A Preliminary Study on the Enzymatic Pattern of Glucose 6-Phosphate Dehydrogenase in the Robusta Species Group of *Drosophila*[1)2)]

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(*With 4 Text-figures and 1 Table*)

The robusta species group belongs to the virilis-repleta section of the subgenus Drosophila (Fig. 1). This species group is represented by the following 11 species: *robusta* and *colorata* recorded from North America, *pullata* from China, *cheda* from China and Korea, *sordidula* and *lacertosa* from Korea and Japan, and *moriwakii*, *okadai*, *neokadai*, *pseudosordidula* and sp. I from Japan. The latter six species

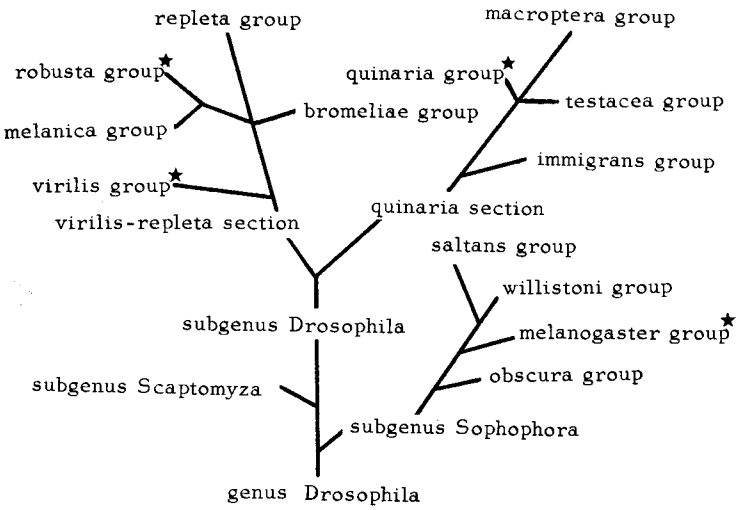

Fig. 1. Diagram of taxonomic relationships within the genus *Drosophila* (modified scheme of Throckmorton 1962). Species group examined in the present study are asterisked.

1) Contribution No. 869 from the Zoological Institute, Faculty of Science, Hokkaido University, Sappro, Japan 060

2) This work was partly supported by a grant from the Japan Society for the Promotion of Science as a part of the Japan-U.S. Cooperative Science Program.

were recorded within the past decade, and this result lead to the development of the systematic study of this group.

Recently, attempts have been made to analyse proteins from different species by gel electrophoresis in a hope to find a possible evolutionary relationship at a molecular level among species. Hubby and Throckmorton (1965) studied adult soluble proteins within the virilis group of Drosophila and obtained data essential for establishing the evolutionary relationship between proteins. A study of a similar scheme has been made by Duke (1966, 1968) who dealt with electrophoresis of the late third instar larval lymph proteins and xanthine dehydrogenases in several species of *Drosophila*. Further there are several workers reporting isozyme patterms and enzymatic polymorphisms in Drosophila (Grell *et al.* 1965, Courtright 1966, Lewontin and Hubby 1966, Johnson *et al.* 1966, Stone *et al.* 1960, 1969, Young *et al.* 1964, Young 1966, Steele *et al.* 1968).

The present authors investigated preliminarily isozyme patterns of the glucose 6-phosphate dehydrogenase (G–6–PD) in five species of the robusta group in order to obtain critical information for understanding the evolutionary relationship of these species.

The authors express their cordial thanks to Professor Sajiro Makino for his helpful suggestion with keen interest. They are also indebted to Professor Eizi Momma for his important advice and to Dr. Samuel H. Hori for his technical guidance.

Materials and Methods

The stocks used for study are listed in Table 1. For electrophoresis each fly was placed on a hollow glass with a small amount of 0.1 % Triton X–100 and 5 mM EDTA in 20 mM Tris-malate-NaOH buffer, pH 7.2, and homogenized with a glass rod. Disc electrophoresis was made according to the method of Orstein and Davis (1962), employing 7.5% acrylamide in the lower gel and 3.3% acrylamide in the spacer and sample gels. Gels were cast in glass tubes (2.5 mm × 50 mm). 0.1ml sample gel containing 0.005–0.01 ml of fly homogenates and 0.03 ml spacer gel were layered above 0.3 ml lower gel. After electrophoresis for about 1 hour in tris-glycine buffer, pH 8.3, supplemented with NADP (in a concentration of 0.1 mg per 100 ml of buffer), with a constant current of 1 mA per column, the gels were stained about 2 hours in the following medium at room temperature (15–20°C). *Stock buffer solution*: 0.2M Tris-malate-NaOH buffer, pH 7.2, 25ml; 0.1N KCN, 10 ml; nitroblue tetrazolium (1mg/ml), 25 ml; and distilled water, 30 ml. Working solution was prepared as follows: stock buffer solution, 9 ml; NADP (10 mg/ml), 0.15 ml; 0.1 M, $MgCl_2$, 0.5 ml; phenazine methosulfate (1 mg/ml), 0.1 ml; and Na Glucose 6-phosphate, (40 mg/ml), 0.2 ml. The pH of the working solution was adjusted to pH 7.2 in order to minimize the effect of nothing dehydrogenase, which shows an increased activity at higher pH. For detection of nothing dehydrogenase activity, glucose 6-phosphate in the working solution was replaced by an equal volume of distilled water. For detail, see Hori *et al.* 1966, and Hori and Kawamura 1968.

