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STUDIES ON THE POLYMORPHISM OF ∝- ESTERASE ACTIVITY IN A FEW MEMBERS OF *NASUTA* SUBGROUP (GENUS: *DROSOPHILA*)

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Polyacrylamide gel electrophoretic technique was used to assay the activity of ∝-esterases in various members of *D. nasuta* subgroup. Five zones of activity designated as EST A to E were recognized. Further 1 to 5 bands in each zone were observed in these species under study. Both similarities and dissimilarities with regard to degree of variation in the isozymes compared with the presumed degree of relationship established by cytological and hybridization studies are discussed.

INTRODUCTION

The *nasuta* subgroup of the *immigrans* species group of the subgenus *Drosophila* consists of a cluster of sibling species. Though WILSON *et al.*, (1969) included eight biologically valid species in this subgroup, the detailed cytogenetical studies including the hybridization experiments made by NIRMALA & KRISHNAMURTHY (1973-1974), RANGANATH & KRISHNAMURTHY (1976) and RANGANATH *et al.*, (1974) enabled them to establish three morphophenotypic complexes on the basis of the extent of silvery markings on the frons. The first is the frontal sheen complex with silvery sheen on the entire frons, and includes *Drosophila nasuta nasuta*, *D. n. albomicana*, *D. n. kepulauana* and *D. kohkoa*. The second is the orbital sheen complex with silvery markings confined to sides of the orbits and include *D. sulfurigaster sulfurigaster*, *D. s. bilimbata*, *D. s. albostrigata*, *D. s. neonasuta*, *D. nixifrons* and *D. pulaua*, while the third includes *D. pallidifrons* without any such markings. On the basis of hybridization experiments and the fixed inversion differences detected between members of the *nasuta* subgroup, RAJASEKARASETTY *et al.*, (1975) were able to establish certain interrelationships between the above said members under study. NAIR *et al.*, (1971) by their studies on the isozyme polymorphism in six members of *mesophragmatica* group of *Drosophila* have demonstrated that there is a correlation between the cytotaxonomic relationship and the isozyme pattern. As the taxonomic status of the original strain of *D. nasuta* in relation to other members of *nasuta* subgroup has been established, the authors wanted to evaluate whether there is any similarity between the earlier cytogenetic findings and the isozyme differences between the above members. Molecular manifestation in the form of isozyme activity by electrophoretic assay has been utilized by several workers to estimate the genetic similarity or dissimilarity between populations or species. JOHNSON *et al.*, (1966) studied esterase differences between taxonomically different species and found a close agreement in the patterns. HUBBY & THROCKMORTON (1968) compared seven isozymes with nineteen systems in triads of closely related species in *Drosophila*

and found that sibling species shared isozymes with identical electrophoretic mobility about 50% on the average and lesser degree of sharing occurred between more distantly related ones. KANAPI & WHEELER (1970) have made preliminary attempt to detect the isozyme differences among few members of the *nasuta* subgroup at a time when the original strain of *D. nasuta* from Seychelles islands was not available to them and thus the cytogenetic status of the then available members were yet to be analysed. As the taxonomic status of the original strain of *D. nasuta* has been established (NIRMALA & KRISHNAMURTHY, 1973; RANGANATH *et al.*, 1974) the present studies on the molecular differentiation in terms of ∝-esterase isozymes were undertaken and the results are presented here.

MATERIALS AND METHODS

Different members of *D. nasuta* subgroup used for the present analysis of esterase isozyme activity, along with their geographic origin are listed in Table I.

TABLE 1. Members of *D. nasuta* subgroup used in the present study along with their geographic origin

1.	*D.n. nasuta*	Coorg (Karnataka, India)
2.	*D.s. neonasuta*	Coorg (Karnataka, India)
3.	*D.n. albomicana* (3146.3)*	Chi-tou, (Taiwan)
4.	*D.n. kepulauana* (3121.3, no. 2)*	Sarawak
5.	*D. Kohkoa* (3256.3, no. 1)*	Gulf of Thailand
6.	*D. pulaua* (3121.5)*	Sarawak
7.	*D.s. sulfurigaster* (3019.8)*	Wau, (New Guinea)
8.	*D.s. albostrigata* (3261.2)*	Mt. Makelins, (Laguna)

* Stocks received from Genetics Foundation, University of Texas at Austin, Texas, U.S.A. The stock number is mentioned in brackets.

