Further observation of paternal transmission of *Drosophila* mitochondrial DNA by PCR selective amplification method

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(Received 19 June 1991 and in revised form 25 November 1991)

**Summary**
By designing 3' ends of primers in PCR (polymerase chain reaction), a specific DNA fragment was selectively amplified in the presence of a 10³-fold excess of highly homologous (sequence difference ca. 2%) opponent DNA. This technique was applied in detecting paternal leakage of mitochondrial DNA (mtDNA) in intraspecific crosses of *Drosophila simulans* and interspecific crosses of *Drosophila simulans* and *Drosophila mauritiana*. The mtDNA types of their progeny were analysed by selective amplification of the paternal mtDNA fragment possessing a polymorphic restriction site and detecting its cleaved fragments. Paternal mtDNA was detected in the progeny of 14 out of 16 crosses. The present result indicates small but frequent inheritance of sperm mtDNA in *Drosophila*, which is supportive to our previous finding.

1. **Introduction**
Inheritance of mtDNA is complicated by the multiplicity of mtDNA within a mitochondrion and a cell. The details of mtDNA segregation between mitochondria and between cells, and selection operating on mtDNA within and between generations are still unclear. Mitochondrial DNA inheritance has been believed to be strictly maternal, although some exceptions are found in plants and animals (Mirfakhrai et al. 1990; Neale et al. 1990; Kondo et al. 1990; Hoeh et al. 1991; Gyllensten et al. 1991). Theoretical population genetics studies have suggested that transmission of even a small amount of paternal mtDNA can provide a sufficient extent of gene flow between otherwise isolated female lineages, which may influence the evolutionary dynamics and history of maternal lineages considerably (Takahata & Maruyama 1981; Chapman et al. 1982; Birky 1983).

One of the first genetic evidences of paternal mtDNA transmission was found in *Drosophila*. By means of Southern hybridization, sperm mtDNA was detected in the progeny of backcrossed lines using different mtDNA types (Kondo et al. 1990). The probe constructed for this experiment required longer exposure of the autoradiograph to detect paternal mtDNA in an amount at least 0.03% of a mixture with maternal mtDNA. A PCR method may provide an easier and more efficient method with which to further analyze the paternal inheritance of mtDNA, owing to its capacity to amplify specific segments from crude and minute quantities of DNA (Saiki et al. 1985; Gyllensten et al. 1990), and its sensitivity which allows one to dispense with radioactive probes (Kaneko et al. 1989). Recent reports point out that perfectly matched primers are favoured over mismatched primers in amplification (Gibbs et al. 1989), and that base pairing at the 3' end of a primer is particularly crucial (Sommer & Tautz 1989). It has been demonstrated that one can discriminate two alleles that differ by a single nucleotide by using primers that have allele-specific nucleotides in their 3' ends (Wu et al. 1989).

In the present study, we designed the 3' end of primers to distinguish two mtDNA types in *Drosophila*. The ability to amplify a specific segment from the perfectly matched mtDNA in the presence of abundant opponent DNA was examined. This was further applied in the study of mtDNA inheritance and the detection of paternally inherited mtDNA in *Drosophila*.

2. **Materials and methods**
Isofemale lines of *Drosophila simulans* (SI303, SI307 and SI265) and *Drosophila mauritiana* (g20) were established from individual inseminated females which were collected in 1979 from Réunion (St Denis) and Mauritius (Port Louis), respectively. SI303 and SI307...
Fig. 1. Design of oligonucleotide primers and expected size of amplified fragment after HpaII digestion. Sequence for the primers were taken from mal(sil III) mtDNA and the region amplified is numbered (3147-4473) as in the sequence of Drosophila yakuba mtDNA (Clary & Wolstenholme 1985). Total length is 1324 bp in sill and 1323 bp in sil III (mal) due to deletions in sill and sil III (mal) mtDNA (Kaneko & Chigusa, unpublished data). Homologous sequence of sil II mtDNA is aligned above each primer sequence. Asterisks indicate the sequence identity with mal(sil III) mtDNA. The 3' ends of both primers differ from the homologous sequences of sill mtDNA enabling selective amplification of mal(sil III) mtDNA.

of template mtDNA and 2-5 units of Taq polymerase, covered with 50 μl of mineral oil. The following condition was used: 20 s at 94 °C, 20 s at 55 °C and 30 s at 72 °C for 30 cycles and 7 min at 72 °C afterwards; 1/10 volume (10 μl) of each PCR product was applied on a gel. The mtDNA type was confirmed by digestion of the amplified fragment with HpaII: ma(sil III) mtDNA is cleaved into 462 and 861 bp whereas 1324 bp of sill mtDNA remains undigested (Fig. 1).

3. Results and discussion

Control experiments were performed using mtDNA from SI303 and g20 as representatives of the maternal sill and the paternal mal(sil III) mtDNA, respectively. The efficiency of amplification was tested first in a series of PCR reactions by decreasing the amount of template mal mtDNA from 6 ng to 0.025 pg. Under the conditions used here, a gradual decrease in the amount of product was observed with amounts of template below 0.3 ng. 25 pg of the template (mal mtDNA) yielded sufficient product for detection after 30 cycles. Approximately 2.5 pg of mal mtDNA was enough, when 5 μl of PCR product was used as template for additional 30 cycles (or a total of 60 cycles) (Fig. 2, lanes 2, 3).

