Incomplete Maternal Transmission of Mitochondrial DNA in Drosophila

Rumi Kondo,* Yoko Satta,† Etsuko T. Matsuura,* Hiromi Ishiwa,‡Naoyuki Takahata† and Sadao I. Chigusa*

**Department of Biology, Ochanomizu University, 2-1-1 Otsuka, Bunkyo-ku, Tokyo 112, Japan, †National Institute of Genetics, Mishima, 411, Japan, ‡Yakult Central Institute for Microbiological Research, Yaho, Kunitachi, Tokyo 186, Japan*

Manuscript received May 1, 1990
Accepted for publication August 1, 1990

ABSTRACT

The possibility of incomplete maternal transmission of mitochondrial DNA (mtDNA) in Drosophila, previously suggested by the presence of heteroplasmy, was examined by intra- and interspecific backcrosses of *Drosophila simulans* and its closest relative, *Drosophila mauritiana*. mtDNAs of offspring in these crosses were characterized by Southern hybridization with two α-^{32}P-labeled probes that are specific to paternal mtDNAs. This method could detect as little as 0.03% paternal mtDNA, if present, in a sample. Among 331 lines that had been backcrossed for ten generations, four lines from the interspecific cross *D. simulans* (female) × *D. mauritiana* (male) showed clear evidence for paternal leakage of mtDNA. In three of these the maternal type was completely replaced while the fourth was heteroplasmic. Since in this experiment the total number of fertilization is known to be 331 × 10 = 3310, the proportion of paternal mtDNA per fertilization was estimated as about 0.1%. The mechanisms and evolutionary significance for paternal leakage are discussed in light of this finding.

MITOCHONDRIA contain multiple copies of their own genome (mtDNAs), which are inherited independently of nuclear genome mostly through cytoplasms of female gametes in higher plants and animals. Although in some animals sperm mitochondria actually enter into an egg when fertilized, mitochondria detected in progeny are exclusively maternally derived [ALBERTS *et al.* (1989) and references therein]. These observations led to at least two models for the mechanisms of maternal inheritance. One postulates a difference in the ability of replication between paternal and maternal mtDNAs, that is that paternal mtDNAs are incapable of enough replication during the development of fertilized eggs. The other invokes the great excess of maternal mtDNAs in a zygote, which means that paternal mtDNA is much more likely to be lost through stochastic processes occurring within a cell lineage [*e.g.*, see BIRKY (1983) for review].

In principle, however, if even a small amount of paternal mtDNA is transmitted and not degraded, it may come to constitute a significant fraction in a cell by a mechanism analogous to random sampling drift in a finite population (*e.g.*, TAKAHATA and MARUYAMA 1981; CHAPMAN *et al.* 1982; BIRKY 1983). There have been, in fact, several attempts to detect incomplete maternal transmission of mtDNA by backcross experiments (LANSMAN, AVISE and HUETTEL 1983; GYLLENSTEN, WHARTON and WILSON 1985) or by examinations of naturally occurring hybridogenic populations (BERMINGHAM, LAMB and AVISE 1986; LAMB and AVISE 1986; AVISE and VRIJENHOEK 1987). Contrary to the expectation, they all failed to detect paternal leakage of mtDNA. One of the reasons may be due to the low resolution of techniques used: most were based on restriction fragment analysis which could not detect paternal mtDNAs if they constituted less than 1% in a sample. LANSMAN *et al.* (1983) therefore used an autoradiographic technique capable of detecting much smaller amounts (0.2%) of paternal mtDNAs in a sample. Despite the high resolution of this experiment, they could not show any paternal contribution and set the upper limit of paternal leakage at about 0.004%. This result appears to have been taken as representative of higher organisms, and complete maternal inheritance has been assumed to be the rule rather than the exception.

