ショウジョウバエのヘテロプラズミー系統におけるミトコンドリアDNAの選択的伝達

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Selective transmission of mitochondrial DNA in heteroplasmic lines for intra- and interspecific combinations in *Drosophila melanogaster*

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ABSTRACT

The transmission of mitochondrial DNA (mtDNA) was investigated in the heteroplasmic lines of *Drosophila melanogaster* at 19°C and at 25°C. The selective transmission of one type of mtDNA was dependent on the temperature at which the lines were maintained. In heteroplasmic lines for an intraspecific combination induced by germ-plasm transplantation using *D. melanogaster* as a germ-plasm donor, the proportion of donor mtDNA decreased in four out of five lines examined, the decreasing rate of which being greater at 25°C than at 19 °C. Donor mtDNA was lost by the 20th generation at 25°C. For an interspecific combination using *D. mauritiana* as a germ-plasm donor, the proportion of donor mtDNA increased and endogenous mtDNA was replaced with donor mtDNA at 25°C. But donor mtDNA was almost lost at 19°C by the 14th generation in all four lines examined. Possible mechanisms involved in the temperature-dependent modes of mtDNA transmission are discussed.

1. INTRODUCTION

Mitochondrial biogenesis is regulated by both nuclear and mitochondrial genomes through coordinate replication and expression of mtDNA, protein assembly and protein import (for a review, see Attardi and Schatz, 1988). The molecular mechanisms for maintaining mitochondria or mitochondrial DNA (mtDNA) in a cell have been studied in nuclear and mitochondrial mutants of unicellular organisms such as yeast and cultured somatic cells (e.g., Yaffe and Schatz, 1984; Fox, 1986; Greenleaf et al., 1986; Tzagoloff and Myers, 1986). Another approach to the study of the regulation of mitochondrial maintenance is to construct a heteroplasmic state usually in hybrid cells and cybrids. The modes of transmission of two types of mtDNA in a cell to its daughter cells have been investigated for a novel combination with nuclear genomes (e.g., Giles et al., 1980; White and Bunn, 1984; Zuckerman et al., 1984). Homoplasmic cells in which endogenous mtDNA has been completely replaced with foreign mtDNA can be used for further analysis of nuclear-mitochondrial interactions at various levels of mitochondrial biogenesis (King and Attardi, 1988, 1989).

In insects and higher animals, the transmission mode of two types of mtDNA

has been studied in some instances of heteroplasmy found in natural populations (Solignac et al., 1984, 1987; Harrison et al., 1985; Rand and Harrison, 1986). Recently, an experimental system for inducing mtDNA heteroplasmy by means of germ-plasm transplantation was developed in *Drosophila* (Matsuura et al., 1989). Using *D. melanogaster* as a recipient species, the germ plasm of *D. melanogaster* and its sibling species *D. simulans* and *D. mauritiana* was incorporated into germline cells of *D. melanogaster* (Hayashi and Murakami, 1988; Matsuura et al., 1989). Similar experimental heteroplasmy was also successfully conducted by transplanting egg cytoplasm between strains of *D. simulans* (de Stordeur et al., 1989). These experimental systems have been found useful for studying the mechanisms for maintaining and transmitting mtDNA in germline cells of insects.

An interesting mode of mtDNA transmission has been observed in such induced heteroplasmic lines in *Drosophila* (Niki et al., 1989). During the maintenance of heteroplasmic lines at 25°C, the proportion of foreign mtDNA derived from *D. mauritiana* increased and then endogenous mtDNA of *D. melanogaster* was completely replaced with foreign mtDNA. Consequently, *D. melanogaster* possessing mtDNA of *D. mauritiana* was obtained. When the same lines were maintained at 19°C, however, foreign mtDNA was not retained (Matsuura et al., 1990). This preferential transmission of *D. mauritiana* mtDNA would thus appear to depend on the particular temperature at which the lines were maintained.

In the present study, to determine the temperature effects on the transmission mode of mtDNA more precisely, the transmission of two types of mtDNA was examined at two different temperatures, 19°C and 25°C, using heteroplasmic lines in *D. melanogaster* re-established from lines previously constructed (Matsuura et al., 1989). The previous results of Niki et al. (1989) and Matsuura et al. (1990) were confirmed even after the temperature was shifted at the time of re-establishing the heteroplasmic lines. Furthermore, for an intraspecific combination, endogenous mtDNA was found to be preferentially transmitted in most cases and the proportion of foreign mtDNA decreased faster at 25°C than at 19°C. Thus it is clear that the selective transmission of one type of mtDNA depends on the temperature at which the heteroplasmic lines are maintained, at least for these combinations of heteroplasmy.