Results and Discussion

Seven bands of G–6–PD activity were observed in zymograms of robusta species group and were referred to as A-G in the order of increasing anodal

Table 1. List of Drosophila species examined

	Species	Strain	Origin	Collected in		Collector
robusta group	*sordidula*	BGES-8	1♀	Botanical Garden, Sapporo, Hokkaido	Aug. '63	Kaneko
		KS-4	〃	near the foot of Mt. Komagatake, southern part of Hokkaido	Aug. '65	〃
	pseudosordidula	KP	〃	〃	〃	〃
		FNP	1♀+6♂	Hidaka-Nukabira, near the Mt. Poroshiridake, Hidaka mountains, Hokkaido	Aug. '67	〃
	lacertosa	CHL-C	mass	Chitou in Formosa	July '68	Takada
		TL-Am	〃	Nyuto-onsen, near the lake Tazawa, Akita prefecture, northern part of Honshu	Aug. '69	Kaneko
		TL-Bm	〃		〃	〃
	moriwakii	TM-B	〃	〃	〃	〃
	okadai	TO-A	〃	〃	〃	〃
		YO-m	5♀+2♂	Yukomanbetsu, in Daisetsu mountains, central part of Hokkaido	Sept. '68	〃
others	*virilis*	Oi-l	4♀+1♂	Okushiri Is.	Aug. '60	〃
	nigromaculata	Un-m	mass	Campus of Hokkaido Univ.	Aug. '60	〃
	melanogaster	Be-M	〃	Beer brewery in Sapporo	Oct. '59	〃

mobility. The zymgorams obtained from flies sacrificed 10 to 20 days after emergence are presented in Figs. 2 and 3. The isozyme patterns for each species are shown as below:

D. pseudosordidula: A-G bands in FNP and KP.

D. sordidula: A, B, D, E and F in BGES-8 and KS-4.

D. okadai: A-G bands in TO-A. E band showed slightly fast anodal mobility. A, B, D, F and G in YO-m.

D. moriwakii: A, B, D, E, F and G in TM-B. Total enzyme activity was apparently less in this strain than in the other strains.

D. lacertosa: A, B, D, E, F and G in CHL-C. E and F band showed slightly fast anodal mobility and strong activity in CHL-C. A, B, D, E, F and G in TL-Am. A-G, E' (extra band on the anodal side of E band) in TL-Bm. Though the latter two strains were sampled in the same locality in this summer, there is a slight difference between them.

Specimens studied in various developmental stages from larva to mature adult displayed no bands except B in larva, imago and a few day-old flies, irrespective of sex. It was shown that the isozyme patterns varied considerably among flies of one and the same strain (Fig. 2). It is uncertain whether such a variation would be attributable to enzyme polymorphism within the strain, or the difference in the physiological condition of individuals. In order to answer the above question, single pair cultures have now been going on. B band was