However, incomplete maternal transmission was suggested when our group found heteroplasmy (the presence of different types of mtDNAs within a cell or individual) in *Drosophila simulans* in Réunion (SATTA *et al.* 1988). The two types of mtDNAs within an isofemale line of *D. simulans*, siII and siIII, differ from each other in many restriction sites distributed over the genome (SOLIGNAC, MONNEROT and MOUNOLOU 1986; SATTA *et al.* 1988), and sequence analysis of these mtDNAs (2500 bp long) revealed that the two sequences differ at about 50 nucleotides or about 2% (SATTA and CHIGUSA 1991). This level of divergence is more than 50% of that for an interspecific comparison between *Drosophila melanogaster* and *D. simulans* (SATTA, ISHIWA and CHIGUSA 1987). This finding clearly ruled out the heteroplasmy produced by a single mutational event and strongly suggested

incomplete maternal transmission of mtDNA in Drosophila. It should be noted that although heteroplasmy has been found in many organisms, the phenomenon could be accounted for by newly arisen mutations without invoking paternal leakage (SOLIGNAC *et al.* 1984; HALE and SINGH 1986; HARRISON, RAND and WHEELER 1985; DENSMORE, WRIGHT and BROWN 1985; BERMINGHAM, LAMB and AVISE 1986; BENTZEN, LEGGETT and BROWN 1988). Although a small paternal contribution of mtDNA in heteroplasmy was suggested in another instance (FERRIS *et al.* 1983), it has not been directly proved.

To confirm paternal leakage of Drosophila mtDNA, we have decided to carry out a large scale backcross experiment which allows us to accurately assess the amount per fertilization. The overall experimental design of the present study was similar to that used in previous studies (*e.g.*, GYLLENSTEN, WHARTON and WILSON 1985), but noteworthy are the following unique features. First, the number of crosses can be regarded as the number of eggs fertilized by single sperms. This is because a single female randomly chosen from the parental line was used for a backcross so that each line was maintained by a single fertilized egg every generation. Since monospemy (the entry of one sperm into the egg cell) is observed in over 95% of fertilizations in Drosophila (HENNIG 1988), paternal mtDNA detected in progeny most likely resulted from accumulating mtDNA that was transmitted by single sperms. Second, compared with the previous work (LANSMAN, AVISE and HUETTEL 1983; GYLLENSTEN, WHARTON and WILSON 1985), the number of backcrossed lines examined was more than ten times as large and therefore enabled us to detect a low frequency of paternal leakage. Third, we prepared probes so as to satisfy the following two conditions. In the heteroplasmic state there must be both paternal and maternal fragments that can hybridize with a specific probe. If the size of these fragments is nearly equal, the intensity of radiation emitted from the minor fragment may be overshadowed by that from the major fragment. Therefore these two fragments must be well separated on the gel. In addition, the efficacy of blotting depends on the size of fragments that are transferred from the gel to the filter. The efficiency is expected to be high if the length of fragment is less than 15 kb (MANIATIS, FRITSCH and SAMBROOK 1982). The two probes 1.69 kb and 3.6 kb long used here satisfy these conditions, allowing us to detect an amount of DNA as small as 20 pg.

In this paper, we show that Drosophila mtDNA is indeed transmitted through sperm, the incidence of which may be so high as to be compatible with the frequency of heteroplasmic individuals observed in natural populations of *D. simulans* (SATTA *et al.* 1988). It is argued that even a small paternal leakage can be evolutionarily significant, providing a significant extent of gene flow between otherwise isolated female lineages and influencing the evolutionary dynamics and history of maternal lineages.

