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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 (*si*II, *si*III and *si*III′) were found, based on the *Hpa* II restriction pattern. Ten of the lines were heteroplasmic for a combination of *si*II and *si*III, 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 (*si*III), which occurs also in *D. mauritiana* as the *ma*I type, was frequently observed, as was a different type of mtDNA (*si*II) common in natural populations of *D. simulans* (Baba-Aïssa *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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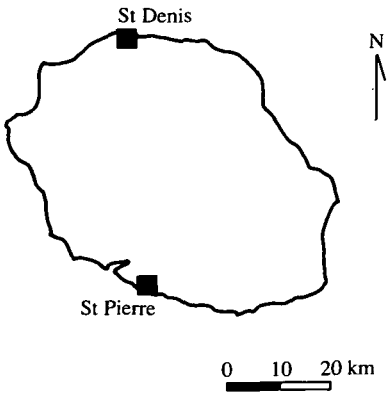

Fig. 1. Collecting sites of *D. simulans* in Réunion.

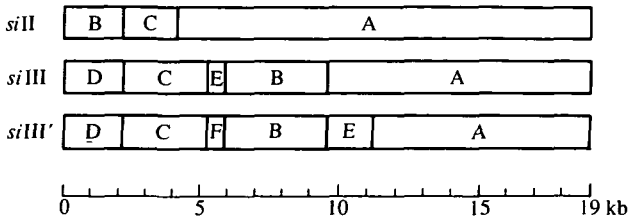

Fig. 2. Linearized *Hpa* II restriction maps of *si*II, *si*III and *si*III′ mtDNA. *si*II and *si*III have already been reported by Solignac *et al.* (1986). The capital letters show relative fragment sizes in the decreasing order.

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* (*si*II) and B fragment of *D. mauritiana* (*ma*I), as shown in Fig. 2, as ^{32}P-labelled probes to detect *si*II and *si*III mtDNA, respectively, the detection level of contamination was as low as 0·035 % for *si*II and 0·075 % for *si*III. At least 300 ng of total mtDNA, extracted from 0·1 to 0·2 g of flies, was sufficient to detect C fragment of *si*II (1·69 kb) and B fragment of *si*III (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, *si*II and *si*III, had been reported by Solignac *et al.* (1986) and found in samples previously collected in 1979 (Satta *et al.* 1988). The third type, *si*III′ in Fig. 2, may possibly be derived from *si*III by base substitution which generates another *Hpa* II restriction

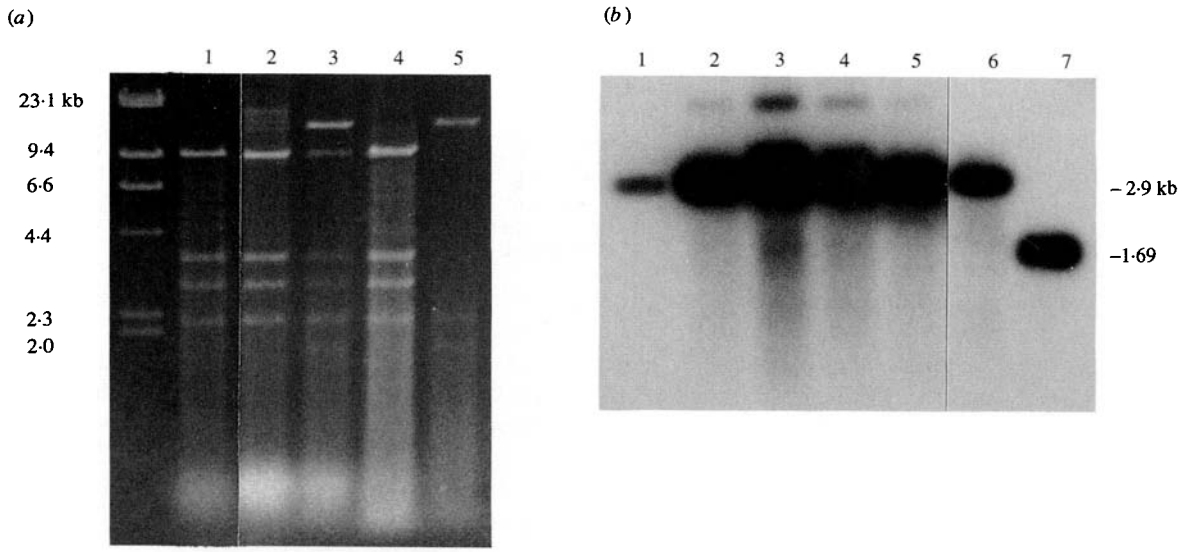

Fig. 3. Examples of the *Hpa* II restriction patterns of mtDNA extracted from isofemale lines and autoradiograph probed by ^{32}P-labelled C fragment of *si*II mtDNA. (*a*) At the left-most lane of the gel, lambda phage DNA digested with *Hin* dIII is shown as the size marker. Lane 1, *ma*I line; lanes 2–4, RE lines; lane 5, *si*II line. In the lane 3, DNA fragments derived from both *si*II and *si*III are present. (*b*) Only C fragments of *si*II and *si*III (*ma*I) are detected in the autoradiograph. Lane 1, *ma*I line; lanes 2–6, RS lines; line 7, *si*II line. In lanes 3 and 4, faint 1·69 kb bands of *si*II mtDNA are present in addition to 2·9 kb bands of *si*III. The exposure was done for 1 day at −80 °C.

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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
*si*II	4 (4)	3 (3)	7 (7)
*si*III	98 (13)	65 (14)	163 (27)
*si*III′	1 (1)	0 (0)	1 (1)
*si*II + *si*III	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 *si*III. 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 *si*III (data not shown).

Table 1 shows the numbers of lines for each cytoplasmic state in the two populations. In both populations, *si*III, which is common in the sibling species *D. mauritiana* as *ma*I, was predominant. The frequency of *si*II, 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-Aïssa *et al.* 1988). Heteroplasmy was observed in one isofemale line of RE from the photographs, as shown in Fig. 3*a*. The proportion of *si*II 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 *si*III lines which seemed that they might be heteroplasmic in the photographs and nine lines of the other cytoplasmic types by Southern hybridization using the ^{32}P-labelled probes. The results are shown in parentheses in Table 1 and examples of autoradiograph are given in Fig. 3*b*. A total of 10 out of 45 lines examined were found to be heteroplasmic for *si*II and *si*III, including the RE line described above and nine RS lines which were misdetermined as *si*III from the photographs. Since the entire *si*III 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 determined from the photograph (data not shown).

The present results are consistent with those previously reported (Satta *et al.* 1988) in that *si*III occurs most frequently and there is heteroplasmy for *si*II and *si*III in the population. However, an additional type of *si*III′ and lower frequency of *si*II (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 *si*II and *si*III 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 heteroplasmy for *si*II and *si*III by cytoplasmic injection in *D. simulans* and found that *si*III was rapidly lost. Satta *et al.* (1988) showed one of the initially heteroplasmic lines to have become homoplasmic for *si*III after 14 generations. The reason for the predominance of *si*III 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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