Recombination of *Chlamydomonas* chloroplast DNA occurs more frequently in the large inverted repeat sequence than in the single-copy regions

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**Summary.** It is well documented that chloroplast DNA (cpDNA) recombination occurs at a relatively high frequency during sexual reproduction of unicellular green algae from the *Chlamydomonas* genus. Like the cpDNAs of most land plants, those of *Chlamydomonas* species are divided into two single-copy regions by a large inverted repeat sequence, part of which encodes the chloroplast rRNA genes. In the present study, we scored the inheritance of polymorphic loci spanning the entire chloroplast genome in hybrids recovered from reciprocal interspecific and F1 crosses between *Chlamydomonas eugametos* and *C. moewusii*, and from these data, estimated the density of recombination junctions within each region of recombinant cpDNAs. Our results indicate that recombination junctions occur at highly variable frequencies across the three main domains of the chloroplast genome. The large inverted repeat sequence was found to exhibit at least a five-fold higher density of recombination junctions compared to one of the single-copy regions, whereas junctions in the latter region were five-fold more abundant relative to those in the other single-copy region. This marked difference in the densities of recombination junctions implies that the extent of genetic linkage between two given chloroplast loci will depend not only on their physical distance, but also on their locations within the genome.

**Key words:** Chloroplast DNA regions – Restriction fragment length polymorphisms – Recombination frequency – Non-Mendelian inheritance – Antibiotic resistance markers

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**Introduction**

Although recombination of chloroplast genetic markers has been widely documented in green algae belonging to the *Chlamydomonas* genus (Gillham 1978; Harris 1989), little is known about the nature and extent of chloroplast DNA (cpDNA) recombination itself. Efforts to correlate genetic and physical maps along the entire *Chlamydomonas* cpDNA molecule have failed so far, because most of the markers used in the construction of the genetic maps are clustered in a small portion of the chloroplast genome (Harris et al. 1987, 1989; Gauthier et al. 1988). In an attempt to resolve this problem, we have recently been tracing the inheritance of physically mapped cpDNA restriction fragment length polymorphisms in interspecific hybrids of *Chlamydomonas eugametos* and *C. moewusii* (Lemieux and Lee 1987; Lemieux et al. 1988).

Fusion of the two parental chloroplasts takes place in *Chlamydomonas* zygotes (Cavalier-Smith 1970), thus allowing recombination between opposite parental chloroplast genomes. Despite this fusion, however, most zygotes transmit exclusively or predominantly the chloroplast genetic markers derived from the mating-type plus (*mt*+) parent. This predominant inheritance of *mt*+ alleles is consistent with studies indicating that the multiple cpDNA copies of the *mt*− parent are preferentially destroyed during the mating process (Kuroiwa et al. 1982; Coleman and Maguire 1983). Chloroplast gene recombination occurs among the progeny of zygotes transmitting the chloroplast alleles from both the *mt*+ and *mt*− parents. In *C. reinhardtii* (Gillham 1978) and *C. eugametos* (Lee and Lemieux 1986) intraspecific crosses as well as in *C. eugametos/C. moewusii* interspecific crosses (Lemieux and Lee 1987), less than 20% of the zygotes transmit chloroplast alleles from both par-
ents with a strong bias in favor of the $mt^+$ alleles, whereas in \textit{C. moewusii} intraspecific crosses (Lee and Lemieux 1986) this class of zygotes, designated biparental, accounts for over 90\% of the population. Segregation of cpDNA takes place rapidly among the mitotic progeny of biparental zygotes, and only 20 postmeiotic mitotic divisions are required to produce cells homoplasmic for parental or recombinant cpDNA molecules.

The chloroplast DNAs of \textit{Chlamydomonas}, like those of most land plants, are circular and are divided into two single-copy regions by a large inverted repeat sequence (20–42 kbp) encoding the chloroplast ribosomal RNAs (Lemieux and Lemieux 1985). At 292 kbp, the \textit{C. moewusii} cpDNA (Turmel et al. 1987) is 97 kbp and 49 kbp larger than its homologues in \textit{C. reinhardii} (Rochaix 1978) and \textit{C. eugametos} (Lemieux et al. 1985 b), respectively. The overall gene organization of the \textit{C. eugametos} and \textit{C. moewusii} cpDNAs is essentially the same (Turmel et al. 1987, 1988), but differs tremendously from that of the \textit{C. reinhardii} cpDNA (Lemieux et al. 1985 a) and the consensus gene order found in most land plant cpDNAs (Turmel et al. 1988). The 49-kbp size difference between the \textit{C. eugametos} and \textit{C. moewusii} cpDNAs is almost totally accounted for by the presence of two large extra sequences in \textit{C. moewusii}: a 21-kbp sequence in the inverted repeat (locus G; Lemieux et al. 1985 c) and a 5.8-kbp sequence in the single-copy region bordering the 16S rRNA genes (locus R; Turmel et al. 1987). Aside from these two major addition/deletion differences, restriction site and fragment length polymorphisms were mapped at 43 locations throughout the two algal genomes (Turmel et al. 1987).

