Inheritance of chloroplasts and mitochondria in Conifers: A review of paternal, maternal, leakage and facultative inheritance

Robert P. Adams
Baylor University, Utah Lab, 201 N 5500 W, Hurricane, UT 84790, USA, email Robert_Adams@baylor.edu

ABSTRACT
The inheritance of chloroplasts and mitochondria in conifers is reviewed. Generally, in the Pinaceae, chloroplasts are paternally inherited and mitochondria maternally inherited. In contrast, most other conifers have paternal inheritance for both chloroplasts and mitochondria. Several examples of leakage are discussed where normally paternal cp or mt are inherited by 1-3% maternal leakage. A few Pinus taxa with mt maternal, have leakage of 1-5% paternal. An unusual case of facultative inheritance is discussed where both forward and reciprocal crosses of Pinus mugo x P. sylvestris resulted in hybrids with P. mugo chloroplasts. Published on-line www.phytologia.org Phytologia 101(2):134-138 (March 21, 2019). ISSN 030319430.

KEY WORDS: inheritance, chloroplasts, mitochondria, conifers

It is estimated that in angiosperms, chloroplasts (cp) are maternally inherited in over 70% of the genera (Hipkins et al. 1994) and about 25% of genera are biparentally inherited (Harris and Ingram (1991). Interestingly, chloroplasts were found to be only maternally inherited in dicot trees according to Rojara and Dancik (1992). However, in conifers, chloroplasts and mitochondria (mt) exhibit considerable variation in their inheritance (Table 1).

In the Pinaceae, chloroplasts are generally inherited paternally (via pollen) and mitochondria generally maternally inherited (Table 1). However, maternal and paternal leakage has often been reported Table 1). Maternal leakage refers to persistence of a few (or sometimes many) chloroplasts and/or mitochondria from the female egg, due to the incomplete degradation during fertilization (Mogensen, 1996). Likewise, paternal leakage is the survival of a few (or many) chloroplasts and/ or mitochondria from the pollen during fertilization (Mogensen, 1996). Mogensen (1996, figs. 24-29, etc.), in his seminal paper, gives lucid details for mechanisms for the destruction of cell organelles and he writes several times how “this mechanism could lead to some ‘leakiness’ of maternal plastids”, “resulting in proembryo cells typically (emphasis added) containing only male cytoplasm”, and “in the Pinaceae, the mechanism of cytoplasmic transmission described above could lead to some leakiness”.

Semidt et al. (1987) reported leakiness in Larix decidua x leptolepis in which 5 hybrids exhibited paternal inheritance, but one of the 6 hybrids had maternal chloroplast DNA, and 2 other hybrids had mixed Bam-HI restriction patterns indicative of recombination between paternal and maternal cp DNAs.

Analyses of mitochondria DNA RFLPs of 125 hybrid seedlings originating from 23 controlled crosses of Pinus banksiana x P. contorta revealed 119 maternal and 6 paternal mitochondria (~5%, Wagner, et al. 1991). Cato and Richardson (1996) utilized cpSSRs to examine inheritance of chloroplast in 206 progeny obtained from controlled pollinations of 4 Pinus radiata families. They found 99% of the progeny inherited their chloroplast paternally (pollen). But, approximately 1% of the progeny contained maternal chloroplasts, thus showing leakage in chloroplast inheritance.
Owens and Morris (1991) studied cytoplasmic inheritance in *Pseudotsuga menziesii* and noted that “most of the proembryo cytoplasm is of paternal origin, but some maternal organelles may be included”. They estimated that about 10% of the final number of mitochondria were carried to the proembryo, along with paternal organelles.

Recently, Kormutak et al. (2017, 2018) have reported a most unusual inheritance of chloroplasts in reciprocal crosses of *Pinus mugo* and *P. sylvestris*. They found *P. sylvestris* (female) x *P. mugo* (male) yielded the typical *Pinus* inheritance of paternal (*P. mugo*) chloroplasts in the hybrids. However, *P. sylvestris* (male) x *P. mugo* (female) resulted in all hybrids with only *P. mugo* chloroplast DNA (maternally inherited!). If this case can be generalized to natural hybridization of *P. sylvestris* and *P. mugo*, then the analysis of hybrids with only *P. mugo* chloroplasts will likely make studies of hybridization and introgression between these species very difficult. In any case, this example does offer a novel mechanism for chloroplast capture.

