Mitochondrial DNA heteroplasmy maintained in natural populations of *Drosophila simulans* in Réunion

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Summary
Mitochondrial DNA (mtDNA) variation in *Drosophila simulans* was studied to determine whether the cytoplasmic state of mtDNA heteroplasmy persists in natural populations in Réunion. For this purpose, 172 isofemale lines, newly collected from two local populations, were examined, among which three types of mtDNA (sill, silll and silll') were found, based on the *Hpa* II restriction pattern. Ten of the lines were heteroplasmic for a combination of sill and silll', as determined by autoradiography. The same type of heteroplasmy had been noted in one of the two local populations 8 years before (Satta et al. 1988). The present results suggest that the heteroplasmic state occurs recurrently in natural populations of *D. simulans* in Réunion.

1. Introduction
Mitochondrial DNA (mtDNA) of animals is maternally inherited (Giles et al. 1980; Lansman et al. 1983) and multiple copies of mtDNA molecules are normally homogeneous both in size and nucleotide sequence within an individual. Studies on mtDNA variation in natural populations of various animals, however, have indicated within-individual heterogeneity in size and restriction site. In most cases, mtDNA molecules of two different sizes coexist in the cells of individual animals (Solignac et al. 1983; Monnerot et al. 1984; Densmore et al. 1985; Harrison et al. 1985; Hauswirth & Clayton, 1985; Bermingham et al. 1986; Hale & Singh, 1986; Wallis, 1987).

Recently, we investigated mtDNA variation in a natural population of *D. simulans* from Réunion, using isofemale lines maintained in laboratories for more than 6 years (Satta et al. 1988). The mtDNA type (silll'), which occurs also in *D. mauritiana* as the mul type, was frequently observed, as was a different type of mtDNA (silll') common in natural populations of *D. simulans* (Baba-Aissa et al. 1988). These two types were occasionally found in a heteroplasmic state in Réunion (Satta et al. 1988). Paternal leakage of mtDNA has been suspected in this case of heteroplasmy, since differences in restriction sites between these two types are difficult to explain by ordinary mutation processes. Analysis of the mtDNA variation in this population should provide useful information for understanding the mechanisms of generation of mtDNA heteroplasmy.

To investigate mtDNA heteroplasmy in *D. simulans* in Réunion, 172 isofemale lines, established from newly collected *D. simulans* from two populations in Réunion, were examined. Three types of *Hpa* II restriction pattern, one of which is new, were found. The heteroplasmic state was also found in ten of these lines even after the elapse of 8 years since the previous collection. Consequently, the present study supplements that of Satta et al. (1988). Based on the results obtained here, the heteroplasmic state appears to arise commonly in *D. simulans* populations in Réunion.

2. Materials and methods
Isofemale lines of *D. simulans* were established from single inseminated females from two natural populations (St Denis and St Pierre) in Réunion in 1987. The locations of the populations are shown in Fig. 1. They had been maintained by mass mating at 19 °C for 1–2 years before being examined. For each isofemale line, mtDNA was extracted from about 0.3 g of adult flies and analysed as described by Satta et al. (1988). From the *Hpa* II restriction pattern, the cytoplasmic state (type of mtDNA) of an isofemale line was unambiguously determined (Figs. 2, 3a). As little as 1% contamination of a different type of mtDNA could be clearly detected on the photograph of a gel taken under UV light after gel staining with ethidium bromide (Matsuura et al. 1989).
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St Denis

St Pierre

0 10 20 km

Fig. 1. Collecting sites of D. simulans in Réunion.

The coexistence of different types of mtDNA was re-examined by Southern hybridization for some of the lines. Using the C fragment of D. simulans (siII) and B fragment of D. mauritiana (mal), as shown in Fig. 2, as 32P-labelled probes to detect siII and siIII mtDNA, respectively, the detection level of contamination was as low as 0.035% for siII and 0.075% for siIII. At least 300 ng of total mtDNA, extracted from 0.1 to 0.2 g of flies, was sufficient to detect C fragment of siII (1.69 kb) and B fragment of siIII (3.6 kb) at these levels, since the amount of DNA detectable by Southern hybridization in the present system was 20 pg after 14 days' exposure. Details of the detection of a minor type of mtDNA will be published elsewhere (Kondo et al. 1990). Hybridization experiments were repeated twice or more, using mtDNA samples from an isofemale line at different generations, to confirm autoradiographic detection at a very low level and also to rule out possibilities of artificial contamination.

Heteroplasmy is here defined as a situation in which two types of mtDNA are present in mtDNA samples from an isofemale line, indicating the original wild-caught female used to establish the line had the two types of mtDNA in its germline cells.