The large inverted repeat sequence of the chloroplast genome participates in both reciprocal and non-reciprocal intramolecular recombination events. Reciprocal intramolecular recombination between the two copies of the inverted repeat sequence occurs at such a high frequency in the cpDNAs of land plants (Palmer et al. 1985) and \textit{C. reinhardii} (Aldrich et al. 1985) that it leads to the formation of an equal proportion of single-copy region orientation isomers. This type of intramolecular recombination, also designated flip-flop recombination, has been observed in \textit{C. reinhardii} cpDNA mutants carrying various deletions of the inverted repeat sequence, thus indicating that it is not restricted to a specific region of this sequence (Palmer et al. 1985). On the other hand, intramolecular gene conversion events are thought to lead to copy-correction of the inverted repeat sequence in heteroplasmic cpDNAs, i.e. cpDNAs whose copies of this sequence differ at several loci (Lemieux and Lee 1987) or cpDNAs in which point mutations (Erickson et al. 1986) or large deletions (Myers et al. 1982) are present in one of the two copies. These gene conversion events also probably account for the symmetrical restoration of inverted repeat sequences during \textit{C. reinhardii} transformation experiments of inverted repeat deletion mutants, with cloned cpDNA sequences encompassing the junction between the inverted repeat and one of the single copy region (Boytonton et al. 1988; Blowers et al. 1989). It should be emphasized that copy-correction of inverted repeat sequences provides the only direct evidence for gene conversion events in \textit{Chlamydomonas} chloroplasts. Although non-reciprocal recovery of chloroplast genetic and physical markers has been reported in intraspecific (VanWinkle-Swift and Birky 1978) and interspecific (Lemieux and Lee 1987; Lemieux et al. 1988) crosses of \textit{Chlamydomonas}, this does not necessarily imply that the recombination mechanism is non-reciprocal, i.e., that it leads to the loss of alleles because of gene conversion events. Even if the mechanism of cpDNA recombination were solely reciprocal, a biased output of certain alleles could be caused by interspecific incompatibilities (Lemieux and Lee 1987) or the random drift of cpDNA sequences (Birky et al. 1981).

We have recently traced the inheritance and recombination of 23 polymorphic cpDNA loci among randomly selected homoplasmic hybrids issued from reciprocal \textit{C. eugametos}/\textit{C. moewusii} interspecific crosses (Lemieux et al. 1988). Analysis of the inheritance patterns revealed that most loci display the allele of the $mt^+$ parent, a result which is consistent with the predominantly uniparental inheritance of individual chloroplast genetic markers in such crosses. Whatever the mating type of the parental strains, however, three loci ($C$, $G$ and $R$) featured only the loci alleles derived from \textit{C. moewusii} or \textit{C. eugametos}; i.e., the 5.8- and 21-kbp extra sequences of \textit{C. moewusii} and an optional group I intron in the large subunit rRNA gene of \textit{C. eugametos}. These unidirectional patterns of inheritance are likely to be the results of gene conversion events. Most recombination junctions identified in $F_1$ hybrid cpDNAs were mapped in the vicinity of the unidirectionally inherited loci, suggesting that co-conversion of alleles at adjacent loci occurs over a relatively short distance. A limited number of recombination junctions were identified within cpDNA regions that are distant from the unidirectionally inherited loci $C$, $G$ and $R$. As these junctions cannot be explained by co-conversion of alleles, they most probably result from the generalized cpDNA recombination system. To facilitate studies of this recombination system, one could attempt to maximize the chances of detecting cells that are recombinant for cpDNA loci by selecting progeny with a chloroplast antibiotic resistance marker derived from the $mt^+$ parent, i.e., the parent that normally contributes fewer cpDNA molecules. Using this approach, Lemieux and Lee (1987) easily recovered, from reciprocal interspecific crosses, hybrids that were recombinant for several of the six polymorphic loci they analyzed within the rDNA operon.