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# Evidence for Random Association between Enzyme Loci in Natural Populations and their Mass Culture Stocks of *Drosophila ananassae*

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## Abstract

**Objective:** The major objective of this study was to see that enzyme loci located on the same chromosome (intra chromosomal) or different chromosomes (interchromosomal) exist in equilibrium or not.

**Material and methods:** In gel analysis was performed following native gel electrophoresis to collect data regarding presence of specific enzyme variants. Based on quantitative data on the frequency of different allozyme combinations, intra- and inter-chromosomal associations between such enzyme loci were studied, in natural populations as well as their mass culture stocks of *D. ananassae*.

**Results:** In natural populations of this species, out of 195 allozyme loci comparisons, non-random associations among different genotypic combinations existed in only four comparisons (2.05%), indicating that these enzyme loci exist in equilibrium. Almost similar results were observed for mass culture stocks, except that the nonrandom cases in mass culture socks were slightly higher (6.67%).

**Conclusion:** The results of this investigation clearly denote that intra- and inter-chromosomal associations between different enzyme loci exist in linkage equilibrium in natural populations as well as in mass culture stocks of *D. ananassae*. The most probable reason for this random association is due to substantial amount of crossing over between the linked enzyme loci and reasonable survival of all possible combinations.

**Keywords:** *Drosophila ananassae*; Linkage disequilibrium; Intra-chromosomal associations; Inter-chromosomal associations; Enzyme loci

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## Introduction

The occurrence of linkage disequilibrium is attributed to differential selection, suppression of crossing over, genetic drift and genetic hitch-hiking. The phenomenon of linkage disequilibrium has been convincingly studied in different species of *Drosophila*. For this purpose chromosome markers like linked inversions, enzyme loci and inversions and segments of DNA and SNPs have been considered. Linkage disequilibrium may be an indicator of genetic drift in natural populations [1,2]. However, Lewontin emphasized on the exclusive role of natural selection in the occurrence of linkage disequilibrium [3]. A number of studies have been conducted in *Drosophila* to detect linkage disequilibrium between enzyme loci, considering that if selection really works, then one might expect linkage disequilibrium to occur. Allozyme-allozyme linkage disequilibrium in natural populations of *D. melanogaster* was studied [4] and he reported no linkage disequilibrium among even tightly linked loci. However, contrasting results in this regard have been documented between loosely linked allozyme loci in laboratory populations of *D. melanogaster* [5]. Japanese populations of *D. melanogaster* for enzyme and chromosomal polymorphisms [6]. They compared allelic frequencies of *Adh* and *α-Gpdh* loci with frequencies of polymorphic inversion InC(2L)B, In(2H)C of the second chromosome and found a significant positive correlation between the frequencies of *Adh S* and In(2L)B, which results due

 

to linkage. They also studied inversion-free populations of this species which were maintained in the laboratory conditions for several generations and reported considerable variation in the frequencies of *Adh* and *α-Gpd* alleles likely to be the consequence of random genetic drift. Studies on linkage disequilibrium between enzyme loci indicate absence of linkage disequilibrium among allozyme loci [4,7,8].

Chromosomal polymorphism particularly inversion polymorphism in association with allozymes has been extensively studied in *Drosophila* [9-13]. Gametic disequilibrium studies were done [14] between second chromosome polymorphic arrangements and seven linked loci, in seven populations of *D. buzzatii* from Argentina who found significant and consistent associations across populations for *Est-1, Est-2, Aldox* and *Xdh*

Norman and Prakash [15] chose *D. persimilis* to test the inter-allelic associations between amylase locus and naturally occurring inversions of its third chromosome. They reported that *Amy1.09* allele occur at a high frequency with whitney (WT) arrangement whereas other common gene arrangements with *Amy1.00*. Non-random association between allozyme loci *Mdh-2*, *Lo-5* and gene arrangements Ui+a+s and Ur+a in natural population of Ttibingen in *D. subobscura* was observed [16]. Similar studies performed by Rodriguez et al. [14] and Barker et el. [17] in *D. buzzatii* for chromosomal arrangements and linked allozyme loci revealed linkage disequilibrium between inversions and enzyme loci. Rodriguez et al. [14] explained that restriction of recombination in heterokaryotypes could be the best reason for linkage disequilibrium between inversion and the enzyme loci located inside the rearranged segments.

