Table 1. Frequency distribution of the species.

Species	Subgenus	No. ♂	No. ♀
D. melanogaster	*Sophophora*	262	198
D. jambulina	〃	9	7
D. malerkotliana	〃	1	-
D. ananassae	〃	1	-
D. takahashii	〃	72	52
D. nepalensis	〃	8	-
D. immigrans	*Drosophila*	39	77

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References: Bock, I.R., and M.R. Wheeler 1972, Univ. Texas Publ. 7213: 1-102; Gupta, J.P., 1973, Dros. Inf. Serv. 50: 112.

Intra-chromosomal association between allozyme loci in *Drosophila ananassae.*

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Non-random association between gene arrangements was referred to as linkage disequilibrium by Lewontin and Kojima (1960) and according to them this happens due to linkage between such arrangements. Chromosomal polymorphism in association with allozymes has been extensively studied in *Drosophila* (Rodriguez-Trelles, 2003; Iriarte *et al.*, 2002; Rodriguez *et al.*, 2000; Kamping and Delden, 1999; Zapata and Alvarez, 1992). Gametic disequilibrium studies have been done between second chromosome polymorphic arrangements and seven linked loci, in seven populations of *D. buzzatii* from Argentina, and a significant and consistent association for *Est-1*, *Est-2*, *Aldox* and *Xdh* has been reported (Rodriguez *et al.*, 2001). They explained that restriction of recombination in heterokaryotypes seems to be the best explanation for the linkage disequilibrium between inversion and enzyme loci located inside the rearranged segments.

Extensive work on allozyme-allozyme linkage disequilibrium in natural populations of *D. melanogaster* has been done involving 36 allozyme pairs (Langley *et al.*, 1974). Among all those, only three showed significant deviation from expectation and only one of them (*Odh-Ao*) could be established to show non-random association. In another study, Langley *et al*. (1977) found no linkage disequilibrium among even tightly linked loci which were nearly 3 cM distant from each other in natural populations of *D. melanogaster*.

D. ananassae, first described by Doleschall (1858), belongs to the *ananassae* species complex of the ananassae subgroup in the melanogaster species group of the subgenus Sophophora and is one of eight cosmopolitan species (Bock and Wheeler, 1972; Tobari, 1993). It occupies a unique status in genus *Drosophila* due to its several genetic peculiarities (Singh, 2010). One of its peculiar features is that of spontaneous crossing over in males, although at much lower frequencies than observed in females (Tobari, 1993; Singh and Singh, 1988). Recently we have started working on the enzyme polymorphism in this species to see genetic diversity in different natural populations derived from various parts of India (Kumar and Singh, 2012; Singh *et al.*, 2013). While studying enzyme polymorphism, we also planned to see association between those enzyme loci which are situated on the same chromosome. In this note we wish to report intra-chromosomal association among three

enzyme loci, *i.e*., *Xdh*, *Acph 1*, and *Acph 2*, present on 2L chromosome arm of *Drosophila ananassae*. The locations of these enzyme loci on polytene chromosomes have been known by sequence analysis from *D. melanogaster* by using BLAST with *D. ananassae* genome sequence (Altschul *et al.*, 1997). The polytene chromosome position of *Xdh* is 28A and *Acph* is 37B on 2L arm (Stephen *et al.*, 2008). The two loci, *i.e*., *Xdh* and *Acph* are approximately 11.5 Mb apart from each other. *Acph 1* and *Acph 2* are clustered together owing to gene duplication during the course of evolution.

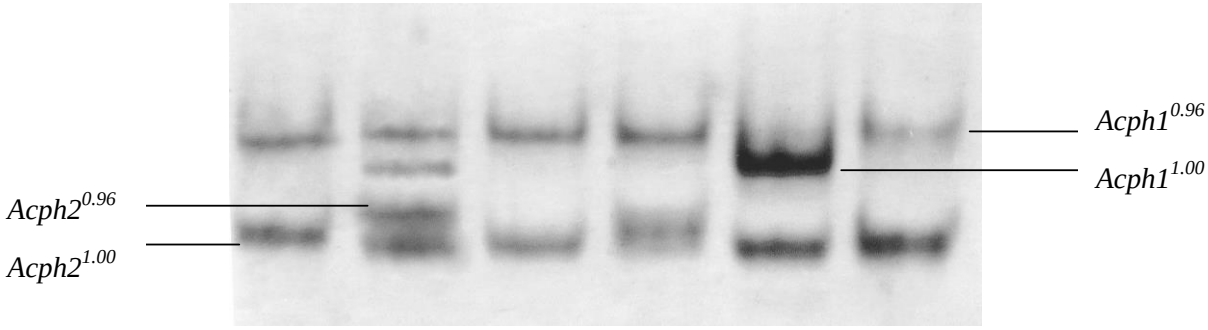

Figure 1. Native polyacrylamide gel of Acid phosphatase enzyme showing two isozyme loci.

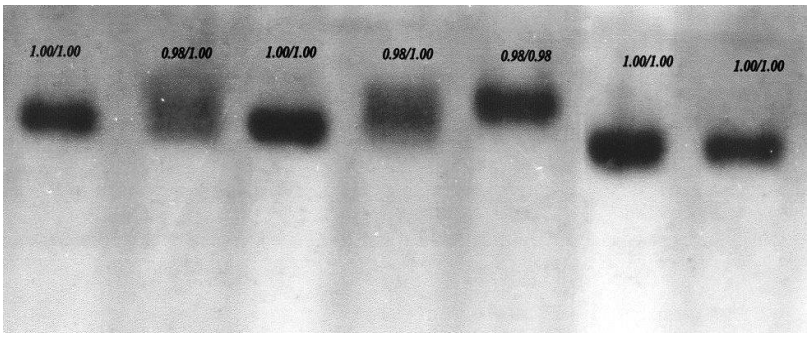

Figure 2. Native polyacrylamide gel showing homozygous $Xdh^{1.00}$, $Xdh^{0.98}$, and heterozygous $Xdh^{0.98/1.00}$ bands for Xanthine dehydrogenase locus.

We are able to record three genotypes for each of the three enzyme loci studied. Since these loci are linked on a single chromosome, intra-chromosomal associations among these three loci can be looked into by seeing the association of genotypes related with two enzyme loci. Due to occurrence of three genotypes for each of the three loci, nine combinations between *Xdh-Acph 1*, *Xdh-Acph 2*, and *Acph1-Acph2* could be ascertained. So far, we have analyzed more than 200 individuals derived from five different natural populations of *D. ananassae* and the trend indicates that there is absence of linkage disequilibrium in all the cases observed so far. The random occurrence of different possible combinations may be due to ample rate of recombination between the enzyme loci and selection playing no role to favor specific association.

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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