The capacity for selective amplification was examined with sill mtDNA in a similar way. There was no sign of the amplified fragment in the whole PCR product from ca. 150 ng of sill mtDNA template after a total of 60 cycles (Fig. 2, lane 7). Therefore, mismatches in the 3' ends of primers prevent amplification of the sill mtDNA under these conditions. However, sill mtDNA could be amplified under less stringent conditions, such as lowering the annealing temperature.

Finally, to test the effect of competition between the two templates, a series of reactions were made containing different amounts of mal and an excess...
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Fig. 2. Restriction analysis of the amplified products. After amplification, mineral oil was completely removed with chloroform, DNA was then precipitated with ethanol. The amplified fragment was purified by gel electrophoresis and digested with Hpa II. It was then applied to 1-5% agarose gel and visualized with ethidium bromide staining; 10 µl of the product was applied on a gel when digested. The content for each lane is as follows: 1, DRIgest III molecular marker (Pharmacia); 2, product of g20 undigested; 3, product of g20 digested with Hpa II; 4, product of sm1CII digested with Hpa II; 5, product of sm1CII digested with Hpa II; 6, product of sm2CII digested with Hpa II; 7, concentrated whole product of SI303 (template = 150 ng) digested with Hpa II (60 cycles); 8, product of SI303 (template = 25 ng) digested with Hpa II (60 cycles); 9, product of SI303 (template = 25 ng) undigested.

(approximately 25 ng) of siII mtDNA. Approximately 25 pg of maI mtDNA yielded sufficient maI mtDNA product after 30 cycles, but, a reaction starting with about 10-fold less (ca. 2.5 pg) maI mtDNA failed to yield a detectable amount of product even after 60 cycles of amplification (Fig. 2, lanes 8, 9). This shows that maI mtDNA can be amplified in presence of at least 10^5 excess of siII mtDNA.

These primers were applied in the detection of paternally inherited mtDNA. In a previous study, paternally derived mtDNA was detected after ten consecutive backcrosses (Kondo et al. 1990). There, backcrossing was performed taking into account two possible effects. First, if a small amount of paternal mtDNA is inherited at each generation, backcrossing may serve to accumulate it to the point of detectability. Second, the nuclear genome of a backcross line is incorporated into germ cells at the first fertilization. Therefore, sperm mtDNA differs from that in the egg, this may suggest the capacity of replication of sperm mtDNA after transmission. The presence of paternal mtDNA in sperm differs from that in the egg, this may suggest the capacity of replication of sperm mtDNA after transmission. The presence of paternal mtDNA during backcross generations is not known, mtDNAs during backcross generations is not known, but again detects paternal leakage of mtDNA in 7 out of 8 lines from both intra- and interspecific crosses. The present result suggests several features of mtDNA inheritance in Drosophila. Firstly, paternally derived mtDNA is inherited to the next generation. Although formation of mitochondria in sperm differs from that in the egg, this may suggest the capacity of replication of sperm mtDNA after transmission. The presence of paternal mtDNA in sperm mtDNA is incorporated into germ cells of the first fertilization. Secondly, paternal leakage not only occurs in interspecific crosses, this may have more evolutionary features of mtDNA inheritance in Drosophila. Firstly, paternally derived mtDNA is inherited to the next generation. Although formation of mitochondria in sperm differs from that in the egg, this may suggest the capacity of replication of sperm mtDNA after transmission. The presence of paternal mtDNA in sperm mtDNA is incorporated into germ cells of the first fertilization. Secondly, paternal leakage not only occurs in interspecific crosses, this may have more evolutionary
significance (Matsuura et al. 1991a). Thirdly, the paternal leakage revealed here may open interesting investigations on the fate of the male mtDNA. Elimination of foreign paternal mtDNA by maternal nuclear DNA has not been seen. Although the proportion of maternal nuclear DNA in F2 progeny is 25% in CI and 50–75% in CII, paternal DNA was detected from all of the CII lines. More extensive examination is needed to determine the effect of nuclear background on mtDNA inheritance (Matsuura et al. 1991b). Finally, the level of paternal leakage is very low, but the paternal input is occurring rather frequently. Given that 25 pg of paternal mtDNA is detectable in a sample, the resolution of the present experiment is estimated to be 0.017% = 25/150000. As a result, paternal mtDNA was detected from 87.5% = 14/16 of the experimental lines. While in our previous study, paternal mtDNA was detected from 1.69% = 4/237 of the experimental lines at the resolution of 0.035% but none at 0.075%. This increase in the rate of detection suggests a frequent inheritance of paternal mtDNA and its low proportion in progeny, which was not detectable using other methods.

This experimental system provides an easy, efficient and direct way to examine the inheritance of mtDNA in Drosophila. Studies on mtDNA transmission using PCR method should give further insight to the understanding of the mode of mtDNA inheritance, and the evolutionary consequence of this gene flow.

We would like to thank Dr N. Takahata and Dr Y. Satta for helpful discussions. We thank the two anonymous reviewers for suggesting ways to make the manuscript clearer and more accurate. This work is supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

References