2. MATERIALS AND METHODS

Establishment and maintenance of heteroplasmic lines

Heteroplasmic lines of *D. melanogaster* (*bw;e*^{11}) were re-established for both intra- and interspecific combinations as shown schematically in Fig. 1. Isofemale lines were newly established at 25°C from single inseminated females of lines constructed previously by germ-plasm transplantation (Matsuura et al., 1989, designated here as original heteroplasmic lines). Mitochondrial DNA of their

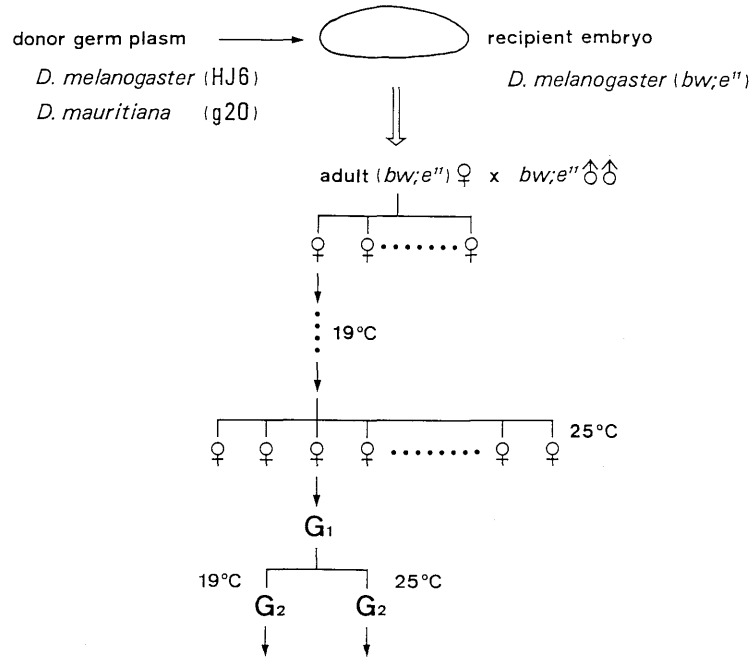

Fig. 1. Procedure for re-establishment of heteroplasmic lines. Original heteroplasmic lines were constructed previously by germ-plasm transplantation (Matsuura et al., 1989) and maintained for more than 15 generations at 19°C. Isofemale lines were established at 25°C and mitochondrial types of the individual females were determined from mtDNA extracted from their progeny (G_1). The progeny of each heteroplasmic line was subsequently divided into two at the second generation (G_2) or the third generation (not shown) and maintained at 19°C and 25°C, respectively, thereafter.

progeny (G_1) was examined and heteroplasmic lines were determined as described previously (Matsuura et al., 1989).

For the intraspecific combination using the HJ6 strain of *D. melanogaster* as a germ-plasm donor, two types of mtDNA were still present in four original heteroplasmic lines at the 17th generation at 19°C (A3, B6, B8 and B9 in Matsuura et al., 1990). A total of 37 isofemale lines was established from the four lines and 16 among them were found heteroplasmic. For the interspecific combination using the g20 strain of *D. mauritiana* as a germ-plasm donor, only one original heteroplasmic line (D6 in Matsuura et al., 1989) possessed two types of mtDNA at the 15th generation at 19°C. Four out of 20 isofemale lines re-established from D6 were found heteroplasmic.

Five lines (A31, B61, B81, B91 and B92) for the intraspecific combination and four lines (D7, D8, D9 and D10) for the interspecific combination were used in this study. The progeny of each heteroplasmic line raised at 25°C was divided into two at the second generation for the A31, B61 and D10 lines and at the third generation for the other lines (B81, B91, B92, D7, D8 and D9). These were

maintained at 19°C and 25°C, respectively, by brother-sister matings using more than 50 flies in each generation.

Analysis of mtDNA

The proportion of donor mtDNA within a line was determined by mtDNA extracted from flies used as parents for the next generation, as described previously (Matsuura et al., 1989). To determine whether the heteroplasmic state also persisted in individual flies, isofemale lines (sublines) were established from single inseminated females at each temperature. More than six sublines were examined in each case and the proportion of donor mtDNA in mtDNA extracted from the progeny was determined. The lack of donor or endogenous mtDNA was confirmed by Southern hybridization using ^{32}P-labeled mtDNA fragment as a probe.