MATERIALS AND METHODS

Strains and backcrosses: Isofemale lines of *D. simulans* (SI232, SI265, SI303, SI307) and those of *D. mauritiana* (g20, g29) were established from individual inseminated females, collected in 1979 from Réunion (St. Denis) and Mauritius (Port Louis). There are three distinct types of mtDNAs in *D. simulans* (siI, siII and siIII) and two in *D. mauritiana* (maI and maII). By *Hpa*II digestion (Figure 1), mtDNAs in lines SI303 and SI307 were classified as siII and those in SI232 and SI265 as siIII, whereas mtDNAs in both lines of *D. mauritiana* (g20 and g29) were classified as maI. These six isofemale lines were homoplasmic, and no differences were detected between these *Hpa*II identical lines even if we used many other restriction enzymes. In addition, the restriction enzyme and sequence analysis revealed that siIII in *D. simulans* and maI in *D. mauritiana* show extremely high homology, the nucleotide differences being only one in a homologous segment 2.5 kb long, or 0.04% (SATTA and CHIGUSA 1991).

In each backcross experiment, a single virgin female was mated with two or three males at 25°. To avoid female contamination, we carefully collected only males from paternal lines. After eggs were laid, the parental individuals were all discarded and the hatched larvae were cultured at 19° until the late pupal stage. Then one female progeny was randomly chosen for the next backcross experiment. After ten consecutive backcrosses, it is expected that at most 0.1% of the nuclear genome is derived from the female progenitor, while all the mtDNA should remain to be unchanged if the inheritance is strictly maternal.

Three different backcross experiments were performed from the above isofemale lines. Two intraspecific crosses were siIII (females) × siII (males) and its reciprocal. An interspecific cross between siII (male) and maI (female) was difficult due to the premating isolation. In the reciprocal cross, however, the rate of successful mating was as high as the intraspecific one, producing fertile female and sterile male progeny. Thus three out of the four possible backcross combinations were used for the present study. In total, 400 backcross lines were constructed, of which 200 were intraspecific crosses [100 for siIII (female) × siII (male) and 100 for its reciprocal] and 200 were interspecific crosses [siII (female) × maI (male)].

Isolation of mtDNA: mtDNA was extracted from each line after ten backcross generations for characterization. Mitochondria from 100 ~ 200 (about 0.1 ~ 0.2 g) adult flies from each line were purified by differential centrifugation and mtDNA was then extracted by SDS-phenol treatment (SATTA *et al.* 1988). The samples were digested with *Hpa*II and separated on 0.8% agarose gels.

Construction of probes: As shown in Figure 1, two *Hpa*II-digested mtDNA fragments were taken from siII and maI (≈ siIII) and each was cloned into the *Eco*RV site of pHY300PLK tet^r region. Plasmid DNA containing this fragment was purified through CsCl-EtBr centrifugation and labeled with [5′-α-^{32}P]dCTP (Amersham, England) following nick translation protocol supplied by BRL (Bethesda Research Laboratories).

Southern blotting and hybridization: Gels were blotted to Hybond filters (Amersham, England). Experiments were made under stringent conditions: hybridization was carried