*D. ananassae* is one of the prevalently occurring *Drosophila* species in India. Its population genetical studies on chromosomal (inversion) and allozyme polymorphism have been one of the major areas of investigation by Indian workers [18-23]. Allozyme studies involving 12 enzyme loci have revealed that Indian natural populations of this species are sub-structured and there is a clinal variation in the distribution of frequency of these enzyme loci [24]. In this paper, we are reporting intra and interchromosomal associations between linked and unlinked enzyme loci to test linkage disequilibrium in *D. ananassae*

## Material and Methods

*D. ananassae* flies were sampled from fifteen different geographical localities of India **(Figure 1)**. **Table 1** shows the places, their abbreviations and time of collection of flies. Individual flies of these places were analyzed for their in-gel assay to collect frequency of different allelic forms of twelve enzyme loci. Flies collected from a place when pooled together, formed the mass culture stock and therefore each natural population was also maintained as mass culture stock. Electrophoretic studies of these mass culture stocks were also performed when they have completed at least 25 generations in the laboratory. These stocks were maintained in the laboratory on simple yeast-agar culture medium at 24 ± 1°C with 12-hour cycle of light-dark period. To study allozyme variations at 12 enzyme loci, a single fly was homogenized in 50 µl 20 mM Tris buffer (pH 7.4) and

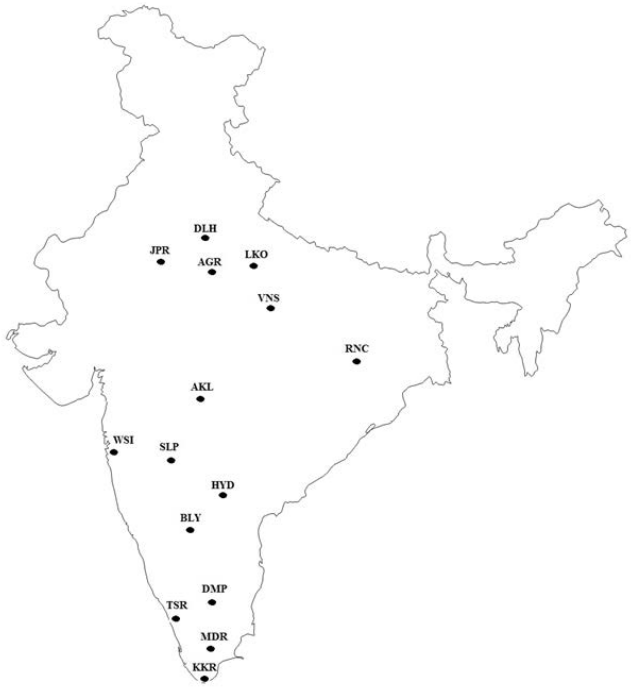


**Figure 1** *D. ananassae* flies sampled from fifteen different geographical localities of India.

**Table 1** The geographical localities, their abbreviations and time of collection of *D. ananassae*.

| Populations | Abbreviation | Time of collection |
|---|---|---|
| Kanniyakumari | KKR | JAN 2013 |
| Madurai | MDR | JAN 2013 |
| Thrissur | TSR | JAN 2013 |
| Dharmapuri | DMP | JAN 2013 |
| Bellary | BLY | JAN 2013 |
| Hyderabad | HYD | JAN 2013 |
| Solapur | SLP | OCT 2012 |
| Washi | WSI | OCT 2012 |
| Akola | AKL | OCT 2012 |
| Ranchi | RNC | OCT 2011 |
| Varanasi | VNS | JUL 2012 |
| Lucknow | LKO | APR 2012 |
| Jaipur | JPR | AUG 2012 |
| Agra | AGR | AUG 2012 |
| Delhi | DLH | AUG 2012 |

the homogenate was centrifuged at 12000 rpm at 4°C for 10 minutes. Supernatant was separated and subjected to 8% native polyacrylamide gel electrophoresis in 25 mM Tris and 250 mM Glycine electrode buffer (pH 8.2) at 200 V for 4-hour at 4°C. In-gel staining for specific enzyme was done [25,26]. The locus and allele designations were done following the standardized genetic nomenclature for enzyme coding loci [27].

Quantitative data on the frequencies of different genotype combinations were analyzed to obtain the number of various intra- and inter-chromosomal associations in the fifteen natural populations and their mass culture stocks of *D. ananassae*. Under

the assumption of random combination of genotypes, their expected numbers were calculated from the marginal totals of R × C contingency table. Since each locus was expressed in three genotypic forms, in total, 9 genotypic combinations for each enzyme pair could be ascertained.