3. RESULTS

Transmission of donor mtDNA in heteroplasmic lines for the intraspecific combination

Five heteroplasmic lines contained donor (HJ6) mtDNA in various proportions (25.6%, 6.0%, 48.5%, 28.4% and 35.6% for A31, B61, B81, B91 and B92, respectively) at the first generation. Changes in the proportion of HJ6 mtDNA at 19°C and 25°C are shown in Figs. 2a and 2b, respectively. At 19°C, it decreased gradually in all except the B81 line, while both endogenous (*bw;e*^{11}) and HJ6 mtDNA were retained in the A31, B91 and B92 lines for more than 20 generations (Fig. 2a). In the same three lines at 25°C, the decrease occurred more quickly than at 19°C, and HJ6 mtDNA was lost by the 20th generation (Fig. 2b). In the B61 line, the proportion of HJ6 mtDNA was low at both temperatures, and HJ6 mtDNA disappeared at the ninth generation at 25°C, earlier than at 19°C.

To determine whether the heteroplasmic state persisted in individuals, sublines of the five lines were examined at both temperatures and the results are shown in Fig. 3. Changes were essentially consistent with the results obtained within a line except the sublines of B81. At 25°C, HJ6 mtDNA in the progeny of an individual fly was gradually diminished during the first ten generations in most cases. At 19°C, the heteroplasmic state was retained in more than half the individual female flies (26/38) even at the 10th and 11th generations. The proportions of HJ6 mtDNA at these generations varied more widely than those at the ninth and tenth generations at 25°C.

In the B81 line, the proportion of HJ6 mtDNA within a line increased at the two temperatures. This increase also appeared dependent on temperature. The changes in the proportion of HJ6 mtDNA in the sublines indicated segregation of endogenous and HJ6 mtDNA into different individual flies, this being distinct at 25°C. Homoplasmic flies for HJ6 mtDNA were obtained in four out of seven

