Narise, S. and M. Sasaki. Josai University, Saitama, Japan. Thermal stability difference among αGpdh allozymes from D. virilis.

Thermal inactivation was compared among three αGpdh allozymes. Three homozygous strains ($\alpha Gpdh^f/\alpha Gpdh^f$, $\alpha Gpdh^m/\alpha Gpdh^m$ and $\alpha Gpdh^s/\alpha Gpdh^s$) for cytoplasmic αGpdh were isolated from single females collected from a natural population in Omaezaki, Japan. Starch gel electrophoretic patterns of the αGpdh from the three strains and their hybrids are shown in Fig. 1.

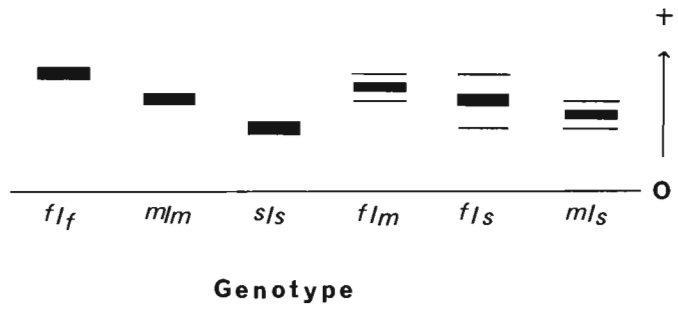

Fig. 1. Electrophoretic patterns of cytoplasmic αGpdh allozymes from homogenates of D. virilis.

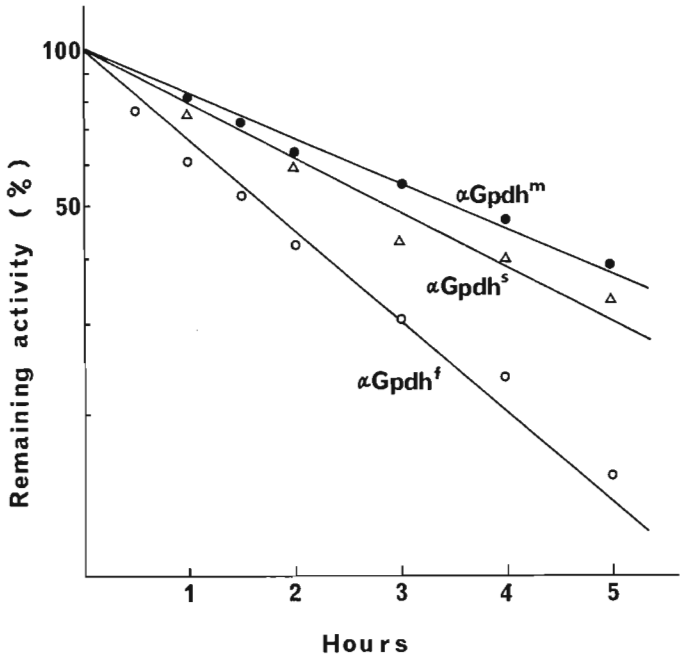

Fig. 2. Thermal inactivation of αGpdh allozymes at 35°C.

The enzyme activity of $\alpha Gpdh^f$ homozygote on the gel did not develop, when the gel was incubated at 55°C for one hour after electrophoresis, while the activities of $\alpha Gpdh^m$ and $\alpha Gpdh^s$ homozygotes were slightly reduced. In both $\alpha Gpdh^f/\alpha Gpdh^m$ and $\alpha Gpdh^f/\alpha Gpdh^s$ heterozygotes, fast moving bands disappeared and intermediate hybrid bands appeared more faintly than did their corresponding slow moving bands, after the same treatment. 92 isofemale lines from natural populations in Omaezaki, Nagoya and Toyama were tested for the thermal stability at 55°C and the same result was obtained, i.e., $\alpha Gpdh^f$ allozymes from every population were thermolabile, whereas $\alpha Gpdh^m$ and $\alpha Gpdh^s$ allozymes were thermostable, and no heterozygote advantage was observed with respect to thermostability of αGpdh.

Three allozymes were extracted from their homozygous strains and purified 130-160 fold by fractionation with ammonium sulfate, DEAE cellulose, Sephadex and hydroxylapatite. When each purified enzyme was examined by polyacrylamide-gel disc electrophoresis, a single band stained for protein was coincided with the αGpdh activity.

Samples of purified enzyme preparations in 0.05 M Tris acetate buffer pH 7.0 were incubated at 35°C and were assayed for activity immediately after various periods of time as indicated in Fig. 2, with the standard assay mixture (0.1 mM DHAP, 0.1 mM NADH, enzyme and 0.1 M Tris acetate buffer pH 6.75 in a total volume of 3.0 ml). Fig. 2 shows a comparison of the thermal stability at 35°C of three αGpdh allozyme activities. As seen in Fig. 2, the activity of $\alpha Gpdh^f$ decreases markedly as compared with the other two. With 45°C treatment, the $\alpha Gpdh^m$ maintained about 60% of the original activity and the $\alpha Gpdh^s$ about 40%. $\alpha Gpdh^f$ lost all activity in 2 min incubation, whereas

These results indicate that $\alpha Gpdh^f$ allele at the αGpdh locus specifies a thermolabile form of αGpdh protein.