In the polytene chromosomes map of *D. ananassae*, four enzyme loci (*Est, Xdh, Me* and *Acph*) have been found to be present on the left arm of second chromosome (2L), whereas, loci of remaining three enzymes (*Mdh, Aph* and *Ao*) are represented on the right arm of third chromosome (3R) **(Figure 2)**.

Since thirteen enzyme loci pairing will be possible for each population, in total 195 (13 × 15) combinations were analyzed for intra and inter-chromosomal enzyme loci associations in natural populations as well as their mass culture stocks of *D. ananassae*. Out of 13 enzyme-enzyme pairing, 12 such pairing was observed for intra-chromosomal associations and only one pairing was studied for inter-chromosomal associations.

## Results and Discussion

Intra chromosomal associations between enzyme loci of left arm of 2nd chromosome (2L) and right arm of 3rd chromosome (3R) and inter-chromosomal associations between enzyme loci of 2L-3R were tested for natural populations and their mass culture stocks of *D. ananassae*. In total, 195 combinations (13 enzyme loci pairs × 15 populations) were checked for deviation from randomness through R × C contingency table for all genotypic combinations. **Table 2** incorporates the values of Chi-square for detection of intra- and inter-chromosomal associations in fifteen natural populations of *D. ananassae*. **Table 3** shows chi-square values obtained after testing intra and inter-chromosomal associations in fifteen mass culture stocks of *D. ananassae*.

The results showed significant deviation from randomness for *Acph1/Acph2* in DMP (17.0) mass culture stocks. *Acph1/Xdh* combination showed significant deviation in HYD and WSI mass culture stocks. *Aph2/*Aph*3* in LKO (11.31) mass culture stock showed nonrandom associations. AKL (11.03), AGR (10.03) and DLH (10.63) showed nonrandom associations for *Ao2/Aph2* gene combination. In BLY mass culture stock, Aph3 showed nonrandom associations with both gene loci of *Ao* (*Ao1* and *Ao2*). *Est3/Est5* gene combinations were found to deviate significantly from randomness in three mass culture stocks, i.e., KKR, VNS and DLH. *Mdh/Me* gene combinations showed nonrandom associations only in the TSR mass culture stock.

Linkage disequilibrium between alleles of two genes arises when any particular combination of alleles has an adaptive superiority over the other allelic combinations of the genes. It is also likely, when two loci are tightly linked with each other and there is very low or no recombination between them. Another phenomenon which may also be responsible for linkage disequilibrium is random genetic drift, whereby certain combinations of alleles become more frequent in a population owing to a bottleneck effect. Therefore, linkage disequilibrium is a result of strong physical linkage, natural selection or random genetic drift. While the contribution of a strong physical linkage may not be ruled out, no two genes are so close enough that the possibility of recombination between them is absolutely zero. Indeed, recombination is even known to occur within a gene and as suggested [28], there is no correlation between linkage and magnitude of linkage disequilibrium.

In this study, intra chromosomal associations between enzyme loci of 2L (*Est, Xdh* and *Acph*) and 3R (*Aph* and *Ao*) and inter chromosomal associations between enzyme loci of 2L and 3R (*Me* and *Mdh*) were studied in natural populations as well as in mass culture stocks of *Drosophila ananassae*. In natural populations, out of 195 allozyme loci comparisons, non-random