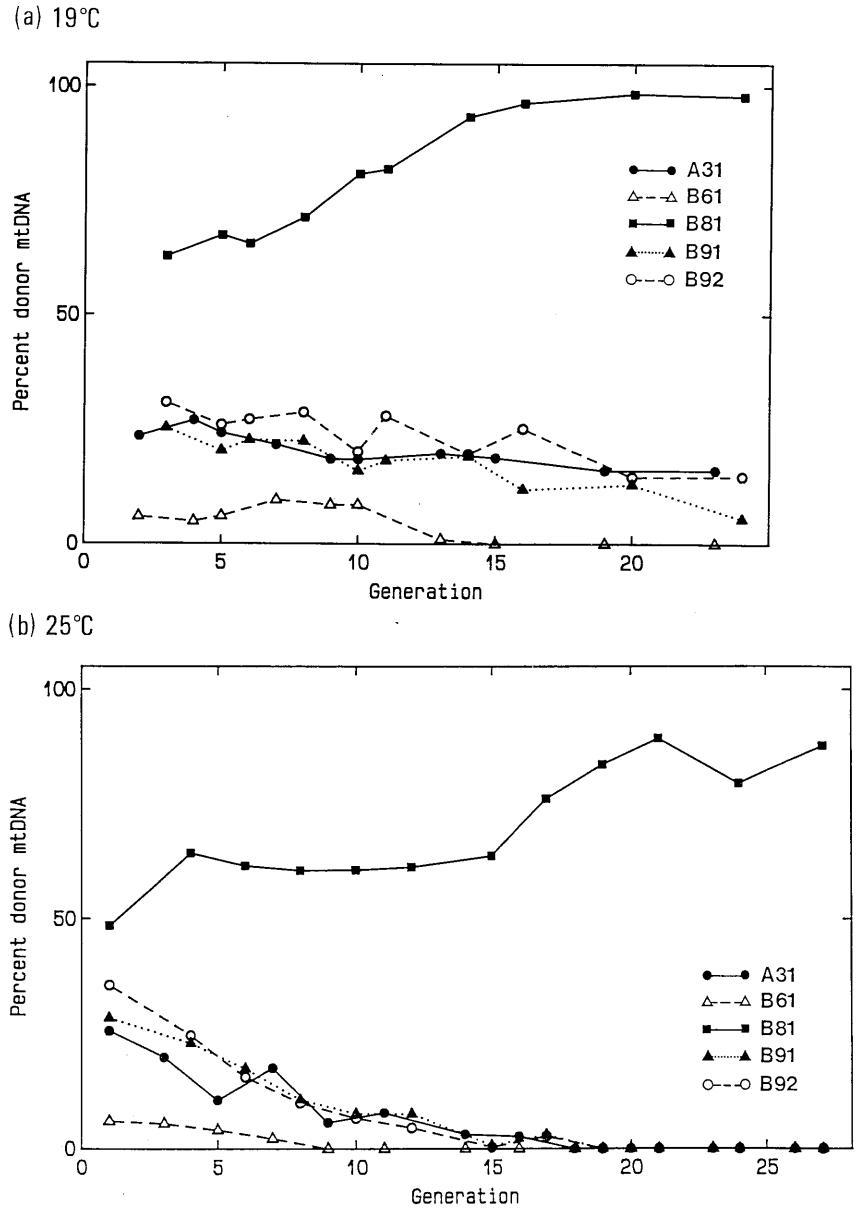

Fig. 2. Changes in the proportion of donor (HJ6) mtDNA in the five heteroplasmic lines for the intraspecific combination. The lines were maintained at 19°C (a) and 25°C (b).

sublines at the 21st generation.

Transmission of donor mtDNA in heteroplasmic lines for the interspecific combination

Four heteroplasmic lines, D7, D8, D9 and D10, contained donor (g20) mtDNA

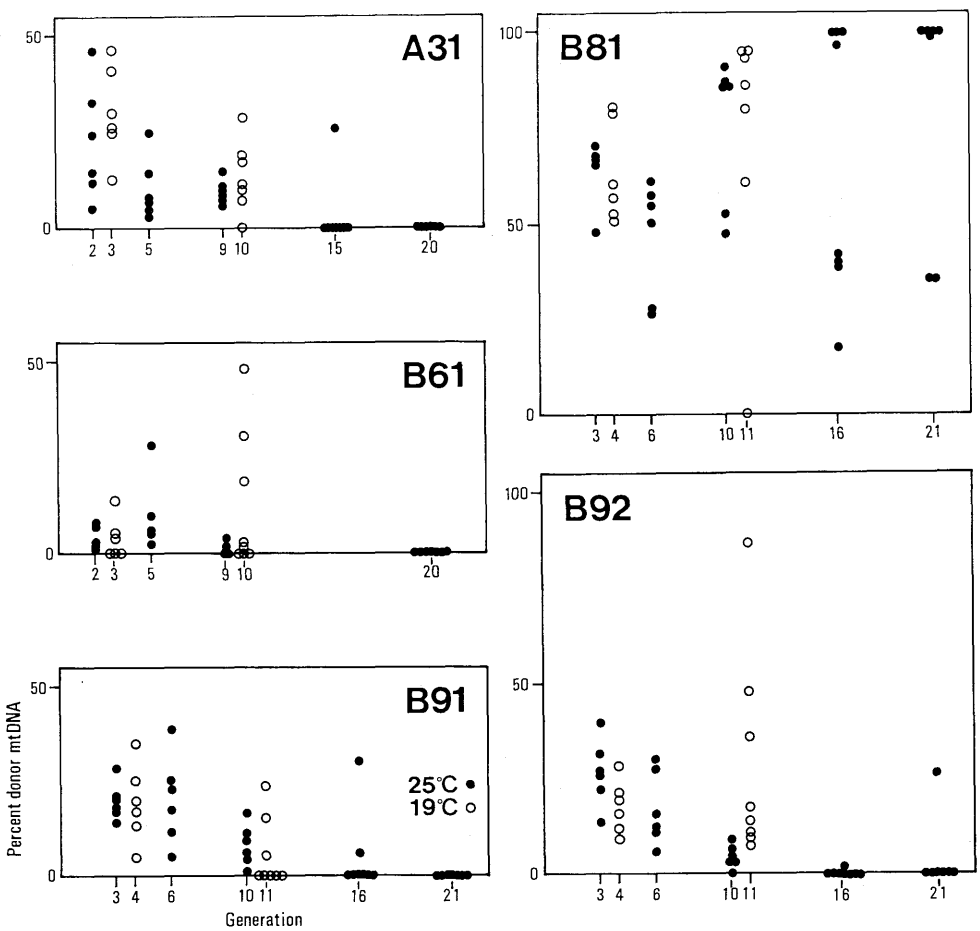

Fig. 3. Changes in the proportion of HJ6 mtDNA in individual females of the five heteroplasmic lines. Open (○) and closed (●) circles show the results at 19°C and 25°C, respectively. Each circle represents the proportion of HJ6 mtDNA within a subline.