3. Results and discussion

The Hpa II restriction patterns of mtDNA for 103 and 69 isofemale lines of D. simulans from St Denis (RS) and St Pierre (RE), respectively, were examined. Three types of this pattern were found; two of which, siII and siIII, had been reported by Solignac et al. (1986) and found in samples previously collected in 1979 (Satta et al. 1988). The third type, siIII' in Fig. 2, may possibly be derived from siIII by base substitution which generates another Hpa II restriction

![Fig. 3. Examples of the Hpa II restriction patterns of mtDNA extracted from isofemale lines and autoradiograph probed by 32P-labelled C fragment of siII mtDNA. (a) At the left-most lane of the gel, lambda phage DNA digested with Hin III is shown as the size marker. Lane 1, mal line; lanes 2–4, RE lines; lane 5, siII line. In the lane 3, DNA fragments derived from both siII and siIII are present. (b) Only C fragments of siII and siIII (mal) are detected in the autoradiograph. Lane 1, mal line; lanes 2–6, RS lines; line 7, siII line. In lanes 3 and 4, faint 1.69 kb bands of siII mtDNA are present in addition to 2.9 kb bands of siIII. The exposure was done for 1 day at −80 °C.](https://doi.org/10.1017/S0016672300029189)
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Table 1. Number of isofemale lines for the four types of cytoplasmic state in the two natural populations of D. simulans in Réunion

| Type   | RS   | RE   | Total |
|--------|------|------|-------|
| sII    | 4 (4)| 3 (3)| 7 (7) |
| sIII   | 98 (13)| 65 (14) | 163 (27) |
| sII'   | 1 (1)| 0 (0)| 1 (1) |
| sII + sIII | 0 (9)| 1 (1) | 1 (10) |
| Total  | 103 (27) | 69 (18) | 172 (45) |

Numbers in parentheses are those of lines determined by hybridization.

site in sIII. This is supported by the finding that other restriction sites such as Bgl II, Eco RI, Hae III and Xho I were the same as those in sIII (data not shown).

Table 1 shows the numbers of lines for each cytoplasmic state in the two populations. In both populations, sIII, which is common in the sibling species D. mauritiana as maI, was predominant. The frequency of sII, which is common and found in only D. simulans populations (Solignac et al. 1986), was low in these two local populations; 4/103 (4%) in RS and 3/69 (4%) in RE (for the recent population survey, see also Baba-Aissa et al. 1988). Heteroplasmy was observed in one isofemale line of RE from the photographs, as shown in Fig. 3a. The proportion of sII in the RE line was estimated to be about 67%, following the method of Matsuura et al. (1989). It was subsequently considered pertinent to re-examine 36 sIII lines which seemed that they might be heteroplasmic in the photographs and nine lines of the other cytoplasmic types by Southern hybridization using the 32P-labelled probes. The results are shown in parentheses in Table 1 and examples of autoradiographs are given in Fig. 3b. A total of 10 out of 45 lines examined were found to be heteroplasmic for sII and sIII, including the RE line described above and nine RS lines which were misdiagnosed as sIII from the photographs. Since the entire sIII line was not examined by hybridization, these values may not indicate actual frequency in the populations. To determine whether the heteroplasmic state also occurs in individual flies of the heteroplasmic isofemale lines, sublines constructed from the heteroplasmic RE line as described by Satta et al. (1988) were examined. One of seven sublines showed the heteroplasmy as described from the photograph (data not shown).

The present results are consistent with those previously reported (Satta et al. 1988) in that sIII occurs most frequently and there is heteroplasmy for sII and sIII in the population. However, an additional type of sIII' and lower frequency of sII (4%) and heteroplasmy (6%) than previously noted (18 and 12%, respectively) were found in this study. In the previous study, only 17 isofemale lines from St Denis in Réunion were used after being maintained in laboratories for more than 6 years. The smaller number of lines examined and long maintenance of the lines may partly be the cause for these differences from the present results.

Heteroplasmy for sII and sIII was noted in natural populations of D. simulans in 1979 and 1987. The nature of the heteroplasmy and its generating mechanisms still remain to be fully clarified. Heteroplasmy in size is apparently stably inherited, based on observations made over a relatively short span of generations (Solignac et al. 1984; Rand & Harrison, 1986). In contrast, the heteroplasmic state induced in D. melanogaster by transplanting germplasm of D. mauritiana (Matsuura et al. 1989) was almost lost within 15–30 generations depending on the line (Niki et al. 1989; Matsuura et al. 1990). de Stordeur et al. (1989) constructed heteroplasm for sII and sIII by cytoplasmic injection in D. simulans and found that sIII was rapidly lost. Satta et al. (1988) showed one of the initially heteroplasmic lines to have become homoplasmic for sIII after 14 generations. The reason for the predominance of sIII in Réunion populations is not known at present. From these observations, the heteroplasmic state found in D. simulans is possibly transient, leading to fixation of one of the two types of mtDNA. Nevertheless, in the present study, the same type of heteroplasmy was found at a frequency of about 6% in the same population even after 8 years. Based on the present data and some previous observations, the hypothesis is proposed that heteroplasmy may occur and disappear recurrently in these natural populations. Experiments to assess the incomplete maternal inheritance of mtDNA as a generating mechanism of heteroplasmy in the present case should be quite urgent and are in progress at our laboratory.

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