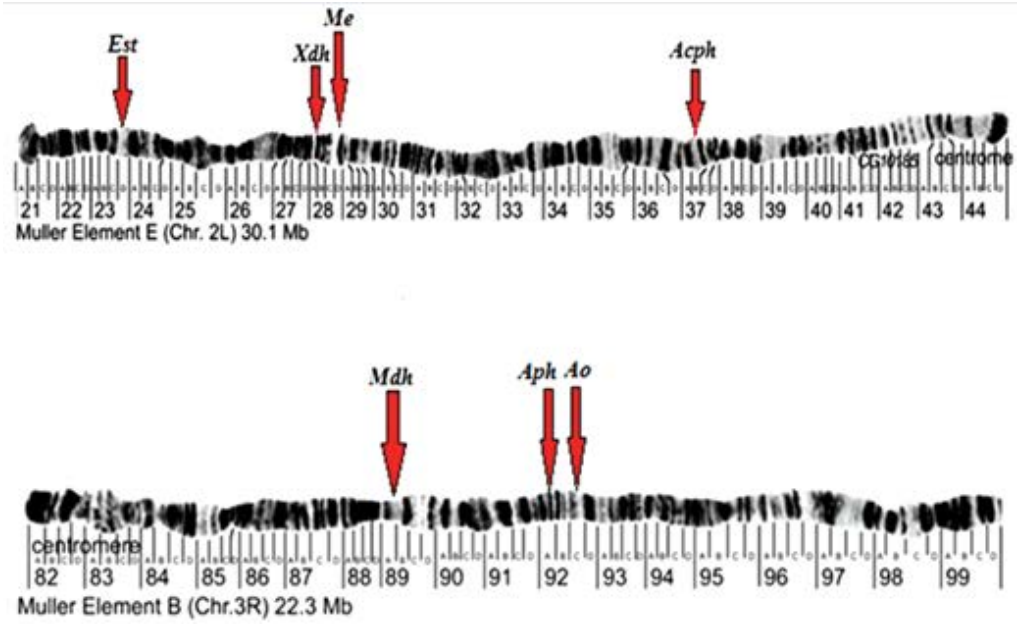


**Figure 2** The polytene chromosome position of seven enzymes on 2L and 3R arms of *D. ananassae.*

**Table 2** Chi-square values for detection of inter- and intra-chromosomal associations in fifteen natural populations of *D. ananassae*.

| Populations | *Acph1/ Acph2* | *Acph1/ Xdh* | *Acph2/ Xdh* | *Aph2/ Aph3* | *Aph2/ Ao1* | *Aph2/ Ao2* | *Aph3/ Ao1* | *Aph3/ Ao2* | *Ao1/ Ao2* | *Est2/ Est3* | *Est2/ Est5* | *Est3/ Est5* | *Mdh/ Me* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| KKR | 6.53 | 1.89 | 0.82 | 2.08 | 3.67 | 1.36 | 1.84 | 3.83 | 2.22 | 6.16 | 7.73 | 1.71 | 5.82 |
| MDR | 2.96 | 8.09 | 2.86 | 0.82 | 0.77 | 1.89 | 4.6 | 5.73 | 3.56 | 5.45 | 8.08 | 1.51 | 1.06 |
| TSR | 5.003 | 0.305 | 3.32 | 1 | 1.88 | 6.37 | 3.86 | 1.4 | 2.39 | 5.29 | 2.78 | 5.35 | 4.72 |
| DMP | 0.11 | 0.55 | 5.51 | 7.26 | 3.74 | 3.83 | 4.88 | 3.01 | 2.42 | 3.16 | 4.64 | 0.79 | 6.43 |
| BLY | 3.61 | 2.59 | 6.54 | 4.76 | 2.94 | 0.53 | 3.95 | 7.13 | 0.49 | 7.24 | 6.86 | 2.61 | 0.84 |
| HYD | 3.49 | 3.51 | 5.53 | 2.64 | 1.85 | 4.92 | 1.52 | 2.37 | 5.66 | 0.94 | 1.27 | 2.47 | 5.07 |
| SLP | 3.203 | 5.968 | 3.82 | 6.79 | 0.43 | 2.1 | 2.47 | 5.26 | 13.96** | 1.2 | 1.72 | 5.26 | 8.26 |
| WSI | 2.701 | 6.41 | 5.53 | 4.18 | 4.15 | 2.94 | 5.98 | 2.94 | 1.25 | 11.37* | 1.71 | 9.26 | 4.29 |
| AKL | 5.62 | 3.36 | 2.38 | 2.53 | 4.23 | 4.77 | 2.59 | 3.1 | 1.84 | 0.95 | 2.54 | 2.46 | 3.8 |
| RNC | 4.55 | 1.98 | 3 | 1.6 | NA | 1.55 | NA | 1.74 | NA | 1.65 | 5.95 | 1.78 | 6.94 |
| VNS | 2.18 | 0.35 | 2.83 | 1.35 | NA | NA | NA | NA | NA | 6.28 | 0.93 | 2.51 | 3.69 |
| LKO | 6.61 | 2.18 | 1.02 | 2.23 | 4.41 | 9.71* | 9.94* | 4.26 | 6.6 | 1.69 | 3.83 | 7.28 | 5.65 |
| JPR | 0.4 | 5.67 | 3.66 | 7.73 | 2.52 | 7.14 | 3.11 | 2.42 | 3.12 | 2.03 | 5.14 | 1.1 | 7.02 |
| AGR | 6.71 | 1.27 | 3.88 | 6.46 | NA | 5.5 | NA | 2.36 | NA | 2.05 | 3.52 | 8.66 | 1.67 |
| DLH | 6.45 | 5.68 | 1.02 | 5.73 | 3.2 | 0.76 | 4.81 | 3.28 | 6.2 | 4.29 | 3.69 | 8.7 | 4.98 |