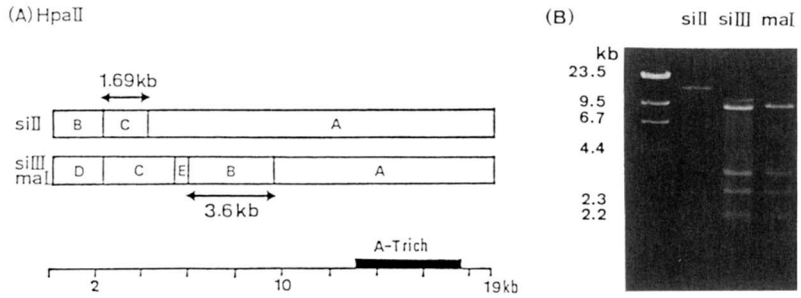

FIGURE 1.—Linearized restriction maps (A) and fragment patterns on agarose gel (B) of *Hpa*II digests of siII, siIII and maI. (A) Capital letters show the relative fragment size in the decreasing order. Bars with arrowheads indicate the fragments used as probes. When we used the C fragment from SI303 (siII) as a probe, it hybridizes with a 1.69-kb fragment of siII and with a 2.90-kb fragment (C fragment in the figure) of siIII. On the other hand, the B fragment from g29 (maI) hybridizes with the 3.6-kb fragment (B fragment) of maI or siIII and with the 15.1-kb fragment (A fragment) of siII. Taking account of the efficiency of the blotting (see text), the 1.69-kb fragment was used as a probe to detect siII mtDNA. For detection of siIII or maI, the 3.6-kb fragment was used. (B) mtDNAs were extracted from parental homoplasmic lines. These DNAs were digested with HpaII and separated on a 0.8% agarose gel. The gel was stained with ethidium bromide. Molecular weight standard is shown on the left-most lane.

out at 68° with 6 × SSC for overnight and the filters were washed three times for 10 min with 2 × SSC, 0.1% SDS at room temperature and twice for 60 min with 1 × SSC, 0.1% SDS at 68°. Dried filters were exposed to Kodak X-Omat films with double intensifying screens for 20 ~ 30 hr at −80°. The filter regions, where the paternal mtDNA be detected, were then cut out and exposed for another 14 days.

Detection of paternal mtDNA: The amount of DNA detectable by the present Southern hybridization was determined by using linearized plasmid DNA as shown in Figure 2. In this system, 200 pg or more DNA was detected for 24-hr exposure (data not shown), whereas another 14-day exposure was required to detect 20 pg DNA (Figure 2B). Under the assumption that the density of the autoradiographic band is proportional to the absolute amount of DNA, the resolution of the present experiment was evaluated as follows. Let y kb be the length of a specific mtDNA fragment. To detect 20 pg of this fragments, $20 \times 19/y$ pg mtDNA was needed since Drosophila mtDNA is *ca.*19 kb long. For instance, when a fragment of $y = 1.69$ kb is chosen, a minimum of 225 pg total mtDNA is necessary for detection. This means that if the fragment is paternally derived, 225 pg paternal mtDNA molecules in a sample can be detected. In case of $y = 3.6$ kb, 105 pg mtDNA is sufficient. Throughout the present experiment, 300 ng of total mtDNA was loaded per lane so that the resolution is estimated as 0.035% = 105/300,000 for a fragment of $y = 3.6$ kb (a *Hpa*II fragment of siIII) and 0.075% = 225/300,000 for $y = 1.69$ kb (a *Hpa*II fragment of siII).

RESULTS

mtDNAs from 331 out of 400 backcross lines were analyzed (Table 1). In 140 intraspecific crosses, 46 siII (female) × siIII (male) crosses and 94 reciprocal crosses, paternal mtDNAs were not detected even after 14-day exposure. On the other hand, four out of 191 siII (female) × maI (male) interspecific crosses showed paternal mtDNA. In three backcross lines of SI303 × g20, SI307 × g20 and SI307 × g29, only paternal mtDNA was detected after 24-hr exposure (Figure 3). To examine the possibility of heteroplasmy, these samples were subjected to another 14-day exposure but maternal mtDNA was not detected (data not shown). Thus, the original maternal mtDNA (siII) appears to be completely replaced by the paternal mtDNA (maI). In the remaining one (SI307 × g29), the paternal mtDNA became apparent only after long exposure (Figure 4), the relative frequency being *ca.* 1%. The latter case indicates that this line became heteroplasmic through paternal leakage and the paternal mtDNA was on the way either to complete fixation or to loss. Thus, although Drosophila mtDNA is believed to be maternally inherited, the present analysis has clearly demonstrate a low but significant level of paternal transmission.