Assays were made for ∝-esterases (EST) using polyacrylamide gel electrophoretic technique of DAVIS (1964), with the following modifications. Homogenates of three day-old single males constituted the material for assay of izozymes. Five stock solutions A, B, C, D and E were prepared following the procedure of Davis (1964). Small pore gel was prepared by mixing one part of solution A, two parts of C, one part of distilled water and four parts of 0.14% ammonium persulphate. This mixture was allowed to polymerise in cylindrical glass tubes in the presence of sunlight. Then the large pore gel solution was prepared by mixing one part of solution B, two parts of D and one part of solution E and layered to a height of 5 mm over the small pore gel. The large pore gel solution was allowed to polymerise in the sunlight. The gel tubes were placed in containers where the basal portion carrying small pore gel was immersed in tray buffer (Boric acid Sodium hydroxide buffer of 0.3M and pH 8.65). The sample homogenate mixed with 0.2 ml of 40% sucrose solution was layered over the large pore gel. This was further layered with the tray buffer in such a way as to completely conceal the electrodes. Electrophoresis was carried out at 4°C with 80 volts current, for 2 hours. Afterwards, the gels were removed from the tubes and were incubated in the staining solution at 37°C. After the enzyme bands appeared the gels were fixed in 7% acetic acid.

The sites of esterase isozymes were stained by the coupling of ∝-naphtol with a diazonium salt (Fast blue RR salt). Here ∝-naphthol was liberated by the activity of esterases from ∝-naphthyl acetate which was added as the substrate.

25 mg of ∝-naphthyl acetate was dissolved in 1 ml of acetone and 1 ml of water and was added to 12.5 ml of 0.1 M phosphate buffer of pH 5.9, to which 25 mg of Fast blue RR salt was added. The whole solution was mixed thoroughly and added to 12.5 ml of 0.1M phosphate buffer of pH 6.5. This constituted the stain.

RESULTS

A comparative study of various zymograms for ∝-esterase activity manifested by different species of *D. nasuta* subgroup under study has revealed the presence of 1 to 5 zones of activity designated here EST A to E. In all species all the zones of activity are not encountered. The number of bands included in each zone of activity ranges from one to five. The variable positions of the bands on the gel are shown in Fig. 1.

The zone A is nearest to anode, while zone E is close to the origin. The E and D zones have one band each. *D. s. albostrigata* has both the zones D and E while *D. pulaua* has only E. Of the two bands which compete to reach the anode, one runs along with the indicator bromophenol blue and this band occupies a position approximately equidistant from the two poles.

broad bands in the C zone and one band in the B zone are seen. In *D. kohkoa* also we recognise two broad bands in C and one band in B but the positions of these bands differ from the previous species. In the case of *D. pulaua*, there is a single band in the zone E, three broad bands in C and one in zone B. In *D. s. sulfurigaster*, there are three broad bands in zone C and two

