

Examination of Mitochondrial and Chloroplast DNA Genetic Variation in carrot, *Daucus carota*

Abstract

Mitochondrial DNA is thought to be maternally inherited, but previous research in some plant species has found evidence of recombination and paternal leakage. Evidence of reassociation between mitochondrial and chloroplast DNA may provide evidence of paternal leakage. Following extraction of DNA from *Daucus carota*, PCR amplification of the DNA using mitochondrial and chloroplast markers was performed. PCR markers of known size variation were examined using gel electrophoresis and used to ascertain individual haplotypes. Evidence of four distinct combinations of the marker variations would yield indirect evidence of paternal leakage and support research related to paternal leakage and heteroplasmy.

Introduction

Previous research on mitochondrial DNA has operated under the assumptions that it is almost exclusively maternally inherited (Sato, 2013) and that it exhibits significantly higher levels of sequence conservation than chloroplast and nuclear DNA. However, sequencing of mitochondrial DNA in some plant species has indicated that sequence variation in mitochondrial DNA is much higher than expected. In addition, inheritance of mitochondrial DNA from maternal and paternal lineages has been demonstrated in a number of plant species, as evidenced by sequence variations in progeny that indicate the incorporation of both maternal and paternal DNA. Paternal leakage is of interest because the incorporation of paternal genes harboring potentially deleterious gene variants into the mitochondrial DNA of progeny could have important consequences. Plant species have characteristic gene markers, often in the form of sequence repeats and single nucleotide polymorphisms, that can be used to indicate the presence of heteroplasmy in plant species. Because mitochondrial DNA is generally thought to be transmitted via maternal inheritance patterns, the presence of varying combinations of alleles at different gene markers provides indirect evidence for the presence of heteroplasmy as a result of paternal leakage.

Genome	Taxa compared	L_S	$K_S \times 100$	Rate*
Plant	Maize/wheat	413	3	0.2– 0.3
	Monocot/dicot	1,219	21	0.8– 1.1
cp	Maize/wheat	934	16	1.1– 1.6
	Monocot/dicot	4,177	58	2.1– 2.9
nuc	Angiosp./bryoph.†	10,242	112	1.4– 1.6
	Spinach/ <i>Silene</i>	123	126	15.8–31.5
	Monocot/dicot	446	161	5.8– 8.1

Figure 1. Mutation rates in mitochondrial, chloroplast and nuclear DNA of different plant species

Materials and Methods

Wild samples of *D. carota* were collected from the Olympic peninsula in Washington and crop and wild samples were collected from Nantucket island. A small amount of leaf material (enough to fit inside a PCR tube) was taken from each sample and roughly inserted into a 2ml tube along with three grinding beads and ground until sufficiently shredded. The protocol for the E.Z.N.A. SQ Plant DNA Extraction Kit was used in order to extract DNA from the leaf samples. To ensure that the quality and concentration of the DNA collected from each leaf tissue sample was high enough to produce usable data, each DNA sample was analyzed utilizing a NanoDrop 2000 spectrophotometer. For those samples that produced low quality DNA, the DNA extraction process was repeated in order to obtain usable DNA samples. In order to identify repeat patterns for the ATP 1 gene in each sample, a 2-primer PCR from Eurofins Genomics was utilized. In order to identify simple nucleotide polymorphisms in the *Atp9* and *Cox1* genes, QPCR was run utilizing assays from Applied Biosystems.

Mitochondrial Gene Markers			
<i>Atp1</i>	<i>Atp9</i>	<i>Cox1</i>	Number of Combinations
F	A	C	2
S	T	T	16
S	T	C	17
F	T	C	5
F	A	T	3
2F	T	C	1
F	C	C	7
S	A	T	1