at 21.2%, 41.1%, 12.3% and 9.7%, respectively, at the first generation. Figs. 4a and 4b show changes in the proportion of g20 mtDNA at 19°C and 25°C, respectively. The proportion of g20 mtDNA decreased in all four lines at 19°C (Fig. 4a), whereas it increased in the four lines during the first 12 generations at 25°C (Fig. 4b). Finally, endogenous mtDNA was replaced with g20 mtDNA in all except the D10 line. Fig. 5 shows the results for the subline analysis at 19°C and at 25°C. Heteroplasmic individuals disappeared at 19°C as the proportion of g20 mtDNA within a line decreased. The heteroplasmic state was retained in individuals at 25°C during generations in which g20 mtDNA was present in a significant proportion in each line. In the sublines of D7, D8 and D9, the frequency of heteroplasmic sublines decreased greatly at the 13th generation

(3/21), and g20 mtDNA was fixed in each individual at 25°C. These findings are consistent with our previous observations (Niki et al., 1989; Matsuura et al., 1990).

In the D10 line, the proportion of g20 mtDNA ceased to increase at the 13th

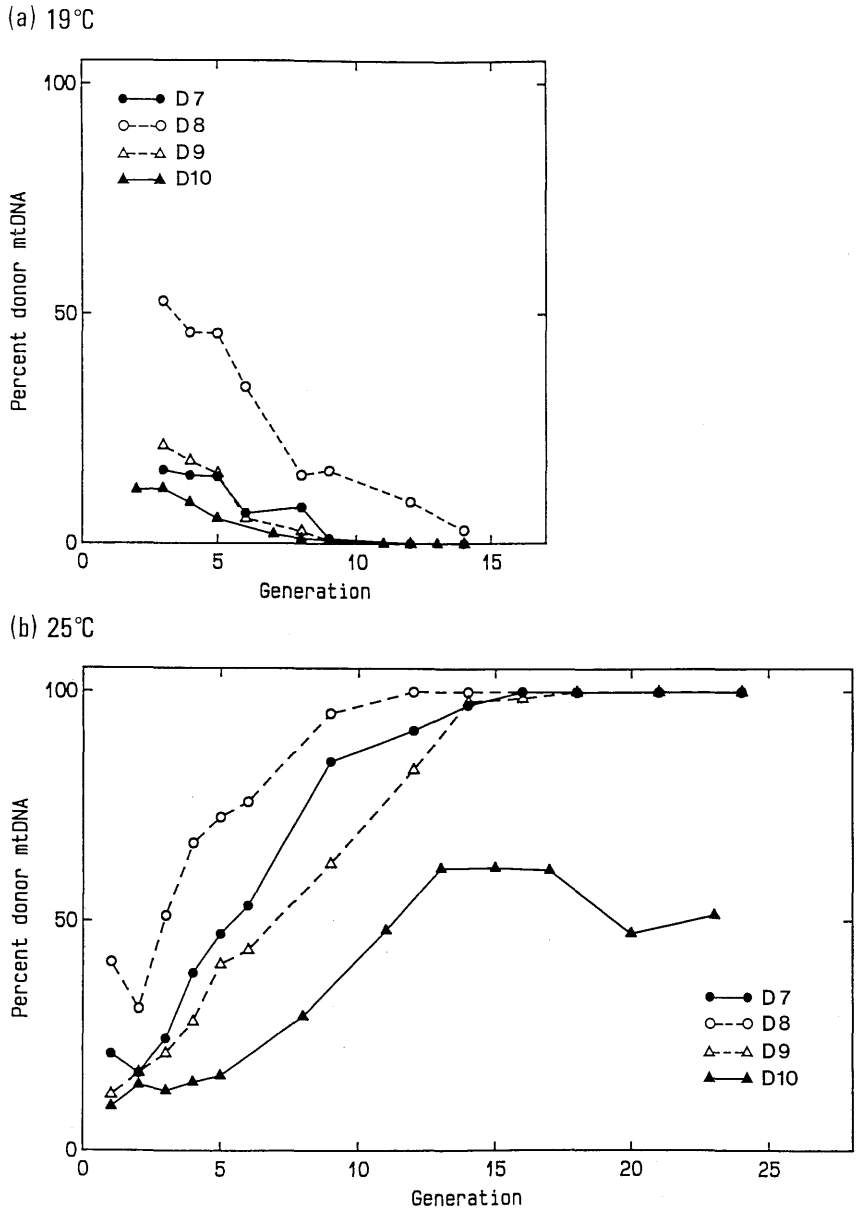

Fig. 4. Changes in the proportion of donor (g20) mtDNA in the four heteroplasmic lines for the interspecific combination. The lines were maintained at 19°C (a) and 25°C (b).

