Inheritance of the Chloroplast Genome in Sorbus aucuparia L. (Rosaceae)

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Chloroplast DNA (cpDNA) inheritance was investigated in Sorbus aucuparia using progenies obtained from six controlled crosses between individuals of known haplotype. Polymerase chain reaction (PCR) amplification, followed by restriction analysis, was used to characterize 248 offspring for either of two polymorphic cpDNA fragments. All offspring exhibited the maternal haplotype, which indicates maternal inheritance of chloroplasts in S. aucuparia. Power analysis of the test showed that the frequency of paternal transmission of chloroplasts, if any, should not exceed 1.84% (with 99% confidence).

The genus Sorbus L. (Rosaceae: Maloideae), mostly distributed in northern temperate regions, comprises about 100 species of trees and shrubs (Clapham et al. 1962). Several of these species are commonly used for decorative planting, and the enormous variation in the color of fruit and autumn foliage provides a tremendous horticultural potential (McAllister 1986, 1996). In particular, several cultivars of Sorbus aucuparia have been selected and clonally propagated: cv. Fructu Luteo (with golden fruits), cv. Aspleniola (which has deeply cut foliage), and cv. Sheerwater Seedling (which has a particularly upright habit). The taxonomy of the genus is complicated by the common occurrence of hybridization, as in other genera of the Maloideae (Campbell and Dickinson 1990). The resulting hybrids often are apomictic and locally distributed (Clapham et al. 1962; Hull and Smart 1984; Máňovský and Bernátová 1996).

Chloroplast DNA (cpDNA) markers have been increasingly used to address plant evolutionary issues. This is because of the markedly different characteristics of cpDNA compared to the nuclear genome, as well as the recent development of efficient molecular tools. The most striking and general differences between chloroplast and nuclear genomes are that the former show uniparental inheritance in most plant species (Birky 1995; Reboud and Zeyl 1994), a clonal mode of evolution (i.e., no recombination), and a slow rate of evolutionary change (Wolfe et al. 1987). These properties have been exploited in studies dealing with a variety of aspects of plant evolutionary biology such as phylogeny (Gielly and Taberlet 1994; Kron et al. 1999; Palmer et al. 1988), interspecific gene flow and hybridization (Ferguson et al. 1999), and plant phylogeography (Petit et al. 1997; Taberlet et al. 1998; Tremblay and Schoen 1999), as well as to get insights into the relative contribution of pollen and seed movement in intraspecific gene flow (McCauley 1995). Although maternal inheritance is the most common mode of transmission of the chloroplast genome in angiosperms, there is evidence of at least partially paternal transmission in many species (e.g., kiwifruit [Chat et al. 1999]; Turnera ulmifolia [Shore and Triassi 1998]; also see Reboud and Zeyl [1994]). It is therefore advisable to check the chloroplast inheritance if such markers are to be used for analyses assuming strict maternal inheritance of this genome. For example, Birky et al. (1989) showed that equilibrium values of organelle diversity within and between populations are sensitive to the degree of paternal transmission.

This study is part of an ongoing research program that is investigating the genetic variation and population structure of S. aucuparia in relation to the life history of the species. The aim of the present research was to test the hypothesis of strict maternal inheritance in S. aucuparia cpDNA in order to use cpDNA markers in population genetic studies. I used the Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) approach (Demesure et al. 1995) because it allows for analysis of a large number of samples, which is necessary to detect rare events of biparental or paternal transmission that might occur. To the best of my knowledge, this is the first study in the Maloideae aiming to test the maternal inheritance of chloroplasts in progeny arrays. The Maloideae, a subfamily of the Rosaceae, comprise several genera of significant economic importance, such as Malus (apple), Pyrus (pear), Cotoneaster, and Pyracantha.