To estimate the extent of paternal leakage, we

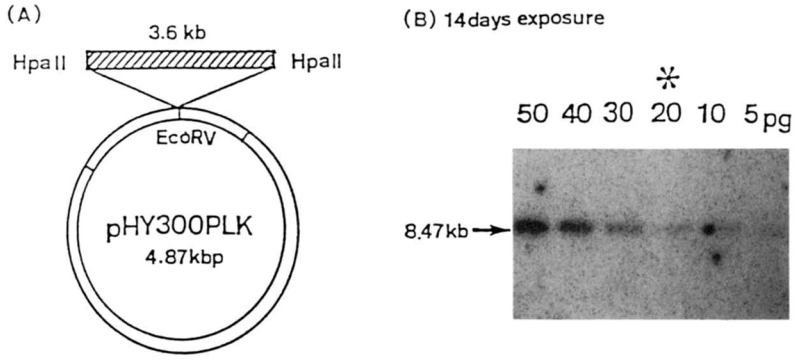

FIGURE 2.—Analysis of the limit of detection with plasmid DNA probe. (A) A diagram of the plasmid used in the analysis. A hatched bar indicates the 3.6-kb *Hpa*II fragment of maI mtDNA cloned into *Eco*RV site of pHY300PLK. (B) A result of autoradiography after 14-day exposure. The plasmid was linearized by digestion with *Hin*dIII, separated on 0.8% agarose gel. DNAs were loaded on each lane in series of 5 ~ 50 pg and blotted for hybridization. The same plasmid DNA labeled with [5′-α^{32}P]dCTP was used as a probe. With this method, 20 pg of DNA are sufficiently detectable.

TABLE 1

Numbers of isofemale lines (*N*) examined and lines (*P*) which show paternal transmission of mtDNA

Types of backcrosses (female)	(male)	*N*	*P*
D. simulans (siII)	× *D. simulans* (siII)	94	0
D. simulans (siII)	× *D. simulans* (siIII)	46	0
D. simulans (siII)	× *D. mauritiana* (maI)	191	4
Total		331	4

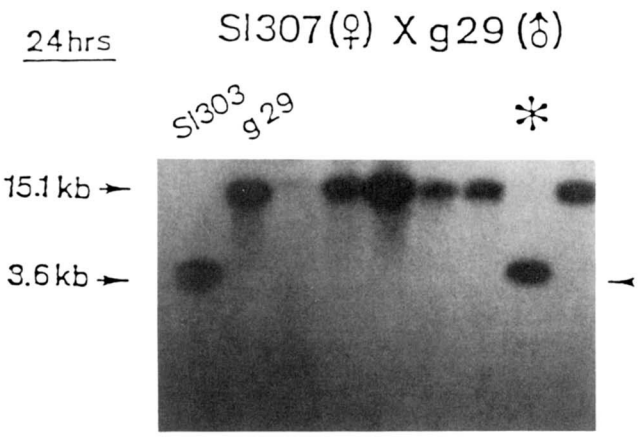

FIGURE 3.—Autoradiograph of *Hpa*II digested mtDNAs from SI307 (female) × g29 (male) backcross lines. Paternal 3.6-kb fragment was detected after 24-hr exposure in one (asterisk) out of seven lines. In this sample a fragment derived from maternal mtDNA could not be detected even after another 14-day exposure, implying that the paternal mtDNA (maI) was fixed in this backcross line.

