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Esterase-4 **locus comprises active and null alleles in** ***Drosophila ananassae*****.**

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Genetic variations in natural populations of *Drosophila* have been studied extensively for many enzymes using electrophoretic mobility (Hubby and Lewontin, 1966; Lewontin and Hubby, 1966; Morton *et al*. 2004). Esterase is one of the most studied enzyme systems in *Drosophila*. However, there is very little literature pertaining to Esterase polymorphism in *D. ananassae* (Johnson, 1971). *D. ananassae* Doleschall (1858) is a cosmopolitan and domestic species. The extent and pattern of inversion polymorphism is well documented in *D. ananassae* (Singh, 1998, 2001; Singh and Singh, 2007). Enzyme polymorphism in *D. ananassae* has also been studied to some extent. Kumar and Singh (2012) observed Xanthine dehydrogenase polymorphism in this species and reported four alleles represented by the *Xdh* locus. Alcohol dehydrogenase (*Adh*) locus has also been shown to be polymorphic in this species (Singh, Kumar and Bhumika, 2013), and on the basis of electrophoretic mobility, two variants, *i.e*., slow and fast, have been found. Esterases (3.1.1.1) are classified as hydrolases, a large and diverse group of enzymes that catalyze the hydrolysis of a wide range of aliphatic and aromatic esters, choline esters, and organophosphorous compounds (Dauterman, 1985). Esterases act on molecules that are completely dissolved in water, hydrolyzing carboxylesterases into alcohol and carboxylate. Over thirty carboxylester hydrolases have been identified in *D. melanogaster* and most are identified as acetyl carboxyl or cholinesterases. About 22 soluble esterase isozymes have been detected by native polyacryamide gel electrophoresis, and more could be resolved if 2-D gel electrophoresis was applied (Oakeshott *et al.*, 1993).

Esterases are highly polymorphic in the genus *Drosophila* (Powell, 1975; Oakeshott *et al.*, 1993). In the present study, Esterase banding pattern of *D. ananassae* was identified using native polyacrylamide gel electrophoresis. In native PAGE α-naphthylacetate is used as substrate and fast blue RR as staining reagent. The *D. ananassae* stock used in this study is GT-ST, a mass culture stock having standard gene arrangement in all the chromosomes derived from flies collected from

Gangtok (India). We have identified *Est-2*, *Est-3*, *Est-4*, and *Est-5* as polymorphic loci. *Est-4* comprises two alleles: active and null.

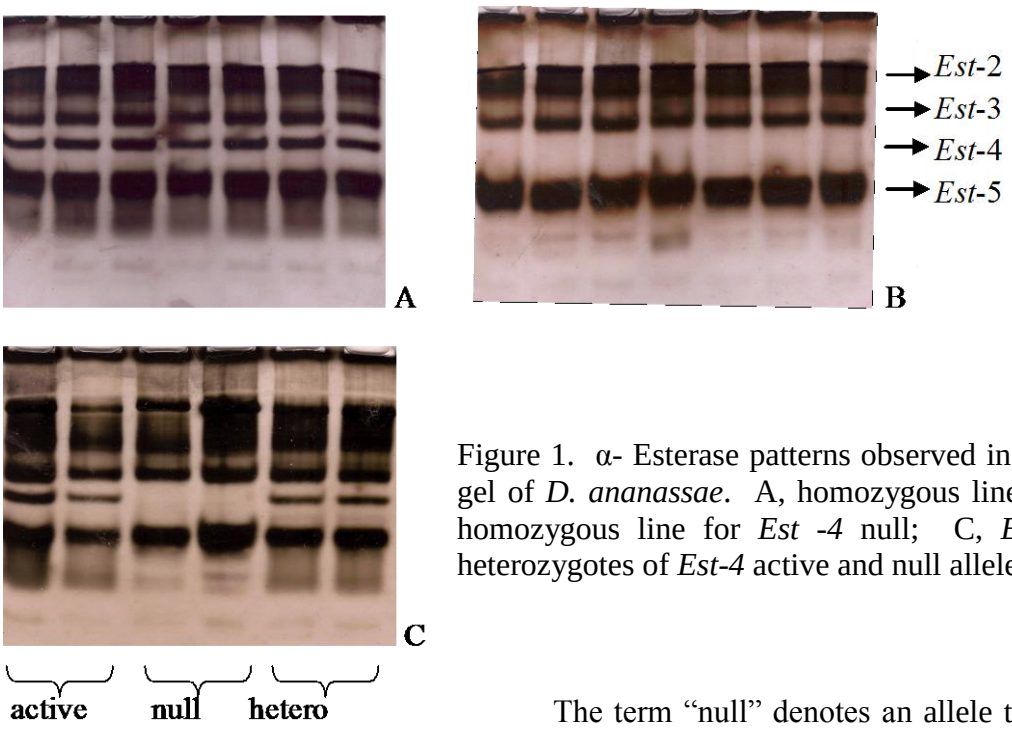

Figure 1. α- Esterase patterns observed in native polyacrylamide gel of *D. ananassae*. A, homozygous line for *Est -4* active; B, homozygous line for *Est -4* null; C, *Est-4* active, null, and heterozygotes of *Est-4* active and null alleles.

The term "null" denotes an allele that specifies a product that shows no catalytic activity as a monomer or heterodimer (depending on the locus) in our *in vitro* gel staining assay.

Survey of electrophoretic variation provided little information about the frequency of the null allele in natural populations. In most surveys null alleles go undetected, and a null-active heterozygote would be indistinguishable from a homozygote for an active allele (Voelker *et al*., 1980). Here, *Est-4* was detected as a polymorphic locus consisting of active and null alleles. Enzyme activity variation is likely to be of more significance as regards fitness than mobility variation, since changes in the activity of enzyme directly affects the biological function of enzymes. We have been able to prepare two separate lines from the GT-ST Stock, one homozygous for *Est-4* active and the other homozygous for *Est-4* null. The two stocks when crossed with each other showed enzyme activity as all the members were of *Est-4* active variant. The two homozygous lines thus prepared and the heterozygous forms derived from their crosses are being analyzed for their role in different aspects of behavior and fitness traits.

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