Materials and Methods

Plant Material

I performed six intraspecific crosses in wild populations located on the Plateau des Tailles (Upper Ardenne, Belgium). I enclosed inflorescences with mesh bags to exclude pollinators. Emasculation was unnecessary since the species has been shown to be self-incompatible using both experimental pollinations (Raspe et al. 2000a) and molecular analysis of the gene encoding self-incompatibility RNases (O. Raspe and J. R. Kohn, unpublished data). It has also been shown that unpollinated bagged inflorescences do not produce any fruit (Raspe et al. 2000a). Therefore it can be assumed that the seeds analyzed in this study were the result of neither selfing nor apomixis. Eight different individuals of known chloroplast DNA haplotype (Raspe et al. 2000b) were used for the performed crosses. The number of offspring analyzed per cross is given in Table 1. Two mutations (indels, i.e., insertions or deletions) were used to track the transmission of the chloroplast genome. In the cross 8.25 × 8.2, the two parents differed for a mutation (an indel of 10 bp) located in a fragment between the tRNA-Cys and tRNA-Asp encoding genes (CD fragment; Raspé
The cpDNA markers are defined by the abbreviated name of the PCR fragment followed by the rank of the restriction fragment in the restriction profile (fragments are ranked by decreasing size).

The haplotype expected for each progeny (i.e., the haplotype of the pollen recipient) is given as the size of the polymorphic fragment (in base pairs).

Table 1. Description of crosses and number of progeny analyzed per cross

<table>
<thead>
<tr>
<th>Pollen recipient</th>
<th>Pollen donor</th>
<th>N progeny</th>
<th>cpDNA marker</th>
<th>Expected haplotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.29</td>
<td>10.40</td>
<td>42</td>
<td>DT1</td>
<td>365</td>
</tr>
<tr>
<td>10.29</td>
<td>10.29</td>
<td>4</td>
<td>DT1</td>
<td>383</td>
</tr>
<tr>
<td>10.29</td>
<td>10.27</td>
<td>38</td>
<td>DT1</td>
<td>365</td>
</tr>
<tr>
<td>11.16</td>
<td>11.45</td>
<td>87</td>
<td>DT1</td>
<td>365</td>
</tr>
<tr>
<td>11.45</td>
<td>11.5</td>
<td>28</td>
<td>DT1</td>
<td>383</td>
</tr>
<tr>
<td>8.25</td>
<td>8.2</td>
<td>49</td>
<td>CD3</td>
<td>318</td>
</tr>
</tbody>
</table>

* The cpDNA markers are defined by the abbreviated name of the PCR fragment followed by the rank of the restriction fragment in the restriction profile (fragments are ranked by decreasing size).

Results and Discussion

Among the 248 progeny tested, none showed the paternal haplotype or both maternal and paternal alleles (heteroplasmic progeny) (Figure 1), which indicates that the chloroplast genome is maternally inherited in *S. aucuparia*. I assessed the power of my analysis using the binomial model presented by Milligan (1992). According to this model, the probability of paternal transmission *P* can be computed with the following equation:

$$ P = 1 - (1 - \beta)^N $$

where *N* is the sample size and $(1 - \beta)$ is the probability of falsely accepting the strictly maternal hypothesis ($\beta$ is the power of the test). My results thus indicate that the probability of paternal transmission is no more than 0.0184, given a sample size of *N* = 248 and $\beta = 0.99$. Therefore I cannot completely rule out rare paternal transmission of at least some chloroplasts. However, even if cpDNA inheritance were strictly maternal, a huge increase in sample size would be required to significantly increase the power of the test [i.e., decrease the maximum level of paternal transmission allowed by the data; see Figure 1 in Milligan (1992)].

Published data on chloroplast inheritance in the Rosaceae are scarce and often incidental. Matsumoto et al. (1997) characterized by an RFLP fragment present in the putative female progenitor (*P. pendula*) but not in the male parent (*P. lannesiana*). To my knowledge, the only study of chloroplast inheritance in the Rosaceae, testing the transmission of a chloroplast marker in the progeny from a controlled cross, is that of Brettin et al. (2000). In their study, all 19 progeny analyzed from a cross between *P. cerasus* cv. Rheinische Schattenmorelle and cv. Erdi Botermo had a restriction fragment characteristic of the former, that is, the female parent.

