Trehan, K.S. and K.S. Gill. Punjab Agricultural University, Ludhiana, India. Isolation and partial purification of allozymes of acid phosphatase from heterozygotes of **Drosophila melerkotliana.**

Isolation of allozymes: Characterisation of heteromultimers, produced in hybrids, is of prime importance to elucidate molecular basis of heterosis and developmental homeostasis. We have developed a simple starch gel electrophoretic technique to fractionate homomultimeric and heteromultimeric allozymes from heterozygotes. Presently this technique has been used to fractionate allozymes of acid phosphatase (a dimeric enzyme) synthesised under the control of Acph-$1^{1.05}$ (fast) and Acph-$1^{0.95}$ (slow) alleles.

Using Tris buffer (Poulik 1957), starch gel of 15% concentration was prepared in 17 cm x 14 cm x 1 cm glass tray (Trehan 1971). After the gel had set, glass strips were removed from 14 cm sides, and from each of these sides, a 2 cm wide gel was cut and removed. A Whatman filter paper (3 mm) strip (henceforth called isolation strip) measuring 6 cm x 1 cm was loaded with 0.1 ml of crude extract (1 g of flies crushed in 2 ml of glass-distilled water), and placed against the middle of one of the cut surfaces of the gel. Two strips (henceforth called marker strips) loaded with the same crude extract were placed one on each side of and at a distance of 1 cm from the isolation strip (Fig. 1). One cm wide gel, cut from one of 2 cm wide gels removed earlier, was placed against the loaded side of the gel. Sufficient care was taken to ensure that no air bubble was left in the interfaces between the strips and the gel surfaces.

The loaded gel tray was connected to the electrode chambers by 1.5 cm thick foam sponge, presoaked in borate buffer (Poulik 1957). The gel was run at 4°C for 4 hr at 300 volts and 30 mA current.

After completion of electrophoresis, the gel was cut longitudinally into three portions, separating the marker portions from the isolation portion (Fig. 2). The former were stained for acid phosphatase and then restored to their original positions to localise the position of three allozymes in the isolation gel. The three demarcated portions of the unstained isolation gel, each containing a different allozyme, were cut, adjusted to same weight, and stored in deep freezer. Ten different gels were subjected to the procedure described above to obtain sufficient amount for each of the fractionated extract. Gel portions containing the same allozyme were pooled. The frozen gels were thawed, homogenised and centrifuged at 10,000 x g for 20 min. The fractionated allozymes were tested for their homogeneity by electrophoresis.

Partial purification of allozymes: Isolation of allozymes, described above, simultaneously achieves their partial purification. Degree of purification was determined by estimating the protein contents and specific activities in the fractionated allozyme extracts and the diluted crude extract. Dilution factor in the allozyme gel portions was determined by taking a portion of the gel having a weight equal to that of the three allozyme gel portions and drying it. Distilled water was then added to the crude extract so that it was diluted to same extent as the allozyme extract. The data obtained for protein content and specific activity are given in Table 1.

The total protein in the three fractionated extracts is 48.52 ± 0.92 ug/ml. Fractionation by electrophoresis has, thus removed 329.27 ± 7.58 ug/ml protein and achieved 7.8 times purification in a single step. It may also be noted that different amounts of protein have been removed in different

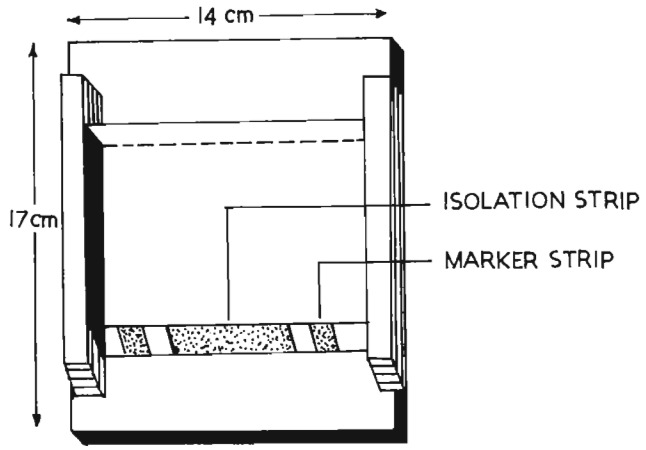

FIGURE 1.