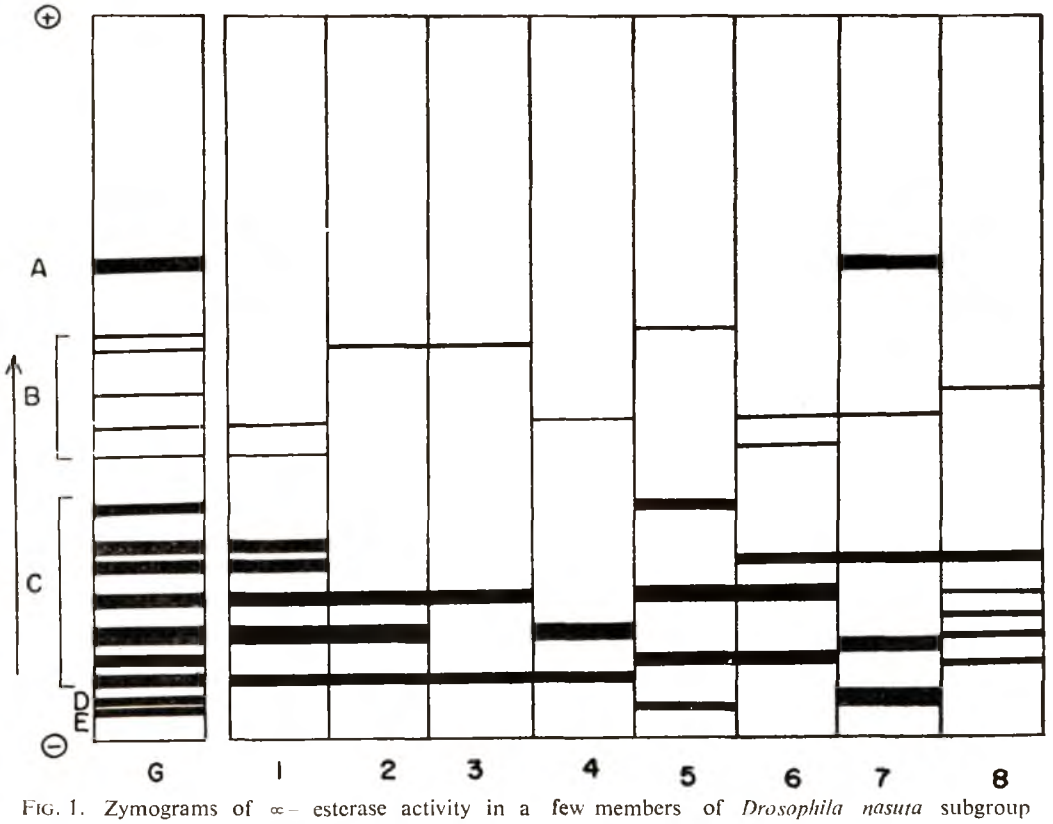

FIG. 1. Zymograms of ∝- esterase activity in a few members of *Drosophila nasuta* subgroup (G) Composite presentation of the five primary zones of activity (1) *D. n. nasuta* (2) *D. n. albomicana* (3) *D. n. kepulauana* (4) *D. kohkoa* (5) *D. pulaua* (6) *D. s. sulfurigaster* (7) *D.s. albostrigata* (8) *D.s. neonasuta.*

In the case of *Drosophila nasuta nasuta,* the A, D and E zones are absent, while the B zone is represented by two bands, zone C is represented by five broad bands. In the case of *D. n. albomicana* there are three broad bands in C zone and only one band in the B zone, while in *D. kepulauana* two bands in zone B. *D. s. albostrigata* exhibits all the five zones of activity, where zones A, B, D and E have one band each and the zone C is represented by two broad bands. The band that moves faster than the dye towards the anode is EST A and is found in 30% of the individuals of this species.

Further, no other species carries this band. *D. s. neonasuta* has one band in B zone and zone C is represented by one broad band and four thin bands.

DISCUSSION

In addition to cytogenetic methods, isozyme studies have been used to study gene differences within Mendelian populations as well as between species. Further, a correlation between classical cytotaxonomic studies and isozyme studies is found to be more meaningful. AYALA & POWELL (1972) have studied enzyme variability in sibling species of the *willistoni* group and discussed the relevance of their results in differentiation of these species.

Of the eight members employed in the present analysis. the hybridization experiments have shown that *D. n. nasuta*, *D. n. albomicana*, *D. n. kepulauana* and *D. kohkoa* belong to the frontal sheen complex; RANGANATH *et al.*, (1974) have shown *D. n. albomicana* as a chromosomal race and *D. n. kepulauana* as a semispecies of *D. n. nasuta*. Further, *D. n. albomicana* is genetically closer to *D. n. nasuta* than to other members of the group because the hybrids are viable and fertile. But *D. n. kepulauana* due to its partial reproductive isolation from *D. n. nasuta* might have undergone a higher level of genetic divergence, while *D. kohkoa* has attained the status of a species because the cross does not give viable and fertile hybrids. Examination of the α– esterase patterns of all the species under discussion, as revealed by the zymograms confirm the findings through hybridization experiments. The banding pattern of the members *D. n. nasuta*, *D. n. albomicana* and *D. n. kepulauana* share some common features particularly in the zone C. The nature of banding of *D. kohkoa* differ from *D. n. albomicana* and *D.n. kepulauana* in this zone, but it possesses similarities with that of *D. n. nasuta* in the zones B and C. Further, among the members of the *sulfurigaster* complex, *D. s. sulfurigaster*, *D. s. albostrigata* and *D. s. neonasuta* have different levels of reproductive isolation reflecting the extent of their genetic affinities. But *D. pulaua* is completely reproductively isolated from these members (NIRMALA & KRISHNAMURTHY, 1973–74; RANGANATH & KRISHNAMURTHY, 1976). *D. s. albostrigata* is the only species which has exhibited activity in all the five zones. It is interesting to note that none of the bands of *D. s. neonasuta* are similar to the bands of *D. s. sulfurigaster*. The zymograms of *D. s. sulfurigaster* and *D. s. albostrigata* present the occurrence of similar activity in zones B and C. The nature of banding of *D. pulaua* has revealed varying degrees of similarities with *D. s. sulfurigaster* and *D. s. albostrigata* but it completely differs from *D. s. neonasuta*.

Thus the extent of genetic differences as revealed by the α – esterase activity in the members of *nasuta* subgroup lends some support to the earlier findings of hybridization experiments. In view of these findings it is felt that one has to employ both cytogenetic as well as isozyme analyses to explore the exact genetic relationships of different species.

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