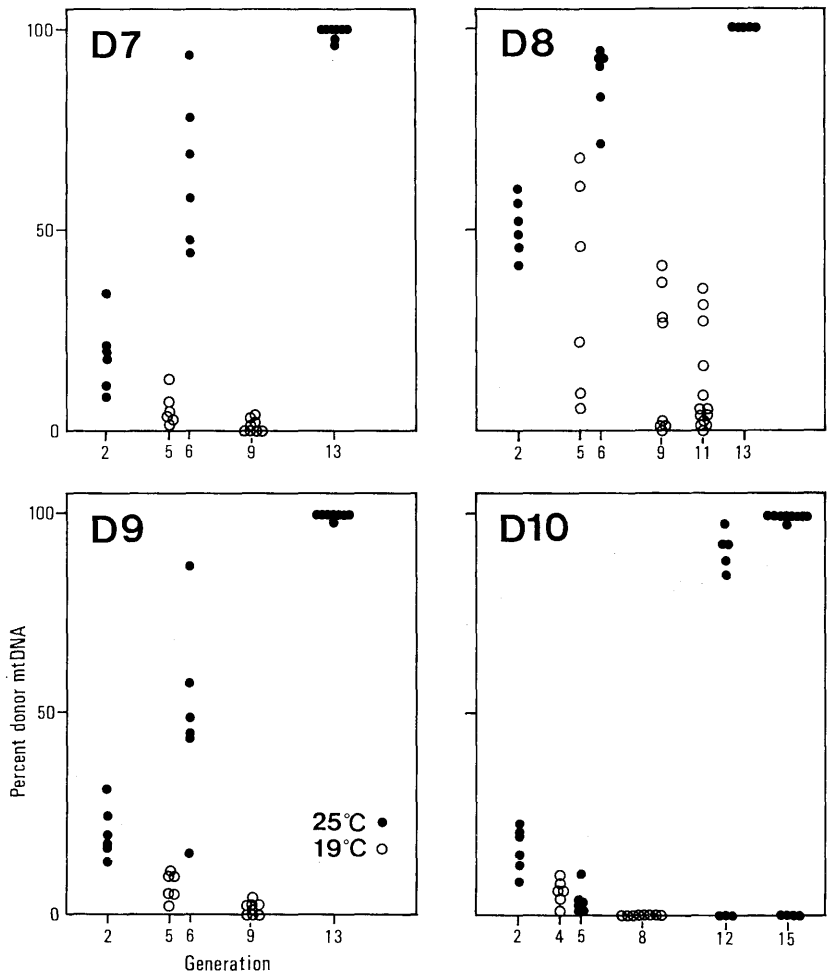

Fig. 5. Changes in the proportion of g20 mtDNA in individual females of the four heteroplasmic lines. Circles are given as in Fig. 3.

generation at 25°C, its proportion remaining about 50% thereafter (Fig. 4b). In the subline analysis of D10, segregation of endogenous and g20 mtDNA was observed at 25°C, and it was virtually complete at the 15th generation. The heteroplasmic state observed in the D10 line was thus due in part to the heteroplasmic state in individual flies at earlier generations up to around the 12th generation, and to the coexistence of two kinds of homoplasmic flies in later generations.

4. DISCUSSION

The present results clearly indicate that one type of mtDNA is selectively transmitted in heteroplasmic state for both intra- and interspecific combinations. Donor mtDNA decreased or increased within an individual as well as within a line, depending on temperature at which the lines were maintained. Here, the heteroplasmic lines were re-established at 25°C from the original ones maintained at 19°C for more than 15 generations. Therefore, the temperature was shifted from 19°C to 25°C at the time of heteroplasmic line re-establishment and this confirms that temperature-dependency is reproducible.

Most lines showed similar changes in the proportion of donor mtDNA in both combinations. Among the nine lines examined, however, two (B81 and D10) appeared exceptional in the mode of transmission of donor mtDNA, as was also noted in our previous studies (Niki et al., 1989; Matsuura et al., 1990). Interestingly, the original heteroplasmic line from which the B81 line was derived showed essentially the same increase. These differences may possibly be due to subtle variation in the nuclear genotype of the recipient, since the recipient strain used here was not an isogenic strain but an isofemale strain. Otherwise, the random process in partitioning of mitochondria would be important. In any case, this variation among lines may be closely related to the mechanisms for mtDNA transmission and verification of this should be made.