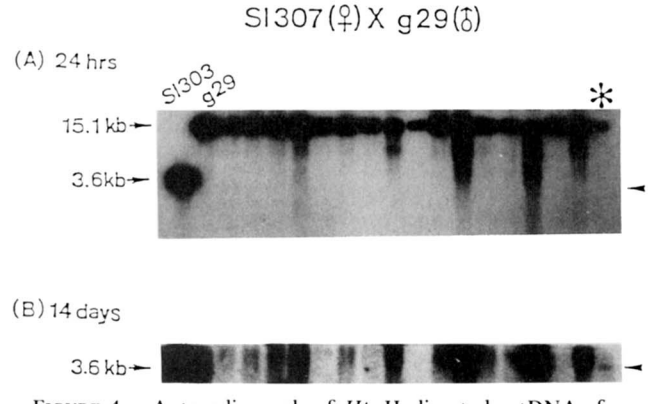

FIGURE 4.—Autoradiograph of *Hpa*II digested mtDNAs from SI307 (female) × g29 (male) backcrossed lines. Approximately 300 ng mtDNA were loaded on each lane. mtDNAs from g29 and SI303 were used as controls to show parental mtDNAs. (A) An autoradiograph of 24-hr exposure. A paternal 3.6-kb fragment (arrowhead in the figure) was not detected in any of 17 backcross lines. (B) The filter region which contains the paternal fragment was cut out and exposed for another 14 days. The line with an asterisk showed the paternal fragment (arrowhead), indicating the paternal leakage in this backcross line.

assume that a constant amount β of paternal mtDNA is transmitted by a sperm in each fertilization. Under this assumption, maternal mtDNA be eventually replaced by paternal one. When there is no preferential replication of paternal or maternal mtDNAs, the mean frequency $\mu(t)$ of paternal mtDNA after the tth backcross generation can be expressed by

$$\mu(t) = 1 - (1 - \beta)^t \approx 1 - e^{-\beta t}. \quad (1)$$

The formula is based upon the assumption that maternal mtDNA remains with probability $1 - \beta$, and it is the same as Equation 8.5.14 in CROW and KIMURA (1970) if the mutation rate is replaced by the extent of paternal contribution. It should be noted that $\mu(t)$ is independent of the strength of random drift within a generation, an expectation under neutrality. We may also be interested in the probability $f(t)$ that paternal mtDNA becomes fixed by the backcrossed lines. Equation 8.5.20 in CROW and KIMURA (1970) gives the approximate formula to be

$$f(t) \approx 1 - (2n_e\beta + 1)e^{-\beta t}. \quad (2)$$

Different from $\mu(t)$, $f(t)$ depends on n_e (per-generation effective size of mtDNA molecules within a cell), but if $4n_e\beta \ll 1$, both formulas are nearly the same. Applying the above formulas to our result, we can obtain the following estimate of β.

Recalling that there are three fixed lines and one heteroplasmic line having 1% paternal mtDNA among 331 lines, we have

$$\mu(t) \approx 3.01/331 \text{ and } f(t) \approx 3/331. \quad (3)$$

Both equations consistently estimated the value of βt as *ca.* 0.01, and substituting $t = 10$ yields the value of β to be 0.001. This means that the assumption $2n_e\beta \ll 1$ is satisfied so $n_e \ll 250$ or it is probably of the order of 10. The significance of the presence of the heteroplasmic line and small value of effective size n_e are discussed in the next section.

DISCUSSION AND CONCLUSION

It is known that in Drosophila, not only the head but the whole sperm enters the egg (HILDERTH and LUCCHESI 1963) so a necessary condition for incomplete maternal inheritance is satisfied. However, it is also known that mitochondria in sperm are morphologically changed. During spermatogenesis in Drosophila, they aggregate and fuse to form one or two *derivatives* that run longitudinally along one side of axoneme for nearly the full length of the sperm tail (HENNIG 1988). Although there has been little information about replication of mtDNAs in mitochondrial derivatives of sperm, the fact we observed shows that they must indeed be capable of regaining the function and ability of replication. In addition, in the early stage of development, pole cells differentiate from other somatic cells at the posterior end of an embryo (within 80 min from the fertilization) and further develop to germ cells (FOE and ALBERTS 1983). It is

therefore necessary that mtDNA coming from sperms moves to the posterior end of the egg and is incorporated into pole cells. Since germ plasm (cytoplasm in the posterior region of an egg) contains many mitochondria, paternal leakage suggests also that there may be cellular mechanism(s) which facilitate their movement into the posterior end.