*P<0.05; **P<0.001; NA-not applicable

**Table 3** Chi-square for detection of intra- and inter-chromosomal associations in fifteen mass culture stocks of *D. ananassae*.

| Populations | *Acph1/ Acph2* | *Acph1/ Xdh* | *Acph2/ Xdh* | *Aph2/ Aph3* | *Aph2/ Ao1* | *Aph2/ Ao2* | *Aph3/ Ao1* | *Aph3/ Ao2* | *Ao1/ Ao2* | *Est2/ Est3* | *Est2/ Est5* | *Est3/ Est5* | *Mdh/ Me* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| KKR | 5.52 | 0.83 | 1.25 | 1.12 | 2.0 | 4.19 | 5.05 | 0.92 | 1.99 | 6.51 | 4.29 | 14.9** | 6.29 |
| MDR | 3.08 | NA | NA | 0.89 | NA | 3.93 | NA | 6.93 | NA | 4.17 | 2.8 | 1.27 | 6.36 |
| TSR | 1.35 | 4.37 | 6.21 | 3.65 | 1.75 | 4.58 | 4.64 | 0.39 | 3.29 | 4.96 | 6.82 | 7.57 | 13.49** |
| DMP | 17.0** | 3.64 | 5.08 | 3.14 | NA | 3.61 | NA | 4.09 | NA | 0.55 | NA | NA | 1.45 |
| BLY | 0.36 | 4.37 | 2.84 | 1.2 | 5.05 | 2.15 | 9.89* | 10.67* | 8.57 | 2.39 | 7.8 | 4.18 | 1.45 |
| HYD | 4.51 | 16.73** | 3.04 | 1.31 | NA | 1.52 | NA | 0.98 | NA | NA | NA | 4.93 | NA |
| SLP | 9.45 | NA | NA | 5.15 | 8.11 | 2.96 | 3.39 | NA | 6.29 | 4.35 | 4.68 | 3.47 | NA |
| WSI | 4.63 | 10.05* | 7.82 | 4.36 | 3.7 | NA | 5.47 | NA | NA | 2.44 | NA | NA | NA |
| AKL | 2.4 | 0.85 | 1.94 | 2.82 | NA | 11.03* | NA | 1.95 | NA | 7.19 | 9.46 | 9.03 | NA |
| RNC | 4.52 | 0.53 | 3.76 | NA | NA | NA | NA | NA | NA | 5.99 | 1.75 | 4.31 | 2.63 |
| VNS | NA | 4.96 | NA | 2.75 | 3.34 | 4.49 | 1.18 | 3.15 | 3.53 | 3.6 | 8.72 | 14.32** | NA |
| LKO | 5.11 | 0.83 | 4.14 | 11.31* | 7.01 | 3.51 | 5.64 | 6.9 | 5.37 | 8.41 | 2.7 | 3.3 | 6.38 |
| JPR | 3.24 | 4.33 | 3.1 | 2.77 | NA | NA | NA | NA | NA | 13.85 | 0.99 | 6.83 | 3.85 |
| AGR | 5.15 | 5.33 | 1.07 | NA | 3.09 | 10.61* | NA | NA | 8.19 | 0.77 | 5.72 | 2.51 | 3.05 |
| DLH | 7.58 | 5 | 3.9 | 2.62 | 6.63 | 10.63* | 8.39 | 3.89 | 7.74 | 3.16 | 4.74 | 15.40** | NA |

*P<0.05; **P<0.001; NA-not applicable

associations of different genotypic combinations could be observed in four allozyme loci comparisons. Inter chromosomal enzyme loci (*Me-Mdh*) also showed random associations of different genotypic combinations. It is therefore evident that intra and inter chromosomal associations of different enzyme loci exist in equilibrium in natural populations. Study conducted for inter-chromosomal associations between enzyme loci of 2L and 3R also revealed similar results. The occurrence of random distribution of all possible genotypic combinations in natural populations and mass culture stocks might be due to substantial amount of crossing over between the concerned enzyme loci and equivalent adaptive importance of all possible combinations. Intra- and inter chromosomal association studies reveal random occurrence of different genotypic combinations in most of the cases in natural as well as laboratory populations demonstrating that there is free recombination between the different pairs of enzyme loci considered and every combination has equal adaptive importance. Hence different genotypic combinations have almost equal representation in the population. Further, selections acting on different enzyme loci are independent of each other.