In a previous article, a surprisingly low level of cpDNA differentiation among *S. aucuparia* populations was reported ($G_{ste} = 0.286$; Raspé et al. 2000b), which is less than half the *G_{ste*} value usually observed in other temperate tree species (see Raspé et al. 2000b for a comparison). It was suggested that this result could be accounted for by the species’ life history (bird-dispersed seeds and colonizing habit). However, the *G_{ste*} estimate was based on the assumption of strict maternal inheritance of the chloroplast genome, and one could argue that the low level of differentiation we observed might be the result of occasional biparental inheritance. Indeed, biparental inheritance would lead to increased gene flow for cpDNA because chloroplasts would migrate in both seeds and pollen. The present study rules out this possibility. Birky et al. (1989, Figure 1) exhibited the same RFLP profile as *M. baccata*, the female parent, whereas it was different from the *M. prunifolia* profile. Similarly Kaneko et al. (1986) showed that five individuals of *Prunus yedoensis* were characterized by an RFLP fragment present in the putative female progenitor (*P. lannesiana*) but not in the male parent (*P. lannesiana*).

Figure 1. Agarose gel showing cpDNA restriction profiles (DT fragment) of parents and progeny from a controlled cross (10.29 × 10.27). The largest (top) fragment differs in the two parents. Lanes: M, molecular weight marker X (Boehringer); [female], pollen recipient (10.29); [male], pollen donor (10.27); 1–13, offspring 1 to 13.
reported that with a level of paternal transmission as low as 2%, $G_{pa}$ was only slightly lower compared to the case where there is no paternal transmission. Therefore, even if paternal leakage of chloroplasts occurred with a frequency of 1.84% (i.e., the maximum allowed by the data), it could not be solely responsible for the low observed differentiation among populations.

In conclusion, I have shown that cpDNA markers can be used as maternally inherited markers in population genetics studies in *S. aucuparia*.

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**References**


**Linkage Between Loci Controlling Nodulation and Testa Variegation in Peanut**

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Linkage of loci controlling nodulation ($N$) and testa variegation ($V$) was studied for cultivated peanut (*Arachis hypogaea* L.). The lines M4-2 (nonnodulating, variegated; $V_N^1,n_1,n_1,n_1,N_N, N_N^1,n_1,n_1,n_1$) and UF 487A (nodulating, nonvariegated; $v_N^1,v_N^1,v_N^1,v_N^1,N_N, N_N^1,v_N^1,v_N^1,v_N^1$) were used as parents in the crosses $M4-2 \times UF 487A$, $M4-2 \times (UF 487A \times M4-2)$, and their reciprocals. Individual plants were evaluated for nodulation and testa variegation in the $F_1$, $F_2$, $F_3$, $F_4$, and $F_5$ generations. Data indicate that the $N$ and $V$ loci are linked with a crossover percentage of 7.1%.

Although the inheritance of numerous traits has been studied in peanut (*Arachis hypogaea* L.), there have been surprisingly few reports of linkage (Knauf and Oziyas-Akins 1995). Patel et al. (1936) reported that growth habit and branching type did not segregate independently. They estimated the rate of crossing over between the genes for growth habit and branching to be 20%. Patil, as reported by Hammons (1973), found that the crossover rate between genes for growth habit and pod recalcification was 40.4% and the crossover rate between genes for stem hairiness and pod reticulation was 31.5%. Cuffe and Hammons (1973) observed an association between alhino seedlings and small seed size. Murthy et al. (1988) reported that four genes for pod shape were linked, and Knaulf et al. (1991) found linkage between loci for orange corolla color and purple testa. Garcia et al. (1996) identified two peanut genes, *Mae* and *Mag*, that conditioned resistance to the root knot nematode *Meloidogyne arenaria* (Neal) race 1 and were tightly linked with a crossover rate of 18%.

Nonnodulating peanuts have been identified in progeny from certain crosses in Florida (Gorbet and Burton 1979), Georgia (Essomba et al. 1991), and India (Dutta and Reddy 1988; Nigam et al. 1980, 1982). Dutta and Reddy (1988) reported that nodulation was controlled by three independent genes, with nodulation being a product of two of these genes and inhibited by a third gene when it was dominant and the others were homozygous recessive ($n_1,n_1,n_1,N_N^1,n_1,n_1,N_N^1,n_1,n_1$). We confirmed this model for inheritance of nodulation, except that in our study there was a parental influence that was observed when $n_1,n_1,N_N^1$ male gametes fused with $n_1,N_N^1$, female gametes or when $n_1,n_1,N_N^1$ male gametes fused with $n_1,N_N^1$, female gametes (Gallo-Meagher et al. 2001). These unions resulted in plants that had reduced nodulation or nonnodulation instead of the expected normal nodulation.

Three alleles have been identified which control red testa color in peanut (Ashri