The phylogenetic distinctiveness of the Pinaceae is demonstrated by the shift in mode from cp (paternal), mt (maternal) in the Pinaceae to cp (paternal), mt (paternal) in other conifer families (Table 1), although the Taxaceae (*Taxus bacata*) may have cp (paternal), mt (maternal), but the literature is unclear as Mogensen (1996) cites Pennell and Bell (1988) who concluded “There is the possibility (emphasis added) that, as in some other gymnosperms, the plastids and mitochondria in the zygote come in part from the male gametophyte”.

*Cunninghamia konishii* (Cupressaceae) seems to be an exception to all conifers (Lu, et al. 2001) in having maternal inherited chloroplasts (Table 1), with the exception of the unusual case of *P. mugo* x *P. sylvestris* (above).

As in the case with the *Pinaceae*, maternal leakage has been reported in other conifer families (Table 1). Ohba et al. (1971) examined the inheritance of the Wogon-Sugi trait (i.e., plants having white-yellowish leaf tips, a chlorophyll mutation) in *Cryptomeria japonica*. They noted “for Wogon-Sugi (,) the trait was mostly (emphasis added) transmitted to the progeny when Wogon-Sugi (plant) was used as pollen parent”. So, apparently, some maternal leakage occurs in *Cryptomeria japonica*.

Recently, Kou et al. (2014) re-examined the classical intergeneric hybrid, Leyland cypress (*Chamaecyparis nootkatensis* x *Cupressus macrocarpa*, now *Callitropsis nootkatensis* x *Hesperocyparis macrocarpa*). Keith Rushforth and I provided historical information on the maternal seed sources of the Leyland cypress analyzed in the study (Tables 2, 3, Kou et al. 2014). Six Leyland cypress cultivars, and putative parents or exemplars were analyzed using sequences from 3 mt genes (*cox1, atpA*, and *rps3*) and 2 cp genes (*matK* and *rbcL*). Four cultivars from seed of *H. macrocarpa* trees (pollen ex *C. nootkatensis*) had *C. nootkatensis* type chloroplasts and mitochondria. However, two cultivars whose seed purportedly came from a *C. nootkatensis* (maternal) tree (x pollen of *H. macrocarpa*), were grown and contained 2 unusual seedlings: Green Spire and Haggerston Grey. These 2 cultivars were found to have *H. macrocarpa* type chloroplasts and mitochondria. Thus, if the historical records on seed source are correct, the six Leyland cypress cultivars contained 4 showing paternal inheritance and 2 exhibited maternal inheritance. Unfortunately, these results rest on historical records of plant growth and plantings, which are, of course, subject to error.

Another Cupressoideae taxon, *Chamaecyparis obtusa*, was studied (Shiraishi et al. 2001) by use of a cpDNA region, trnD-trnY. In 361 progenies, 352 (97.5%) had the same haplotype as their male parent (paternal) and 9 (2.5%) had the haplotype of their female parent (maternal), showing that although chloroplasts are mostly inherited paternally in *Chamaecyparis obtusa*, there is maternal leakage.
Table 1. Inheritance of cp (chloroplasts) and mt (mitochondria) in conifers. ns = not studied.