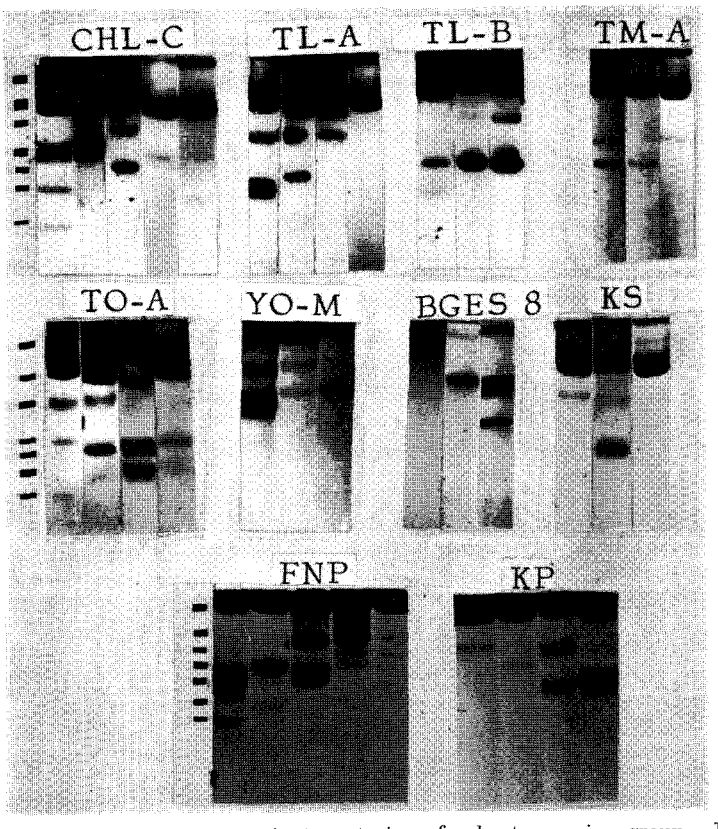

Fig. 2. G–6–PD isozyme patterns in ten strains of robusta species group. For strains see Table 1.

the only one common to every individual irrespective of their origin and sex. It showed a moderate activity of nothing dehydrogenase activity. It is of interest that the electrophoretic mobility seems to differ slightly between the robusta group and the virilis group, but not between the robusta group and

the quinaria or melanogaster group (Fig. 4). Though further experiments for reconfirmation have now been in progress, some interesting speculation would be still possible on the evolutionary relationship among those species groups of Drosophila.

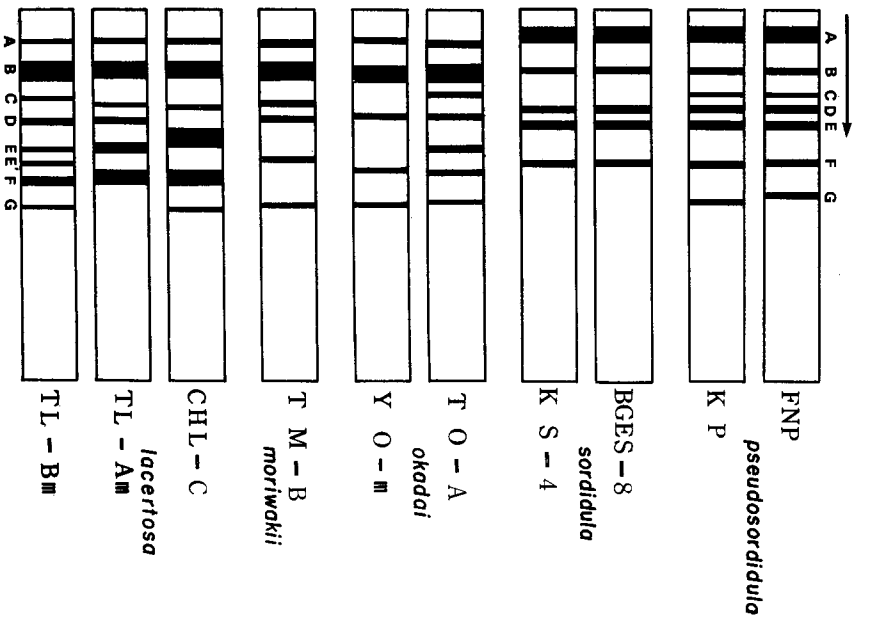

Fig. 3. Average mobility of the bands in ten strains of robusta species group. C-F bands in each strain tend to vary slightly in the relative electrophoretic mobility.

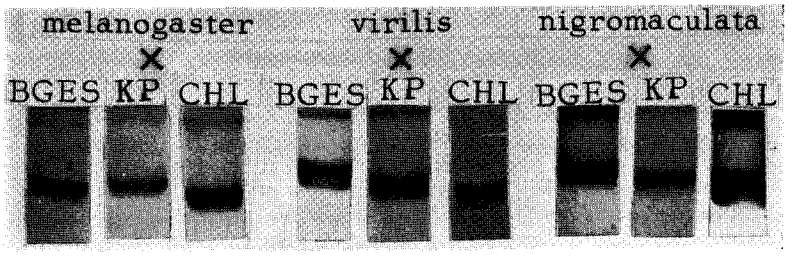

Fig. 4. B band in *in vitro* hybridization between *D. pseudosordidula* (robusta group) and each of the following three species: *D. nigromaculata* (quinaria group), *D. virilis* and *D. melanogaster*.

Summary

Flies of 10 strains from the following five species of robusta group from Asia, *D. sordidula*, *D. pseudosordidula*, *D. lacertosa*, *D. moriwakii* and *D. okadai*, showed

a significant variation in isozyme patterns of glucose 6-phosphate dehydrogenase. There was no strain-specific isozyme pattern even in the same strain. The band termed B was common to all the flies here examined, and its electrophoretic mobility seemed to differ slightly between the flies of robusta group and those of virilis group.

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