It is an interesting finding that mtDNA derived even from the same species as the recipient species could be distinguishable from endogenous mtDNA at the higher temperature. In experimental heteroplasmy between two *D. simulans* cytoplasmic races (*si*II and *si*III, Solignac et al., 1986), though not confirmed under different temperature conditions, *si*II mtDNA had an advantage over *si*III mtDNA in reciprocal combinations (de Stordeur et al., 1989). From the present and other results, the propagation and transmission of mtDNA is not necessarily random, as noted in heteroplasmy based on mtDNA size variation in insects (Solignac et al., 1984; Rand and Harrison, 1986). Our preliminary data for some other combinations of heteroplasmy also indicate the modes of transmission of donor mtDNA to be determined not only by a donor strain but by donor and recipient strain combinations as well. Besides the type of mitochondrial genome itself, nuclear-mitochondrial communication should be in some way related to the selective transmission of mtDNA.

The transmission of mtDNA was found to be temperature-dependent, and this should be a very important feature for understanding the factors for regulating the propagation and transmission of mitochondria and mtDNA. Based on mtDNA sequence analysis, one of processes in mtDNA replication may possibly be such a factor. In *Drosophila* mtDNA, replication starts within the non-coding A+T-rich region (Goddard and Wolstenholme, 1978, 1980) that is most variable in the genome within and between species (Fauron and Wolstenholme, 1980a, 1980b;

Reilly and Thomas, 1980; Satta et al., 1990). In the other region of mtDNA, nucleotide differences were only 4% and 3% for the *ND2* and *COI* genes, respectively, even between *D. melanogaster* and *D. mauritiana* (Satta et al., 1987). Although no sequence data on replication origin are presently available for mtDNA used in this study, two types of mtDNA, whether originating from the same or different species, possibly differ in their sequences of the A+T-rich region and compete in replication efficiency, with temperature being a determining factor.

Little is known regarding the mitochondrial biogenesis in *Drosophila*. Thus, that some specific nucleotide sequences or amino acids encoded in mtDNA may be responsible for the temperature-dependent mtDNA transmission is a possibility that should be considered. The temperature-dependency should be examined in greater detail, and nuclear and/or mitochondrial factors controlling mtDNA transmission be identified, using different heteroplasmy for reciprocal intra- and interspecific combinations. Genetical as well as molecular analyses of mtDNA transmission in the present system of *Drosophila* should greatly contribute to elucidate the regulation of mitochondrial transmission in insects and eucaryotic cells.

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REFERENCES

ATTARDI, G. and SCHATZ, G. (1988) Biogenesis of mitochondria. *Ann. Rev. Cell Biol.* **4**, 289–333.

DE STORDEUR, E., SOLIGNAC, M., MONNEROT, M. and MOUNOLOU, J.-C. (1989) The generation of transplasmic *Drosophila simulans* by cytoplasmic injection: Effects of segregation and selection on the perpetuation of mitochondrial DNA heteroplasmy. *Mol. Gen. Genet.* **220**, 127–132.

FAURON, C. M.-R. and WOLSTENHOLME, D. R. (1980a) Extensive diversity among *Drosophila* species with respect to nucleotide sequences within the adenine+thymine-rich region of mitochondrial DNA molecules. *Nucl. Acids Res.* **8**, 2439–2452.

FAURON, C. M.-R. and WOLSTENHOLME, D. R. (1980b) Intraspecific diversity of nucleotide sequences within the adenine+thymine-rich region of mitochondrial DNA molecules of *Drosophila mauritiana, Drosophila melanogaster* and *Drosophila simulans*. *Nucl. Acids Res.* **8**, 5391–5410.

FOX, T. D. (1986) Nuclear gene products required for translation of specific mitochondrially coded mRNAs in yeast. *Trends in Genet.* **2**, 97–100.

GILES, R. E., STROYNOWSKI, I. and WALLACE, D. C. (1980) Characterization of mitochondrial DNA in chloramphenicol-resistant interspecific hybrids and a cybrid. *Som. Cell Genet.* **6**, 543–554.

GODDARD, J. M. and WOLSTENHOLME, D. R. (1978) Origin and direction of replication in mitochondrial DNA molecules from *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **75**, 3886–3890.

GODDARD, J. M. and WOLSTENHOLME, D. R. (1980) Origin and direction of replication in mitochondrial DNA molecules from the genus *Drosophila*. *Nucl. Acids Res.* **8**, 741–757.

