.8% agarose gels in 1 × TBE 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 × SSC and dried by baking in a vacuum oven at 80°C for 1 h.

### Source of probe, hybridization, and autoradiography

The probe used for hybridization was a heterologous *Petunia* clone, P10 (Sytsma and Gottlieb 1986). Whole plasmids containing cpDNA inserts were labelled with [$P^{32}$] using the Decaprime kit (Ambion, Austin, Tex.) and hybridized overnight at 42°C. Membranes were washed, rinsed, and placed on X-ray film for 1–2 days at −80°C.

**Table 1** Carrot inbred lines evaluated, and BP10 plastid status observed

| Inbred | Parentage | P10 status[a] | |
|---|---|---|---|
| | | A-line | B-line |
| B2126 | B2566 × B3475 | L | U |
| B2170 | B2566 × B5238 | L | U |
| B2226 | Synthetic | L | U + L |
| B2254 | B9304 × B3180 | L | U |
| B2566 | B9304 × B493 | L | U |
| B3475 | M10138 × M5931 | L | L |
| B4367 | Synthetic | L | U |
| B5238 | B4367 × PI419042 | L | U |
| B5280 | B6345 × B10138 | L | L |
| B5494 | B2566 × B3475 | L | U |
| B6253 | F524 × B4367 | L | L |
| B6274 | Nts × B2340 | L | U |
| B6373 | B4367 × PI419042 | L | U |
| B7262 | PI173687 × M10138 | L | L |
| B9264 | PI419042 crosses | – | L[b] |
| B9304 | W93 × Keiler Rote | L | U |
| B9695 | B4367 × B3430 | L | U |
| F524 | Waltham Hicolor | – | L[b] |

[a] L, BP10L; U, BP10U
[b] C line

### Data collection

The probe P10 identified bands in carrot of which those in the 1.3- to 2.0-kbp range were evaluated for this study. Presence or absence of bands in individual plants was scored.

## Results and discussion

### Variation of the cpDNA marker

Polymorphism in the cpDNA was observed among carrot lines probed with the heterologous *Petunia* clone P10 (Fig. 1). In addition to four monomorphic bands, male-fertile inbreds had either the approximately 1.8-kbp band (BP10U) or the approximately 1.4-kbp band (BP10L) (Table 1). All the male-sterile carrot inbreds in this study had BP10L as did 6 of 18 male-fertile lines including one of the two C-lines (Table 1). Only male-fertile lines had BP10U. All of the inbreds in Table 1 went through at least one generation of self-pollination, except for B2226 where 4 or more plants were involved in each generation of mass selection. B2226 segregated for BP10 with 2 plants having BP10U and 1 having BP10L. None of the plants evaluated in this study had both bands. Thus, all carrot inbreds developed from 1 plant during the breeding process had only one of the two BP10 phenotypes. In the one instance mentioned

**Table 2** Carrot hybrids evaluated, and BP10 plastid status observed

| Hybrid | BP10 Status[a] | | |
|---|---|---|---|
| | Parental | Maternal | Observed |
| Male-sterile × fertile crosses | | | |
| B2566A × B6274B | L × U | L | L[b] |
| B5280A × B9695B | L × U | L | L |
| B9304A × B2126B | L × U | L | L |
| B9304A × B2254B | L × U | L | L |
| B9304A × B5238B | L × U | L | L |
| (B9304A × B2126B) × B2126B | (L × U) × U | L | L |
| (B9304A × B2254B) × B2170B | (L × U) × U | L | L |
| (B9304A × B5238B) × B6274B | (L × U) × U | L | L |
| (B9304A × B5238B) × B6373B | (L × U) × U | L | L |
| (B9304A × B5238B) × B9264B | (L × U) × U | L | L |
| B9695A × B3475B | L × L | L | L |
| (B9695A × B3475B) × B9695B | (L × L) × U | L | L |
| Fertile × fertile crosses | | | |
| $F_1$ hybrids | | | |
| B2566B × B9304B | U × U | U | U |
| (B2566B × B6274B) × B9304 | (U × U) × U | U | U |
| B5238B × B5280B | U × L | U | U |
| B9304B × B7262B | U × L | U | U |
| $F_2$ hybrids | | | |
| B7262B × B9304B | L × U | L | L[c] |
| B9304B × B7262B | U × L | U | U[d] |

[a] L, BP10L, U, BP10U
[b] 300 $F_1$ plants evaluated
[c] 22 $F_2$ plants evaluated
[d] 8 $F_2$ plants evaluated