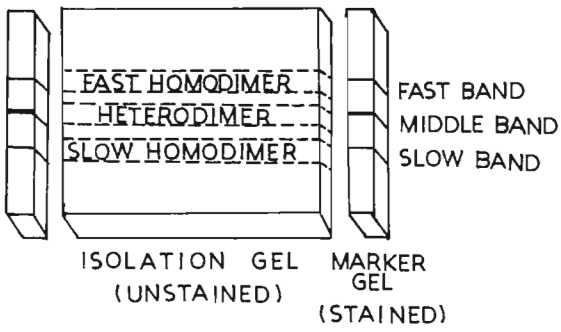

FIGURE 2.

Table 1. Protein contents and specific activities in crude extracts, fractionated allozyme extracts from heterozygotes and partially purified extracts from homozygotes.

	Heterozygotes		Fast homozygotes		Slow homozygotes	
Extract	Prot.cont.	Sp.Act.	Prot.cont.	Sp.Act.	Prot.cont.	Sp.Act.
Crude	379.50±12.60	12.01	379.50±12.60	5.54	379.50±12.60	8.94
Fractions						
Fast homodimer	12.87±0.29	26.83				
Slow homodimer	19.90±0.30	32.25				
Heterodimer	15.85±0.33	74.06				
Partially purified			23.29±0.56	73.17	30.75±0.48	113.72

fractions. The total specific activity in the fractionated extracts is 138.24 uM of napthol-released/min/mg protein and that in crude extract is 12.01. Thus partial purification has resulted in 11.50 times increase in specific activity.

Partial purification of allozymes from homozygotes: The technique described above is applicable and was used for achieving partial purification of allozymes from homozygotes. Partial purification of crude extract from fast homozygotes resulted in removal of 356.4 ± 12.04 ug/ml of protein and thus obtaining 16.29 fold purification for fast homodimer. However, in case of slow homozygotes, less protein was removed (348.75 ± 12.2 ug/ml) than that in fast homozygotes and 12.06 fold purification was observed for slow homodimer. It may be noted that the average protein content is same in the crude extracts from three genotypes, i.e., 379.50 ± 12.68.

References: Trehan, K.S. 1971, M.Sc. Thesis, Panjab Univ, Chandigarh; Poulik, M.D. 1957, Nature 180:1477.

Tsacas, L.* and F.P. Saitta.+ * - Laboratoire de Biologie et Génétique Evolutives, Gif-sur-Yvette, France. + - University of Swaziland, Kwaluseni. [Saitta-present address: 58 Butler St., Pittston, Pennsylvania 18640 USNA). Drosophilids in Swaziland, Southern Africa.

In the most recent publications dealing with the African Drosophilidae (Tsacas 1980 and Tsacas et al. 1981), there is no record of the presence of this family in Swaziland. In 1982, one of us (F.P.S.) made some collections in this country and 6 species were identified: **Drosophila buzzatii** Patterson & Wheeler, **D.hydei** Sturtevant, **D.** sp. of the **latifasciaeformis-finitima** complex, now under study, **Zaprionus collarti** Tsacas, **Z.ghesquierei** Collart. **D.buzzati** was collected from **Opuntia** pads, the two species of the **latifasciaeformia-finitima** complex from the fermenting fruits of **Ficus sansibarica** while the remaining species were collected from fermenting pineapple. Swaziland is the southernmost locality for **Z.ghesquierei.** Up to now, seven species are known from Mozambique and 39 from South Africa, the two neighbouring countries. A large number of species probably remains to be collected in Swaziland as well as in the two other countries.