GREENLEAF, A. L., KELLY, J. L. and LEHMAN, I. R. (1986) Yeast *RPO41* gene product is required for transcription and maintenance of the mitochondrial genome. *Proc. Natl. Acad. Sci. USA* **83**, 3391–3394.

HARRISON, R. G., RAND, D. M. and WHEELER, W. C. (1985) Mitochondrial DNA size variation within individual crickets. *Science* **228**, 1446–1448.

HAYASHI, J.-I. and MURAKAMI, J. (1988) Identification of cytoplasmically transferred mitochondrial DNA in female germlines of *Drosophila* and its propagation in the progeny. *Mol. Gen. Genet.* **211**, 381–385.

KING, M. P. and ATTARDI, G. (1988) Injection of mitochondria into human cells leads to a rapid replacement of the endogenous mitochondrial DNA. *Cell* **52**, 811–819.

KING, M. P. and ATTARDI, G. (1989) Human cells lacking mtDNA: Repopulation with exogenous mitochondria by complementation. *Science* **246**, 500–503.

MATSUURA, E. T., CHIGUSA, S. I. and NIKI, Y. (1989) Induction of mitochondrial DNA heteroplasmy by intra- and interspecific transplantation of germ plasm in *Drosophila*. *Genetics* **122**, 663–667.

MATSUURA, E. T., CHIGUSA, S. I. and NIKI, Y. (1990) Differences in the modes of transmission of foreign mitochondrial DNA in heteroplasmic lines for intra- and interspecific combinations in *Drosophila melanogaster*. *Jpn. J. Genet.* **65**, 87–93.

NIKI, Y., CHIGUSA, S. I. and MATSUURA, E. T. (1989) Complete replacement of mitochondrial DNA in *Drosophila*. *Nature* **341**, 551–552.

RAND, D. M. and HARRISON, R. G. (1986) Mitochondrial DNA transmission genetics in crickets. *Genetics* **114**, 955–970.

REILLY, J. G. and THOMAS, Jr., C. A. (1980) Length polymorphisms, restriction site variation, and maternal inheritance of mitochondrial DNA of *Drosophila melanogaster*. *Plasmid* **3**, 109–115.

SATTA, Y., ISHIWA, H. and CHIGUSA, S. I. (1987) Analysis of nucleotide substitutions of mitochondrial DNAs in *Drosophila melanogaster* and its sibling species. *Mol. Biol. Evol.* **4**, 638–650.

SATTA, Y., MATSUURA, E. T. and CHIGUSA, S. I. (1990) Mitochondrial DNA polymorphism in *Drosophila melanogaster*. In: *Population Biology of Genes and Molecules*, (ed.: N. Takahata and J. F. Crow), pp. 57–73. Baifukan, Tokyo.

SOLIGNAC, M., GÉNERMONT, J., MONNEROT, M. and MOUNOLOU, J.-C. (1984) Genetics of mitochondria in *Drosophila*: mtDNA inheritance in heteroplasmic strains of *D. mauritiana*. *Mol. Gen. Genet.* **197**, 183–188.

SOLIGNAC, M., GÉNERMONT, J., MONNEROT, M. and MOUNOLOU, J.-C. (1987) *Drosophila* mitochondrial genetics: Evolution of heteroplasmy through germ line cell divisions. *Genetics* **117**, 687–696.

SOLIGNAC, M., MONNEROT, M. and MOUNOLOU, J.-C. (1986) Mitochondrial DNA evolution in the *melanogaster* species subgroup of *Drosophila*. *J. Mol. Evol.* **23**, 31–40.

TZAGOLOFF, A. and MYERS, A. M. (1986) Genetics of mitochondrial biogenesis. *Ann. Rev. Biochem.* **55**, 249–288.

WHITE, F. A. and BUNN, C. L. (1984) Segregation of mitochondrial DNA in human somatic cell hybrids. *Mol. Gen. Genet.* **197**, 453–460.

YAFFE, M. P. and SCHATZ, G. (1984) Two nuclear mutations that block mitochondrial protein import in yeast. *Proc. Natl. Acad. Sci. USA* **81**, 4819–4823.

ZUCKERMAN, S. H., SOLUS, J. F., GILLESPIE, F. P. and EISENSTADT, J. M. (1984) Retention of both parental mitochondrial DNA species in mouse-Chinese hamster somatic cell hybrids. *Som. Cell Mol. Genet.* **10**, 85–92.