Table 1. Allele combinations for mitochondrial gene markers in *D. carota*



Results

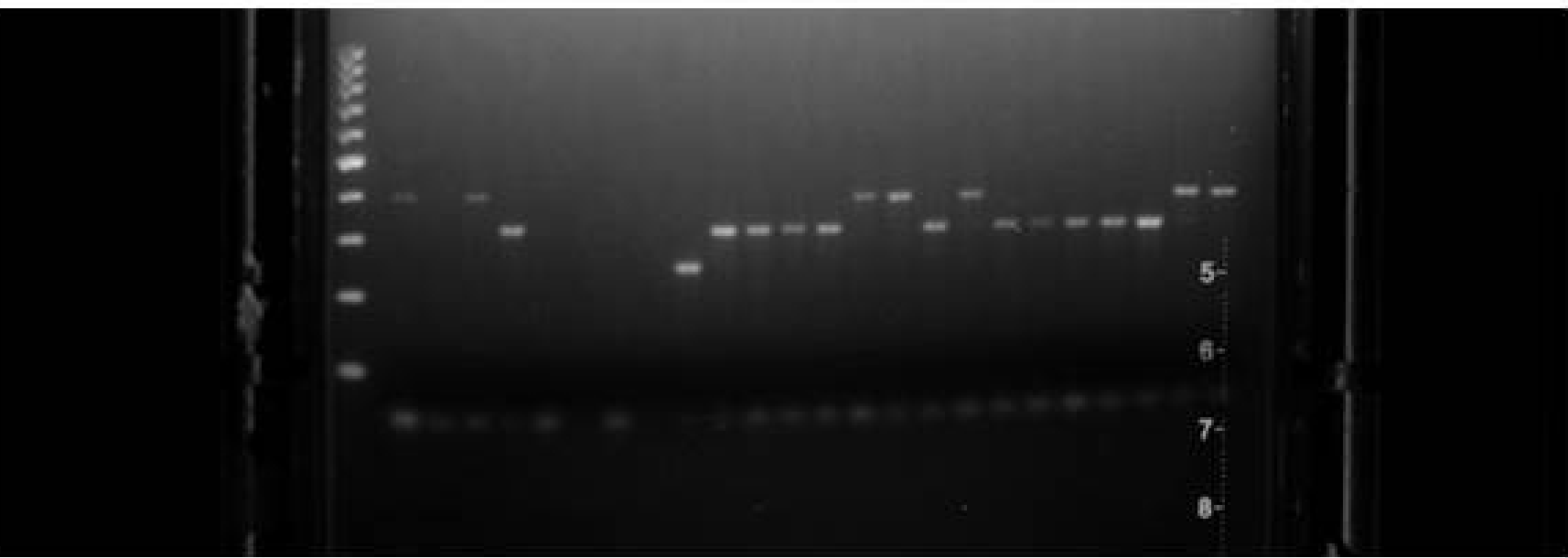


Figure 2. Agarose Gel for *Atp1* gene in *D. carota*



Chloroplast Genome Alleles	
Alleles	Number Sampled
440	8
437	5
438	13
445	13
433	2

Table 2. Alleles for chloroplast genome in *D. carota*

Discussion

Mitochondrial DNA has traditionally been thought to contain highly conserved gene sequences and, thus, little variation in progeny would exist even if recombination occurs. In addition, mitochondrial DNA was traditionally thought to be almost exclusively inherited from the maternal line; the lack of sequence variation in conjunction with maternal inheritance should produce genotypes with very little variation. The chloroplast genome is also thought to exhibit low rates of sequence evolution (Mason-Gamer, R. et al., 1995) and maternal inheritance. Our data indicates that a high degree of sequence variation exists in both the mitochondrial and chloroplast DNA (Tables 1 and 2) *D. carota*. This variation provides the potential for us to observe heteroplasmy and any paternal leakage of chloroplast or mitochondrial DNA.

The presence of three distinct alleles in the *Atp1* gene (S, F, 2F) was observed (Figure 2). Three alleles were observed in the *Atp9* gene, and two alleles were observed in the *Cox1* gene (Table 1). Of 18 possible allele combinations from these three gene markers, 7 combinations were observed (Table 1). The “four gamete test” tests for the presence of genetic recombination. Comparing two genes that have both undergone mutation independently of one another, one would expect to find three gene combinations present in a population. Comparing the sequence data for the *Atp1* gene and the single nucleotide polymorphism data for the *Cox1* genes, the observed presence of a fourth combination pair indicates that recombination has likely occurred. Because the mutation rate for mitochondrial DNA is very low, the presence of this fourth recombination pairing is most likely the result of recombination—something that would not be observable if there had not been heteroplasmy in the individuals.

References

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- Mason-Gamer, R. J., K. E. Holsinger, and R. K. Jansen. (1995). Chloroplast DNA haplotype variation within and among populations of *Coreopsis grandiflora* (Asteraceae). *Molecular Biology and Evolution*. 12:371–381.

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