During this developmental process, the copy number n of mtDNA may change in a germ cell lineage. However, the presence of heteroplasmy observed in this experiment as well as in the previous survey (SATTA *et al.* 1988) hints that the effective size n_e is not particularly small (see also SOLIGNAC *et al.* 1984). This size depends not only on n but also on the number λ of cell divisions per generation. If n changes in time and there are λ cell divisions in a generation, the harmonic mean of n should be divided by λ to obtain the per-generation effective size n_e as shown in TAKAHATA and MARUYAMA (1981) [see also (3.13.4) in CROW and KIMURA 1970]. We have estimated that n_e is much smaller than 250, but the harmonic mean of n can be fairly large, provided that the number of cell divisions per generation λ is 10 or more in Drosophila. If n_e is of the order of 10 as mentioned, n should be about 10 times larger and the persistence time of heteroplasmy be about 10 generations. It was puzzling that two isofemale lines has been heteroplasmic for such a long time as 6 years in the laboratory (SATTA *et al.* 1988). If our estimate of the persistence time is correct and if there are 10 generations per year, the only likely explanation for the maintenance is that those lines were subjected to a balance between constant production of heteroplasmy due to paternal leakage and constant segregation of homoplasmy due to within-generation drift.

Although we have assumed that there is no bias in replicating paternal and maternal mtDNAs in a zygote, some recent papers showed that this is not always the case. MIRFAKHRAI, TANAKA and YANAGISAWA (1990) reported the uniparental transmission of mtDNA in cellular slime mold. It produces two kinds of gametes, but unlike higher animals both gametes have almost the same volume. In spite of their apparent equal contribution to a zygote, uniparental transmission was observed and it was suggested that there are genes responsible for mitochondrial inheritance associated to mating types. More surprisingly, NEALE, MARSHALL and SEDEROFF (1989) showed the strictly paternal transmission of mitochondria in redwoods, a complete replacement of maternal mtDNA by pollen mtDNA in F_1 progeny. In gymnosperm fertilization, paternal plastids and mitochondria are transmitted but the proportion is considerably small as in higher animals. It is therefore likely to postulate selective amplification of paternal mitochondria in this case [see NEALE, MARSHALL and SEDEROFF (1989) and references therein]. Such biased amplification is important only when there is a significant amount of paternal leakage and this will be discussed later.

The fact that the transmitted paternal mtDNAs in our experiment all came from interspecific backcrosses may deserve special attention, although we could not reject the hypothesis that this might be fortuitous: there was no statistical difference ($P > 0.5\%$) and, moreover, it was noted that 97% of the nuclear genome of a backcross line should be replaced by that of the donor male by the fifth generation and hence, unless leakage occurred only by that time, the difference in inter- and intraspecific crosses would not have had any difference. To study whether or not there is indeed some unrecognized preference or selective silencing of mtDNA, however, a technique developed by MATSUURA, CHIGUSA and NIKI (1989) may be useful. They constructed heteroplasmic Drosophila by transplanting germ plasm. When germ plasm was injected into the posterior end of an egg, foreign mitochondria were successfully incorporated into pole cells. Unless foreign mtDNAs are not selectively destroyed in a host cell soon after injection, such incorporation can be expected and the heteroplasmic state should result. They noted that there was a tendency for foreign mitochondria to increase and be completely fixed in some instances (NIKI, CHIGUSA and MATSUURA 1989). Although we cannot decisively judge whether this finding can be applied only to interspecific combinations, the injection technique seems promising for further studying the transmission genetics of Drosophila mtDNA in general and the transmission bias, if present, in particular.

Finally, we would like to briefly discuss effects of paternal leakage on evolution of mtDNA in terms of (i) variation within a population, (ii) differentiation of subpopulations, (iii) linkage disequilibrium, and (iv) substitution rate.