<table>
<thead>
<tr>
<th>Family</th>
<th>cp</th>
<th>mt</th>
<th>ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinaceae</td>
<td></td>
<td></td>
<td>review, Mogensen, 1996.</td>
</tr>
<tr>
<td><em>Larix decidua</em> x <em>leptolepis</em></td>
<td>pat¹</td>
<td>mat</td>
<td>DeVerno et al. 1991; Schmidt et al. 1987</td>
</tr>
<tr>
<td><em>Picea abies</em></td>
<td>ns</td>
<td>mat</td>
<td>Grivet, et al. 1999</td>
</tr>
<tr>
<td><em>Picea sp.</em></td>
<td>pat</td>
<td>mat</td>
<td>Sutton et al. 1991</td>
</tr>
<tr>
<td><em>Pinus sp.</em></td>
<td>pat</td>
<td>mat</td>
<td>Wagner et al. 1987; Neale and Sederoff, 1988, 1989</td>
</tr>
<tr>
<td><em>Pinus banksiana</em> x <em>contorta</em></td>
<td>ns</td>
<td>mat²</td>
<td>Wagner et al. 1991</td>
</tr>
<tr>
<td><em>Pinus echinata</em>, <em>Pinus elliottii,</em> <em>Pinus palustris,</em> and <em>Pinus taeda</em></td>
<td>pat</td>
<td>ns</td>
<td>Wagner et al. 1992</td>
</tr>
<tr>
<td><em>Pinus radiata</em></td>
<td>pat³</td>
<td>ns</td>
<td>Cato and Richardson, 1996</td>
</tr>
<tr>
<td><em>Pinus sylvestris</em>(female) x <em>P. mugo</em> (male)</td>
<td>pat</td>
<td>ns</td>
<td>Kormutak, et al. 2017, 2018</td>
</tr>
<tr>
<td><em>Pinus mugo</em> (female) x <em>P. sylvestris</em> (male)</td>
<td>mat</td>
<td>ns</td>
<td>Kormutak, et al. 2017, 2018</td>
</tr>
<tr>
<td><em>Pinus taeda</em></td>
<td>pat</td>
<td>mat</td>
<td>Neale and Sederoff, 1988, 1989</td>
</tr>
<tr>
<td><em>Pseudotsuga menziesii</em></td>
<td>pat⁴</td>
<td>mat⁴</td>
<td>Marshall and Neale, 1991; Wagner et al. 1989</td>
</tr>
<tr>
<td><em>Callitris</em> (4 species)</td>
<td>pat</td>
<td>ns</td>
<td>Sakaguchi, et al. 2014</td>
</tr>
<tr>
<td><em>Leyland cypress</em> - <em>Callitropsis nootkatensis</em> (= <em>Chamaecyparis nootkatensis</em>) x <em>Hesperocyparis</em> (= <em>Cupressus</em>)</td>
<td></td>
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<tr>
<td><em>macrocarpa</em></td>
<td>pat⁴</td>
<td>mat⁴</td>
<td>Koh, et al. 2014</td>
</tr>
<tr>
<td><em>Calocedrus decurrens</em></td>
<td>pat</td>
<td>mat</td>
<td>Neale, Marshall and Harry, 1991</td>
</tr>
<tr>
<td><em>Chamaecyparis obtusata</em></td>
<td>pat⁶</td>
<td>ns</td>
<td>Shirashi et al. 2001</td>
</tr>
<tr>
<td><em>Chamaecyparis obtusa</em> x <em>pisifera</em></td>
<td>pat</td>
<td>pat</td>
<td>Kondo, et al., 1998</td>
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<tr>
<td><em>Chamaecyparis lawsonia</em></td>
<td>pat</td>
<td>pat</td>
<td>Chesnoy, 1973</td>
</tr>
<tr>
<td><em>Platycladus orientalis</em></td>
<td>pat</td>
<td>pat</td>
<td>Chesnoy, 1969</td>
</tr>
<tr>
<td><em>Hesperocyparis arizonica</em> x <em>H. macrocarpa</em></td>
<td>pat</td>
<td>ns</td>
<td>Adams et al. 2018</td>
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<tr>
<td><em>Juniperus ashei</em>, <em>J. pinchotii</em>, <em>J. virginiana</em></td>
<td>pollen</td>
<td>pollen</td>
<td>Mohanty et al. 2016, ultrastructural presence of cp and mt in pollen was confirmed by TEM and DNA.</td>
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</table>
Mohanty et al. (2016) examined the pollen of *Juniperus ashei*, *J. pinchotii* and *J. virginiana* by TEM (Transmission Electron Microscopy) and verified the presence of chloroplasts and mitochondria in pollen in these three species. They also perfected a method to extract DNA from single pollen grains and verified specific cpDNA markers that distinguished these three species. Unfortunately, they did not have access to hybrids from controlled crossings of junipers, so they could not comment on the inheritance of chloroplasts and mitochondria in *Juniperus*.

However, Scion Ltd., New Zealand has made controlled crossings in a closely related genus, *Hesperocyparis*. Adams et al. (2018) recently analyzed 18 hybrids from a single, controlled cross, *H. arizionaica* (male) x *H. macrocarpa* (female), and all 18 had perfect *H. arizionaica* (paternal) chloroplast DNAs, confirming paternal inheritance of chloroplasts in *Hesperocyparis* (Table 1).

In conclusion, from this brief review of the inheritance of chloroplasts and mitochondria in conifers, it seems very apparent that the literature is divided by the earlier, genetics works (mostly before 1996) and the newer, more molecular reports (1996- present), that will superseded by NextGen technologies, sequencing individual DNA stands, that will very likely reveal unusual modes of variation. We will surely discover more unusual mechanisms of chloroplast and mitochondria inheritance similar to the case of facultative selection reported by Kormutak et al., (2017, 2018).

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**LITERATURE CITED**