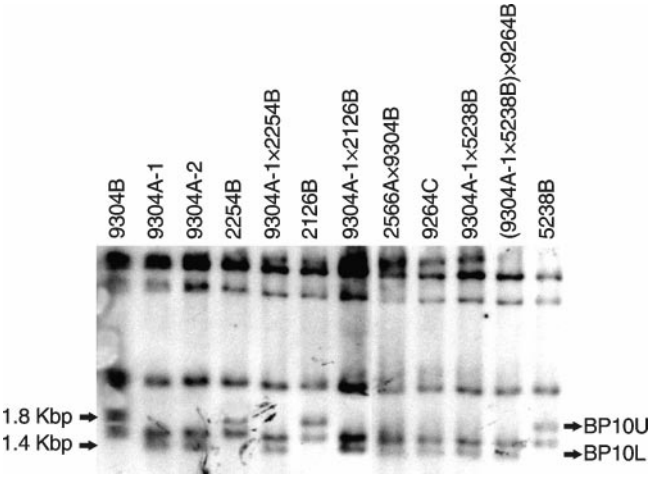


**Fig. 1** Autoradiogram of carrot DNA from male-sterile (A-lines), fertile counterparts (B- and C-lines), and their hybrids probed with *Petunia* chloroplast clone P10. Each lane represents DNA from a single plant

above (B2226) where 4 or more plants were used to advance each generation in inbred development, both BP10 phenotypes were found among the different inbred plants.

The pedigree of the carrot inbreds suggested three sources of origin of this mutation (assuming strict maternal inheritance): B4367, B6274, and B9304. B4367 was the female parent of B2144, B5238, B6373 and B9695. B9304 (or its progeny, B2566) was the female parent of B2126, B2170, B2254, and B5494. No derivatives of B6274 were evaluated. These inbreds come from diverse genetic backgrounds both in terms of root shape (Nantes, Danvers, and Imperator) and geographic origin (Europe and Asia). Equally diverse are the inbred backgrounds for BP10L: B3475, B5280, B7262, F524, and its derivative, B6253.

Thus, we can conclude that BP10U is associated with male-fertility in some carrot inbreds. All the male-steriles were of the petaloid type and had the BP10L phenotype. An analysis of brown anther male-steriles would be interesting, especially in light of the reports by Borner et al. (1995) and Steinborn et al. (1995) which describe a similar apparent polymorphism in *Bgl*II-digested DNA of a brown anther sterile carrot line.

## Inheritance of the cpDNA marker

All of the 11 informative (male-sterile) × (male-fertile) crosses, both informative (fertile) × (fertile) crosses, and all the $F_2$ plants had the BP10 phenotype of their maternal parent (Table 2), even in the six 3-way crosses and, in the case of the $F_2$s, after two generations. No exceptions were observed. Three crosses involved parents of the same BP10 phenotype and were non-informative. All this data indicated maternal plastid transmission.

## Occasional biparental inheritance

Our results indicated maternal plastid inheritance but did not rule out occasional paternal transmission. Smith (1989) cautioned that observations on maternal inheritance for a relatively small sample of hybrid plants should be used only as evidence that regular biparental inheritance of organelles with a significant proportion of paternal organelles does not occur. To determine the statistical confidence (power) of the hypothesis of maternal plastid inheritance, we evaluated a larger population of 300 plants of the cross B2566A × B6274B with DNA of 10 bulked plants in each of 30 sets. No evidence of paternal transmission was observed. We had earlier found that DNA mixtures of (15 BP10L):(1 BP10U) clearly exhibited both bands (data not presented). Thus, a rare BP10U paternally transmitted band would have been apparent if present even in 1 of the 10 bulked plants of a set. We tested the power of the maternal inheritance hypothesis using the binomial expansion of $(p + q)^n$ ($p$ = probability of detection of paternal inheritance; $q$ = probability of non-detection of rare paternal inheritance; $n$ = number of $F_1$ plants) and assuming $q = 0.01$ (Milligan 1992). Since no paternal plastid bands were observed in these 300 plants we can state with 95% certainty that plastid transmission in carrot is maternal. Higher numbers of progeny would need to be evaluated for a higher level of confidence.

## Implications for plant breeding

Cytoplasmic male-sterility (CMS) is a strictly maternally inherited trait in carrot (Peterson and Simon 1986) associated with rearrangements in the mitochondrial genome (Pingitore et al. 1989) as has been found in other plants (Hanson 1991). Borner et al. (1995) and Steinborn et al. (1995) also studied mitochondrial RFLPs in the progeny of the intersubspecies cross, *D. carota maximus* × *D.c. sativus* as well as other interspecific and intersubspecific crosses. They concluded that inheritance of the mtDNA, like the cpDNA, was also strictly maternal. Certain broad-based carrot breeding populations, including certain old cultivars, land races, and derivatives of cultivated × wild carrot crosses, are mixed for male-fertility but, to date, no mtDNA polymorphisms have been identified to differentiate male-fertile from CMS plants in these populations. Until mtDNA markers are developed that are able to unambiguously screen for CMS, selection for the BP10U phenotype in mixed populations should provide for a means for early selection of male-fertility in polymorphic populations.

Our identification of a maternally inherited plastid mutation in carrot also provides an opportunity to better determine the origins of carrot cytoplasm. Both the lineage of carrot cultivars and the contribution of wild carrot to cultivated types may be clarified with an evaluation of this mutation.

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