41. *Drifting Polymorphism of the Number of Multiplication at the Glycerol-3-phosphate Dehydrogenase Locus in Natural Populations of Drosophila melanogaster*

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Since the advent of molecular cloning techniques, we have seen that eukaryote chromosomes have many multiplicated genes. The genome seems to be always subject to duplication pressure (Kimura 1983, 1989; Ohta 1988). We reported that the *sn*-glycerol-3-phosphate dehydrogenase (GPDH; *sn*-glycerol-3-phosphate: NAD^+ 2-oxidoreductase, EC 1.1.1.8) locus of *Drosophila melanogaster* is partially duplicated and the duplication is polymorphic in natural populations both in Japan and in the USA (Koga *et al.* 1988). Furthermore, triplicated *Gpdh* loci were independently found in the Aomori population of northern Japan and in the Ogasawara population of southern Japan (Takano *et al.* 1989). Since the difference between duplicated and triplicated loci can only be detected by the intensity of hybridization of the same band morph, this prompted us to reexamine the previous report (Koga *et al.* 1988) and to further survey the distribution of the polymorphism in the other area both in Japan and elsewhere.

Materials and methods. *Drosophila strains.* The three populations, the Raleigh, the Texas and the Ishigakijima population were the same as those in Koga *et al.* (1988). We reexamined whether the lines which were classified as duplications in Koga *et al.* (1988) are really duplications or triplications. In the case in which the second chromosome has a lethal gene, the line was made hemizygous for *Df(2L)GPDHA*. About a hundred isogenic lines for the second chromosome were established from each of the Katsunuma (51 lines), the Osaka (77 lines) and the Nagasaki (73 lines) population in Japan. The isofemale lines were used for the Reunion (7 lines) population in the Indian Ocean. The isofemale lines were also used for the Botswana southern African population (52 lines).

Detection of Gpdh duplications and triplications. The presence of duplications or triplications was determined as follows: Genomic DNA extracted from each chromosome line was digested with the restriction enzyme XbaI and processed for Southern analysis. The probe used is a fragment encompassing an approximately 8 kb region bearing the complete single gene. As can be seen from the restriction map in Fig. 1, chromosomal lines with non-duplicated *Gpdh* gene yield two XbaI fragments bearing *Gpdh* sequences, a 7.0-kb fragment and a 6.1-kb fragment. Those with a *Gpdh* duplication or triplication should yield an additional 4.5-kb fragment. Since its multiplication is the same in size, the chromosome line with a triplication has double doses of a 4.5-kb fragment compared with that of a duplication and therefore, the intensity of hybridization with the probe is much stronger in a triplication than in a duplication. These differences are easily distinguishable as is shown in Fig. 2B in Takano *et al.* (1989).

Results. *Distribution and frequency of duplications and triplications.* We

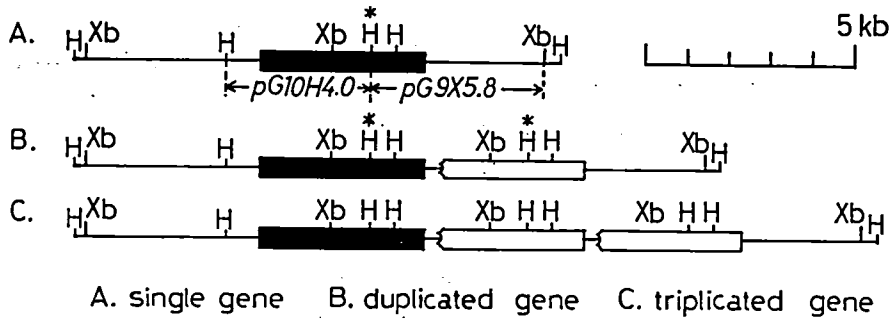

Fig. 1. Schematic restriction map of the single (A), duplicated (B), and triplicated (C) *Gpdh* genes. The filled boxes indicate translated region and the open boxes are partial duplicates and triplicates. Xb and H stand for Xba I and Hin dIII restriction sites. * indicates restriction sites some of which are polymorphic in natural populations. *pG10H4.0* and *pG9X5.8* are the probes used in Southern analysis.

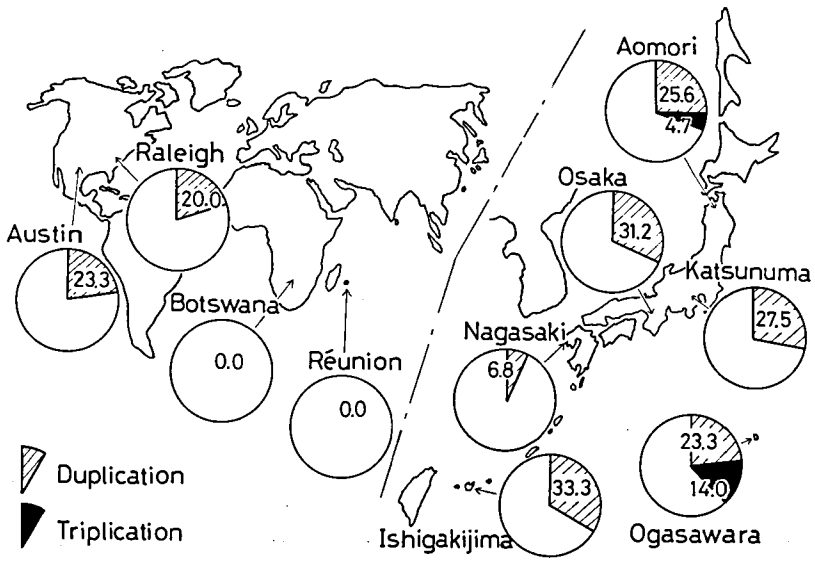

Fig. 2. Distribution of the duplicated and triplicated *Gpdh* genes. The hatched and filled area shows frequencies of duplications and triplications, respectively.

detected no triplications in the lines which we had previously examined (Koga *et al.* 1988). In addition, we found no triplications in three local Japanese populations other than the Aomori and the Ogasawara population. The frequency of duplications is less than forty percent and are not very different from those of the USA. The average frequency of duplications including triplications in the Japanese and the American population is 26.5%. In contrast, we could find neither a duplication nor a triplication in the Reunion and the Botswana population. The distribution and the frequencies of duplications and triplications are shown in Fig. 2.

Discussion. In this study, we have faced with the interesting case of polymorphism of the copy number of a gene. Three things can be said about this polymorphism. First, the original melanogaster population must have been monomorphic in the centro-African area before it migrated into other areas of the world. This appears to be true since only the Botswana, African population and the Reunion population, which is located near the African mainland, are lacking both duplications and triplications. Second, wide spread polymorphism might have been driven by the paramount power of random genetic drift alone. The 4.5-kb duplication of the *Gpdh* gene is presumably a kind of pseudogene, since it deletes the first and the second exons. This means that neither a duplication nor a triplication confers any advantage to the fitness of its carrier and therefore, this suggests that by the power of random genetic drift alone the frequency can reach a rather high level. Third, continual gene conversion (Smithies and Powers 1986) must be occurring as a source of genetic variation, which serves to produce raw material for evolution. This view is supported by the fact that triplications have been independently found only in the Aomori population and the Ogasawara population, which are more than a thousand kilometers apart.

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