When we test association between loci, it is important to realize that random mating does not absolutely restore the population to equilibrium in each generation. It is rather recombination and random assortment that push genotype frequencies towards equilibrium. While the presence of linkage disequilibrium may be attributed to physical linkage, its absence as found in our study is testimony to the fact that there is free recombination in appreciable frequency between the concerned loci. [18], studied linkage association between different karyotypic combinations in *D. ananassae* populations and reported linkage disequilibrium to exist more in laboratory populations as compared to natural

populations. This was attributed to the enhanced role of drift in the laboratory populations.

Similar study conducted with mass culture stocks also show lack of intra and inter chromosomal associations between different enzyme loci. However, in mass culture stocks, out of 195 comparisons, 13 showed non-random distribution of genotypic combinations. The occurrence of more cases of non-random associations in mass culture stocks may be due to random genetic drift. The incidence of random distribution of all possible genotypic combinations in both natural and laboratory populations (for intra and inter chromosomal associations) might be due to substantial amount of crossing over between the concerned enzyme loci and equal survival of all possible combinations. *D. ananassae* is a species which exhibits some unique features, one of them being spontaneous male meiotic recombination [19]. Thus, in *D. ananassae* the recombination rate is higher than the other species of the *Drosophila*, where males are not known to show recombination during meiosis. This may be one of the reasons for the absence of linkage disequilibrium between the concerned enzyme loci in the present study.

During the study on genetic differentiation among the natural populations of this species, we could record higher frequency of homozygotes than heterozygotes and the observed heterozygotes always remained less than their respective expected heterozygotes. It's quite likely that individuals with homozygosity will show higher frequency of crossing over producing gametes of all expected combinations. Thus occurrence of different genotypic combinations in equilibrium is fairly possible in natural populations. Non-random occurrence of different gene combinations for inter-chromosomal associations in natural populations might be due to independent assortment of genes.

Similar study has been undertaken [29] to detect linkage disequilibrium between allozyme loci by including thirty six allozyme pairs in natural populations of *D. melanogaster*. Their results indicate the existence of random associations between the loci. In another study conducted by same group of workers [30] in natural populations of *D. melanogaster* absence of linkage disequilibrium has been reported even between those loci which were tightly linked. The present study also supports the findings [30] as the different genotypic combinations occur randomly for intra as well as interchromosomal enzyme loci. Presence of few non-random associations in some of the laboratory populations also support the earlier work [5] who, found linkage disequilibrium between loosely linked allozyme loci in laboratory populations of *D. melanogaster*. Chromosomal and allozyme associations have been detected by many workers in different species of *Drosophila* [6,9-13]. Rodriguez et al. [14] studied linkage disequilibrium between second chromosome polymorphic arrangements and seven linked loci, in seven populations of *D. buzzatii* from Argentina and found significant and consistent associations across populations for *Est-1, Est-2, Aldox* and *Xdh*. They opined that restriction of recombination between inversion heterokaryotypes could be the main reason for the occurrence of linkage disequilibrium between inversion and enzyme loci located inside the rearranged segments. However, epistatic interactions between *Xdh* (located outside of the inversion) and loci tightly linked to inversions could be the most likely explanation for the association between *Xdh* and chromosomal inversions.

## Conclusion

The results of present study indicate that there is random association between the linked enzyme loci situated on 2L and 3R chromosomes of *D. ananassae*. The occurrence of random distribution of all possible genotypic combinations in both natural populations and their mass culture stocks of *D. ananassae* may be due to considerable amount of recombination between the concerned enzyme loci and also survival of all possible combinations. Further, enzyme loci situated on different chromosomes (inter-chromosomal) also existed in random association, demonstrating that the unlinked loci assort independently and the various recombinant types have fair chance of survival.

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