The inbreeding effective number, N_e, of mtDNA in a panmictic population consisting of N_f females and N_m males is approximately given by

$$N_e = \frac{N_f N_m}{\alpha^2 N_m + \beta^2 N_f}$$

where $\alpha = 1 - \beta$, and the genetic variability in the mitochondrial population is determined mainly by N_e and the mutation rate under neutrality [see TAKAHATA (1985) and BIRKY, FUERST and MARUYAMA (1989) for review]. It is expected that if β is as small as 10^{-3} and unless the sex ratio is strongly biased toward male, a small amount of paternal leakage does not much affect the genetic variability. It is conceivable, however, that if N_e is large and the number of cell divisions per generation is relatively small, even a small amount of parental leakage can significantly increase the variability not only within a population

but in an individual (TAKAHATA and MARUYAMA 1981). This appears not to be the case in Drosophila (BABA-AÏSSA *et al.* 1988) so that the sequence variation of mtDNA within and between individuals should not be much influenced by paternal leakage.

When a population is divided into subpopulations with migration among them, the local differentiation depends largely on the effective migration rate

$$m_e = \alpha m + \beta m^*$$

where m and m^* are female and male migration rates (TAKAHATA and PALUMBI 1985). Hence it is clear that unless $m^* \gg m$, there is little effect of sexually unequal inheritance of mtDNA on local differentiation (TAKAHATA and PALUMBI 1985; BIRKY, FUERST and MARUYAMA 1989). At present, there is no evidence for different migration rates between males and females in Drosophila.

It is generally thought that mtDNA does not produce recombinants and therefore it is a suitable material to study matriarchal lineages (AVISE *et al.* 1987; CANN, STONEKING and WILSON 1987). In fact, the recombination in mammalian somatic fused cells has not been observed (HAYASHI *et al.* 1982; ZUCKERMAN *et al.* 1984), a situation being entirely different from that in the biparental inheritance as in yeast (BIRKY 1978; BIRKY *et al.* 1982). There can be two explanations for the apparent lack of recombination: (i) The inheritance is more or less strictly maternal so that mtDNA in an individual tends to be clonal, resulting in no effects of recombining, and (ii) the recombination does not occur at all. In the lack of data on suppression of mtDNA recombination, it seems to us that the strictly maternal inheritance is the more likely explanation. From this view, our result suggests that mtDNA in Drosophila may have been undergoing recombination, provided that mitochondria can fuse and divide. It was shown, under neutrality, that the variance of linkage disequilibrium depends much on the value of β (TAKAHATA 1983) so that it is likely that different parts of mtDNA have different evolutionary histories if β is significantly large. Therefore there is no warrant for the identity between genealogy of Drosophila mtDNA and female ancestry.

Paternal leakage also has a large effect on the substitution rate of mutants when some selection operates within a generation. The effect is similar to that of gene conversion and therefore if there is an appreciable paternal contribution, within-generation selection can be a strong evolutionary force (TAKAHATA 1984). If paternal leakage in Drosophila occurs as frequently as estimated in this paper, study on selection that might operate on mtDNAs mediated through differential replication or selective silencing of different mtDNAs becomes more important in evolutionary thinking.

It is thus concluded that the estimated value 0.1% of paternal leakage is evolutionarily significant and consistent with the findings that heteroplasmy exists in two isofemale lines maintained for 6 years in a laboratory condition (SATTA *et al.* 1988) and also in natural populations of *D. simulans* in Réunion with frequency *ca.* 6% (data not shown). These observtions can now be reasonably interpreted. Heteroplasmy can never be immune to segregation and it takes only some ten generations to return to homoplasmy. Therefore heteroplasmy in the laboratory as well as in natural populations must be a result of constant production due to paternal leakage. There is no *a priori* reason to think that this conclusion applies only to Drosophila and, in fact, data on incomplete maternal transmission of mtDNAs in other higher animals have been gradually accumulating (R. W. CHAPMAN; W. M. BROWN, personal communication). If this is the case, we need to be more careful about the transmission genetics of mtDNA in considering the evolutionary mechanism and history.

We thank ROSS CROZIER and two anonymous reviewers for their useful comments and constructive criticisms. This work was supported by Grants-in-Aid for Overseas Scientific Survey and for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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Communicating editor: A. G. CLARK